ENERGY IMBALANCE AND LUNG DAMAGE IN CYSTIC FIBROSIS

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Contents

1. Acknowledgements

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2. List of original papers

This thesis is based on the following papers:

I. Dorlochter L, Roksund O, Helgheim V, Rosendahl K, Fluge G. Resting energy expenditure and lung disease in cystic fibrosis. J Cyst Fibrosis 2002;1:131-136.

II. Dorlochter L, Nes H, Fluge G, Rosendahl K. High resolution CT in cystic fibrosis--the contribution of expiratory scans. Eur J Radiol 2003;47:193-198.

III. Dorlochter L, Helgheim V, Roksund OD, Rosendahl K, Fluge G. Shwachman-Kulczycki score and resting energy expenditure in cystic fibrosis. J Cyst Fibrosis 2003;2:148-151.

IV. Dorlochter L, Aksnes L, Fluge G. Faecal elastase-1 and fat-soluble vitamin profiles in patients with cystic fibrosis in Western Norway. Eur J Nutr. 2002;41:148-152.

3. Abbreviations

BIA	Bio-electrical impedance analysis
BMD	Bone mineral density
CCR	Conventional chest X-ray
CFCS	Cystic Fibrosis Clinical Score
CFRDM	CF-related diabetes mellitus
CFTR	Cystic-fibrosis-transmembrane-conductance-regulator
DEXA	Dual-energy X-ray absorptiometry
DHA	Docosahexaenoic acid (omega-3 fatty acid)
DIOS	Distal intestinal obstruction syndrome
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid (omega-3 fatty acid)
GER	Gastroesofageal reflux
FE-1	(Human) Faecal elastase-1
FEV_1	Forced expiratory volume in one second (percent of predicted)
FVC	Forced vital capacity (percent of predicted)
HRCT	High-resolution computer tomography
IC	Indirect calorimetry
MBW	Multiple breath inert gas washout
NIH score	National Institutes of Health score
PEG	Percutaneous endoscopic gastrostomy
PFT	Pulmonary function tests
PI	Exocrine pancreatic insufficiency
PS	Sufficient exocrine pancreatic function
PSA	Pseudomonas aeruginosa
REE	Resting energy expenditure (percent of predicted)
SK score	Shwachman-Kulczycki score

4. Introduction

4.1 Background

Cystic fibrosis (CF) is the most common life-limiting autosomal-recessive disease in Caucasians with a prevalence of 1:2500 - 3500 living birth in most European countries and the United States (1;2). Approximately 250 patients with CF are living in Norway today and 50% are older than 18 years.

CF was distinguished from celiac disease and recognised as an own entity in 1938 (3). DiSant' Agnese and colleagues identified elevated sweat chloride and sodium concentrations to be the major tool in diagnosing CF in the early fifties, and since then, the sweat iontophoresis has been a cornerstone of diagnosis (4). Successful identification of the CF gene was accomplished in 1989 and localised on chromosome 7 (5-7). The CF gene encodes a large, single-chain protein that forms a membrane bound chloride channel, the cystic-fibrosis-transmembrane-conductance-regulator (CFTR). More than 1200 different mutations have been found so far (8). However, approximately 70% of all CF patients are homozygous for the ΔF508-mutation (9). Mutations of the CF gene can either result in reduced amount of CFTR protein, transport hindrance of the protein to the plasma membrane or chloride channel dysfunction (10). Although these mutations affect epithelial ion and water transport through cell membranes of exocrine organs, the phenotypic expression varies greatly.

Basically, the impaired epithelial ion and water transport result in mucus of higher viscosity. This viscous mucus is accumulating in excretory ducts of the organs that express CFTR in particular, the respiratory, the gastrointestinal, the hepatobiliary and the reproductive tract. Mucus accumulation associated with progressive obstruction leads to subsequent destruction of these organs.

Fatty diarrhoea and failure to thrive in combination with therapy resistant productive cough is characteristically prominent in children. The classic “diagnostic triad” consists of abnormal sweat chloride concentration (>60 mmol/L) and pulmonary and exocrine pancreatic disease. A sibling or first cousin with CF as well as identification of two CFTR mutations are other important indicators of CF (11). Transepithelial

nasal potential difference measurements are useful in assisting in the diagnosis of atypical CF; however, this diagnostic approach is not available in Norway. Recent reports indicate that computer tomography of the paranasal sinuses may give additional information in cases of diagnostic uncertainty (12;13).
In the longer run repetitive bacterial lung infections, progressive bronchiectasis, decreasing alveolar gas exchange and malnutrition are responsible for high morbidity and mortality among CF patients (14-16). Early in life, patients with CF become infected with a limited spectrum of bacteria, where *Pseudomonas aeruginosa* (PSA) becomes the predominant organism (17). Once chronic (endobronchial) PSA lung infection is established, more rapid deterioration of lung function begins and continues until death. Moreover, CF-related diabetes mellitus (CFRDM) is a frequent complication of CF and is associated with declining pulmonary function and increased mortality (18;19).

There is no cure of the primary defect yet. The major goal is controlling the heterogenic symptoms and preventing complications effectively in order to slow down or halt disease progression. Managing CF is complex and rather challenging for everyone involved; the family in particular, numerous medical staff, different authorities on community level, as well as personnel in Kindergarten and in school. Care of these patients demand almost constant supervision from their direct environment, but they need to be treated as normal children all the same.
Regular airway clearance, treatment of lung infection and nutritional therapy are the cornerstones of CF care. Current therapies consist of mucolytic agents, numerous chest physiotherapy techniques, inhaled bronchodilators, antibiotics and nutrional therapy like high-fat/high-energy diet, supplementation of pancreatic enzymes and essential nutrient supplementation. Aggressive treatment regimes and close patients' surveillance have contributed to improved life quality and expectancy the last decades. The median life expectancy has improved dramatically from six month in 1940, to 18 years in 1976 and to over 30 years these days (20) and is continuously improving. However, gene therapy, as the ultimate solution that addresses the primary chloride channel defect directly, still remains a challenge for the future.

4.2 Surveillance of lung disease

Chronic pulmonary disease is the major cause of morbidity in the majority of patients and more than 90% of all deaths in CF are related to pulmonary disease. Therefore, early detection of damage to the lung parenchyma is of major interest in the management of CF. Traditionally, conventional chest X-ray (CCR) and pulmonary function tests (PFT) play an important role in the follow-up of these patients. Hyperinflation of the lungs and airway obstruction increase as the disease progresses, and forced expiratory volume in one second (FEV_1) is widely used both as follow-up and prognostic factor (21).

Recently, pulmonary high-resolution computed tomography (HRCT) has been advocated as a precise diagnostic method, which gives the opportunity to detect even slight disease progression. HRCT visualises the airway involvement characteristically and makes this procedure superior to CCR for diagnosis and follow-up of patients with CF (22-24). Different systematic HRCT scores have been proposed to classify these findings, of which Bhalla's is the most commonly used (25). HRCT scans confer detailed information of the distribution and severity of CF specific airway and lung parenchymal disease (26;27) and scoring of the pathological findings carry the opportunity to quantify structural lung damage. Moreover, there are promising reports of composite HRCT/PFT scores and quantitative air trapping measurements to be more sensitive in discriminating early or mild CF lung disease (28;29). Lately, multiple breath inert gas washout (MBW) has been advocated as a promising method to evaluate ventilation maldistribution. Using MBW, the sensitivity to detect early CF lung disease in children with CF was higher than traditional spirometry (30-32).

However, the benefit of HRCT examinations has to be weighed up against the radiation dosage when performing HRCT-examinations. Therefore, the decision for pulmonary HRCT scans should be taken for each patient individually when used beyond scientific research.

4.3 Clinical scoring of disease severity

Categorization of clinical scoring has contributed to the success of clinical trials and helped towards a better understanding of this heterogeneous and complex disease. Scoring systems have reduced intuitive subjective assessments and provided a means of quantifying the rate of decline in clinical status (33). Scores have been widely adopted in attempts to standardize recordings of the diverse manifestations of CF in order to alert to any subtle deviation from clinical stability.

There are a number of clinical scoring systems, of which perhaps the best known is the Shwachman score (34). Shwachman and Kulczycki invented this score as early as in 1958, with general activity, physical findings, nutritional status and CCR as main categories. The assessment is semi-quantitative and allows a simple, however rough orientation of the patients general condition (excellent, good, mild, moderate, severe). Other clinical scores like the Doershuk score (35), the Cooperman score (36), the NIH score, also known as Taussig score (37) or the CFCS (38) are considered as more advanced and modern clinical scores compared to the original SK score. Moreover, there are numerous scores, which are based on chest radiograph scores alone, like the Chrispin-Norman score (39), Berner score (40), Brasfield score (41), Wisconsin score (42) and the Northern score (43), and there is still an ongoing debate which of these scores is the most appropriate method to evaluate the clinical condition (44).

However, despite this great variety of scoring alternatives and several limitations of the SK score itself, the SK score is still the most used clinical score to assess and monitor over-all disease severity in patients with CF. Nevertheless, scoring does not necessarily reflect the reality in this complex disease and scores in general have to be used with an awareness of their limitations.

Clinical scoring is no substitute for close clinical appraisal and adequate interpretation of pulmonary function tests and chest imaging methods.

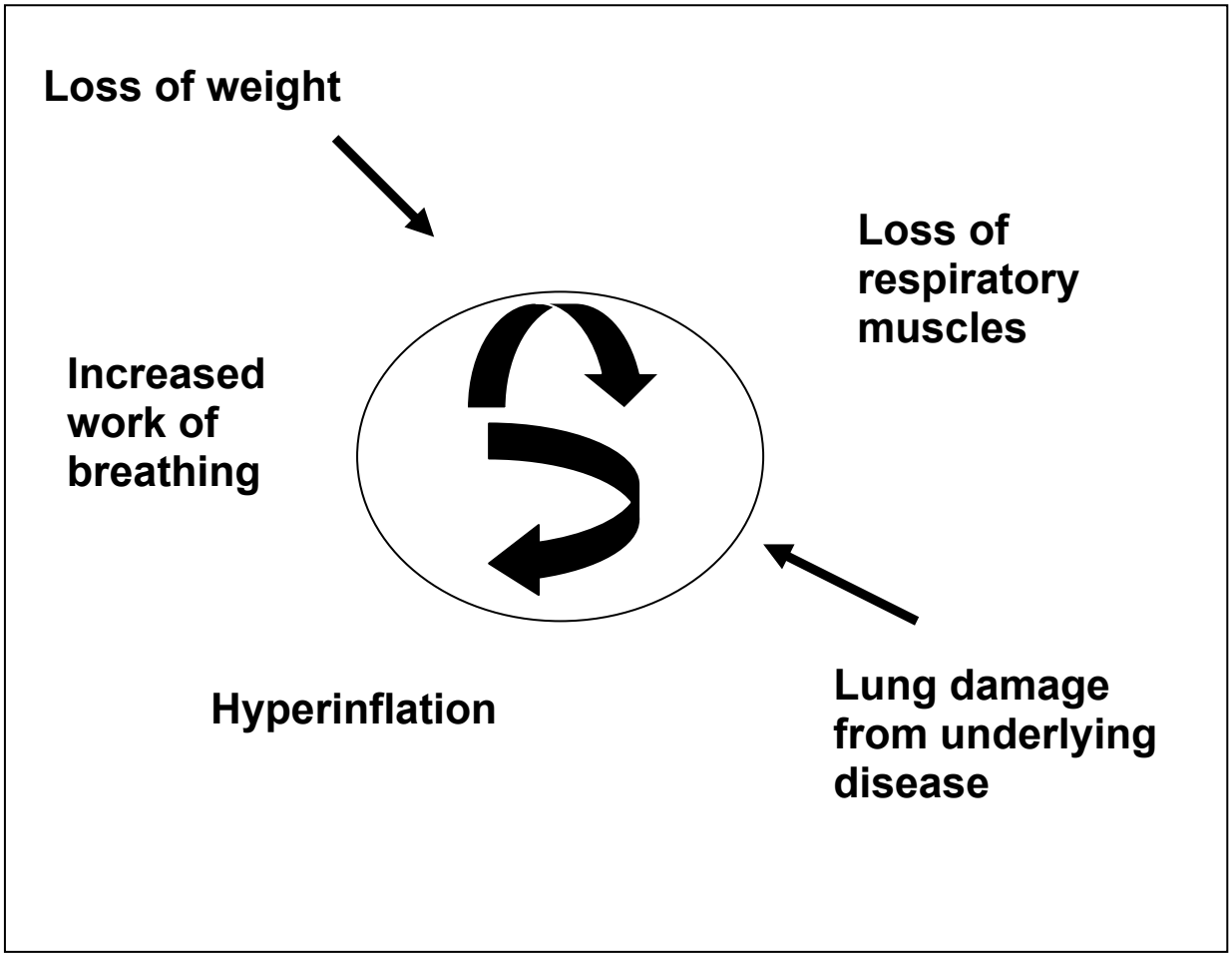
4.4 Energy deficiency

Patients with CF are at high risk for malnutrition, which may result in accelerated progression of the disease and increased morbidity. Therefore, optimal nutrition is an integral part of CF care. Long term follow-up of CF patients has shown significantly better survival in those who achieved normal nutritional status (45;46). During the last 20 years, it has become increasingly clear that nutritional status has an impact on lung disease and lung function in CF patients (47-52). Recently, Peterson and colleagues found that children who weigh more and who gain weight at an appropriate and uninterrupted rate have a better FEV_1 outcome (53).

In addition to increased energy losses secondary to the exocrine pancreatic insufficiency, factors like inappropriate energy intake or elevated resting energy expenditure (REE, basal metabolism) contribute to energy imbalance in CF. Generally, REE accounts for 60-75% of total energy expenditure in humans, leaving 10-25% to physical activity and 10% for diet-induced thermogenesis (54). REE itself can be raised like in CF, leading to higher energy consumption and thus contribute to deterioration of the nutritional status. Respiratory tract inflammation and bacterial lung infection are considered to be the most important factors which contribute to elevated REE in CF.

Schöni and colleagues (55) developed a model to describe the progressive lung destruction in people with CF (Figure 1). Increased cardiac output due to a fixed pulmonary capillary bed and raised work of breathing reflect a hypermetabolic state which results in respiratory muscle fatigue. Shorter time to recover for respiratory muscles is a consequence of hyperinflation, and leads itself to diaphragm fatigue. Malnutrition on the other hand results in loss of muscle mass of the respiratory muscles.

Figure 1: The destructive circle in CF (mod. Schöni 2000)



Therefore, Schöni and co-workers pointed out weight loss and lung damage as crucial components in the pathogenesis of CF lung destruction. Focus of intervention should concentrate on both components as early as possible. Oral nutritional supplementation, feeding through enteral tube or percutaneous endoscopic gastrostomy (PEG) are options that characterize modern, offensive nutritional therapy (56), and they have to be taken into consideration when decrease or any halt in weight gain is present.

4.5 Faecal elastase-1 (FE-1) and fat-soluble vitamins

Steatorrhea is common in patients with CF and reflects a clinical manifestation of maldigestion and malabsorption. By late childhood, up to 90% of all patients have developed exocrine pancreatic insufficiency (PI), which results in massive faecal energy loss and therefore deteriorates nutritional status and energy balance (57). The assessment of maldigestion in patients with CF can be performed by measuring (human) faecal elastase-1. This is a simple, reliable and non-invasive test to evaluate exocrine pancreatic function (58-61). Results are not influenced by enzyme supplementation, and this method is characterized by high sensitivity (>90%) and specificity (99%).

Pancreatic insufficiency, variable degrees of bile salt disturbance and hepatobiliary dysfunction make fat-soluble vitamin deficiency common in CF. Profiles of vitamins A (retinol), D (25-hydroxyvitamin D) and E (alpha-tocopherol) are considered to be helpful in the assessment of gastrointestinal absorption and nutritional status in CF, and therefore analysis of fat-soluble vitamins are performed annually in most CF centres like in ours (62).

Low vitamin A levels are frequent in patients with CF and are associated with disturbed dark adaptation, poorer clinical outcome (63), impaired lung function (64) and ocular surface changes (65). There are reports which demonstrated vitamin A deficiency regardless of exocrine pancreatic function, nutritional status (66), genotype (62) or development of lung disease (67).

Children and young adults with CF may suffer from bone demineralisation, an imbalance between bone deposition and resorption (68;69). Concurrent with the increased lifespan, up to three-quarters of adult patients with CF have osteopenia or osteoporosis (70). Vitamin D deficiency is considered to play an important role in this context and is more likely to be found among patients living in northern latitudes.

The classical symptoms of hypovitaminosis E like haemolytic anaemia and neuropathies are rare in CF. Nevertheless, vitamin E is a powerful antioxidant protecting against oxidative stress which derives from chronic respiratory inflammation. A consequence of vitamin E deficiency is impaired resistance of lipoproteins and cell membranes to oxidation (71). A positive correlation between

plasma vitamin E levels and FEV_1 in patients with CF was recently reported (72). This gives raise to the speculation that early administration of vitamin E may have a role in the protection of lung tissue against oxidative damage. Recently, Koscik and coworkers have shown that prolonged alpha-tocopherol deficiency in infancy is associated with lower cognitive performance (73).

5. Aims of the thesis

1. To assess the impact of lung parenchyma damage (quantified by a HRCT score) on resting energy expenditure in patients with CF and correlate these findings with FEV_1.

2. To examine whether expiratory HRCT scans might add information about the degree of mosaic perfusion in patients with CF and whether this modified HRCT score correlates with pulmonary function.

3. To assess disease severity as evaluated by the Shwachman-Kulczycki score and to correlate these findings with REE and FEV_1 in patients with CF.

4. To evaluate FE-1 values and fat-soluble vitamin profiles in patients with CF, and to correlate exocrine pancreatic function to fat-soluble vitamin profiles. To assess the difference between fat-soluble vitamin profiles in patients with impaired versus patent exocrine pancreatic function. To study, if fat-soluble vitamin deficiency at diagnosis is effectively treated by vitamin supplementation.

6. Material and methods

Haukeland University Hospital serves as a regional centre for CF patients in Western Norway (950 000 citizen). The regional incidence of CF is approximately 1 in 4500 live births (2). This cross sectional cohort study was performed between January 1999 and August 2000 where patients were enrolled consecutively as part of their comprehensive assessment.

6.1 Patients

In 1999, seventy-one patients with CF have been living in the three counties that form the Western region of Norway (Hordaland, Rogaland, Sogn & Fjordane). Adult patients enrolled in this study were followed-up at the Department of Pulmonology, Haukeland University Hospital. Twenty-one adult patients from this region have been followed fully or partly at The National Centre for CF in Oslo according to tradition and were not included in this study. Five adult patients attending our hospital refused to participate in the study. Sixteen patients younger than six years were excluded due to necessity for cooperation under the technical procedures, leaving 29 patients as the basis for this thesis (15 females, median age 13 years).
For paper III, we enrolled our two five year old patients as well, who were able to perform indirect calorimetry. For paper IV, we used retrospective data from the patient charts at diagnosis and longitudinal data from the annually routine follow-up as well in patients below six years.

The CF diagnosis was made by demonstration of repeated elevated sweat chloride concentrations and clinical manifestations compatible with the diagnosis in all patients (Rosenstein 1998). Our patients were screened for the ΔF508-mutation and several other mutations known to occur in our region (del I507, 394delTT, 3659delC, 4005+2t>c, G542X, G551D, N551D, N1303K, R117C, R117H, R553X, W128X). Nine patients were found to be homozygous for ΔF508 (31%); the remaining 20 patients did either have ΔF508/other mutation (8 patients), two other mutations (1 patients), one (4 patients) or no detectable mutation (7 patients).

DNA analysis was unfortunately incomplete in our hospital in 1999/2000. CF patients with unidentified mutations are getting re-evaluated these days at The Centre for Medical Genetics and Molecular Medicine, Haukeland University Hospital in Bergen. A few more mutations have recently been detected among our study patients. We decided therefore to actualise the DNA data presented in this chapter, although the original data in the papers are slightly different concerning DNA analysis. General patient data, characterizing our study population, are shown in table 1.

Table 1: General patient data; mean values, SD. Ranges are shown in parentheses.

	N=29	
Age (years)	16 ± 8,5	(6-40)
Chloride sweat in mmol/L (Eq/L)	101 ± 26	(62-157)
Body mass index (kg/m^2)	17,8 ±3	(12,6-25,8)
Shwachman-Kulczycki score	75,4 ±15,7	(28-95)
FVC – forced vital capacity[#]	95,0 ±21,9	(47-153)
FEV_1 - forced expiratory volume in one second[#]	81,3 ± 24,1	(25-131)

[#]in percent of predicted

Our patients attend the outpatient department every 2nd- 3rd month or more often when needed. Eight patients (28%) have chronic lung infection with *Pseudomonas aeruginosa*, and they are given 12-day courses of intravenous antibiotic treatment every 3rd month.

Twenty-one patients (72%) have been taking pancreatic enzyme supplementation and the amount of lipase was between 5000 and 10000 IE per kilogram bodyweight per day. All patients had received conventional therapy for CF for at least six months and they were in a clinically stable condition. One patient was treated with insulin because of CFRDM. Written informed consent was obtained from patients and/or their families. The regional ethics committee approved our study protocol.

Table 2 categorizes the four parts of our study. Unfortunately, the number of subjects varied between the study parts. Coincidentally, HRCT pictures got lost during the process, or HRCT scans during expiration were forgotten by the technicians. There were some technical problems with the procedures such as insufficient HRCT scan quality.

Table 2: Overview of study designs

	Study I	**Study II**	**Study III**	**Study IV**
Experimental group	20 CF patients 6-34 years	17 CF patients 6-34 years	28 CF patients 5-40 years	35 CF patients 2-40 years
Limitation	9/29 missed [#]	12/29 missed [#]	3/29 missed [#] 2 new recruited	Study enlarged for younger patients
Control group	16 healthy controls 8-33 years		16 healthy controls 8-33 years	
Pulmonary imaging method	HRCT, scored according to modified Bhalla* CCR	HRCT, scored according to modified Helbich* CCR	CCR	
Pulmonary function test	FEV_1	FEV_1	FEV_1	
Resting energy expenditure	IC		IC	
Clinical score	SK score		SK score	
Assessment of maldigestion				FE-1
Assessment of malabsorption				Fat-soluble vitamins

[#] HRCT pictures got lost, scans during expiration were forgotten, technical problems with the procedures and insufficient HRCT scan quality.

* See description of HRCT under Methods

6.2 Methods

6.2.1 High-resolution computer tomography (HRCT)

Pulmonary HRCT was performed (HiSpeed CT, General Electrics, Wisconsin, USA) using 1-mm-thinn sections and 10-mm intervals during inspiration, followed by 1-mm scans at 20-mm intervals during expiration (level and width settings of approximately –600/1500). A paediatric radiologist evaluated the HRCT images without knowledge of the clinical condition, and classified the findings according to a modified scoring system based on Bhalla's and colleagues (25), table 3a. The morphological criteria were based on description of localisation and degree of pathological changes. According to Bhalla we evaluated bronchiectasis, peribronchial thickening, mucus plugging, sacculations, bullae, emphysema and collapse or consolidation. In addition, the presence and degree of mosaic perfusion in inspiration was evaluated according to Helbich and colleagues (74), table 3b. The score ranges from 0-27 points, where 0 represents normal lung parenchyma.

Finally, we evaluated the presence and degree of mosaic perfusion in expiration (patchy lung attenuation resulting from regional differences in lung perfusion), table 3c. Our modified scoring system thus includes eleven items, and the maximum score is 29 (paper II).

Table 3a: Pulmonary HRCT scoring system for CF according to Bhalla (25)

Category	0	1	2	3
Severity of bronchiectasis	Absent	Mild (luminal diameter slightly greater than diameter of adjacent vessel)	Moderate (luminal diameter two to three times the diameter of the vessel)	Severe (luminal diameter more than three times the diameter of the vessel)
Severity of peribronchial wall thickening	Absent	Mild (thickening less or equal to diameter of adjacent vessel)	Moderate (thickening greater than and up to twice the diameter of adjacent vessel)	Severe (thickening more than two times the diameter of adjacent vessel)
Extent of bronchiectasis	Absent	1-5	6-9	> 9
Extent of mucous plugging*	Absent	1-5	6-9	> 9
Extent of sacculations and abscesses*	Absent	1-5	6-9	> 9
Generations of bronchial divisions involved (bronchiectasis or plugging)	Absent	Up to the fourth generation	Up to the fifth generation	Up to the sixth generation and distal
Severity of bullae	Absent	Unilateral (not more than 4)	Bilateral (not more than 4)	More than 4
Severity of emphysema*	Absent	1-5	> 5	
Severity of collapse or consolidation	Absent	Sub segmental	Segmental or lobar	

Table 3b: Hebich`s modification of Bhalla`s scoring system; adding evaluation of inspiratory mosaic perfusion

Category	0	1	2	3
Severity of mosaic perfusion in inspiration*	Absent	1-5	> 5	

Table 3c: Our modification of Bhalla`s scoring system, adding evaluation of both inspiratory and expiratory mosaic perfusion

Category	0	1	2	3
Severity of mosaic perfusion in inspiration*	Absent	1-5	> 5	
Severity of mosaic perfusion in expiration*	Absent	1-5	> 5	

* Numbers are the number of bronchopulmonary segments

6.2.2 Conventional chest X-ray (CCR)

Conventional chest x-ray was performed the same day as pulmonary HRCT (anterior-posterior, 125 kV). The same paediatric radiologist evaluated CCR according to the SK score. CCR-assessment was performed without knowledge of clinical status or pulmonary HRCT findings.

6.2.3 Pulmonary Function Tests (PFT)

Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured by a Vmax 22 spirometer (Sensormedics, Anaheim, USA). The results were compared to a standard reference set (75) and expressed as percent of predicted value.

6.2.4 Shwachman-Kulczycki score (SK score)

The overall clinical condition was evaluated by the Shwachman-Kulczycki score (34), table 4. A maximum of 25 points and a minimum of one point are awarded on a five-point scale to each of the following categories: general activity, physical examination, nutritional status and CCR findings. This results in a global score from 4-100, low values reflect poor clinical status (severe= 40 and below; moderate= 40-55; mild= 56-70; good=71-85; excellent=86-100).

Table 4: The Shwachman-Kulczycki score: Overall clinical evaluation of patients with CF

Grading	Points	General activity	Physical examination	Nutrition	CCR findings
Excellent (86-100)	25	Full normal activity, plays ball, goes to school	Normal; no cough, puls and respiration normal, clear lungs, good posture	Maintains weight and height at > 25th perc., well formed stools; good muscle mass and tone	Clear lung fields
Good (71-85)	20	Lacks endurance and tiers at the end of the day; good school endurance	Resting pulse and respiration normal; rare coughing or clearing of throat; no clubbing, clear lungs; minimal emphysema	Weight and height approx. 15th to 20th perc.; stools slightly abnormal; fair muscle tone and mass	Minimal accentuation of bronchovascular markings, early emphysema
Mild (56-70)	15	May rest voluntarily during the day; tires easily after exertion; fair school attendance	Occasional cough, perhaps in morning upon rising; respiration slightly elevated, mild emphysema, coarse breath sounds, rarely localized rales early clubbing	Weight and height above 3rd perc.; stools usually abnormal, large and poorly formed, very little if any abdominal distension; poor muscle tone with reduced muscle mass	Mild emphysema with patchy atelectasis and increased bronchovascular markings
Moderate (41-55)	10	Home teacher; dyspnoeic after short work; rests a great deal	Frequent cough, usually productive, chest retraction; moderate emphysema; may have chest deformity; rales usually present; clubbing 2 to 3+	Weight and height below 3rd perc.; poorly formed, bulky fatty offensive stools; flabby muscles and reduced mass; abdominal distension mild to moderate	Moderate emphysema; widespread areas of atelectasis with superimposed areas of infection; minimal bronchiectasis
Severe (<40)	5	Orthopnoeic, confined to bed or chair	Severe coughing spells, tachypnoea with tachycardia and extensive pulmonary changes; may show signs of right-sided cardiac failure, clubbing 3 to 4+	Malnutrition marked; large protuberant abdomen; rectal prolapse; large foul frequent, fatty movements	Extensive changes with pulmonary obstructive phenomena and infective lobular atelectasis and bronchiectasis

6.2.5 Indirect Calorimetry (IC)

We performed standard computerised open-circuit indirect calorimetry (Vmax, Sensor Medics, California, USA) after 12 hours fasting, with at least four hours restriction of using β-2 inhalative agents. The patients were placed under the canopy hood in a relaxed supine position. Respiratory gas exchange was monitored for 10 minutes to allow acclimatisation, followed by a subsequent 20-30 minutes measurement period. REE was calculated using the abbreviated Weir formula (76) on a steady state period. The steady state period was defined by a period of at least 5 consecutive minutes where oxygen uptake and ventilation varied by less than 10%, and respiratory quotient varied by less than 5%. We also examined 16 healthy subjects (7 female, median age 12 years, range 8-33 years) with indirect calorimetry in order to verify our predicted values of REE. High REE values represent increased metabolism.

6.2.6 Faecal elastase-1 (FE-1)

FE-1 was measured as part of assessment of the exocrine pancreatic function. FE-1 was determined with a commercial sandwich ELISA kit (ScheBo Tech GmbH, Wettenberg, Germany), which uses two monoclonal antibodies against different specific epitopes of human pancreatic elastase. Measurement of the optical density was performed with Multiscan EX (Labsystems, Espoo, Finland). Concentrations of elastase-1 above 200 µg/g faeces were considered as normal, values between 100-200 µg/g faeces were reflecting moderate exocrine pancreatic insufficiency, and values below 100 µg/g faeces were taken as a sign of severe deficiency.

6.2.7 Fat soluble vitamins

Determination of the serum or plasma levels of retinol (vitamin A), 25-hydroxyvitamin D, and alpha-tocopherol (vitamin E) are the most frequently used parameters to evaluate status of vitamin A, D, and E, and also to assess the gastrointestinal

absorption of the vitamins. This method, described by Aksnes (77), is a simple and sensitive method for simultaneous determination of these vitamins in 0.5 ml human serum or plasma. The vitamins were extracted from serum by methanol/iso-propanol (80/20, v/v) and n-hexane. The n-hexane phase was evaporated and injected to a reversed-phase (C-18) high-performance liquid chromatography system. Elution was performed with methanol/water (85:15, v/v) for 25-hydroxyvitamin D and retinol, and after that by methanol/water (98:2, v/v) for alpha-tocopherol. The eluate was monitored by a UV detector at 265 nm for detection of the vitamins. Baseline separation was obtained for all vitamins, and the system also permitted separate determinations of the D2 and D3 forms of 25-hydroxyvitamin D. The limit of detection and interassay variation for determination in 0.5 ml serum were 6.0 nmol/L and 6.2% for 25-hydroxyvitamin D2, 6 nmol/l and 6.1% for 25-hydroxyvitamin D3, 0.035 μmol/L and 5.0% for retinol, and 1.2 μmol/l and 5.5% for alpha-tocopherol.
Normal range for vitamin A: >200 μg/L, 25-OH vitamin D: 25-130 nmol/L, vitamin E: >5 mg/L.

6.2.8 Statistical methods

Data analysis was performed using a commercially available software package (SPSS). Descriptive statistics included mean, minimum and maximum values, standard deviations and median. Differences in REE between healthy subjects and CF patients were tested using a Student's *t* test. The correlation between HRCT score and REE, HRCT score and FEV_1 and REE and FEV_1 and the correlation between SK score and REE were assessed by the Pearson correlation coefficient. Partial correlation adjusting for chronic *PSA* lung infection was used to assess the impact of *PSA* on the HRCT score and REE. Partial correlation adjusting for age was used to assess the impact of age on the modified HRCT score and FEV1. Due to small number of cases, we firstly decided to limit our adjustment to just one factor, and secondly, we agreed not to run a regression analysis.
Differences in FE-1 between patients with patent or impaired exocrine pancreatic function were tested by Student's *t* test. The relation between fat-soluble vitamins and FE-1 were assessed by the Pearson correlation coefficient. All reported p values are two-tailed and a $p < 0.05$ was considered significant.

7. Summary of the papers

Paper I: Resting energy expenditure and lung disease in cystic fibrosis

Dorlochter L, Roksund O, Helgheim V, Rosendahl K, Fluge G.
J Cyst Fibrosis 2002;1:131-136.

20 patients performed pulmonary high-resolution computed tomography (HRCT) and assessment of resting energy expenditure (REE). HRCT was scored using a standardised method.

The mean HRCT score in our study group was 8,4 ranging from 1 to 22 (median 7,5). Bronchiectasis was the most frequent finding (17 of 20 patients, 85%). Peribronchial thickening was the second most common finding and was found in 15/21 patients (75%). Mucus plugging was present in 14 patients (70%). The mean REE value was 108,4% of predicted vs. 96,5% of predicted of 16 healthy subjects (t= -2,75; 95% CI -20,7 to -3,1; p= 0.009). There was a significant correlation between HRCT score and REE (r=0,661; p= 0.002), HRCT score and FEV_1 (r= -0,851; p< 0.001) and REE and FEV_1 (r= -0,454; p= 0.044). High resting energy expenditure was correlated with high HRCT score (extensive lung damage). After adjusting for chronic *PSA* lung infection, the correlation was slightly less, for REE (r= 0,577; p= 0.01) and for FEV_1 (r= -0,596; p= 0.007), but still significant. These findings confirm a close interaction between lung parenchyma damage and elevated REE in people with CF. Therefore, any increase in REE should raise suspicion of progress in lung impairment.

Paper II: High resolution CT in cystic fibrosis – the contribution of expiratory scans.

Dorlochter L, Nes H, Fluge G, Rosendahl K.
Eur J Radiol 2003;47:193-198.

To examine whether expiratory HRCT scans could add information about the degree of mosaic perfusion in patients with CF, we performed pulmonary HRCT in 17 CF patients both in- and expiratory. HRCT was scored by using a standardised method.

The mean HRCT score was 8,2 (1-21, median 8). 11 out of 17 patients demonstrated a pathological mosaic perfusion in expiration (65%), while only three patients showed mosaic perfusion in inspiration (18%). The degree of expiratory mosaic perfusion was graded as severe in nine patients and moderate in two. There was a significant correlation between our modified HRCT score and lung function as measured by FEV_1 (r= -0,778; p< 0.001). After adjusting for age, the correlation was almost the same (r= -0,735; p= 0.001). Mosaic perfusion in expiration was a common pathological HRCT finding in our study group. It is reasonable to believe that mosaic perfusion which only occurs during expiration represents a lower degree of pathology than mosaic perfusion seen in both in- and expiration. The clinical significance of these findings need further evaluation.

Paper III: The Shwachman-Kulczycki score and resting energy expenditure in cystic fibrosis.

Dorlochter L, Helgheim V, Roksund OD, Rosendahl K, Fluge G.

J Cyst Fibrosis 2003;2:148-151.

We aimed to assess disease severity in 28 patients with CF by evaluating the Shwachman-Kulczycki (SK) score and to correlate these findings with resting energy expenditure (REE). The overall mean SK score for our CF population was 75,3 ± 15,7 (range 28-95), which means that our CF patients on average have had a good general condition. Mean value of REE was 109,1 ± 13,6% of predicted in our CF population. In healthy subjects a mean value of 96,5 ± 8,7% of predicted was found, which was significantly lower compared to the cases (t= -3,3; 95% CI -20,3 to -5,0; p= 0.002). There was a close correlation between the SK score and REE (r= -0.576; p= 0.001), the SK score and FEV_1 (r= 0,796; p< 0.001) and REE and FEV_1 (r= -0,403; p= 0.034). High REE was correlated with a low SK score. After adjusting for chronic *PSA* lung infection, the correlation was slightly less, for REE (r= -0,488; p= 0.01) and for FEV_1 (r= 0,629; p< 0.001), but still significant.

In our younger patients (N= 18, age <16 years) the mean SK score was 80,1 ± 11,2 compared to 66,8 ± 19,5 in patients above 16 years of age (t= 2,3; 95% CI 1,2 to 25,1; p= 0.03). There was no significant correlation between the SK score and REE in our young patients, whereas, a significant correlation of the SK score and FEV_1

was present in this group (r= 0.656; p= 0.003). Furthermore, we have found a close correlation between SK score and pulmonary HRCT, even after adjustment for *PSA* (unpublished data, r= 0,872; p< 0.001). Our results emphasise the clinical value of the SK score, which is easy to assess in a clinical setting.

Paper IV: Faecal elastase-1 and fat-soluble vitamin profiles in patients with cystic fibrosis.

Dorlochter L, Aksnes L, Fluge G.

Eur J Nutr. 2002;41:148-152.

To assess maldigestion and malabsorption, we performed 212 consecutive analyses of fat-soluble vitamin profiles and 35 analyses of FE-1 in 35 patients with CF. In 17/35 patients fat-soluble vitamin profiles were also assessed at diagnosis. Mean values of FE-1 for all CF patients were 256,9 µg/g faeces (SD 445,2; median 24,1). CF patients considered to have maldigestion (N=24) showed a mean value of 19,9 µg/g faeces (SD 15,8; median 18,7), those without pancreas affection had a mean value of 773,9 µg/g faeces (SD 494,4; median 728,9; Z=-4,7; p< 0.001). Vitamin E profiles in patients with exocrine pancreatic insufficiency (PI) was low in 5/9 patients at diagnosis (median 3,6 mg/L), one of these showed additional vitamin A and D deficiency. Two patients with patent exocrine pancreatic function suffered from hypovitaminosis at diagnosis (hypovitaminosis D and combined A/E deficiency). Supplementation of pancreatic enzymes and vitamins normalized profiles in this group at follow-up.

While on appropriate supplementation, there was almost no difference in fat-soluble vitamin profiles regardless pancreatic function. There was no significant correlation between exocrine pancreatic function as measured as FE-1 and fat-soluble vitamin profiles, neither in patients with impaired nor in those with patent pancreatic function.

Severe degree of PI was common in our CF patients. Among 17 patients assessed at diagnosis, 9 had PI. Low values for vitamin E were found in five out of these. Once treated, they became normal. At follow-up, values were mostly in normal range regardless pancreatic function.

8. General discussion

The main aim of the present study was to improve understanding of the complex interaction between energy imbalance and lung damage in CF. Parts of the study focused on disease severity both by scoring lung parenchyma damage and by evaluation of the patients' overall clinical condition, others on energy expenditure in rest as well as parameters of maldigestion and malabsorption. With the recognition of the close link between nutritional status and pulmonary function in CF, treatment and prevention of malnutrition have become a major focus in the modern therapeutic approach of patients with CF (78;79).

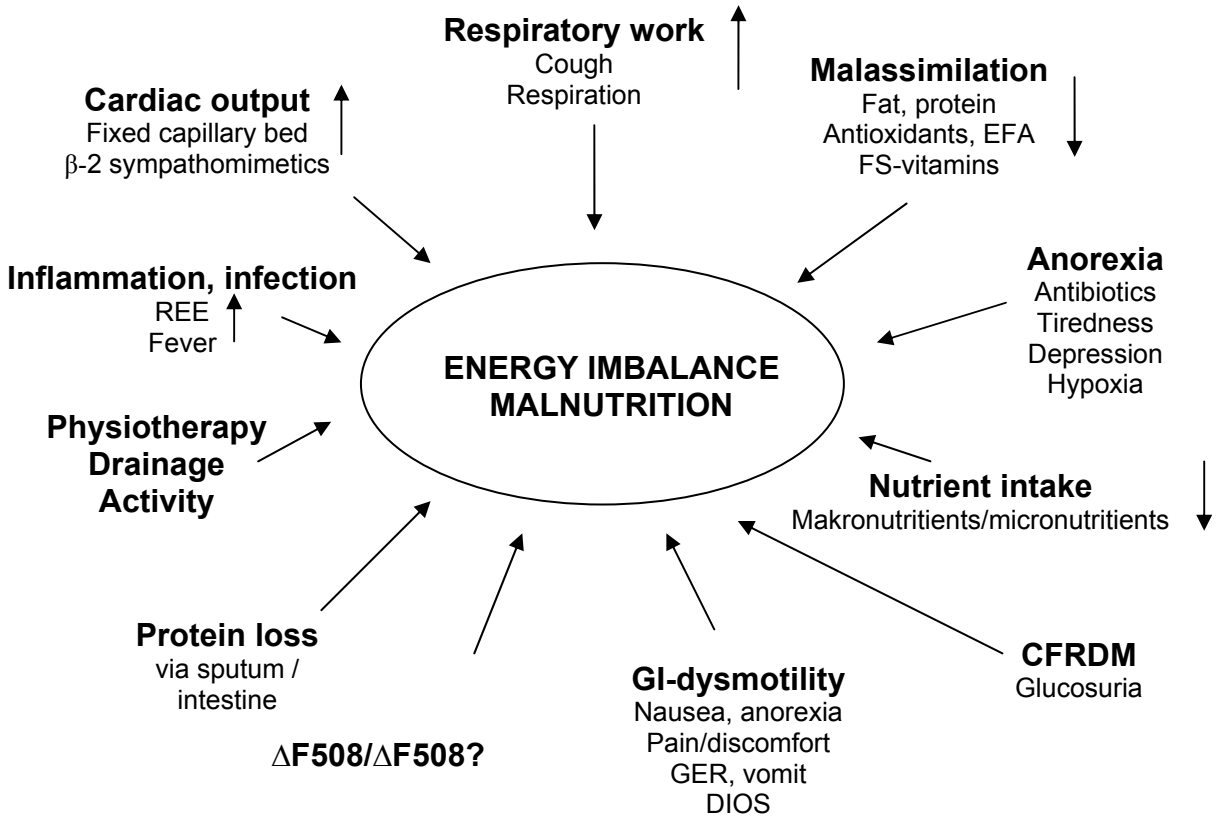
8.1 Energy imbalance

Chronic energy imbalance (long-lasting catabolism) with its clinical manifestation as malnutrition is unfavourable in CF. It deteriorates life quality and is, among other factors, responsible for high morbidity and mortality (45). Wasting has shown to be a predictor of survival (80).

In simple terms, energy imbalance results from a discrepancy between energy intake and energy needs.

Energy losses and elevated energy expenditure are reasons for higher energy needs in CF, which, unfortunately, is not always compensated by appropriate intake of energy. Exocrine pancreatic insufficiency and pulmonary inflammation as well as decreased nutrient intake are considered to be crucial factors in the development of energy imbalance. Therefore it is of great interest to address parameters which directly or indirectly influence energy imbalance in CF. To contribute to better understanding in this context, we focused on different topics of energy imbalance in the present study. Figure 2 is an attempt to provide a survey of these close interactions, placing energy imbalance in the centre. Each factor is dependent on the subjects' current health situation and shows broad variation during course of the disease.

Figure 2: Interdependent factors that influence energy imbalance in cystic fibrosis



Evaluation of energy expenditure in rest was one of our study aims, and we found distinct elevated values in our CF patients. Furthermore, we found close correlations between REE, HRCT score and FEV_1. High REE was correlated with high HRCT score and low FEV_1, which means impaired pulmonary function (extensive lung damage). Fried and colleagues found similar results, a strong correlation between declining pulmonary function and increased REE. Once FEV_1 falls below 75% of predicted values, the REE raises dramatically. In well-nourished CF males with well-preserved lung function there is only little, if any increase in REE (81). However, Girardet et al. demonstrated elevated REE in CF infants free of clinical symptoms (82). Bowler and colleagues found increased REE in CF patients with mild lung involvement as well (83).

Whether the genotype plays a role in this context, remains unclear. Several studies revealed higher REE in patients who are homozygous for ΔF508 (84;85). Recent research has not documented any primary defect of energy metabolism in subjects with CF (86;87). Those of our patients, who were homozygous for ΔF508, showed

higher REE compared to the other patients. However, this difference did not reach statistical significance, probably due to low number of individuals.

Regarding maldigestion, we have experienced FE-1 as a valid tool to discriminate between sufficient and insufficient exocrine pancreatic function. As presented by Walkowiak and colleagues (88), this method is useful in evaluating exocrine pancreatic function over time as well. Because CF patients with patent pancreatic function may be at risk of developing pancreatic insufficiency, they might benefit from longitudinal FE-1 measurements to detect pancreatic deterioration as early as possible. Moreover, there are recent reports that moderate decreased FE-1 values may correlate to residual function of the exocrine pancreas (89;90). Pancreatic enzyme supplementation is traditionally based on clinical symptoms, as well as analysis of faecal fat excretion accompanied by a simultaneous 72-hour fat intake record. Whether enzyme supplementation should be based solely on FE-1 examination, remains to be seen. Lately, Baker and colleagues pointed out, that patients with patent exocrine pancreatic function as well should be thoroughly taken care of in respect to weight gain and malnutrition. They found indeed, surprisingly better growth parameters in patients with PI compared to patients with PS (91;92).

Fat-soluble vitamin deficiency is common in CF (62). Hypovitaminosis D and K in particular, contribute among other factors to the occurrence of bone disease in CF. Due to increased lifespan, this is of growing concern. Evidence is accumulating that vitamin K also plays an important role in maximising and maintaining bone mineral density (70;93;94). Although not implemented in the present study, we have already included analysis of vitamin K in a follow-up study of our cohort.

Vitamins A, D and E given in appropriate dosages, combined with pancreatic enzymes, ensured normal profiles in our patients. Officially recommended supplementation of vitamin A and D in Norway during infancy and childhood may explain why so few patients had vitamin deficiencies at diagnosis. However, due to bad compliance, some patients may have reduced levels, and routine serial monitoring of fat-soluble vitamins in patients with CF are recommended (62).

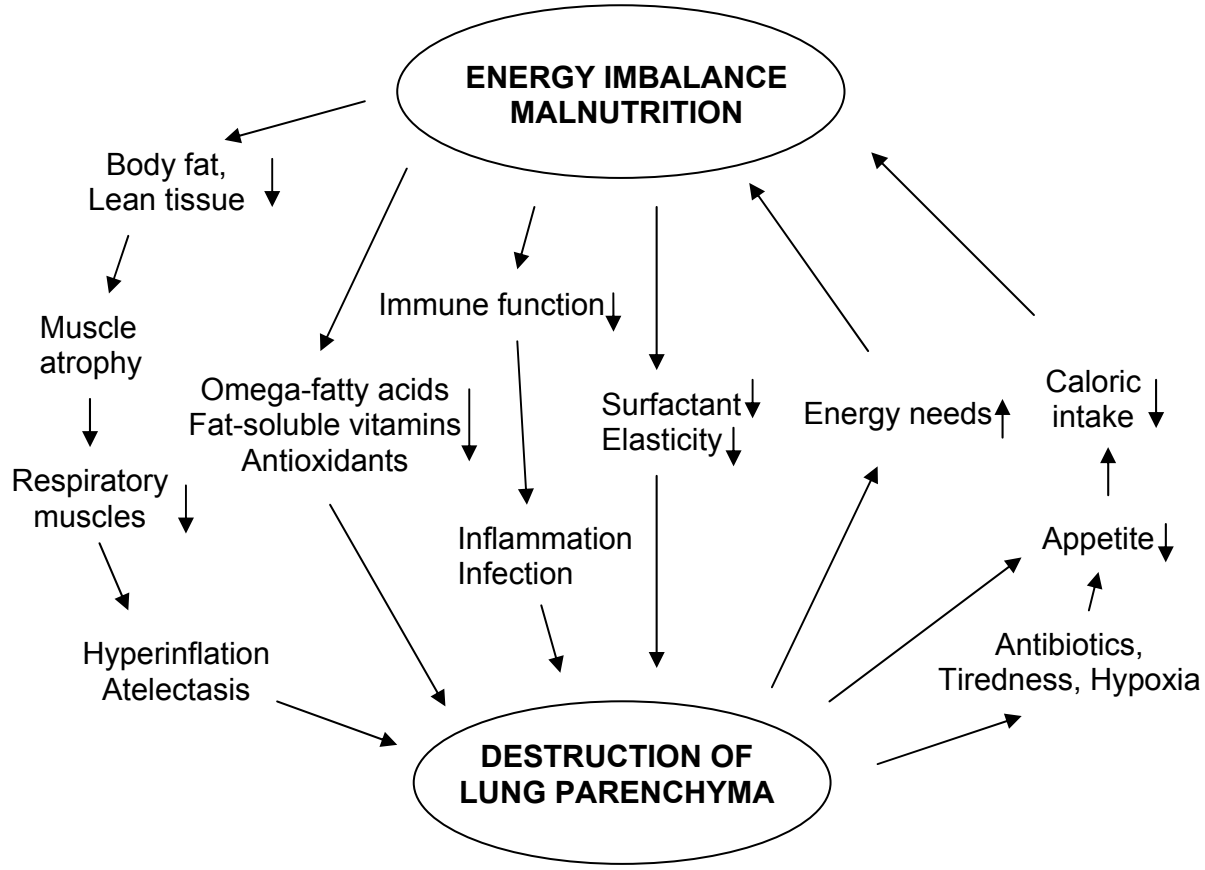
8.2 Links between energy imbalance and lung damage

As part of the present study, we have evaluated mosaic perfusion both in inspiration and expiration by using HRCT. This finding is considered to be an early manifestation of CF lung disease. We believe that mosaic perfusion that only occurs during expiration, represents a lower degree of pathology than mosaic perfusion seen in both in- and expiration. Other studies focused on volumetric HRCT imaging to provide structural and functional lung analysis at the same time. These new methods generate three-dimensional data sets and they can detect regional CF lung changes before changes in global pulmonary function measurements (28;95).

Several studies have focused on the impact of energy deficit on lung function, and correlations have been demonstrated between lean body mass (96), exocrine pancreatic function (97) as well as REE and lung function (81). As part of our study protocol, we intended to evaluate body composition by bio-electrical impedance analysis (BIA) and skin fold measurements. Although scientifically approved earlier (98;99), our experience with these methods was rather disappointing. Measurements were showing a broad range of variation, both intra- and interpersonal, and we therefore decided to exclude this potentially interesting topic from our study. Using dual-energy X-ray absorptiometry (DEXA) is the method of choice today but the software for paediatric patients was not available at Haukeland University Hospital at study time. However, we intend to look at body composition and bone mineral density in our CF cohort with DEXA in our follow-up studies.

Figure 3 is a simplified model, which places findings of our study in a larger pathophysiological context of the close interactions between energy imbalance and lung parenchyma damage in cystic fibrosis.

Figure 3: The interactions between energy imbalance and lung parenchyma damage in cystic fibrosis



Visualizing lung pathology by HRCT improves understanding of this complex disease in general, but HRCT scans are promising for clinicians in particular. The condition of the lungs is depicted more precisely than by the traditionally performed CCR or PFT. Thus HRCT can give raise to change of airway clearance techniques and provides the clinician last not least a tool to increase patients' engagement in times when motivation is inappropriate. Scoring of pulmonary HRCT findings gives the opportunity to follow single patients over time (27), compare patients of a cohort in a cross-sectional manner like in the present study, or to evaluate treatment effects in clinical intervention studies (28). Either way, the clinician gets additional information regarding the most life-limiting factor in CF, namely lung disease.

It is rather astonishing, that Shwachman's overall clinical score from 1958 is correlating excellently with the pulmonary HRCT score we used in our study ($r=0,872$; $p<0,001$; data not presented here), as well as it showed good correlation to REE and FEV_1. Although there are several drawbacks connected to the SK score, it

is easy to perform in a clinical setting, gives broad clinical information of the patients' condition and has the opportunity to monitor disease progression.
There is still no obvious explanation of a causal relationship between the basic chloride channel defect and the association between energy imbalance and lung function (55). However, there seems to be a distinct interaction between these parameters. What are the starters for the destructive circle in CF? Is it the pulmonary disease, the imbalance of energy needs and intake, or is it the basic defect itself? Either way, it is of major interest to prevent energy imbalance by focusing on energy losses and expenditure as well as on aggressive nutritional support. One should be highly aware of raised energy needs when dealing with worsening lung function in CF. Prevention and early intervention are most successful in combating nutritional failure.

8.3 General considerations

Due to necessity for cooperation under the technical procedures, we have based this thesis on the investigation of CF patients older than six years (N=29), which accounts for approximately 13% (29/215) of all Norwegian CF patients at this age. We consider our group as representative in terms of pancreatic function, disease severity, treatment options and follow-up, as all Norwegian CF patients follow common national guidelines. However, our cohort is just a minor part of the Norwegian CF cohort, and therefore we must be careful not to generalize from our findings.
Moreover, the percentage of identified CF mutations in our cohort is rather low. As described earlier, DNA analysis in our hospital in 1999/2000 was incomplete and it is still topic of ongoing work. In the meantime, a few more mutations have been detected, resulting at present time in 18/29 patients with identified mutations (62%). In all other cases, the combination of clinical manifestations and abnormal sweat chloride concentrations was the basis for the CF diagnosis.

Some Norwegian peculiarities have to be addressed when discussing our study in a larger context.

There might be a difference regarding nutritional habits in Norwegian CF patients compared to patients from other countries. Official nutritional guidelines in Norway advice vitamin A and D supplementation from four weeks of age and throughout childhood, because of the northern climate and limited exposure to sunlight during the winter months. Our patients having PI are advised to take a double dose of a multivitamin preparation daily, and all patients take vitamin E daily. The doses are adjusted according to monitoring of blood levels of the fat soluble vitamins D, A, and E. Daily intake of cod liver oil has been encouraged. The recommended five millilitres of cod liver oil contain 1200mg omega-3 fatty acids, including 600mg DHA and 400mg EPA in addition to vitamin A, D_3 and E. 52% of our patients take cod liver oil daily.

Secondly, Norwegian people have traditionally a high consumption of fish and fish products, especially people living along the coastline, where Bergen is located. According to Fluge and colleagues (100), average fish consumption in Norwegian young adults has been 270g a week, and more than half of our patients (15/29) were eating a fishmeal at least once a week.

CF patients in Norway seem to run a more favourable course than in many other countries. The prevalence of chronic *PSA* lung infection in Norway is 35 per cent (101), and in the patients cohort from western Norway presented here the prevalence is 28%, which is rather low, compared to Denmark (45%) (102) or the USA (60%) (103).

Finally, mutations in Norwegian CF patients are not fully revealed yet. One mutation described at the Department of Medical Genetics in our hospital (4005+2T>C, Helge Boman, personal communication) has not been found in CF patients outside Norway. This mutation seems to protect against exocrine pancreatic insufficiency, even when occurring together with the ΔF508 mutation. There might well be other mutations protecting against exocrine pancreatic insufficiency and carrying a better prognosis than usually among CF patients elsewhere.

9. Main conclusions

We found a close interaction between lung damage and elevated REE in our patients with CF. Prevention of a negative energy balance is an integral part of the surveillance of patients with CF. One should be aware of progress in lung impairment when increased REE is found and secondly, deterioration of pulmonary function raises the energy needs even more.

Mosaic perfusion in expiratory HRCT scans was a common finding in our study group. Our modified HRCT score correlated well with lung function measured by FEV_1. It is reasonable to believe that mosaic perfusion, only occurring during expiration, represents a lower degree of pathology than mosaic perfusion seen in both in- and expiration.

The use of the SK score in evaluation of overall disease severity and progression in CF is still valid, especially in older patients. Our results emphasise the clinical value of the SK score, which is easy to assess in a clinical setting in contrast to many other methods.

Severe degree of exocrine pancreatic insufficiency is common in patients with CF. There was no correlation of FE-1 levels to fat-soluble vitamin status. Fat-soluble vitamins (A, D, E) given in appropriate dosages combined with pancreatic enzymes ensured normal profiles in our patients with CF. Officially recommended supplementation of vitamin A and D in Norway during infancy and childhood may explain why so few patients had vitamin deficiencies at diagnosis. Because CF patients with patent pancreatic function may be at risk of developing PI, they might benefit from FE-1 measurements as part of longitudinal follow-up to detect pancreatic deterioration as early as possible.

10. References

1. Lewis PA. The epidemiology of cystic fibrosis. In: Hodson ME, Geddes DM, eds. Cystic Fibrosis. London: Arnold, 2000:13-25.

2. Lucotte G, Hazout S, de Braekeleer M. Complete map of cystic fibrosis mutation delta F508 frequencies in Western Europe and correlation between mutation frequencies and incidence of disease. Hum Biol 1995; 67:797-803.

3. Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease. Am J Dis Child 1938;56:344-399.

4. DiSant` Agnese PA, Darling RC, Perera GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas; clinical significance and relationship to the disease. Pediatrics. 1953;12:549-563.

5. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 1989 8;245:1059-1065.

6. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989 8;245:1066-1073.

7. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. Science 1989;245:1073-1080.

8. Cystic Fibrosis Mutation Database, 2006. http://www.genet.sickkids.on.ca/cftr/

9. Cutting GR, Curristin SM, Nash E, Rosenstein BJ, Lerer I, Abeliovich D, Hill A, Graham C. Analysis of four diverse population groups indicates that a subset of cystic fibrosis mutations occur in common among Caucasians. Am J Hum Genet. 1992;50:1185-1194.

10. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993;73:1251-1254.

11. Rosenstein BJ, Cutting GR. The diagnosis of cystic fbrosis: a consensus statement. J Pediatr 1998;132:589-595.

12. Eggesbo HB, Sovik S, Dolvik S, Eiklid K, Kolmannskog F. Proposal of a CT scoring system of the paranasal sinuses in diagnosing cystic fibrosis. Eur Radiol. 2003;13:1451-1460.

13. Eggesbo HB, Sovik S, Dolvik S, Kolmannskog F. CT characterization of inflammatory paranasal sinus disease in cystic fibrosis. Acta Radiol 2002;43:21-28.

14. Kerem E, Reisman J, Corey M, Canny GJ, Levison H. Prediction of Mortality in Patients with Cystic Fibrosis. N Engl J Med 1992;326:1187-1191.

15. Wilson DC, Pencharz PB. Nutrition and cystic fibrosis. Nutrition. 1998;14:792-795.

16. Marshall SG, Rosenfeld M, Ramsey BW. Respiratory System. In: Hodson ME, Geddes DM, eds. Cystic Fibrosis. London: Arnold, 2000:204-217.

17. Høiby N, Frederiksen B. Microbiology of cystic fibrosis. In: Hodson ME, Geddes DM, eds. Cystic Fibrosis. London: Arnold, 2000:83-107.

18. Lanng S. Glucose intolerance in cystic fibrosis patients. Paediatr Respir Rev 2001;2:253-259.

19. Brennan AL, Geddes DM, Gyi KM, Baker EH. Clinical importance of cystic fibrosis-related diabetes. J Cyst Fibrosis 2004;3:209-222.

20. Fluge G. Cystisk fibrose i Helseregion Vest. Tidsskr Nor Lægeforen 2000;120:568-571.

21. Kerem E, Kerem B. The relationship between genotype and phenotype in cystic fibrosis. Curr Op Pulm Dis 1995;1:450-456.

22. F. Santamaria, G. Grillo, G. Guidi, A. Rotondo, V. Raia, G. de Ritis, P., Sarnelli, M. Caterino, L. Greco. Cystic fibrosis: When Should High-resolution Computed Tomography of the Chest Be Obtained? Pediatrics 1998;101:908-913.

23. Helbich TH, Heinz-Peer G, Eichler I, Wunderbaldinger P, Götz M, Wojnarowski C, Brasch RC, Herold CJ. Cystic Fibrosis: CT Assessment of Lung Involvement in Children and Adults. Radiology 1999; 213:537-544.

24. Brody AS, Sucharew H, Campbell JD, Millard SP, Molina PL, Klein JS, Quan J. Computed tomography correlates with pulmonary exacerbations in children with cystic fibrosis. Am J Respir Crit Care Med 2005;172:1128-1132.

25. Bhalla, N. Turcios, V. Aponte, M. Jenkins, B.S. Leitman, D.I. McCauley, D.P. Naidich. Cystic Fibrosis: Scoring System with a Thin-Section CT. Radiology 1991;179:783-788.

26. Brody AS, Klein JS, Molina PL, Quan J, Bean JA, Wilmott RW. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. J Pediatr 2004;145:32-38.

27. Brody AS, Tiddens HA, Castile RG, Coxson HO, de Jong PA, Goldin J, Huda W, Long FR, McNitt-Gray M, Rock M, Robinson TE, Sagel SD. Computed tomography in the evaluation of cystic fibrosis lung disease. Am J Respir Crit Care Med 2005 15;172:1246-1252.

28. Robinson TE. High-resolution CT scanning: potential outcome measure. Curr Opin Pulm Med 2004;10:537-541.

29. Long FR, Williams RS, Adler BH, Castile RG. Comparison of quiet breathing and controlled ventilation in the high-resolution CT assessment of airway disease in infants with cystic fibrosis. Pediatr Radiol 2005;35:1075-1080.

30. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. Eur Respir J 2003;22:972-979.

31. Aurora P, Gustafsson P, Bush A, Lindblad A, Oliver C, Wallis CE, Stocks J. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. Thorax 2004;59:1068-1073.

32. Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, Stroobant J, Carr S, Stocks J; London Cystic Fibrosis Collaboration. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005;171:249-256.

33. Conway SP Littlewood JM. Cystic fibrosis clinical scoring systems. In: Dodge JA, Brock DJH, Widdicombe JH, eds. Cystic fibrosis - Current topics. John Wileys and Sons, 1996:339-358.

34. Shwachman H, Kulczycki LL. Long term study of 105 patients with cystic fibrosis. Am J Dis Child 1958;96:6-10.

35. Doershuk CF, LeRoy WM, Tucker AS, Nudelman H, Eddy G, Wise M, Spector S. A five year clinical evaluation of a therapeutic program for patients with cystic fibrosis. J Pediatr 1964;65:677-693.

36. Cooperman EM, Park M, McKee J, Assad JP. A simplified cystic fibrosis scoring system (a preliminary report). Can Med Assoc J 1971;105:580-582.

37. Taussig LM, Kattwinkel J, Friedewald WT, Di Sant'Agnese PA. A new prognostic score and clinical evaluation system for cystic fibrosis. J Pediatr 1973;82:380-390.

38. Kanga J, Kuhn R, Craigmyle L, Haverstock D, Church D. Cystic fibrosis clinical score: a new scoring system to evaluate acute pulmonary exacerbation. Clin Ther 1999;21:1343-1356.

39. Chrispin AR, Norman AP. The systematic evaluation of the chest radiograph in cystic fibrosis. Pediatr Radiol 1974;2:101-105.

40. Kraemer R, Tschäppeler H, Rüdeberg A, Stoll E, Rossi E. Verlauf und quantitative Erfassung des pulmonalen Befalls bei zystischer Fibrose. Schweiz Med Wochenschr 1979;109:39-45.

41. Brasfield D, Hicks G, Soong S, Peters J, Tiller R. Evaluation of scoring system of the chest radiograph in cystic fibrosis: a collaborative study. Am J Roentgenol 1980;134:1195-1198.

42. Weatherly MR, Palmer CG, Peters ME, Green CG, Fryback D, Langhough R, Farrell PM. Wisconsin cystic fibrosis chest radiograph scoring system. Pediatrics 1993;91:488-495.

43. Conway SP, Pond MN, Bowler I, Smith DL, Simmonds EJ, Joanes DN, Hambleton G, Hiller EJ, Stableforth DE, Weller P. The chest radiograph in cystic fibrosis: a new scoring system compared with the Chrispin-Norman and Brasfield scores. Thorax 1994;49:860-862.

44. Terheggen-Lagro S, Truijens N, van Poppel N, Gulmans V, van der Laag J, van der Ent C. Correlation of six different cystic fibrosis chest radiograph scoring systems with clinical parameters. Pediatr Pulmonol 2003;35:441-445.

45. Pencharz PB, Durie PR. Pathogenesis of malnutrition in cystic fibrosis, and its treatment. Clin Nutr 2000;19:387-394.

46. Milla CE. Association of nutritional status and pulmonary function in children with cystic fibrosis. Curr Opin Pulm Med 2004;10:505-509.

47. Vaisman N, Pencharz PB, Corey M, Canny GJ, Hahn E. Energy expenditure of patients with cystic fibrosis. J Pediatr 1987;111:496-500.

48. Corey M, McLaughlin FJ, Wiliams M, Levison H. A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. J Clin Epidemiol 1988;41:583-591.

49. Steinkamp G, Wiedemann B. Relationship between nutritional status and lung function in cystic fibrosis: cross sectional and longitudinal analyses from the German CF quality assurance (CFQA) project. Thorax 2002;57:596-601.

50. Konstan MW, Butler SM, Wohl ME, Stoddard M, Matousek R, Wagener JS, Johnson CA, Morgan WJ; Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. Growth and nutritional indexes in early life predict pulmonary function in cystic fibrosis. J Pediatr 2003;142:624-630.

51. Staab D. Cystic fibrosis -- therapeutic challenge in cystic fibrosis children. Eur J Endocrinol 2004;151 Suppl 1:S77-80.

52. Walkowiak J, Przyslawski J. Five-year prospective analysis of dietary intake and clinical status in malnourished cystic fibrosis patients. J Hum Nutr Diet 2003;16:225-231.

53. Peterson ML, Jacobs DR Jr, Milla CE. Longitudinal changes in growth parameters are correlated with changes in pulmonary function in children with cystic fibrosis. Pediatrics 2003;112:588-592.

54. Sinaasappel M, Stern M, Littlewood J, Wolfe S, Steinkamp G, Heijerman HG, Robberecht E, Doring G. Nutrition in patients with cystic fibrosis: a European Consensus. J Cyst Fibrosis 2002;1:51-75.

55. Schöni MH, Casaulta-Aebischer C. Nutrition and lung function in cystic fibrosis patients: review. Clin Nutr 2000;19:79-85.

56. Gunnell S, Christensen NK, McDonald C, Jackson D. Attitudes toward percutaneous endoscopic gastrostomy placement in cystic fibrosis patients. J Pediatr Gastroenterol Nutr 2005;40:334-338.

57. Borowitz D. Update on the evaluation of pancreatic exocrine status in cystic fibrosis. Curr Opin Pulm Med 2005;11:524-527.

58. Soldan W, Henker J, Sprössig C. Sensitivity and specificity of quantitative determination of pancreatic elastase-1 in feces of children. J Pediatr Gastroenterol Nutr 1997;24:53-55.

59. Leus J, VanBiervliet S, Robberecht E. Detection and follow up of exocrine pancreatic insufficiency in cystic fibrosis: a review. Eur J Pediatr 2000;159:563-568.

60. Cade A, Walters MP, McGinley N, Firth J, Brownlee KG, Conway SP, Littlewood JM. Evaluation of faecal pancreatic elastase-1 as a measure of pancreatic exocrine function in children with cystic fibrosis. Pediatr Pulmonol 2000;29:172-176.

61. Walkowiak J, Nousia-Arvanitakis S, Cade A, Kashirskaya N, Piotrowski R, Strzykala K, Kouniou M, Pogorzelski A, Sands D, Kapranov N. Fecal elastase-1 cut-off levels in the assessment of exocrine pancreatic function in cystic fibrosis. J Cyst Fibrosis 2002;1:260-264.

62. Feranchak AP, Sontag MK, Wagener JS, Hammond KB, Accurso FJ, Sokol RJ. Prospective, long-term study of fat-soluble vitamin status in children with cystic fibrosis identified by newborn screen. J Pediatr 1999;135:601-610.

63. Rayner RJ, Tyrrell JC, Hiller EJ, Marenah C, Neugebauer MA, Vernon SA, Brimlow G. Night blindness and conjunctival xerosis caused by vitamin A deficiency in patients with cystic fibrosis. Arch Dis Child 1989;64:1151-1156.

64. Carr SB, Dinwiddie R. Annual review or continuous assessment? J R Soc Med 1996;89 S27:3-7.

65. Mrugacz M, Tobolczyk J, Minarowska A. Retinol binding protein status in relation to ocular surface changes in patients with cystic fibrosis treated with daily vitamin A supplements. Eur J Pediatr 2005;164:202-206.

66. Lancellotti L, D'Orazio C, Mastella G, Mazzi G, Lippi U. Deficiency of vitamins E and A in cystic fibrosis is independent of pancreatic function and current enzyme and vitamin supplementation. Eur J Pediatr 1996;155:281-285.

67. Bines JE, Truby HD, Armstrong DS, Carzino R, Grimwood K. Vitamin A and E deficiency and lung disease in infants with cystic fibrosis. J Paediatr Child Health 2005;41:663-668.

68. Henderson RC, Madsen CD. Bone mineral content and body composition in children and young adults with cystic fibrosis. Pediatr Pulmonol 1999;27:80-84.

69. Grey V, Lands L, Pall H, Drury D. Monitoring of 25-OH vitamin D levels in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2000;30:314-319.

70. Hecker TM, Aris RM. Management of osteoporosis in adults with cystic fibrosis. Drugs 2004;64:133-147.

71. Winklhofer-Roob BM, Ziouzenkova O, Puhl H, Ellemunter H, Greiner P, Muller G, van't Hof MA, Esterbauer H, Shmerling DH. Impaired resistance to oxidation of low density lipoprotein in cystic fibrosis: improvement during vitamin E supplementation. Free Radic Biol Med 1995;19:725-733.

72. De Vriese S, Robberecht E, De Gussem J, Christophe A. FEV1 correlates with plasma vitamin E in young cystic fibrosis (CF) patients: an argument for early administration? Netherlands J Med 54:S61,A144.

73. Koscik RL, Lai HJ, Laxova A, Zaremba KM, Kosorok MR, Douglas JA, Rock MJ, Splaingard ML, Farrell PM. Preventing early, prolonged vitamin E deficiency: an opportunity for better cognitive outcomes via early diagnosis through neonatal screening. J Pediatr 2005;147:S51-56.

74. Helbich TH, Heinz-Peer G, Fleischmann D, Wojnarowski C, Wunderbaldinger P, Huber S, Eichler I, Herold CJ. Evolution of CT findings in patients with cystic fibrosis. Am J Roentgenol 1999;173:81-88.

75. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 1993;6:Suppl.16;5s–40s.

76. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1-9.

77. Aksnes L. Simultaneous determination of retinol, alpha-tocopherol and 25-hydroxyvitamin D in human serum by high-performance liquid chromatography. J Pediatr Gastroenterol Nutr 1994;18:339-343.

78. Schibli S, Durie PR, Tullis ED. Proper usage of pancreatic enzymes. Curr Opin Pulm Med 2002;8:542-546.

79. Olson DL, Schwenk WF. Nutrition for patients with cystic fibrosis. Nutr Clin Pract. 2004;19:575-580.

80. Sharma R, Florea VG, Bolger AP, Doehner W, Florea ND, Coats AJ, Hodson ME, Anker SD, Henein MY. Wasting as an independent predictor of mortality in patients with cystic fibrosis. Thorax 2001;56:746-750.

81. Fried MD, Durie PR, Tsui LC, Corey M, Levison H, Pencharz PB. The cystic fibrosis gene and resting energy expenditure. J Pediatr 1991;119:913-916.

82. Girardet JP, Tounian P, Sardet A, Veinberg F, Grimfeld A, Tournier G, Fontaine JL. Resting energy expenditure in infants with cystic fibrosis. J Pediatr Gastroenterol Nutr 1994;18:214-219.

83. Bowler IM, Green JH, Wolfe SP, Littlewood JM. Resting energy expenditure and substrate oxidation rates in cystic fibrosis. Arch Dis Child 1993;68:754-759.

84. Zemel BS, Kawchak DA, Cnaan A, Zhao H, Scanlin TF, Stallings VA. Prospective evaluation of resting energy expenditure, nutritional status, pulmonary function, and genotype in children with cystic fibrosis. Pediatr Res 1996;40:578-586.

85. Thomson MA, Wilmott RW, Wainwright C, Masters B, Francis PJ, Shepherd RW. Resting energy expenditure, pulmonary inflammation, and genotype in the early course of cystic fibrosis. J Pediatr 1996;129:367-373.

86. Pencharz PB, Jackson R. There is no evidence of a primary defect in energy metabolism in subjects with cystic fibrosis. J Pediatr 2002;140:498-489.

87. Bines JE, Truby HD, Armstrong DS, Phelan PD, Grimwood K. Energy metabolism in infants with cystic fibrosis. J Pediatr 2002;140:527-533.

88. Walkowiak J, Nousia-Arvanitakis S, Agguridaki C, Fotoulaki M, Strzykala K, Balassopoulou A, Witt M, Herzig KH. Longitudinal follow-up of exocrine pancreatic function in pancreatic sufficient cystic fibrosis patients using the fecal elastase-1 test. J Pediatr Gastroenterol Nutr 2003;36:474-478.

89. Cohen JR, Schall JI, Ittenbach RF, Zemel BS, Stallings VA. Fecal elastase: pancreatic status verification and influence on nutritional status in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2005;40:438-444.

90. Walkowiak J, Lisowska A. Re: fecal elastase: pancreatic status verification and influence on nutritional status in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2006;42:117; author reply 118.

91. Baker SS, Borowitz D, Duffy L, Fitzpatrick L, Gyamfi J, Baker RD. Pancreatic enzyme therapy and clinical outcomes in patients with cystic fibrosis. J Pediatr 2005;146:189-193.

92. Baker SS, Borowitz D, Baker RD. Pancreatic exocrine function in patients with cystic fibrosis. Curr Gastroenterol Rep 2005;7:227-233.

93. Winklhofer-Roob BM. Cystic fibrosis: nutritional status and micronutrients. Curr Opin Clin Nutr Metab Care 2000;3:293-297.

94. Wilson DC, Rashid M, Durie PR, Tsang A, Kalnins D, Andrew M, Corey M, Shin J, Tullis E, Pencharz PB. Treatment of vitamin K deficiency in cystic fibrosis: Effectiveness of a daily fat-soluble vitamin combination. J Pediatr 2001;138:851-855.

95. Bonnel AS, Song SM, Kesavarju K, Newaskar M, Paxton CJ, Bloch DA, Moss RB, Robinson TE. Quantitative air-trapping analysis in children with mild cystic fibrosis lung disease. Pediatr Pulmonol 2004;38:396-405.

96. Rochat T, Slosman DO, Pichard C, Belli DC. Body composition analysis by dual-energy x-ray absorptiometry in adults with cystic fibrosis. Chest 1994;106:800-805.

97. Gaskin K, Gurwitz D, Durie P, Corey M, Levison H, Forstner G. Improved respiratory prognosis in patients with cystic fibrosis with normal fat absorption. J Pediatr 1982;100:857-862.

98. Borowitz D, Conboy K. Are bioelectric impedance measurements valid in patients with cystic fibrosis? J Pediatr Gastroenterol Nutr 1994;18:453-456.

99. Groeneweg M, Tan S, Boot AM, de Jongste JC, Bouquet J, Sinaasappel M. Assessment of nutritional status in children with cystic fibrosis: conventional anthropometry and bioelectrical impedance analysis. A cross-sectional study in Dutch patients. J Cyst Fibrosis 2002;1:276-280.

100. Fluge O, Omenaas E, Eide GE, Gulsvik A. Fish consumption and respiratory symptoms among young adults in a Norwegian community. Eur Respir J 1998;12:336-340.

101. Fluge G, Ojeniyi B, Hoiby N et al. Typing of Pseudomonas aeruginosa strains in Norwegian cystic fibrosis patients. Clin Microbiol Infect 2001;7:238-243.

102. Johansen HK, Kovesi TA, Koch C, Corey M, Hoiby N, Levison H. Pseudomonas aeruginosa and Burkholderia cepacia infection in cystic fibrosis patients treated in Toronto and Copenhagen. Pediatr Pulmonol 1998;26:89-96.

103. Fritzsimmons SC. Cystic Fibrosis Foundation Patient Data Registry Annual Data Report 1996, Bethesda, Maryland.