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# CYSTIC FIBROSIS: FOOD, MICROFLORA AND HEALTH - THE GOLDEN TRIANGLE -

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## SUMMARY

Cystic fibrosis remains a life long, life limiting disease, with median survival in Europe and the United States steadily increasing and now into the third decade. The primary cause of death remains respiratory failure brought on by the constant cycle of infection and inflammation occurring in the lungs. Improved survival is related to an aggressive three-pronged approach: Good nutrition, targeted antibiotic therapy and increasing lung clearance. Inflammation appears to play a role in the intestinal tract as well as the lungs. Correction of inflammation both in the gastrointestinal tract and the lungs may be possible with concentration on correcting micronutrient deficiencies, especially those linked to the antioxidant defence systems. However, there is still only limited evidence in this area. The environment in the gut is also affected by the frequent courses of antibiotics given to patients with cystic fibrosis and early work has started to look at correction of imbalance in flora using antibiotics and probiotics, with theoretic benefits further a field in the lung. Apart from improved body mass index and its direct effect on increased survival, the benefits of good nutrition and micronutrients run over into other areas such as bone metabolism and cognitive function. This review attempts to emphasis these important areas in the overall disease process of cystic fibrosis.

## INTRODUCTION

Cystic Fibrosis is the commonest autosomal recessive condition in the European Caucasian population. In the United Kingdom (UK) there are over 7500 people with cystic fibrosis with a birth rate of 1 in 2415 (*Dodge* et al., 1997). The basic defect found is in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene (*Kerem* et al., 1989). It is now recognised that there are over 1000 different mutations of this gene (*The Cystic Fibrosis Genotype-Phenotype Consortium*, 2004), with the commonest 32 mutations found in over 96% of the UK population and indeed the most prevalent of these, a deletion of phenylalanine at position 508 (ΔF508), accounting for over 74% of the deletions (*McCormick* et al., 2002).

The primary function of the CFTR is as a chloride channel, (*Linsdell*, 2006) in health it is positioned on the apical membrane of epithelial cells (*Tsui*, 1995). The subsequent imbalance in electrolytes and water flow from this defective channel is clinically relevant in several body systems: the

lung, the pancreas, the gut, the liver, the sweat glands and the vas deferens in males. All lead to important problems for the individual with cystic fibrosis, the most studied of which is the abnormality in the lungs. Here, the relentless cycle of infection and inflammation, caused by airway surface fluid dehydration and mucus plugging of airways, has a profound effect on both the morbidity and mortality associated with this disease. Over 90% of deaths in cystic fibrosis are from respiratory failure with the median age of survival in the USA being reported as 36.8 years (Cystic Fibrosis Foundation, 2005 database figures, personal correspondence).

There is extensive work on the inflammation and infection cycles within the lungs and this article will only briefly cover this before exploring the problems in the cystic fibrosis gut: inflammation, malabsorption, nutrition and the links with health.

## INFLAMMATION AND THE LUNGS

Inflammation in the cystic fibrosis lung is well described (*Courtney*, 2004; *Schiotz*, 1978) with debate about whether the CFTR defect leads to inflammation even without the presence of infection. In 1995 two papers clearly demonstrated the presence of lung inflammation as early as 4 weeks of age in populations of neonatally screened infants with cystic fibrosis. In these studies markers of inflammation such as Interleukin 8 (IL-8) and neutrophils were raised, both with and without the presence of bacterial or viral infection (*Khan* et al., 1995; *Armstrong* et al., 1995). Neutrophils appear to be the predominant cell involved in the inflammation of the airway. More recently *Armstrong* et al. (2005) have again looked at bronchoalveolar lavage (BAL) fluid in infants with cystic fibrosis diagnosed by neonatal screening. They show that the increased inflammation only appears in the presence of bacterial or viral infection and that over subsequent years inflammatory response decreases in the absence of infection. This suggests that it is the infection promoting this inflammatory response, rather than an innate effect of the CFTR.

This theory has been questioned again recently. It has been known for some time that increased levels of arachidonic acid (AA) are present in the airways of patients with cystic fibrosis (*O'Driscoll* et al., 1984). AA is a protagonist in the inflammatory pathway and like other raised inflammatory markers in cystic fibrosis it has been thought to be a reactive process following infection. Work by *van Heeckeren* et al. (1997) using *Pseudomonas* beads in mouse airway suggests that there is an inflammatory response above and beyond that expected, they and others feel that CFTR is responsible for this up regulation.

It is impossible not to mention the role of *Pseudomonas aeruginosa* infection here. The cystic fibrosis lung appears particularly susceptible to infection by *Pseudomonas aeruginosa*. Work by *Davies* et al. (1997) implies that the CFTR defect itself is responsible for increased binding of *Pseudomonas aeruginosa* to the cystic fibrosis airway, they found correction of the CFTR lead to decreased *Pseudomonas aeruginosa* adherence. We know that chronic colonisation of the airway with *Pseudomonas aeruginosa* leads to a more rapid decline in lung function (*Pamukcu* et al., 1995) and the American Cystic Fibrosis Foundation Data from 2002 showed that median sur-

vival, when chronically colonised with *Pseudomonas aeruginosa,* is 10 years younger than in those patients not growing it in their sputum.

## INFLAMMATION AND THE GUT

The cystic fibrosis knock out mice models are notorious for their severe and early gut involvement, making study of therapies and strategies in the lungs hard. It does, however, give us a useful starting point to look for evidence of abnormal inflammation in the gut.

Several authors have shown the presence of inflammation in the gut of cystic fibrosis knock out mice. *Norkina* et al. (2004a) found that the gut of sacrificed Peptamen fed cystic fibrosis knockout mice showed up regulation of genes governing innate immunity but not adaptive immunity. The most up regulated gene they found was RELMB/FIZZ2, associated with cell growth and inflammation. In this study, expression of genes involved in lipid metabolism was found to be down regulated, especially cytochrome P450. They authors hypothesised that the increased inflammation in the gut may cross over to the systemic circulation and cause priming of the immune system leading to the over response to subsequent infections in the lungs.

Croft and colleagues produced a triad (*Croft* et al., 1995, 1996, and *Smyth* et al., 2000) of papers looking at gut inflammation in patients with cystic fibrosis. They described a process of looking at inflammatory markers in the fluid obtained by whole gut lavage (WGL) (*O'Mahony* et al., 1990), in the same way that inflammatory markers in the fluid from BAL are examined. In the largest of their studies looking at 21 patients with cystic fibrosis and 12 controls it was evident that the cystic fibrosis patients had raised levels of proteins derived from the plasma: Albumin, and IgG. Increases in the secretory immunoglobulin, IgM and the cellular constituents, eosinophil cationic protein and neutrophil elastase were also found. A key cytokine in cystic fibrosis: IL-8 and the lesser studied IL-1β were both also found to be raised. This was a similar finding to a previous study looking at inflammatory markers (IL-8) in the faeces of patients with cystic fibrosis (*Briars* et al., 1995). Like investigators of the inflammation in the lungs, they concluded that the CFTR itself had inherent pro-inflammatory properties and was likely to be responsible for the raised gut inflammatory markers (*Smyth* et al., 2000).

More recently, a small study looking at 30 children with cystic fibrosis confirms gut inflammation in 27 of them, showing significantly raised levels of faecal nitric oxide and calprotectin (both markers of inflammation) when compared to controls. Their levels were comparable to a second control group suffering from inflammatory bowel disease (*Bruzzese* et al., 2004).

The presence of chronic inflammation in the intestine of cystic fibrosis patients (*Raia* et al., 2000) would also go some way to explaining the increase in bowel cancer found in cystic fibrosis (*Schoni* et al., 1996). Increased oxidative stress is implicated in non-cystic fibrosis bowel cancer (*Skrzydlewska* et al., 2005) and may also play a role in cystic fibrosis. Other forms of cancer are not increased, specifically lung cancer, suggesting that CFTR is not responsible for the increased risk of cancer.

## ABNORMAL MICROFLORA

Infection, both acute and chronic has been shown to be responsible for the inflammation found in the cystic fibrosis lung (*Armstrong* et al., 2005). Is there evidence that there is bacterial overgrowth in the gut? An increase in small intestine bacteria by up to 40 fold in CF knockout mice (*Norkina* et al., 2004b) has been shown using PCR (16S) techniques. Subsequent treatment with broad-spectrum antibiotics (ciprofloxacin and metronidazole) decreased this bacterial load down to levels similar to control mice, decreased the expression of inflammatory genes and also led to improved growth. This group also showed that a 3-week course of antibiotic treatment decreased the amount of small intestinal mucus found in these mice (*De Lisle* et al., 2006). The improved growth (as measured by weight gain) after decreasing bowel bacterial load may have implications for patients with cystic fibrosis. However, the evidence of bacterial overgrowth in cystic fibrosis is actually quite sparse. One previous study in 54 children with cystic fibrosis examined the prevalence of bacterial overgrowth using breath tests, it showed that it is more common than in children with non-CF related bowel disease: 32% vs. 7%. It also showed slower transit times in the cystic fibrosis group as a whole and when divided into those with overgrowth and those without (*Lewindon* et al., 1998).

Reasons for this apparent bacterial overgrowth have not been studied, it could be postulated that the same mechanisms up regulating inflammation at the lung epithelium are occurring in the intestine. Why increased inflammation occurs in the lung is not that well understood (*Courtney* et al., 2004) and it is likely to be a while before an understanding of the process in the intestine becomes clear. However, some theories, especially related to micronutrient roles are discussed later.

## PROBIOTICS

Imbalance of natural flora within the intestine from bacterial overgrowth (*Norkina* et al., 2004b) and the repeated use of antibiotics to treat chest exacerbations must lead to some discussion of the use of probiotics in patients with cystic fibrosis. The theory behind their use already exists in other disease models, with a reduction in antibiotic associated diarrhoea and *Clostridium difficile* diarrhoea now being confirmed by meta-analysis (*McFarland*, 2006). *C. difficile* is more prevalent in the cystic fibrosis population being reported as between 22 and 32% (*Welkon* et al., 1985; *Peach* et al., 1986). However, in these two studies its presence was reported as being asymptomatic with no associated diarrhoea.

Bruzzese's paper (*Bruzzese* et al., 2004) describing gut inflammation in cystic fibrosis is the only study also looking at probiotic use for patients. They showed a significant reduction, after using *Lactobacillus* GG, in both calprotectin (n=10) and faecal nitric oxide (n=5). Cystic fibrosis mouse work has focused on the effect of *Lactobacillus casei* on lung clearance of *Pseudomonas aeruginosa* (*Alvarez* et al., 2001) again with a significant improvement in the treated group. *Miake* et al. (1985) showed a protective effect using *Lactobacillus casei* on peritoneal cavity infection with *Pseudomonas* in non-cystic fibrosis mice. These studies give some hope for further work in this area.

## NUTRITION

The interplay between the gut and the lung is very important; the majority of people with cystic fibrosis are pancreatic insufficient, requiring supplementation with pancreatic enzymes to help with absorption of food. Since *Corey* et al (1988) reported improved survival and weight of patients on a high fat, high energy diet rather than the traditional low fat diet there has been a shift to aggressive nutritional management.

Registry data from Germany (*Steinkamp* and *Wiedemann*, 2002) looking at 3298 patients over a 2-3 year time period confirms this original article. They defined patients as malnourished if they had a weight for height percentile of <90% and showed progression of the proportion of malnourished patients with advancing age. More importantly they demonstrated worsened lung function when malnourished, which was particularly evident in the adolescent age range. They were able to demonstrate an improvement in lung function of 2.1 % on gaining > 5% weight. This was a similar finding to our own data following the introduction of gastrostomy feeding in a small group. This showed an improvement in weight for height with the introduction of feeds (92% vs. 98%) and an arrest in the yearly rate of lung function decline ($FEV_1$ -5% vs. 4%) (*Collyer* et al., 2000). Unfortunately, the recent multicentre CALICO Trial of oral supplementation in moderately malnourished children with cystic fibrosis has not replicated these results (*Poustie* et al., 2006). They were unable to show an increase in weight in this randomized trial, so effects on lung function in relation to weight gain were not possible to determine.

Looking to the mouse model again, *van Heeckeren* et al. (2004) tried to tease out whether nutrition had a specific effect on host response to lung infections. They compared three groups of mice: Wild type, cftr knock out and gut corrected cftr knockout mice (expressing human CFTR primarily within the gut). *Pseudomonas aeruginosa*-laden agarose beads were placed in the bronchi of each group after various enriched diets and in a separate experiment after adding the essential fatty acid Docasahexaenoic acid (DHA). They found no differences in lung inflammation (post sacrifice BAL samples) or on early death within the groups, suggesting nutrition and DHA imbalances alone, are not able to explain the different inflammatory responses in CF and wild type mice.

## ESSENTIAL FATTY ACIDS AND CURCUMIN

The essential fatty acid (EFA), Docasahexaenoic acid was used in van Heeckeren's study (*van Heeckeren* et al., 2004) because of several studies pointing to the imbalance of EFA in patients with cystic fibrosis (*Bhura-Bandali* et al., 2000; *Strandvik* et al., 1988; *Carlstedt-Duke* et al., 1986) and especially with the knowledge that low levels of DHA are seen in relation to the severe CFTR mutations (*Strandvik* et al., 2001). A pivotal paper reporting the correction of pancreatic duct abnormalities in knock-out cftr mice following supplementation with 40mg day of DHA has lead to increased research in this area (*Freedman* et al., 1999). Pilot data on the safety of oral DHA supplementations (*Lloyd-Still* et al., 2005) and proof of concept (*Jumpsen* et al., 2006) are now available and there are ongoing studies of supple-

**Table 1**: Role of vitamins in cystic fibrosis

| Vitamin | Role |
|---|---|
| Vitamin A | Immunity (*Reifen*, 2002)<br>Respiratory epithelium integrity (*Biesalski* and *Nohr*, 2003)<br>Vision (*Fulton* et al., 1982; *Huet* et al., 1997) |
| Pro-vitamin A (b carotene) | Antioxidant defences (*Lepage* et al., 1996) |
| Vitamin D | Bone metabolism (*Haworth* et al., 2004) |
| Vitamin K | Bone Metabolism (*Beker* et al., 1997; *Weber*, 2001) |
| Vitamin E | Antioxidant defences (*Wood* et al, 2001)<br>Neurological integrity (*Bye* et al., 1985) |
| Vitamin C | Antioxidant defences (*Winklhofer-Roob* et al., 1997)<br>Immunity (*Wintergerst* et al., 2006) |

mentation of DHA in newborn infants with cystic fibrosis.

Aside from the frenzied research in trying to find a cure for cystic fibrosis by gene therapy, equally vigorous attempts are being made to find substances that can correct the function of the faulty CFTR rather than replace it. There was therefore some excitement a few years ago when *Egan* and colleagues (2004) reported that a simple food substance, curcumin appeared to correct the basic defect, as demonstrated by correction of nasal potential difference in nasal epithelium in a hamster model. Sadly, this has not been replicated and doubt as to its future as a treatment is now rife (*Dragomir* et al., 2004).

## ROLE OF MICRONUTRIENTS

It is well established that there are deficiencies of the fat-soluble vitamins in cystic fibrosis early in life (*Bye* et al., 1985; *Sokol* et al., 1989), this is in part due to the malabsorption of fats due to pancreatic enzyme deficiency. Routine supplementation of fat-soluble vitamins is now well established. The role of the fat-soluble vitamins in disease process is myriad and some are summarised in Table 1.

## ANTIOXIDANT ROLE

The argument for oxidant antioxidant imbalance in cystic fibrosis is well documented (*Brown* and *Kelly*, 1994a). Several micronutrients play an important role in the antioxidant defences (Table 2). Evidence of decrease in these antioxidants and increased oxidative stress has been shown in several studies looking at antioxidant blood levels (*Langley* et al., 1993), breath

**Table 2:** Examples of links between dietary constituents and antioxidant defence

| Nutrient | Role |
|---|---|
| Cu | Superoxide dismutase (SOD), caeruloplasmin |
| Fe | Catalase, SOD |
| Mg | Co-factor for multiple enzymes |
| Mn | SOD |
| Nicotinamide | DNA repair, energy source |
| Proteins | Amino Acids for synthesis of antioxidant enzymes, metal binding proeins, albumin |
| Riboflavin | Glutathione reductase |
| Se | Glutathione peroxidases |

pentane levels (*Bilton* et al., 1991) and markers of oxidative stress in the urine (8 hydroxyguanosine) (*Brown* et al., 1995) and plasma: 8-isoprostaglandin F 2α (8-iso $PGF_{2\alpha}$) (*Collins* et al., 1999), thiobarbituric acid-reactive substances (T-bars) (*Benabdeslam* et al., 1999, *Brown* and *Kelly*, 1994b) and malondialdehyde (*Rust* et al., 1998).

Linking micronutrients to lung or general health in cystic fibrosis is hard as there is little research in this area. *Bines* et al. (2005) suggest that at 1 year follow up of a group of 39 screened infants with cystic fibrosis there was no link to increased lung inflammation (BAL inflammatory markers) in the 20 infants that had been found to have low retinol at diagnosis. Our own work suggests that there may be a link between vitamin A and lung function in a group of 78 clinically stable children with cystic fibrosis: a low vitamin A level gave an odds ratio of 5 (95% CI 1.7-14.4) to predict an $FEV_1$ < 50% (predicted for height and sex) (*Carr* and *McBratney*, 2000). Follow up of these children over a 3 year period suggested a continuation of this independent link in a regression model, between vitamin A and $FEV_1$ (*Ranganathan* et al., 2002). A note of caution, however, vitamin A is negatively correlated to markers of inflammation (CRP) and depressed levels in cystic fibrosis may just be an expression of acute inflammation in some cases (*Greer* et al., 2004).

When moving on from simple observational studies that look at associations to cause and effect studies the literature becomes thinner. A recent small double blind trial of multiple antioxidants therapy (vitamin A, E, C b-carotene and Selenium) in 46 patients with cystic fibrosis showed good increases in the measured blood levels of these antioxidants and glutathione peroxidase but failed to show any improvement in oxidative stress as measured by 8-iso $PGF_{2\alpha}$ (*Wood* et al., 2003). An earlier open label study in 12 children given β-carotene for two months showed a decrease in the lipid peroxidation marker malondialdehyde (*Lepage* et al., 1996). A short study of

vitamin E supplementation also showed an improvement in the tolerance of low density lipoprotein to oxidation (*Winklhofer-Roob* et al., 1995).

Apart from the Wood trial which showed a slight correlation between change in lung function and selenium and 8-iso $PGF_{2\alpha}$ (*Wood* et al., 2003), these studies show proof of concept rather than any other clinically relevant outcomes such as fewer chest exacerbations, improved weight or increased survival. This is again demonstrated by a recent randomised, blinded, crossover study in a group of 22 patients. They were supplemented with a micronutrient mixture containing, minerals, trace elements, vitamins, fats (linoleic acid) or with placebo, the study failed to show any effect on pulmonary function or muscle performance (*Oudshoorn* et al., 2007).

Excitingly, an old drug N-acetylcysteine (NAC) is having a resurgence and not just as a mucolytic. It is a strong antioxidant and a precursor of glutathione. Glutathione (GSH) is found in large quantities in the epithelial lining fluid (ELF) of the lung and plays an important role in antioxidant defence. It also appears that the efflux of GSH may be controlled by CFTR, giving another reason for imbalance in antioxidant defences (*Hudson*, 2004). A recent phase 1 trial in 18 patients with cystic fibrosis has shown supplementation with high oral doses of NAC decreases lung inflammation as evidenced by lower sputum elastase, and neutrophil counts (*Tirouvanziam* et al., 2006). Similarly, proof of concept has also been shown in trials of inhaled glutathione (*Bishop* et al., 2005).

## OTHER EFFECTS OF MICRONUTRIENTS ON HEALTH

Moving away slightly from the antioxidant effects of micronutrients we must take note of a recent report suggesting that early deficiency of Vitamin E in a non-screened group of infants with cystic fibrosis led to impaired cognitive function at age 6-14 years when compared to their screened peers (*Koscik* et al., 2005). There are several case reports in the literature related to side effects of this and other fat soluble vitamin deficiencies in cystic fibrosis: varying from night blindness (*Huet* et al., 1997), to bleeding (*Verghese* and *Beverley*, 2003) to peripheral neuropathies (*Bye* et al., 1985).

Another important complication of cystic fibrosis is osteoporosis which tends to be preceded by osteopenia (*Elkin* et al., 2001. *Haworth* et al., 1999). This does not appear to be related to the CFTR defect but rather a side effect of having cystic fibrosis: the prevalence rises with increasing age (*Buntain* et al., 2004). Its cause is probably a combination of malabsorption of the micronutrients related to bone metabolism, especially the fat soluble vitamins D and K, but also of decreased muscle bulk, immobility and occasional use of steroids. A placebo controlled study supplementing calcium and vitamin D for a year in adult patients failed to reach statistical significance, but did show a trend towards decreasing bone turnover (*Haworth* et al., 2004). A recent article supplementing 20 children with cystic fibrosis for a year with vitamin K has shown improvement in biochemical markers of bone health, without alteration to their vitamin D intake or exercise regimens (*Nicolaidou* et al., 2006).

## CFTR AND MICRONUTRIENTS

Finally, going full circle back to the CFTR, micronutrients are beginning to stir interest for their potential roles in modulation of the CFTR function. Vitamin C (ascorbic acid) which is found in the respiratory epithelial lining fluid appears to help in the CFTR regulation of chloride flow as evidenced by changes in potential difference, in both *in vivo* and *in vitro* studies (*Fischer* et al., 2004). Zinc in combination with ATP appears to help activate an alternative, calcium dependent chloride channel. In a cystic fibrosis mouse model this allowed restoration of chloride permeability across the nasal epithelia (*Zsembery* et al., 2004).

Short chain fatty acids, easily absorbable orally, are being looked at in airway cell models for their potential ability to up regulate both the CFTR and other chloride channels, some success having being shown with 2,2-dimethyl-butyrate and alpha-methylhydrocinnamic acid (*Nguyen* et al., 2006).

## CONCLUSION

This review has provided a brief guide from the basic defect in cystic fibrosis, through the malfunctioning of the CFTR protein leading to increased inflammation and infection and the knock on effect this has at the all important lung and intestine surfaces. The malabsorption from lack of pancreatic enzyme leaves the majority of patients with cystic fibrosis the everyday challenge of maintaining good overall nutrition, in the knowledge that poor intake will lead to greater morbidity and likely early death. It has shown the complex interplay of CFTR, micronutrients and gut flora in the overall picture, especially in relation to lung health. Hopefully it has confirmed the need for careful thought about achieving the best dietary approach to aid in the everyday struggle to lengthen survival in cystic fibrosis.

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