UNIVERSITY OF SOUTHAMPTON

INFLUENCE OF NUTRIENT INTAKE ON COLONIC FUNCTION AND STOOL COMPOSITION IN ENTERALLY FED CYSTIC FIBROSIS PATIENTS

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ABSTRACT

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INFLUENCE OF NUTRIENT INTAKE ON COLONIC FUNCTION AND STOOL COMPOSITION IN THE ENTERALLY FED CF POPULATION

by Joan Gavin

Cystic Fibrosis (CF) patients need to consume a high energy diet to satisfy their increased metabolic demands and those with long term inadequate energy intakes are given overnight enteral feeds to prevent growth retardation. These feeds and this type of diet are low in dietary fibre and those patients with the lowest fibre intakes are reported to have the most frequent abdominal symptoms

The aim of this study was to determine whether increasing dietary fibre intake could alter colonic function and stool output therefore reduce the incidence of abdominal symptoms for this CF group. If a causal relationship was identified this would have clinical implications for the dietetic management of enterally fed CF patients. The study was designed as two phases. The first phase assessed habitual stool losses and frequency of gastrointestinal symptoms in the enterally fed CF group compared with healthy individuals, and the second phase assessed the difference in stool losses and symptoms when the CF group were given either a fibre supplemented feed or a placebo feed.

The enterally fed CF group had more frequent symptoms and larger stool losses than the healthy group but these were unrelated to their low dietary fibre intake. The fibre supplemented feed did not increase stool losses or reduce the incidence of symptoms compared with the placebo feed. As no causal relationship was identified, the fibre supplemented feed could not be recommended in dietetic management of symptoms for these patients.

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INTRODUCTION

The primary purpose of the diet is to provide sufficient nutrients to satisfy the metabolic demands of the body and enable the deposition of tissue during growth in children. If the metabolic demand of cellular processes increase, such as in disease, energy and nutrient intake must also increase. This may be difficult to achieve if disease is affecting appetite for food. In addition, impairment of digestive or absorptive processes may limit the availability of energy intake to the body. The amount of energy in stool and urinary losses is usually relatively small in healthy individuals (equivalent to less than 5% of energy intake). However, in diseases such as Cystic Fibrosis (CF), where maldigestion and malabsorption occur, stool losses may be significantly increased (typically 20 – 80% of energy intake in untreated patients). Such marked losses would substantially limit the availability of energy from the diet. As a consequence the dietary requirement for energy is increased further and may result in supplementary feeding for those patients with consistently poor appetites and inadequate energy intakes for growth. Enterally fed CF patients represent approximately 10% of the CF population in the UK. The current recommendation for the dietary management of this patient group is to provide an energy dense enteral feed but the majority of commercially available high energy feeds do not contain dietary fibre.

Maintenance of bowel habit requires the presence of colonic substrates for bacterial fermentation. In the healthy colon this is principally dietary fibre and a smaller component of maldigested / malabsorbed dietary residue and endogenous secretions. When healthy individuals consume a consistently low dietary fibre intake they are reported to complain of gastrointestinal symptoms e.g. abdominal pain or discomfort, due to a prolonged colonic transit time and small stool weights (Cummings 1986). When non-enterally fed CF patients consume a high energy diet, dietary fibre intake is compromised and those with the lowest fibre intakes are reported to complain of the most frequent gastrointestinal symptoms (Gavin *et al* 1997). In the enterally fed CF patients,

the effect of a low dietary fibre intake on the frequency of gastrointestinal symptoms and stool output has yet to be determined. The objective of this study therefore, was to determine whether the relationship between a low NSP intake and abdominal symptoms was causal and consequently, whether a higher fibre intake could prevent gastrointestinal symptoms in the enterally fed CF group by influencing colonic function and stool composition. If increasing fibre intake can relieve symptoms there are clinical implications for the dietetic management of the enterally fed CF patient. Feeds supplemented with fibre are commercially available but are infrequently used in CF patients because the effects on stool composition and gastrointestinal symptoms have not been previously characterised. By providing a specific quantity of dietary fibre to the CF colon on a daily basis it should be possible to investigate the influence of fibre on bowel habit, stool composition and energy availability.

As this study is primarily concerned with the role of dietary fibre, the literature review (Chapter 1) is concerned with the metabolic handling of dietary carbohydrates rather then a range of nutrients. The metabolism of dietary carbohydrates is reviewed in both healthy individuals and CF patients. Chapter 2 presents the methods used to characterise habitual stool composition and colonic function in an enterally fed CF group and a healthy group (Phase 1) and an enterally fed CF group receiving a fibre-free feed or a fibre enriched feed (Phase 2). Chapter 3 presents the results from the two phases of the study and Chapter 4 presents the discussion.

CHAPTER 1 LITERATURE REVIEW

1.1 METABOLISM OF DIETARY CARBOHYDRATES

Traditionally, dietary carbohydrates were divided into two groups; "available carbohydrates" such as starch and sugars providing energy to the body upon digestion and absorption, and "unavailable carbohydrates" collectively termed as dietary fibre which remain relatively undigested and pass into the colon.

More recently, a detailed classification of dietary carbohydrates has been developed based on chemical analysis, and this is summarised in Table 1.1.

TABLE 1.1 CLASSIFICATION OF DIETARY CARBOHYDRATES (Englyst & Hudson 2000)

Class	Components
Free sugars	Mono- and disaccharides Sugar alcohols
Short-chain carbohydrates	Oligosaccharides and inulin
Starch	Rapidly digestible starch (RDS) Slowly digestible starch (SDS) Resistant starch (RS)
Non-starch polysaccharides (NSP)	Cell-wall NSP in plant foods Other NSP

Green plants synthesise sugars that can be polymerised to form polysaccharides. The sugars provide the energy for the plant cells whilst the polysaccharides are stored (Englyst & Hudson 2000). Plants sugars e.g. fructose, glucose and pentose are in a monosaccharide form.

Starch is stored in granules in the roots and seeds of plants, and as glycogen in animal tissues. Starch grains contain two polysaccharides derived from glucose: amylopectin and amylose (Southgate 1995). The former is a highly branched molecule with approximately 12 glucose units in each branch joined by α 1 - 6 linkages and the latter is a long unbranched chain of glucose units with α 1 - 4 linkages (Gray 1975; Cummings & Englyst 1995; Southgate 1995). Glycogen is similar to amylopectin in structure as it also contains α 1 - 6 linkages. Dextrins are degradation products of starch whereby the glucose chains have been broken down to smaller units by partial hydrolysis (Gray 1992). Dextrins are the main source of carbohydrate in enteral feeds.

In the Western diet starch accounts for 24% of energy intake (Gregory *et al* 1990) and sugars 17 – 25% of energy intake (Dept of Health 1989). Upon ingestion starches and sugars are digested by salivary and pancreatic enzymes into simple sugars and then absorbed providing a source of energy to meet the metabolic demands of the body (Englyst & Hudson 2000). It is generally assumed that in the healthy individual all starches and sugars are completely processed within the small intestine but a proportion of starch depending on its physical form actually escapes digestion (resistant starch) and enters the colon (Cummings & Englyst 1995).

Other components of the plant cell walls such as cellulose, hemicellulose, oligosaccharides and pectin are not digested by alimentary enzymes and pass through the intestine virtually unchanged where they are partly fermented and the remainder excreted in stool (Cummings 1981b; Englyst &

Cummings 1987b; Southgate 1995). In addition to maldigested and malabsorbed starches, they are therefore termed as dietary fibre.

1.1.1 DIGESTION AND ABSORPTION OF CARBOHYDRATES

Amylase, an enzyme secreted by the salivary glands, begins the digestion of starch polysaccharides into disaccharides in the mouth but this enzyme is inactivated by stomach acidity (Macdonald 1980). When the stomach contents reach the small intestine they are neutralised by bicarbonate secreted from the pancreas, and pancreatic amylase continues the process of starch digestion (Southgate 1995). Starch is hydrolysed by this enzyme to short-chain dextrins and maltose. Disaccharidases in the brush border of the intestinal epithelium (Borgstrom *et al* 1957; Miller & Crane 1961) split the dextrin and the disaccharidases maltase, sucrase and lactase convert maltose, sucrose and lactose into monosaccharides, glucose, fructose and galactose (Southgate 1995). These are transported across the epithelial cells against a concentration gradient and enter the portal vein (Macdonald 1980). Galactose and glucose are absorbed faster than fructose (Gray 1992). The main monosaccharide absorbed is glucose and after leaving the gastrointestinal tract in the portal vein it is transported to the liver where it is utilised in two different ways (Southgate 1995) either stored in the form of glycogen or oxidised into fat before subsequent utilisation.

1.1.2 DIETARY FIBRE

Dietary fibre was first described as "plant cell wall polysaccharides and lignin that resist digestion by the enzymes of the human gut" (Trowell 1972). This definition is open to criticism because the exact site of digestive processes on fibre in man is still unclear, and there is considerable variation in the degree to which each type of fibre is degraded in individuals (Cummings 1981b; Stephen *et al* 1983; Forsum *et al* 1990; Brunsgaard *et al* 1995). Cummings & Englyst (1987) later defined dietary fibre as "consisting of a group of cell wall and related substances which resist digestion in the upper small intestine".

The definition of fibre has since been clarified by chemical analysis of the structure; a complex carbohydrate consisting of at least 20 monosaccharide sugar residues with different physical and chemical properties. This includes the proportion of dietary starches and sugars by virtue of their physical form that resist digestion and absorption (resistant starch) and pass into the colon for bacterial fermentation or excretion. The British Nutrition Foundation Task Force (1990) suggested that the term dietary fibre should therefore be replaced by a definition of the physiological effects of the plant polysaccharides. More recently, Bar (1994) suggested that all carbohydrates that reach the intestine will be fermented therefore should be included in the definition of dietary fibre. The current definition of dietary fibre now exists as non-starch polysaccharides (NSP) lignin and resistant starch. The NSP can be further sub-divided to distinguish between cellulose and non-cellulosic polysaccharides (NCP) (Cummings & Englyst 1987) such as hemicellulose, pectin, inulin, guar, plant gums, oligosaccharides, mucilages. Cellulose is a polymer of glucose linked by β 1 - 4 bonds whilst hemicelluloses are branched polymers of pentose and hexose sugars. Pectins, gums and mucilages are complex mixtures of polysaccharides. Non-cellulosic polysaccharides contain both water insoluble and water soluble components (Englyst *et al* 1982). References to dietary fibre in this thesis will be collectively termed as NSP.

1.1.3 SUMMARY

In the healthy individual starches and sugars are either completely digested and absorbed in the small intestine providing a source of energy to meet the metabolic demands of the body or pass undigested and malabsorbed into the colon. Plant cell wall components also pass through the intestine to the colon for bacterial fermentation or excretion. In combination with maldigested / malabsorbed starches and sugars, these cell wall polysaccharides are collectively known as NSP.

1.2 BACTERIAL FERMENTATION IN THE HEALTHY COLON

1.2.1 COLONIC BACTERIAL FLORA

The large intestine is an extremely complex ecosystem that contains more than 400 different species of bacteria (Finegold *et al* 1983). There are on average 10^{11} to 10^{12} bacteria per gram dry weight of stool (Moore & Holdeman 1974; Finegold *et al* 1977). The normal human intestinal flora has primarily an anaerobic metabolism, but facultative anaerobes are also present. The numerically predominant anaerobes are Bacteroides (30%), Bifidobacteria (25%), and Eubacteria (Finegold *et al* 1983; Cummings & Macfarlane 1991; Gibson *et al* 1995). The species of bacteria that exist in the colon reflect the variety of fermentable substrates presented to the bacteria. These include saccharolytic species that ferment complex carbohydrates, proteolytic bacteria, methanogens, and other bacteria that grow on the products of fermentation such as hydrogen, lactate, succinate (Gibson *et al* 1995). The ability to compete for limiting nutrients and adhesion sites on food particles and on the colonic mucosa determines the number of different species, so that those that are unable to compete are eliminated from the system. The gut flora partake in a number of metabolic processes with the role of maintaining homeostasis through fermentation (Cummings & Macfarlane 1991; Gibson *et al* 1995).

1.2.2 BACTERIAL FERMENTATION

Fermentation is defined as an anaerobic process whereby bacteria break down dietary and other substrates to obtain energy for growth and maintenance of cellular function (Cummings & Englyst 1987). Miller & Wolin (1979) have derived an equation for fermentation of complex carbohydrate in the human colon from studies on the rumen of animals:

$$34.5\ C_6H_{12}O_6 \longrightarrow 48CH_3COOH + 11CH_3CH_2COOH + 5CH_3(CH_2)_2COOH$$
$$23.75\ CH_4 + 34.25CO_2 + 10.5H_2O + \text{ENERGY}$$

The three main products of fermentation are short chain fatty acids (SCFA) (acetic, propionic and butyric acid) gases (carbon dioxide, hydrogen and methane) and energy. Hydrogen is produced in large quantities during fermentation (Cummings 1981a; Gibson *et al* 1988) and approximately one-fifth of the hydrogen is absorbed into the bloodstream and excreted on breath (Levitt 1971) and the rest expelled as flatus. This fermentation equation is generic and can therefore be applied to a collective range of bacterial species. Individual species however, may have different fermentation strategies producing different proportions of SCFA and growth patterns. Furthermore, the availability of environmental co-factors e.g. pH, other than the type and quantity of fermentable substrates available (Table 1.2), will also influence the fermentation strategy.

Table 1.2 THE PRINCIPAL SUBSTRATES AVAILABLE FOR FERMENTATION BY BACTERIA IN THE LARGE INTESTINE OF HEALTHY PERSONS CONSUMING WESTERN DIETS (Cummings & Macfarlane 1991)

SUBSTRATE	AMOUNT (g/d)	ORIGIN
Non-starch polysaccharides	8-18	All plant foods
Resistant starch	8-40	Starch foods
Oligosaccharides	2-8	Legumes,root vegetables, artichokes
Unabsorbed sugars	2-10	Lactase deficiency
Dietary protein	3-9	Whole seeds and grains
Pancreatic enzymes and other gut secretions	4-6	
Mucus	2-3	
Sloughed epithelial cells	Unknown	

1.2.3 BACTERIAL FERMENTATION OF MALABSORBED STARCH AND NSP

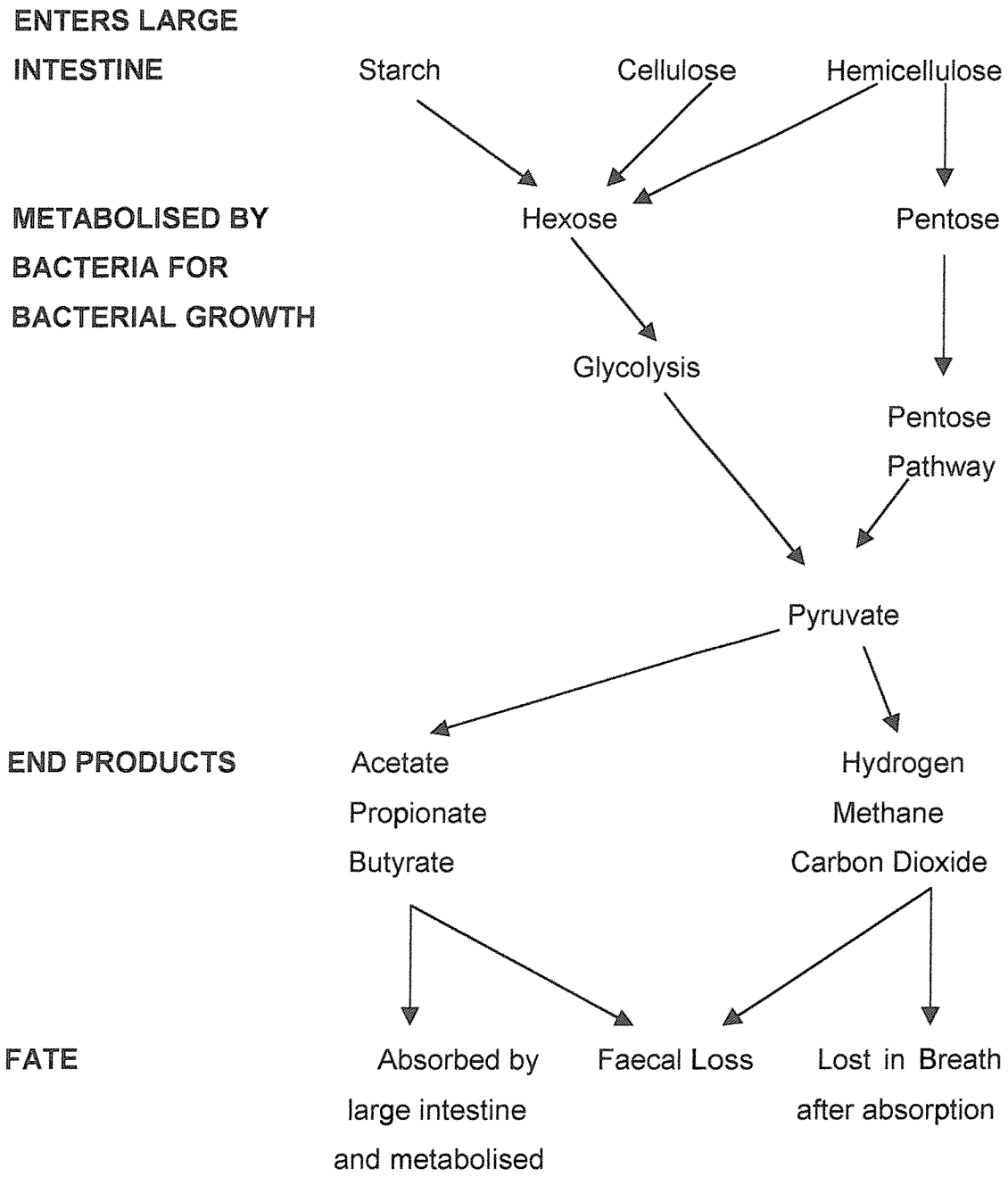
Starch is a storage polysaccharide found in cereal grains, potatoes, legumes and some root vegetables. Some types of starch resist digestion by amylase and are therefore termed resistant starch (Englyst & Cummings 1987b). Resistant starch is the predominant substrate presented to the colon for fermentation (Cummings & Macfarlane 1991). Much of our understanding of the nature of dietary carbohydrate reaching the colon has been derived from studies measuring the effluent of ileostomy patients.

Resistant starch can be classified into three different types (Englyst *et al* 1992a;

- Type I: Starch that is physically inaccessible to digestion by enzymes e.g. grains seeds, corn ,peas
- Type II: Highly crystallised starch granules e.g. bananas, peas and beans present a small surface area that is inaccessible to enzymes
- Type III: Retrogradation of starch structure caused by manufacturing processes e.g. freezing and cooling results in resistance of the structure to digestion.

Resistant starch and NSP are hydrolysed by cell-associated bacterial polysaccharidases and glycosidases in the colon to their component sugars, hexoses and pentoses, and amino acids (McNeil 1984). The stages in the breakdown of dietary carbohydrates are illustrated in Figure 1.1.

FIGURE 1.1 STAGES IN THE BREAKDOWN AND ASSIMILATION OF CARBOHYDRATE IN THE HUMAN LARGE INTESTINE (McNeil 1984)



1.2.4 BACTERIAL FERMENTATION OF NON-DIGESTIBLE OLIGOSACCHARIDES AND INULIN

Oligosaccharides and inulin are resistant to the action of salivary, pancreatic and intestinal hydrolases and stomach acid (Roberfroid *et al* 1993). In the large intestine bifidobacteria preferentially ferment these substrates. They are polymers of fructose bound by β 1-2 osidic bonds and one extremity is occupied by either a β D-fructose or an α D-glucose (Gibson *et al* 1995). Oligofructose is a product of partial hydrolysis of inulin, and both occur naturally in some foods. The average daily intake of both substrates is 2 - 12 g/person/day (Roberfroid *et al* 1993). Not all strains of bifidobacteria ferment oligofructose to the same extent, but once the oligofructose is hydrolysed, β D-fructose serves as a substrate for the Bifidus pathway by which bifidobacteria oxidise carbohydrates (Roberfroid *et al* 1993). The enzyme fructose-6-phosphate phosphoketolase is specific to this pathway and to bifidobacteria, so this enzyme can be used to identify the presence of the bacteria. *In vitro* fermentation of oligofructose yields 40% SCFA (primarily acetate), 15% lactate and 5% CO_2 and the remaining 40% of energy is used for bacterial growth of bifidobacteria (Roberfroid *et al* 1993).

1.2.5 BACTERIAL FERMENTATION OF PROTEIN

Protein breakdown involves hydrolysis of protein to peptides by bacterial endopeptidases. Hydrolysis provides nitrogen for growth of saccharolytic bacteria and amino acids for fermentation by asaccharolytic species. The bacteria involved in proteolysis are peptococci, acidaminococcus, fusobacteria, eubacteria and clostridia (Macfarlane *et al* 1986). End products include SCFA, branched chain fatty acids, ammonia, phenols, indoles, organic acids, alcohols, CO_2 and H_2. Fermentation of protein has been shown to provide 17% of SCFA produced in the caecum and 38% of SCFA in the sigmoid / rectum (Macfarlane & Macfarlane1997).

1.2.6 BACTERIAL FERMENTATION OF MUCINS

Colonic mucins are glycoproteins and the carbohydrate component is either an acidic mucopolysaccharide (containing uronic acids and hexosamines) or an oligosaccharide (containing L-fucose). Measurement of hexosamine in ileal flow in the fasting subject corresponds to a mucin contribution of 3 - 4 g into the large intestine daily (Stephen *et al* 1983). Little is known about the ability of bacteria to degrade mucin polysaccharides. Hoskins and Boulding (1982) attempted to define the bacteria involved by studying mucin degradation in liquid anaerobic faecal cultures. This identified a functionally distinct bacterial subset with extracellular glycosidases and L-fucosidases, averaging 1% of the normal gut flora capable of degrading mucin carbohydrate. The majority of species capable of fermenting mucins are from the bacteroides genera (Salyers *et al* 1977). Extracellular enzymes are especially beneficial in CF where the mucus is highly viscous, because they reduce the viscosity sufficiently to permit attack by cell-associated enzymes. In addition, CF mucus is reported to be hexose-rich (Wesley *et al* 1983) therefore providing large quantities of fermentable substrate for bacterial growth.

1.2.7 BACTERIAL METABOLISM OF SUGARS

The sugars lactose, raffinose, and stacchyose escape digestion in the upper intestine and pass into the colon (Southgate 1995). The sugar alcohols sorbitol and xylitol and a number of synthetic carbohydrates are used as sweeteners in foods and are poorly absorbed passing into the colon. Both sugars and sugar alcohols are efficiently metabolised by colonic bacteria (Tadesse *et al* 1980; Englyst & Hudson 2000)

1.2.8 BACTERIAL METABOLISM OF FATS

Little is known about the mechanisms involved in bacterial metabolism of malabsorbed fats. As NSP is the only dietary fraction shown to have a significant effect on stool bacterial mass, maldigested / malabsorbed fat would appear to contribute primarily to fecal bulk, potentially causing gastrointestinal symptoms, rather then influencing bacterial metabolism (Englyst & Hudson 2000). Smith & Bryant (1979) however proposed that some bacterial fermentation does take place as long chain fats are hydrolysed to glycerol and unsaturated fatty acids are hydrogenated. Hydroxy - fatty acids are reported to have the opposite effect to SCFA (Dodge 1996). They restrict blood flow to the colonocytes and inhibit stimulation of water and sodium from the colonic lumen potentially causing mucosal and submucosal damage (Dodge 1996).

1.2.9 SUMMARY

The species of colonic bacteria are dependent upon the variety of substrates presented to the colon. The numerically predominant species are saccharolytic as the principle substrates are carbohydrate based. The principal products of fermentation of NSP, maldigested / malabsorbed nutrients, endogenous products and mucins are SCFA, carbon dioxide, methane, hydrogen and energy

1.3 SCFA PRODUCTION

The fermentation of NSP, resistant starch and oligosaccharides accounts for 83-95% of the SCFA production in the large intestine (Nordgaard & Mortensen 1995). The remainder of SCFA are produced by fermentation of mucin, protein and other endogenous products. Difficulties in accessing the colon in living subjects have prevented direct measurements of SCFA production. Estimates of SCFA content in stools may act as a proxy for colonic fermentation, but it has been reported that approximately 95% of SCFA are absorbed as they pass along the transverse colon therefore stool output represents only a small proportion of production (McNeil *et al* 1978; Cummings 1981a). Studies of pH changes in the colon show that bacterial fermentation is greatest in the caecum and ascending colon where substrate is most available, although poorly fermented fibres such as wheat bran can be fermented in the descending colon (Cummings *et al* 1987; McIntyre *et al* 1991).

An indirect method of calculation of SCFA production is based on the growth requirements of intestinal bacteria (McNeil 1984). 0.1 mol ATP is needed to generate 1 g of bacteria. The average stool bacterial mass on a British diet is 15 - 20 g bacteria per day, therefore 1.5 - 2.0 mol ATP are needed to replace daily stool output. 50 – 65 g hexose is required to produce 1.5 – 2 mol ATP and generate 15 – 20 g bacteria. Approximately 15 - 30% of 50 – 65 g hexose will be NSP and the rest will be maldigested / malabsorbed sugars and starch. 10 g carbohydrate will yield 75 mmol SCFA therefore 50 – 65 g hexose will yield approximately 400 - 500 mmol SCFA. Fermentation of the hexose component within mucus will make a small contribution to SCFA production in healthy individuals, but this may be increased in CF where endogenous mucus production is excessive. This method of calculation of SCFA production assumes that stool bacteria is representative of colonic bacterial content. As bacterial turnover is high within the colon (Mandelstam *et al* 1982)

this may not be the case, so the calculation might actually underestimate SCFA production.

1.3.1 ABSORPTION OF SCFA

SCFA are rapidly absorbed across the colonic mucosa, into the portal vein and transported to the liver. This allows the host to salvage a source of energy. The relative energy contribution made by colonic fermentation may be small when gross energy intake is high. For individuals on a marginal intake the relative contribution will be greater and may be crucial to weight gain and growth. The precise mechanism by which SCFA are absorbed is not well understood, except that the process is concentration dependent and SCFA transport is associated with the appearance of bicarbonate ions and stimulation of sodium absorption and is independent of bulk water flow (Cummings 1981a). SCFA are the driving force for movement of water out of the colonic lumen and consequently may constitute an important protection against diarrhoea (Silk 1987). SCFA are metabolised to carbon dioxide and ketone bodies and are precursors for lipid biosynthesis in the colonic mucosa, important in mucosal integrity and growth (Roediger 1989). Butyrate is preferentially metabolised by colonocytes providing 70% of the energy for these processes. The addition of butyrate to cell and tissue cultures *in vitro* renders the cell less susceptible to malignant damage therefore butyrate may also have a protective mechanism (Silk 1987; Gibson *et al* 1995). Propionate is transported to the liver to modulate hepatic carbohydrate and lipid metabolism. Acetate also escapes colonic metabolism and serves as a fuel for muscle (Cummings & Macfarlane 1991).

1.3.2 EXCRETION OF SCFA

McNeil *et al* (1978) reported that in the healthy adult colon approximately 95% SCFA are readily absorbed from the intestinal lumen, therefore only 5% are present in the stool. Fleming & Rodriguez (1983) suggested that although NSP might influence the excretion of SCFA, individuals have a variety of responses in terms of the quantity of SCFA excreted. In addition, the degree of excretion of SCFA could be affected by different levels of SCFA production, caused by different microbial populations and differences in enzymatic or microbial activities even in the same populations (Fleming & Rodriguez 1983). The degree to which fermentation of endogenous products influences SCFA production and excretion has not yet been identified.

1.3.3 EFFECT OF ANTIBIOTICS ON SCFA PRODUCTION

Studies investigating the effect of antibiotics on microflora have been largely undertaken on healthy subjects (see section 1.5.2 for discussion of the effect of antibiotics in CF). The severity with which antibiotics cause changes in the healthy intestinal microflora is dependent on their concentrations and antimicrobial spectra. Both oral and intravenous administrations of antibiotics can impair colonic fermentation but the mechanism for this is not clear. Ampicillin, Clindamycin, and Vancomycin have been reported to suppress SCFA production (Hoverstad *et al* 1986b) whilst Erythromycin is only partially capable of this effect. Oral administration of Clindamycin decreases bacterial fermentation, reducing the stool concentration of acetate to 50%, and propionate and butyrate to 10% of pre-treatment values (Hoverstad *et al* 1986b). The residual carbohydrate content accumulates in the colon as unfermented osmotically active saccharides and may stimulate gastrointestinal symptoms or diarrhoea (Clausen *et al* 1991).

1.3.4 SUMMARY

Butyrate is the principle SCFA involved in maintenance of mucosal integrity and bacterial proliferation. SCFA are absorbed across the colonic mucosa allowing the host to salvage an additional source of energy, therefore contributing to weight gain and growth especially in individuals with marginal dietary intake. Absorption of SCFA stimulates water and sodium absorption to create a formed stool. SCFA production may be reduced in patients receiving regular antibiotics as specific colonic bacteria are eliminated. This might precipitate gastrointestinal symptoms or loose stools as a result of osmotically active unfermented carbohydrates remaining in the colon.

1.4 ROLE OF NSP ON BOWEL HABIT IN THE COLON OF HEALTHY INDIVIDUALS

1.4.1 NSP INTAKE IN HEALTHY INDIVIDUALS

The reference figure for healthy subjects is 18 g NSP per day based on the quantity required to increase stool weights by 25 per cent to reduce the number of individuals in the UK with stool weights below 100 g per day (Dept of Health 1991). This does not however take into consideration maldigested / malabsorbed starches that have a similar physiological effect to NSP. The average NSP intake in healthy adults in the UK is 11 - 13 g/day and vegetables rather than cereals now supply the main source of NSP (Bingham *et al* 1985). There are no reference figures for NSP intake for children in the UK except that they should have proportionately lower intakes to adults (Dept of Health 1991). Williams *et al* 1995 (on behalf of the American Academy of Paediatrics) recommends that children should consume "age + 5 grams" and adolescents "age + 10 grams" to prevent colonic dysfunction and disease in adulthood. There are little data on NSP intakes in healthy children. A mean NSP intake of 14.7 g/d was demonstrated in a group of healthy children aged 7 - 18 years (Ellis *et al* 1992).

1.4.2 THE ROLE OF NSP IN PREDICTING STOOL WEIGHT AND GASTROINTESTINAL SYMPTOMS IN HEALTHY INDIVIDUALS

In the UK, median stool weight is approximately 100 g/day with 95% of the adult population passing between 30 - 260 g faeces per day (Department of Health 1991). Stool weights below 150 g/day have been associated with increased risks of bowel cancer (Burkitt 1971b). There is a simple linear relationship between NSP intake and stool weight over a range of intakes from 4 - 32 g/day, other factors being constant, but NSP intakes of greater than 32 g/day do not produce further stool weight increase (Cummings 1986). On NSP-free diets, stool weights are reduced to 30 - 60 g/day and volunteers complain of abdominal symptoms (Cummings 1986). There are four distinct

properties of NSP that create an increase in stool weight and may prevent gastrointestinal symptoms (Cummings 1984):

- Plant cell walls that resist degradation by gut flora (due to the presence of lignin) are of low digestibility and are able to retain water within their cellular structure, which aids colonic movement and creates a heavier stool
- Degradation by microflora of highly digestible NSP stimulates bacterial growth and this contributes to stool weight.
- Due to shortened transit time, there is less water absorbed by the colon therefore greater wet weight of stools
- Gas production from fermentation becomes trapped within faeces and increases stool bulk.

The quantity of NSP added to the diet can be directly related to increased stool weight. When Cummings *et al* (1978) fed subjects 22 g of apple NSP for 3 weeks, those with the smallest stool weights had the smallest increase in stool weight. The average daily increase was 37 – 99 g/day, which was a direct proportional increase on the original weights (65 – 194 g/day).

Another study demonstrates that the type of NSP consumed affects stool composition in different ways. Stephen & Cummings (1980) fed volunteers 18 g/day of wheat bran for three weeks. Stool weight increased by 102 g/day; 48% of this weight was due to the water-holding effect of the excreted undigested bran and 10% due to the increased bacteria in the stool. When these volunteers were then given 18.3 g/day cabbage NSP for three weeks, stool weight only increased by 54.3 g/day;12% of this weight was accounted for by excreted NSP, and 35% of the increase was attributed to an increased stool bacterial mass. Wheat bran (a poorly digested NSP due to a large particle size) therefore has a greater effect on stool weight than cabbage NSP, which is a smaller particle size and has a larger surface area for bacterial fermentation, therefore affects stool weight by increasing bacterial

mass. Although subject numbers were small in both studies, the effect of NSP on the colon was reproducible.

1.4.3 ROLE OF NSP ON TRANSIT TIME IN HEALTHY INDIVIDUALS

On average it takes 24 - 72 hours for NSP to pass through the digestive system. Soluble NSP may increase the viscosity of stomach contents causing delayed gastric emptying (Spiller 1996). Average colonic transit time is 18 - 64 hours (Bond & Levitt 1976). A raised intake of NSP shortens colonic transit time consequently increasing bacterial mass and turnover so energy required for maintenance of bacteria is reduced and more energy is available for bacterial growth (Stephen 1985). As fermentation of NSP will be increased, more bacteria will therefore be excreted in the stool. Shortened transit time also results in reduced water reabsorption in the colon therefore a stool with larger water content.

1.4.4 ROLE OF NSP IN MAINTENANCE OF COLONIC BACTERIA IN HEALTHY INDIVIDUALS

Colonic bacteria are reported to contribute up to 45 - 50% of wet stool weight in a healthy adult depending on the quantity of fermentable substrate presented to the colon (Stephen & Cummings 1980; Cummings & Macfarlane1991). When adult subjects were fed a NSP-free diet, a diet supplemented with NSP as bananas, and a habitual diet, bacteria represented 24%, 42.6% and 19.1% of stool dry weight respectively (Murphy 1991). In the case of the NSP-free diet (also resistant starch–free), energy required for the maintenance of the bacteria must have been supplied by the fermentation of endogenous products as the amount of dietary residue reaching the colon was small.

1.4.5 ROLE OF NSP IN LIPID ABSORPTION IN HEALTHY INDIVIDUALS

NSP has been shown to impair lipid digestion and absorption *in vitro* through several mechanisms:

a) NSP binds to pancreatic lipase resulting in partial loss of the enzyme activity involved in fat digestion *in vitro.* Wheat bran, oat bran, cellulose and to a lesser extent pectin demonstrated this effect *in vitro* (Lairon *et al* 1985; Verbeek *et al* 1995).

b) Soluble NSP *in vitro* enhances the viscosity of intestinal contents restricting the peristaltic process that promotes transport of bile salts to unmicellised fat. Alternatively soluble NSP physically traps lipids within the particle structure so the lipid is unavailable for micelle formation (Eastwood & Morris 1992; Spiller 1996).

An enhanced lipid excretion has also been seen *in vivo* but the exact mechanism is unclear. When 45 g/day wholewheat products were introduced into the diets of healthy subjects stool lipid losses increased significantly from 3 to 4.5 g/day (Cummings *et al* 1976). The increase may have been a consequence of indigenous lipid contained in the NSP products, but when pectin (a lipid free NSP component) is administered at 15 g/day, stool lipid losses double (Kay & Truswell 1977). As pectin is a fermentable substrate bacterial mass is ultimately increased. A greater stool lipid could therefore be attributed to an increased bacterial lipid and not due to any effect of NSP upon fat digestion or absorption from food (Murphy 1991). It may even be a combination of both effects.

1.4.6 ROLE OF NSP IN STOOL ENERGY LOSSES IN HEALTHY INDIVIDUALS

Energy within the stool is derived from a number of sources. The components of stool dry weight are undigested/ malabsorbed residue such as protein, lipid, resistant starch, NSP and endogenous products e.g. mucus and colonic bacteria (Stephen & Cummings 1980b). The average daily stool energy in healthy adults is reported to be equivalent to 3 - 5% of the gross energy intake (Southgate & Durnin 1970) and stool energy per gram is estimated at 25.9 kJ/g; 6.2 kcal/g dry weight of stool (Rubner 1902). The average daily stool energy for children between the ages of 6 -10 years is reported to be equivalent to 3.5% of the gross energy intake and stool energy per gram is estimated at 20.5 kJ/g; 5 kcal/g dry weight of stool and 5.3 kJ/g; 1.3 kcal/g wet weight of stool (Murphy 1991).

Studies have reported that average stool energy losses are greater with higher intakes of NSP in comparison to low and normal intakes (Southgate & Durnin 1970; Kelsay *et al* 1978; Stephen *et al* 1983). A larger stool energy loss may result in an energy deficit in those individuals who are on marginal energy intakes. If this energy deficit remains uncorrected, it may cause weight loss and retarded growth. A study on healthy adult subjects fed a NSP-free diet reported that stool energy was equivalent to 3.2% of gross energy intake, but when bananas were added, stool energy increased to 7.2% of gross energy intake (Murphy 1991). This was principally due to increased bacterial growth stimulated by a fermentable source of NSP (from 24% to 43% of stool dry weight).

1.4.7 SUMMARY

The physiological effect of NSP can influence stool weight, transit time, bacterial mass, lipid digestion and absorption, and stool energy. Soluble NSP has less influence on stool weight but a greater effect on stool bacterial mass whilst insoluble NSP principally affects stool weight and transit time. Improving colonic function through addition of insoluble and soluble NSP to the diet might therefore reduce the risk of gastrointestinal symptoms and bowel disease in those individuals with low stool weights, bacterial mass and slow colonic transit time. The disadvantage of NSP fortification is that it might increase stool energy losses by reducing lipid digestibility but this will only have a detrimental effect on weight gain and growth for those who consume a marginal energy intake.

1.5 GASTROINTESTINAL DYSFUNCTION IN CYSTIC FIBROSIS (CF)

CF is an autosomal recessive gene defect in the transmembrane conductance regulator (CFTR). The most common gene mutation is homozygous delta F508 (World Survey 1990). The gene defect is expressed in many organs and tissues throughout the body including the pancreas where obstruction of the ducts by viscous secretions and fibrosis of the pancreas causes insufficiency (Littlewood 1995). The secretion of proteolytic enzymes such as trypsin and chymotrypsin, lipase, colipase, and amylase are consequently reduced from the pancreas, potentially resulting in maldigestion of protein, carbohydrate, and fat (Davidson 1995). There does, however, remain a high concentration of enzymes in the salivary glands especially lingual lipase, which is acid resistant and may be responsible for a small proportion of residual duodenal lipolytic activity in CF patients (Fredrikzon & Blackberg 1980). Carbohydrates appear to be well absorbed despite a deficiency of pancreatic amylase (Littlewood & MacDonald 1987). This is because of the quantity of salivary amylase that remains, and also due to the normal disaccharidase activities of the small intestinal mucosa (Antonowicz *et al* 1978). With the addition of enteric coated microsphere enzyme therapy, it is possible to simulate digestion provided an "adequate" number of enzymes are taken and compliance is good.

In addition, the small and large bowel have been shown to have abnormal histology (Freye *et al* 1964) whereby excessive viscous mucus is extruded from distended goblet cells and this may obstruct the absorption of nutrients. The saccharide component of this abnormally viscous mucus is more densely glycosylated than non-CF mucin, therefore potentially increases bacterial fermentation in the CF colon (Wesley *et al* 1983). Pancreatic insufficiency also reduces the secretion of bicarbonate into the intestinal lumen (Wong *et al* 1982) which compromises neutralisation of the acid entering the duodenum from the stomach. An acidic environment compromises function of the enteric

coated enzymes, therefore digestion of fats may take place further down the intestine than appropriate for absorption resulting in steatorrhoea (Littlewood 1995). In addition, reduced secretion of bile from the bile ducts of the liver results in an inadequate supply of bile salts so that any digested fat is unable to be solubilised into fat micelles for absorption (Littlewood & MacDonald1987). This combined with excessive faecal bile acid losses contributes to a reduced bile acid pool, and correlates with the severity of maldigestion and malabsorption (Weber *et al* 1973).

1.5.1 STOOL LOSSES RESULTING FROM GASTROINTESTINAL DYSFUNCTION IN CF

For the healthy individual, stool is composed primarily of NSP, bacteria and dietary residue (Stephen & Cummings 1980b). There are little data available on stool composition in CF. The principal study (Murphy 1991) compared stool output from 16 CF patients on pancreatic enzyme therapy with 20 healthy subjects. Stool weights for CF patients were twice that for healthy subjects, and stool energy losses were equivalent to 5 - 20% of gross energy intake in the CF group, approximately three times larger than the healthy group. Both groups consumed similar NSP and energy intakes. The energy content of wet stool was estimated at 8.0 kJ/g in CF patients (Murphy 1991) compared to 5.3 kJ/g for the healthy children.

In the same study (Murphy 1991) stool lipid losses were 12 - 15 g/day for CF patients, compared to 2.2 g/day for healthy children. Bacterial mass accounted for approximately 35% and 25% of the dry mass of the stool for CF patients and healthy subjects respectively. The quantity of fermentable substrate required to maintain the bacterial mass was significantly greater than the NSP consumed by CF patients. This implies that additional substrates other than NSP such as maldigested / malabsorbed nutrients and endogenous mucus were fermented. The effect of a low NSP intake on stool

weight, bacterial mass and energy loss in CF patients has not yet been identified.

1.5.2 GASTROINTESTINAL SYMPTOMS IN CF

A gastrointestinal symptom is defined as an abnormal sensation or emotional expression accompanying gastrointestinal dysfunction (Svedlund *et al* 1988). Few studies have identified the incidence and potential causes of lower gastrointestinal symptoms in CF patients. In one study of a regional clinic 11% of patients reported occasional abdominal pain (Littlewood 1995). There are several unproven theories that exist as to the cause of abdominal symptoms in CF. The use of high strength broad spectrum antibiotics in CF may play a role in the development of abnormal colonic function and gastrointestinal symptoms. However, there has been little attempt to document or address this issue in CF patients. Inadequate doses of pancreatic enzyme can also cause maldigestion and malabsorption of fat that may precipitate gastrointestinal symptoms such as abdominal pain, distension and constipation. These symptoms may develop into Distal Intestinal Obstructive Syndrome (DIOS) and Fibrosing Colonopathy (Littlewood 1995). In such cases, their aetiology can also be linked to colonic dysfunction caused by an inadequate NSP intake. A recent study by Gavin *et al* (1997) reported that CF patients with the lowest NSP intakes had the most frequent and severe abdominal pain suggesting that reduced bacterial metabolism was the cause of impaired colonic function in CF.

A CF patient may have a habitual daily mild abdominal pain that they accept as "normal" therefore do not acknowledge as a symptom. A tool has been developed to aid recording of symptoms for identification of the potential causes. This tool is a self-assessment bowel habit questionnaire (Lyyra 1997) and encourages the patient to subjectively document the frequency and severity of their symptoms (Appendix 1). Using this questionnaire Lyyra (1997) reported that 50% of CF patients and 63% of controls did not have

abdominal symptoms and there was little association between the occurrence of symptoms and NSP intake. This study however, involved only eight CF patients who volunteered to complete the questionnaire.

1.5.3 FIBROSING COLONOPATHY (COLONIC STRICTURES)

Fibrosing colonopathy is a gastrointestinal complication that has been recently described (Smyth *et al* 1994). The causes of this condition have not yet been identified, but various theories include excessive lipase intake (Smyth *et al* 1994), an immunological disorder (Lee & Durie 1997) or the presence of malabsorbed fat in the colon (Dodge 1996). An added concern is that these patients consume only half the NSP intake of age-matched controls (Gavin *et al* 1997) potentially compromising SCFA production therefore mucosal integrity and growth remain unprotected. This theory of reduced bacterial metabolism and SCFA production resulting in "colonic malnutrition" in CF has yet to be proved.

1.5.4 DISTAL INTESTINAL OBSTRUCTIVE SYNDROME (DIOS)

DIOS (meconium ileus equivalent) is partial or complete obstruction of the small or large intestine or both (Littlewood 1995). In CF, it results from thickened adherent mucus in layers in the terminal ileum and ascending colon. The prevalence of DIOS is reported to be 10 - 47% of patients with CF (Anderson *et al* 1990). No cause has yet been identified. Predisposing factors for the condition are frequent respiratory exacerbations, (Dalzell *et al* 1990), dehydration, a rapid change in pancreatic enzyme dosage (O'Halloran *et al* 1986) or a slow colonic transit time because of a low NSP intake (Gavin *et al* 1997). There are however no previous studies on the effect of NSP on transit time in the CF colon to confirm the latter hypothesis. Treatment normally takes the form of gut motility drugs (Koletzo *et al* 1990) rather than increasing the NSP content of the diet as the effect of additional NSP in the CF colon is unknown.

1.5.5 CONSTIPATION

The characteristics of constipation in CF are marginally different to DIOS Littlewood 1995). The main feature is increasing colicky central abdominal pain caused by distension of the colon relieved by defecation. There may be reduced stool frequency, and occasional rectal loading resulting in overflow incontinence. The cause of constipation in CF is unclear although a slow transit time due to a low NSP intake has been suggested (Gavin *et al* 1997). Anecdotal evidence in one clinic reports that a sudden increase in enzyme dosage or a change to a more effective enzyme preparation might have resulted in faecal accumulation in the colon, although the mechanism of this effect is unclear (Littlewood 1995). Treatment normally takes the form of bulk laxatives rather than increasing the NSP content of the diet.

1.5.6 SUMMARY

Increased mucus production, pancreatic and bicarbonate insufficiency, and bile duct blockage result in maldigestion and malabsorption of ingested nutrients in CF. Stool losses are larger for CF patients than healthy subjects when consuming similar NSP intakes but the effect of a low NSP intake on stool output has not been identified. A low NSP intake has been proposed as a cause of gastrointestinal symptoms in CF although many other unproven theories exist.

1.6 GROWTH RETARDATION IN CF

Maldigestion and malabsorption of nutrients has an impact on weight gain and growth in CF. CF children are reported to be smaller and thinner than the healthy child population. A cross sectional survey of weight, height and body mass index measures in 3056 CF patients in the UK showed that during the first decade of life, weight and height are maintained at 0.5 standard deviations below the standards for the general population (Morison *et al* 1997). At the onset of adolescence CF children exhibit delayed puberty, with 10 - 40% of children showing significant growth retardation (Dodge 1996). Growth in CF depends on the interaction of multiple factors, including severity of lung function, appetite, frequency of respiratory infections, degree of maldigestion and malabsorption and the extent to which corrective treatment modifies these factors. With the use of pancreatic enzyme therapy however, maldigestion and stool energy losses can be limited and with the prophylactic use of antibiotics, the frequency and severity of respiratory infections can be reduced. Resting energy expenditure has been reported to be 22% greater in CF patients compared to gender-matched healthy individuals with a similar lean body mass (Wootton *et al* 1991) and may potentially be a primary contributing factor to retarded growth. A reduced physical activity however compensates for an increased energy expenditure therefore total daily expenditure is not markedly raised beyond that of healthy counterparts (Wootton *et al* 1991). The contributing factors to retarded growth therefore are, either an inadequate energy intake or a combination of inadequate intake and uncontrolled maldigestion / malabsorption, or a normal intake but uncontrolled maldigestion / malabsorption.

Final weight and height are greater in those who have been relatively free from chest infections whilst there is marked stunting of growth in those with a long history of lung disease in childhood (Corey *et al* 1988). Corey *et al* (1988) also demonstrated that a high fat dietary intake counteracted maldigestion

and malabsorption for a group of CF patients in Toronto creating marked differences in growth and survival compared to CF patients in Boston who were maintained on a low fat diet. This was despite similar lung function in both groups of patients. Pencharz *et al* (1984) reported that growth retardation in CF was caused primarily by an energy deficiency alone as all biochemical parameters of nutritional status were within a normal range and positive nitrogen balance still existed in a group of children below the ideal range of weight for height. Miller *et al* (1982) also reported CF children to be smaller and thinner but with a proportional lean body mass indicating that severe malnutrition did not exist in this patient group. An increased work of breathing and frequent respiratory infection (Nagabhusan & Rao 1978) may also prevent body fat from being deposited during growth in CF.

1.6.1 ENERGY INTAKE IN CF

The most recently documented "recommendation" of energy intake for CF patients is 120 -150% of the Recommended Daily Allowance (Dept of Health 1979 - RDA) (Roy *et al* 1984; Littlewood & MacDonald 1987; MacDonald 1996). This figure is based on "the energy cost needed to correct poor growth patterns, pubertal delay, and to compensate for stool energy losses" i.e. estimated energy requirement. The figure of 120 –150% RDA would therefore be more appropriately defined as 120 –150% Estimated Average Requirement (EAR) as EAR (Dept of Health 1991 - Dietary Reference Values) defines the estimated reference value of average energy requirement for healthy groups whilst RDA defines the recommended average energy intake of healthy groups. For the purposes of this review however RDA will be presented where it is quoted in the literature, but use of the term RDA in future chapters of this thesis will be replaced by EAR.

Several studies have assessed the ability of CF patients to achieve an intake of 120 – 150% RDA for age. Energy balance studies have shown that weight gain does not occur until CF children consume an energy intake in excess of 100 - 110% RDA for age (Parson *et al* 1983). A longitudinal three year study

of 3 day dietary intakes (four times a year) showed CF children to consume more energy than control children (100 - 107% RDA for age versus 90% RDA for age). This energy intake was not enough to prevent declining weight and height measures over the three year study (Kawchak *et al* 1996). In another study, median energy intake of a group of CF children was 92% RDA for age compared to age and gender matched controls who consumed 91% RDA for age. If the energy intake is expressed relative to body weight, energy intakes were 34% greater than controls. Despite eating more energy, 30% of the CF patients exhibited weights more than 2 standard deviations below the median and 17% exhibited heights 2 standard deviations below the median (Wootton *et al* 1991; Ellis *et al* 1992). This implies that CF children have an increased energy requirement, as larger energy consumption in comparison with healthy subjects is unable to demonstrate adequate weight gain and growth. In addition, the results highlight the inadequacy of assessing energy intake against a recommendation corrected only for age. As weight for age is frequently lower than weight for actual age for CF patients, an intake of 120 – 150% RDA for actual age may be too high to realistically achieve. A CF consensus equation (Ramsey *et al* 1992 – Appendix 2) has been developed that incorporates body weight, an estimated metabolic rate, a disease coefficient and a coefficient of fat absorption to account for maldigestion / malabsorption to calculate energy requirements for CF patients. Comparison of this equation with estimated energy requirement from the doubly labelled water technique, and the figure of 120 - 150% RDA for age, shows an improved prediction of energy requirement using the CF consensus equation (Reilly *et al* 1999).

In general CF patients are unable to achieve an intake of 120 – 150% RDA for age and the reasons are multifactorial. Frequent periods of ill-health inducing temporary anorexia and nausea from swallowed sputum may reduce a patients hunger and energy intake until antibiotics are received. These frequent episodes may induce a long-term energy deficit that creates retarded growth. In addition the constant pressure to eat a high fat diet from parents

and medical staff may eventually result in a child refusing food compromising weight gain and growth (Stark *et al* 1995). Frequent abdominal symptoms may also reduce food intake especially of high NSP containing foods in the knowledge that they may alter stool characteristics and create social embarrassment for younger patients.

1.6.2 NSP INTAKE IN CF PATIENTS

There is little information available about NSP intakes in CF and there is dispute over whether the reference figure for NSP intakes for healthy individuals is also applicable to CF patients. The current nutritional guidelines for the management of CF patients encourage a high energy and fat intake only. In the UK recommendations for other nutrients in the CF diet do not yet exist.

The nature of a high energy diet limits the number of NSP-rich foods that can be consumed as these are generally less energy dense. Gavin *et al* (1997) highlighted this fact by demonstrating that NSP intake, assessed on completion of 5-day food diaries of CF patients was half that of age-matched controls (7.0 g vs 14.7g). Lyyra (1997) reported a similarly low NSP intake for CF patients. In the dietetic management of CF patients with long term inadequate energy intakes and growth retardation enteral feeds are recommended. These products do not routinely contain a source of NSP therefore this group potentially have lower NSP intakes than their non-enterally fed CF counterparts. Are these patients therefore at greater risk of abdominal symptoms as a result?

1.6.3 TREATMENT OF GROWTH RETARDATION IN CF

Growth retardation results from prolonged or excessive reliance on endogenous body fuels through inadequate oral intake. Guidelines for the dietetic management of CF patients unable to meet energy requirements and

whose ideal weight for height is less than 90% is to introduce high energy supplement drinks (MacDonald 1996). Few patients can manage to continue with these supplements for long periods due to "taste fatigue" and food intake is often displaced by supplements so that only minor increases in energy intake are achieved (Wootton *et al* 1991). For those patients whose ideal weight for height falls to 75% enteral feeding is recommended and 40 - 50% RDA for energy provided as an overnight feed (MacDonald 1996). The composition of enteral feeds is based on combined European and American macronutrient and micronutrient requirements and the UK Dietary Reference Values (Dept of Health 1991). At manufacturing level micronutrients are added to the feed based on the quantity required per 100 kcal for subjects over 20 kg body weight. Traditionally these feeds have been NSP-free to maximise the energy density per 100 ml.

Enteral feeding is invariably a long term daily treatment for weight loss and the quantity of feed recommended remains constant until the individual is approaching their ideal weight for height. This current practice of feeding a constant volume ignores daily fluctuations in appetite. As a consequence, there is a concern that feeding excessive calories via a feeding tube will override normal mechanisms that control appetite (Wootton *et al* 1991). Any nutrient given in excess of requirements will be placing a metabolic demand on an individual whose metabolism might already be stressed by a respiratory infection (Wootton *et al* 1991). This may result in poor utilisation of the nutrients consumed and excess energy will be deposited as adipose tissue.

1.6.4 SUMMARY

An inadequate energy intake and an increased energy requirement can cause retarded growth in CF. Treatment for long term energy deficit involves high energy supplements or enteral feeds. Traditionally, enteral feeds are deficient in NSP and oral NSP intakes may be particularly low for the enterally fed CF group. This stimulates concern for the dietetic management of these patients

over whether NSP supplemented feeds should be used routinely with this group in an effort to maintain colonic function and prevent abdominal symptoms.

1.7 THE ROLE OF NSP IN ENTERALLY FED HEALTHY VOLUNTEERS

NSP-free enteral feeds have been used for the treatment of nutritionally compromised patients for several decades. Prolonged use however, is reported to reduce stool wet weight and frequency (Slavin *et al* 1985) and increase whole gut transit time (Slavin *et al* 1985; Walters *et al* 1997). In recent years NSP containing feeds have become commercially available although their clinical benefit remains unclear as the majority of studies have only been completed on healthy subjects. The reported effect of NSP on the healthy colon of enterally fed subjects is now discussed.

1.7.1 TYPES OF NSP IN ENTERAL FEEDS

Traditionally enteral feeds have not contained NSP as it creates an increased viscosity making delivery via a feeding pump difficult (Patil *et al* 1985). Different types of NSP however have variable effects on viscosity (Van Soest 1984) and more recently soy polysaccharide has been selected as the most suitable component for NSP containing enteral feeds.

The ability of soy polysaccharide to simulate the physiological effects of both insoluble and soluble NSP is questionable. When healthy subjects were enterally fed 30 g per day of soy polysaccharide (via a nasogastric tube) with no oral diet for 10 days stool wet weight increased and colonic transit time decreased (Slavin *et al* 1985). Heymsfield *et al* (1988) showed that healthy individuals reported larger stool weights with correspondingly larger intakes of soy polysaccharide feed compared with an NSP-free formula when fed for two weeks. Other studies (Lubke *et al* 1987; Fischer *et al* 1985; Patil *et al* 1985) providing a range of 21 - 30 g per day of soy based feeds have not been able

to support these results even though the recommended NSP intake for the UK is only 18 g per day. All these studies were over a short feeding period of between 4 - 14 days and this may have been an insufficient length of time for the colon to adapt to an increased fermentable substrate. For NSP to affect stool composition and colonic function, a one month period is required for colonic adaptation (Brunsgaard *et al* 1995). This time period was however demonstrated in rats therefore the length of time for adaptation may be longer in humans. An adaptation period exists because colonic bacterial enzymes are substrate inducible so daily exposure to a specific quantity of NSP may alter stool weight and composition with time. In addition a regular intake of NSP over a longer period of time will stimulate fermentation and turnover of a range of bacterial species potentially influencing colonic function to a greater or lesser extent. In support of the theory of colonic adaptation, a one year study of colonic function indices on the addition of 18-25 g/day soy polysaccharide liquid diet to mentally retarded patients resulted in a significant increase in stool weight and bowel frequency (Liebl *et al* 1990).

Soya polysaccharide consists of β - linkages of more than one type of monosaccharide. As soy polysaccharide is a rapidly fermented small particle sized, single source of predominantly non-cellulosic NSP, an increase in stool weight will be the result of stimulated bacterial growth within the colon. This type of NSP has a smaller effect on stool weight compared to a larger particle, poorly fermented substrate such as wheat bran (Stephen & Cummings 1980). A single source of NSP will therefore only have marginal benefit on stool weight. Using a mixed NSP source of variable fermentability and solubility might have a greater influence on stool weight. Sunvold *et al* (1995) studied the effect of a mixed NSP blend (75% poorly fermented and 25% rapidly fermented NSP) compared to a 100% soy enriched feed. Stool wet weight was significantly greater in the NSP blend group due to the additional water-holding properties of the larger oat particle.

Another study in healthy subjects using a mixed NSP blend however failed to show any effect on stool weight compared with an NSP-free feed although colonic transit time was decreased (Walters *et al* 1997). This may have been due to a different study design, a different proportion of mixed NSP, or an insufficient feeding period. In all these studies healthy subjects were used and no oral diet was consumed. The only source of nutrients was the enteral feed so the effect of added NSP could be seen more clearly. A high starch basal diet in combination with an enteral feed has been shown to obscure the effects of NSP because of the contribution of malabsorbed starch to colonic fermentation (Howard *et al* 1995a).

1.7.2 ROLE OF A NSP SUPPLEMENTED FEED ON ABDOMINAL SYMPTOMS IN HEALTHY VOLUNTEERS

The addition of NSP to a previously NSP-free feed might initially cause gastrointestinal complaints due to the increased production of gases from bacterial fermentation. Slavin *et al* (1985) asked healthy subjects on a soy enriched feed (30 g/d) to record daily symptoms. No significant difference was seen in subjective measures of bowel function. Patil *et al* (1985) and Walters *et al* (1997) also found an NSP supplemented feed to be equally well tolerated

1.7.3 ROLE OF A NSP SUPPLEMENTED FEED IN TREATMENT FOR DIARRHOEA AND CONSTIPATION IN HEALTHY VOLUNTEERS

In the practical situation, diarrhoea can be defined as the passage of too frequent stools or too loose a consistency. Diarrhoea may be caused by many factors such as intolerance of high osmotic feeds, concomitant antibiotic therapy or metabolic stress (Silk 1987). Antibiotics may compromise the bacterial fermentation processes and reduce SCFA production, which alters water and sodium absorption resulting in a watery stool. Administration of 10 g pectin (a rapidly fermented fibre) to each litre of a NSP-free feed reduced

diarrhoea in healthy individuals but in hospitalised patients results have been inconclusive (Zimmaro *et al* 1989).

Constipation has been defined as a stool frequency of less than three times per week of a stool weight less than 50 g per day (Cummings 1984; Stephen 1985; Department of Health 1991). Subjects receiving NSP-free feeds as a sole source of nutrition are reported to have a habitual stool weight of 40 - 70 g/day and a stool frequency of 0.5 - 0.7 stools per day (Patil *et al* 1985; Slavin *et al* 1985). Low stool weights have been associated with gastrointestinal symptoms and colonic disease (Burkitt 1971b) therefore increasing stool weight is a recently implemented initiative in the dietetic management of patients receiving long-term enteral feeds. There are however little published data on the effect of NSP supplemented feeds on colonic function of enterally fed CF patients to support the use of these feeds in the clinical situation.

1.7.4 SUMMARY

Long term use of NSP-free feeds is reported to cause colonic dysfunction. Single source NSP and mixed NSP containing feeds have become commercially available, but the benefit of these feeds in trial studies is inconclusive. Most of the studies have been completed on healthy individuals with the enteral feed as the sole source of nutrition. Further studies are therefore required to clarify the effects of a NSP feed on colonic function in various patient groups. The results may however be obscured by maldigested / malabsorbed residue if an oral diet is consumed or the functional integrity of the colon is affected.

1.8 THE HYPOTHETICAL ROLE OF NSP ON BOWEL HABIT IN ENTERALLY FED CF PATIENTS

Dietary education for the CF patient is focused on a high fat intake to compensate for energy losses and increased energy requirements. Those patients who are unable to achieve a sufficiently high energy intake may require enteral feeds to increase their intake to meet metabolic demands. These enteral feeds are traditionally NSP-free to maximise the energy density within the feed. Gavin *et al* (1997) reported that a high energy diet compromised NSP intake and CF patients with the lowest NSP intakes were reported to have the most frequent abdominal symptoms. The potential effects of a NSP enriched enteral feed on the bacterial metabolism and bowel habit of CF patients is now described.

1.8.1 STOOL WEIGHT, TRANSIT TIME AND GASTROINTESTINAL SYMPTOMS

Healthy subjects on NSP-free feeds are reported to have low stool weights and a reduced stool frequency (Patil *et al* 1985; Slavin *et al* 1985). Low stool weights precipitate gastrointestinal symptoms and are associated with colonic cancer in non-enterally fed healthy subjects (Burkitt 1971b; Cummings 1986). CF patients are reported to have an increased risk of digestive tract cancer (Lloyd-Still 1990; Neglia *et al* 1995). Enterally fed CF patients with a low NSP intake and NSP-free feeds may therefore potentially have lower stool weights and a prolonged transit time increasing the risk of gastrointestinal symptoms and colonic cancer. A mixed NSP feed may be beneficial by shortening transit time, increasing stool weight and relieving abdominal symptoms.

1.8.2 STOOL ENERGY

A raised NSP intake increases stool energy losses in healthy individuals by increasing stool weight (Southgate & Durnin 1970; Kelsay *et al* 1978;Stephen *et al* 1983). A NSP supplemented feed might therefore increase stool energy losses for the CF patients but these losses may be insignificant compared with the large energy intake consumed by the enterally fed CF group.

1.8.3 STOOL LIPID

Addition of NSP to the enteral feed may increase stool lipid in two ways: impair lipid digestion (Lairon *et al* 1985; Verbeek *et al* 1995) and absorption (Eastwood & Morris 1992; Spiller 1996), or stimulate bacterial proliferation which has an indigenous lipid component (Kay & Truswell 1977; Murphy 1991). A reduction in lipid digestibility and consequently energy availability may however be insignificant in the enterally fed CF population where energy intake often exceeds requirement (Wootton *et al* 1991).

1.8.4 STOOL BACTERIA AND SCFA PRODUCTION

Enterally fed CF patients with lower NSP intakes might have a lower bacterial mass due to the reduced availability of fermentable substrate. Frequent antibiotic treatment may further reduce specific bacteria. As a result SCFA production may be compromised impairing colonic nutrition and function resulting in gastrointestinal symptoms. The addition of a mixed NSP feed may help to maintain a stable bacterial flora and consistent SCFA production, especially throughout antibiotic treatment.

1.8.5 SUMMARY

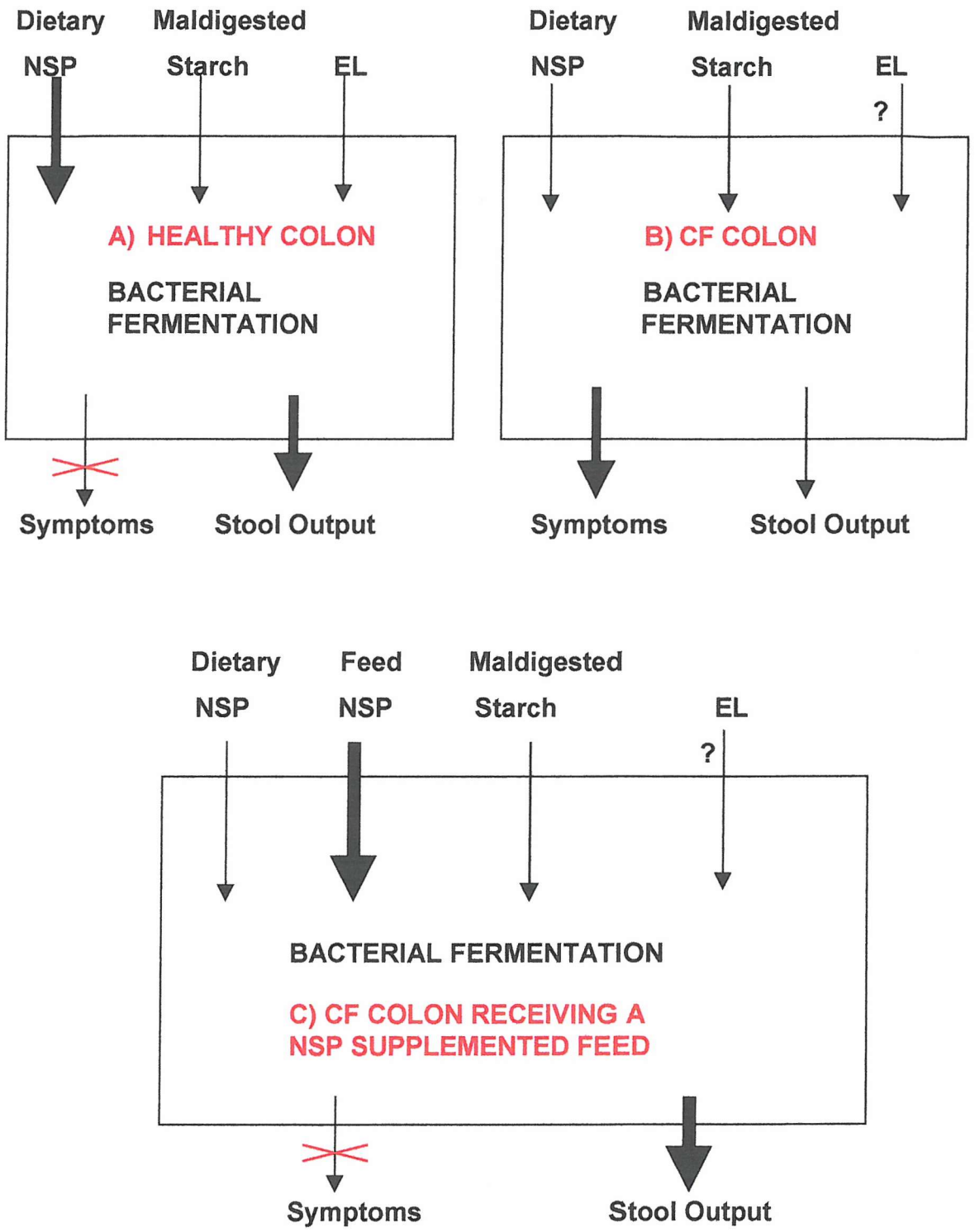
The high energy diet recommended as treatment for CF patients compromises NSP intake, and this is reported to be detrimental to colonic function resulting in abdominal symptoms (Gavin *et al* 1997). The enterally fed group are therefore more at risk of abdominal symptoms due to their low NSP intake and NSP-free feeds. A NSP supplemented enteral feed could potentially relieve symptoms by increasing stool weight and reducing colonic transit time. Lipid digestibility and energy availability might be compromised as a result, but this may be insignificant as energy intake from feed often exceeds energy requirement for this group.

The metabolic pathway of dietary carbohydrates in the healthy colon and the CF colon is summarised in Figure 1.2. In the healthy colon (Figure A) NSP has a primary role in bacterial fermentation and stool composition, and an adequate intake enhances stool weight preventing gastrointestinal symptoms. Endogenous losses and maldigested / malabsorbed nutrients are also presented to the colon but their role in bacterial fermentation and stool composition in the healthy subject is much smaller.

In the CF colon (Figure B) smaller quantities of NSP are presented to the colon but in addition, unquantified amounts of maldigested / malabsorbed nutrients and endogenous mucus. If the role of NSP in the CF colon were assumed to be similar to its role in the healthy colon, a low NSP intake would reduce stool output causing gastrointestinal symptoms.

Figure C illustrates the hypothetical role of a NSP supplemented feed in the CF colon. The additional NSP reinstates NSP as the primary influence on stool composition and bowel habit increasing stool output therefore preventing gastrointestinal symptoms.

FIGURE 1.2 METABOLIC PATHWAY OF DIETARY CARBOHYDRATES IN A HEALTHY AND CF COLON WITH AND WITHOUT A NSP SUPPLEMENTED FEED



EL = Endogenous losses

1.9 CONCLUSIONS

The metabolism of dietary carbohydrates is dependent on their availability for digestion and absorption. Starch and sugar provide energy to meet the metabolic demands of the body whilst maldigested / malabsorbed starch and NSP pass through the small intestine into the colon for bacterial fermentation or excretion. In the healthy individual the carbohydrates presented to the colon are principally NSP therefore there is a strong association between NSP intake, stool weight and the occurrence of gastrointestinal symptoms. In the CF colon the role of NSP is assumed to be similar to its role in the healthy colon as a low NSP intake has been related to the frequency and severity of symptoms (Gavin *et al* 1997) although the effect on stool output has not yet been identified.

In conclusion, this review of the literature revealed limited research into the cause of gastrointestinal symptoms in CF and the metabolic handling of dietary carbohydrates in CF. Gavin *et al* (1997) demonstrated a simple association between a low NSP intake and gastrointestinal symptoms but the need to determine whether the relationship was causal was emphasised, so the present study was developed to address this issue. Gavin *et al* (1997) used a group of CF patients receiving a typical CF high energy diet for their study but there is a group of patients (the enterally fed CF group receiving NSP-free feeds) who are more susceptible to gastrointestinal symptoms because of a potentially lower NSP intake. This group has not been previously investigated. If a causal relationship does exist between a low fibre intake and gastrointestinal symptoms, then it should be more apparent in this patient group. In the present study the following questions were addressed:

1. Does energy and NSP intake for a group of enterally fed CF patients differ from healthy individuals?

2. Do gastrointestinal symptoms and stool composition relate to NSP intake in a group of healthy individuals and enterally fed CF patients?

3. Does the administration of a NSP supplemented feed to a group of enterally fed CF patients relieve gastrointestinal symptoms?

4. Does the administration of a NSP supplemented feed to a group of enterally fed CF patients affect stool composition and energy availability?

1.10 AIMS OF RESEARCH

The study hypothesis proposed that a low NSP intake would result in a reduced stool output and would be the causal factor of abdominal symptoms for the enterally fed CF patients. Addition of a NSP supplemented feed would therefore increase stool output and relieve symptoms for this group. If a causal relationship could be demonstrated it had important clinical implications for the dietetic management of enterally fed CF patients. The primary aim of the study was therefore to determine whether the reported relationship between a low NSP intake and symptoms (Gavin *et al* 1997) was directly linked to colonic bacterial metabolism in a group of enterally fed CF patients. The study was designed as two distinct phases of work with the following objectives:

1. To investigate the differences in energy intake, NSP intake, stool losses, and the frequency of bowel habit, in a group of CF patients consuming their habitual NSP-free feed and oral diet, and a reference group of healthy individuals consuming habitual diet (Questions 1 & 2)

2. To investigate the differences in energy and NSP intake, stool losses, and the frequency of bowel habit in a CF group consuming a habitual diet and a NSP supplemented feed, and a CF group consuming a habitual diet and a placebo feed (Questions 3 & 4)

CHAPTER 2 METHODOLOGY

2.1 CHARACTERISATION OF THE CF PATIENTS AND HEALTHY SUBJECTS

There are 240 patients in the Regional CF clinic (150 children and 90 adults) and 23 of these patients receive supplementary overnight enteral feeds to maintain weight gain and growth. Of this group, there were only ten CF children (aged 7-16 years) and two CF adults (aged 26 and 30 years) who met the inclusion criteria and were willing to participate in the study. The criteria stipulated that patients had a confirmed diagnosis of Cystic Fibrosis, were consuming a non-elemental overnight enteral feed and were not receiving gut motility medications. They had to be clinically well and had not received antibiotics for four weeks prior to assessment.

Eight healthy children (aged 7-16 years) were recruited to the study to provide reference data against which the CF patient data could be interpreted. These children were school friends or siblings of the CF patients so were not matched for fat free mass or body weight. Unfortunately a reference group for the CF adults was unavailable.

Informed consent was obtained from all the patients and subjects or their parent/guardian. The study protocol had the approval of the Joint Research Ethics Committee of Southampton and South West Hampshire.

2.2 STUDY PLAN

The Study Plan and Information Sheet (Appendix 3) were explained to the CF patients during recruitment at an outpatient appointment in their local hospital. The study was a parallel randomised double-blind study, composed of two distinct phases of work;

PHASE 1: The objectives for this phase were to assess habitual bowel habit, nutrient intake and stool composition for CF patients and healthy subjects for comparison. An assessment of nutrient intake was made from a 5 day weighed food diary concomitant with a 3 day stool collection and a bowel habit diary. All patients prior to Phase 1 completed a retrospective bowel habit questionnaire with regard to the previous two months. Anthropometric measures were also taken including weight, height, triceps skinfold measures and mid arm circumference to assess nutritional status.

Following the initial assessment period, the CF patients were randomly assigned by an independent investigator to one of two enteral feeds (different to their usual feed) in addition to their habitual oral diet for the next four weeks. By chance an unequal number of patients received the trial feed. Five patients were assigned to the placebo feed and seven patients were assigned to the NSP supplemented feed. No other dietary advice was given to the CF patients throughout the trial period. No change in enzyme dosage was recommended.

The enteral feeds were similar in composition to those typically used by the patients but modified as;

- A feed containing 1g per 100ml of mixed NSP (in the form of resistant starch (0.1 g/100 ml), oat grain (0.4 g/100 ml), and inulin (0.5 g/100 ml) – Fresenius, Germany)
- A feed devoid of the above ingredients (Fresenius, Germany)

Five CF patients required antibiotics between Phases 1 and 2. These patients received an additional two weeks of the trial feed before completing Phase 2.

PHASE 2: This phase involved CF patients only. The objectives for this phase were to assess the effect of a NSP supplemented feed on bowel habit, nutrient intake and stool composition compared with a placebo feed. A second assessment of nutrient intake was made from a 5 day weighed food diary concomitant with a 3 day stool collection and a bowel habit diary. A retrospective bowel habit questionnaire was completed with regard to the four week feeding period. A second assessment of anthropometry was also completed.

2.3 PRIMARY OUTCOME VARIABLES

To identify habitual colonic function in health and CF (Phase 1) and the effect of a NSP supplemented feed on the CF colon (Phase 2) the following primary outcome variables were assessed:

a) Energy and nutrient intake and energy availability for the body
b) The frequency and severity of gastrointestinal symptoms and relationship to stool indices and nutrient intake.
c) Stool indices e.g. weight, bacterial mass, lipid and energy content

2.4 ANTHROPOMETRY AND BODY COMPOSITION

The weight and height of each CF patient and healthy subject were measured by the same observer using a balance weighing scale reading to 100 g and a free standing portable digital stadiometer reading to 1mm respectively. Both items of equipment were validated regularly against a standard kilogram weight and a standard metre rule. The measures were compared to standards of normal growth developed from longitudinal studies to detect any retardation (Tanner & Whitehouse 1966a/b; Freeman *et al* 1990). Ideal weight for height was calculated for children by dividing actual weight by the weight equivalent to the height centile and multiplying by 100. The result was applied to the

following classification (Waterlow & Rutihauser 1974); 90 -110% weight for height (ideal weight range), 85 - 90% weight for height (underweight), 80 - 85% weight for height (mild malnutrition), 75 - 80% weight for height (moderate malnutrition), < 75% weight for height (severe malnutrition). Body Mass Index (BMI) was calculated for the adults as weight (kg) divided by height (m^2) and applied to the following classification (Anon 1983): < 20: long term hazard to health, 20 – 24.9: desirable BMI.

Triceps skinfold thickness (TST) measurements were taken three times by the same observer on each patient/subject using Holtain callipers at the mid-point of the triceps muscle. The average of the three values was taken to validate reproducibility. Mid-arm circumference (MAC) was also measured using a steel tape measure. The same observer took three values and the average value was calculated and compared to normal standards for mid arm circumference (Friscancho 1981) and skinfold thickness (Tanner & Whitehouse 1975). Fat free mass was measured using bioelectrical impedance. Mid-arm muscle circumference (MAMC) was then calculated using the equation below;

$$\text{MAMC (mm)} = \text{MAC (mm)} - (0.3142 \times \text{TST (mm)})$$

2.5 DIETARY ASSESSMENT

A food diary and digital kitchen scales were provided for each patient/subject and a demonstration on how to use the scales given in the home environment. Typed instructions were also given. The food diary had specific categories for the description of the food consumed, the quantity of food eaten, and quantity of food left behind. For the CF patients, there was also a category to specify the quantity of enzymes taken for meals and snacks. In addition, a feed diary was given to the CF patients to record the volume received on a daily basis. This is because some CF patients had 1-2 nights where no feed was consumed, and it was important that each patient received

a minimum quantity over the entire feeding period. All the patients and subjects were closely monitored and given support throughout the study period. It was emphasised that normal dietary habits must be continued throughout the study period.

The Soehnle food weighing scales were battery operated with a 1000 g capacity measuring in 1g divisions over the first 64 g and 2 g divisions over the remainder with a tare facility. When the recording period was finished the diary was checked with the patient/ subject and parent if necessary for accuracy. All dietary records were successfully completed by the patients/subjects and were analysed for nutrient content per gram using a computerised database – Compeat 5 (Nutrition Systems Ltd, London). This programme incorporates food composition figures from McCance and Widdowson, and the Royal Society of Chemistry. The specific methods used to calculate gross energy and NSP intakes from the computer analysis are discussed in more detail in Sections 2.5.1 and 2.5.2

2.5.1 GROSS ENERGY INTAKE

Gross energy intake was calculated by multiplying the average nutrient intake in grams by specific energy factors determined from the energy yield of nutrients of food measured by ballistic bomb calorimetry (Miller & Payne 1959). The gross energy values of the nutrients are carbohydrate (17.5 kJ/g; 4.2 kcal/g), lipid (39.3 kJ/g; 9.4 kcal/g), protein (23.6 kJ/g; 5.7 kcal/g) and alcohol (29.7 kJ/g; 7.1 kcal/g). Digestible and metabolisable energy were calculated from gross energy values. Digestible energy was the amount of energy available for digestion and was calculated as the difference between gross energy intake and stool energy losses. Metabolisable energy was the amount of energy available for metabolism. It was calculated as the difference between gross energy and the energy content of stool and urine. The metabolisable energy factor for NSP was included in the calculations of energy intake because the contribution of NSP to stool output was measured. The value for urinary energy loss as a percentage of gross energy intake was estimated at 5% (Southgate & Durnin 1970; Cummings *et al* 1978; Jackson *et*

al 1992). Due to the confines of this study, urinary energy losses were not measured so an estimated metabolisable energy was calculated. Metabolisable energy intake results were then compared with the Estimated Average Requirement (EAR) for Energy for age (Dept of Health 1991 – Dietary Reference Values).

2.5.2 NSP INTAKE

The food analysis computer program (Compeat 5) used the Englyst method to calculate the grams of NSP consumed on an average daily basis. The Englyst method (1979) measured NSP only therefore did not account for maldigested / malabsorbed starches that have a similar physiological effect as NSP.

2.6 DETERMINATION OF FREQUENCY AND SEVERITY OF GASTROINTESTINAL SYMPTOMS

The bowel habit questionnaire and diary used in the study were first developed by Lyyra (1997) and are presented in Appendix 1. Symptoms in the questionnaire and diary were categorised using the simple method employed by a previous study to determine the occurrence and severity of abdominal symptoms in CF patients (Gavin *et al* 1997): 1 = no symptoms, 2 = occasional or mild / moderate symptoms, 3 = frequent and / or severe symptoms. The questionnaires and diaries were also qualitatively assessed for a stool appearance indicative of constipation or maldigestion / malabsorption.

2.7 STOOL COLLECTION

Stools were collected for 3 days during the study concomitant with the weighed food diary. All stools were collected between carmine markers, from the appearance of the first marker in the stool to the appearance of the second marker given three days later. All patients/subjects were asked to take their carmine markers at breakfast time or before 9.00 a.m. on the relevant days.

2.7.1 PREPARATION OF STOOLS FOR ANALYSIS

Stools were collected into polythene bags and immediately frozen using dry ice kept in coolboxes until transfer was arranged to the hospital freezer. Stools were thawed, weighed and homogenised with a known quantity of distilled water for 1 - 2 minutes using a kitchen jug homogeniser (Moulinex, France). 10% of each sample was pooled from the 3 day collections of each subject. Pooled portions of between 20 - 25g of stool homogenate were freeze-dried for each patient/subject. The length of time taken to freeze-dry stool homogenate ranged from 24 - 48 hours depending on the amount of water present in the stool homogenate sample and the number of samples added to the freeze dryer at any one time. Stool water content was calculated from stool weight allowing for water added prior to homogenisation and loss in weight due to freeze-drying. Stool dry weight was determined as stool weight minus the weight of stool water.

2.7.2 DETERMINATION OF STOOL BACTERIAL MASS

The bacterial content of freeze-dried stool was determined by repeated fractional centrifugation using a modification of Stephens & Cummings method (1980b). Freeze-dried samples of 0.5 g were resuspended by shaking with 30 ml formlysaline and 0.3 ml sodium lauryl sulphate and then filtered through muslin three times. Stool suspensions were centrifuged at 30000 g for 30 mins. Repeated resuspension and centrifugation fractionates the stool into its main components; large plant material, small plant material and the bacterial fraction.

2.7.3 DETERMINATION OF STOOL LIPID AND STOOL BACTERIAL LIPID

A modification of the Folch technique (1956) was used for the extraction of both stool and bacterial lipid. A 50 mg dry stool sample was analysed in duplicate for each patient/subject. The stool samples were aliquots from a 1 day stool collection from the patients/subjects whilst the bacterial lipid samples were aliquots from pooled samples of bacteria from each group e.g. CF group, reference group, placebo group, NSP supplemented group. Pooled bacterial samples have been used in previous work in CF patients providing similar results to individual samples (Murphy 1991).

The 50 mg stool samples were saponified with 1.75 ml chloroform/methanol and hydroxybutyrate to extract the lipid. Samples were then acidified with 500 μl acetic acid to maximise extraction of fatty acids. After centrifugation, the solvent layer was washed 3 times with salt solution to remove water soluble impurities. The final solvent portion was mixed with 200 μl ethanol, dried under nitrogen flow and samples weighed. The modified Folch method will give larger stool lipid results than a similar study (Murphy 1991) where the Van de Kamer method (1949) was used. This is because the Folch method is gravimetric therefore incorporates all types of fat in the measurement e.g.

triglyceride, fatty acids, phospholipid whilst the Van de Kamer method is titrametric accounting for fatty acids only.

Preparation of stools for analysis and measurement of bacterial mass and lipid content was undertaken by colleagues Kirsi Laiho and Angela Hounslow.

2.7.4 DETERMINATION OF STOOL ENERGY AND STOOL BACTERIAL ENERGY

The stool energy content was measured by ballistic bomb calorimetry (Gallenkamp, UK) on samples of freeze-dried stool. Benzoic acid, thermochemical grade, was the reference source for the calibration of the instrument. Aliquots of 0.25 g freeze-dried stool were combusted and measured for energy content in triplicate and an average value taken. Aliquots for each patient/subject were used to measure stool energy but aliquots from patient/subject groups were used to measure bacterial energy

2.8 STATISTICAL ANALYSIS

Data was analysed using the statistics package SPSS (Version 9.0). Normal distribution could not be assumed due to the small numbers recruited to the study and the large variability between CF patients, therefore medians and ranges were used to describe the data. Statistical significance was assumed at the 5% level. Spearman rank correlations were used to identify relationships between nutrient intake and stool variables. The Mann-Whitney U test was used to determine the statistical significance of the difference between the respective groups.

2.9 COMMENTS ON THE METHODOLOGY

2.9.1 DESIGN OF THE STUDY

A parallel design was selected for this study for three reasons. Firstly, the CF patients consumed a variety of enteral feeds prior to the study. To assess habitual nutrient intake, stool composition and bowel habit for comparison with healthy subjects, it was important that the patients remained on their usual feed for Phase 1. Secondly, the trial feeds contained marginal differences in protein, fat and carbohydrate components to their habitual feeds which might have created an altered colonic function independent of NSP, so Phase 1 results could not be compared with Phase 2. Assuming the differences were equal between the placebo and NSP supplemented groups, the groups results could be compared within Phase 2. Thirdly, if a crossover design was selected, the length of washout period before commencing a second feed is unknown so any residual effect of the first feed might obscure the effect of the second.

2.9.2 THE NSP SUPPLEMENTED FEED

The NSP supplemented feed was a commercially available product in Germany and it was selected for the trial due to the specific quality and quantity of NSP components.

a) Quantity of fibre: Recommendations for NSP intake (Williams *et al* 1995) are "age + 5 grams" for children, "age + 10 grams" for adolescents and 18 g/d NSP for adults (Dept of Health 1991). As enterally fed CF patients consumed NSP intakes significantly lower than recommendations, addition of large quantities of NSP within a short space of time to meet recommended levels might have created intestinal discomfort. A 1 g/100 ml NSP supplemented feed therefore provided at least "age in grams" of NSP for the majority of CF children without the risk of causing discomfort. The final NSP intake was dependent on the volumes of feed consumed according to individual energy requirement and was controlled independently of the study.

b) Quality of fibre: A large proportion of commercially available enteral feeds contain soya polysaccharide, a type of NSP not normally found in large quantities in the UK diet. The trial feed contained two NSP components frequently found in the habitual diet e.g. resistant starch and oat fibre. A prebiotic, inulin, was the third NSP component therefore the feed provided 70% soluble and 30% insoluble fibre. A mixed NSP blend was selected as it has been shown to have a greater effect on stool output than a feed containing a single source of NSP (Sunvold *et al* 1995)

CHAPTER 3 RESULTS

3.1 RESULTS FOR CF PATIENTS AND HEALTHY SUBJECTS: PHASE 1

3.1.1 POPULATION CHARACTERISTICS

The individual characteristics of both healthy and reference groups are *presented individually in Appendix* 4.

The ten CF children (two boys and eight girls) had a median age of 10.9 years. Their weights ranged from 20.5 to 48.0 kg and their heights from 116.0 to 154.4 cm. Median weight for height was 106% and median fat free mass was 26.2 kg. Standard deviation scores ranged from – 2.5 to – 0.1 for weight and – 3.2 to – 0.4 for height. Whilst median weight for height was within the normal range (Waterlow & Rutihauser 1974) median standard deviation scores demonstrated abnormal growth. Weights for the two CF adults (both female) were 43.5 kg and 58.1 kg and their heights were 150.5 cm and 169.0 cm. BMI was 19.1 kg/m^2 and 20.3 kg/m^2 and fat free mass was 30.8 kg and 42.0 kg. It is generally assumed that adults are able to consume more food and consequently have a larger stool output, but this was not the case for the CF population so stool and nutrient results for CF children and adults were analysed as a combined group. As nutrient data were not associated with age, body weight or fat free mass the nutrient data remained absolute.

As the eight healthy children (including three boys) were not matched for body size, they were a reference group only. The reference group had a median age of 12.4 years. Their weights ranged from 29.8 to 70.0 kg and their heights from 133.4 to 173.4 cm. Median weight for height was 99% and median fat free mass was 35.1 kg. Standard deviation scores ranged from – 0.1 to 1.4 for weight and – 1.2 to 1.7 for height. Both median weight for height and median standard deviation scores demonstrated normal growth. As the CF children

were smaller and thinner than the reference group, anthropometric measures were expected to be proportionately lower but fat free mass, mid arm and mid arm muscle circumferences were markedly lower ($P < 0.05$). The characteristics of both groups are presented in detail in Table 3.1 and 3.2

TABLE 3.1 CHARACTERISTICS OF THE CF POPULATION

PATIENT	DOB	AGE AT DIAGNOSIS	GENOTYPE	GASTROSTOMY INSERTION	[1]% FEV_1
1	27.7.91	16 MONTHS	δF508 Homozygous	FEB 1996	72%
2	15.8.90	3 WEEKS	δF508 Homozygous	DEC 1997	85%
3	30.9.67	BIRTH	δF508 Homozygous	JAN 1995	55%
4	25.6.72	BIRTH	δF508 Homozygous	JAN 1998	66%
5	7.7.84	6 MONTHS	δF508 Homozygous	AUG 1996	90%
6	30.4.88	BIRTH	δF508 Homozygous	FEB 1996	90%
7	17.3.82	13 MONTHS	δF508 Homozygous	JAN 1997	76%
8	28.5.83	3 YEARS	δF508 Homozygous	APRIL 1996	46%
9	19.1.87	BIRTH	δF508 Homozygous	JUNE 1994	90%
10	20.7.88	BIRTH	δF508 Homozygous	JUNE 1994	71%
11	30.3.92	17 MONTHS	δF 508 G551D	MARCH 1999 NASOGASTRIC	70%
12	2.8.84	18 MONTHS	UNKNOWN	MARCH 1998	49%

[1] **FEV_1**: A measure of lung function. Forced expiratory volume blown in 1 second.

TABLE 3.2 CHARACTERISTICS OF THE CF AND REFERENCE GROUPS

Values are given as medians with range in parenthesis. An asterix denotes statistical significance at $P < 0.01$. A # denotes statistical significance at $P < 0.05$. The standard deviation score for weight and height for the children is in red. As there were only two CF adults both values are given for each variable.

VARIABLES	CYSTIC FIBROSIS CHILDREN (N=10)	CYSTIC FIBROSIS ADULTS (N=2)	HEALTHY CHILDREN (N = 8)
DECIMAL AGE	10.9 (7.0-16.3)	26.2, 30.9	12.4 (7.8 – 16.5)
WEIGHT (kg)	32.1 (20.5 – 48.0) -0.8 (- 2.5 to – 0.1)	43.5, 58.1	47.2 (29.8 – 70.0) 0.7 (- 0.1 to 1.4)*
HEIGHT (cm)	131.0 (116.0 – 154.4) -1.2 (- 3.2 to – 0.4)	150.5, 169.0 (Not Applicable)	151.4 (133.4 – 173.4) 0.3 (- 1.2 to 1.7)*
TRICEPS SKINFOLD THICKNESS (mm)	13.1 (8.0 – 21.3)	13.9, 23.5	11.1 (8.9 – 24.6)
MID ARM CIRCUMFERENCE (mm)	214.5 (170.0 – 233.0)	208.0, 258.0	238.0 (191.0 – 298.0)#
MID ARM MUSCLE CIRCUMFERENCE (mm)	162.3 (128.2 – 183.9)	164.3, 184.2	204.6 (158.6 – 258.8)#
WEIGHT FOR HEIGHT (%) / BMI (kg/m^2)	105.5 (91.0-149.0)	19.1, 20.3	99.0 (93.0-123.0)
FAT FREE MASS (kg)	26.2 (15.6 – 42.0)	30.8, 42.0	35.1 (23.6 – 57.3)#

3.1.2 ENERGY, NSP AND FAT INTAKES FOR CF AND REFERENCE GROUPS

Median gross energy intake (GEI) was 24.0% greater for the CF group (consuming feed and food) compared with the median GEI for the reference group (Figure 3.1). Estimated metabolisable energy intake (MEI) was calculated as GEI minus stool and estimated urinary energy losses. MEI as a percentage of GEI was 86.9% (70.2% to 91.2%) for the CF group and 91.6% (88.2% to 93.1%) for the reference group (Table 3.3).

Median NSP intake for the reference group was double the CF group (14.6 g/d; 8.6 - 21.5 g/d) compared with (5.8 g/d; 3.3 - 8.4 g/d; $P < 0.01$) (Figure 3.2). Applying the assumed heat of combustion factor for NSP this provided 0.3 MJ/d (0.2 - 0.4 MJ/d) and 0.1 MJ/d (0.1 - 0.2 MJ/d; $P < 0.01$) respectively. NSP energy as a percentage of GEI was three times greater for the reference group (3.2%; 1.2 to 3.5%) compared with (0.9%; 0.5 to 1.2%; $P < 0.01$) for the CF group.

Median dietary fat intake was greater for the CF group than the reference group (102.1 g/d; 69.4 - 157.8 g/d) compared with (53.1 g/d; 35.5 - 108.8 g/d; $P < 0.01$) (Table 3.4). Applying the assumed heat of combustion factor for fat this provided 4.0 MJ/d; 2.7 - 6.2 MJ/d compared with 2.7 MJ/d; 2.1 - 4.3MJ/d; $P < 0.01$ respectively, equivalent to 32.4% (24.8% to 38.8%) GEI and 29.9% (27.4% to 34.9%; NS) GEI. Median protein intake was greater for the CF group than the reference group (93.9 g/d; 51.4 – 133.8 g/d) compared with (62.8 g/d; 51.1 – 92.5 g/d; $P < 0.05$) (Table 3.4). Applying the assumed heat of combustion factor for protein this provided 2.2 MJ/d; 1.2 - 3.2 MJ/d compared with 1.5 MJ/d; 1.2 - 2.2 MJ/d; $P < 0.05$) respectively, equivalent to 17.3% (13.8% to 20.4%) GEI and 17.6% (14.9% to 20.5%; NS) GEI.

FIGURE 3.1 GROSS ENERGY INTAKE FOR CF AND REFERENCE GROUPS

Values are presented in increasing rank order.

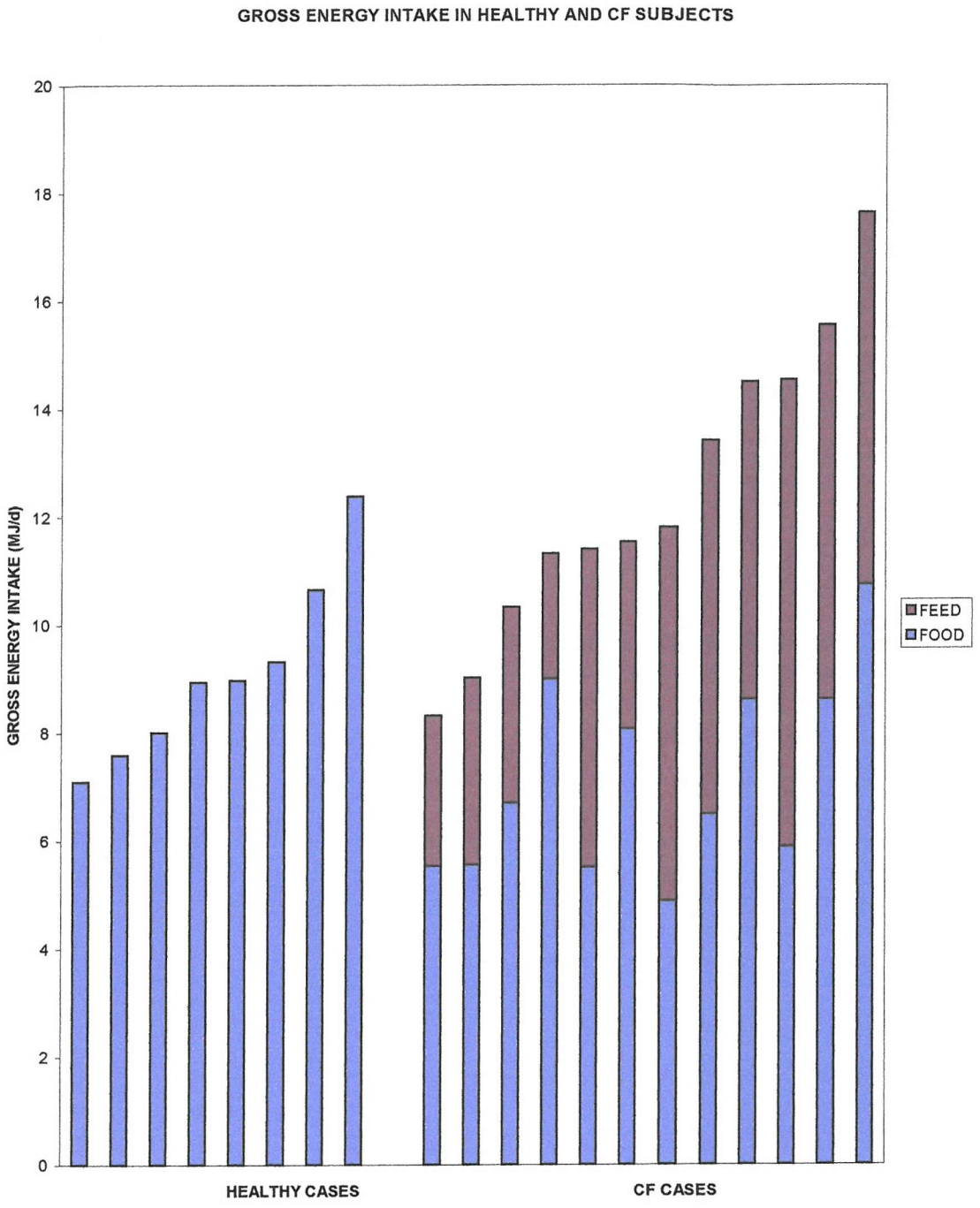


TABLE 3.3 DEFINITIONS OF ENERGY PRESENTED IN CF AND REFERENCE GROUPS

Values are given as medians with range in parenthesis. Gross energy intake represented total intake (feed and food) for CF patients and food intake only for healthy subjects. An asterix denotes statistical significance at $P < 0.01$. An # denotes statistical significance at $P < 0.05$. The CF group had greater stool energy losses therefore energy available for digestion and metabolism was significantly compromised for this group.

ENERGY INDICES	CF GROUP (N=12)	REFERENCE GROUP (N=8)
GROSS ENERGY INTAKE (GEI) (MJ/d)	11.7 (8.3 – 17.6)	9.0 (7.1-12.4)*
APPARENT DIGESTIBLE ENERGY INTAKE (DEI) – MJ/d	10.9 (7.4 – 16.1)	8.7 (6.8 – 12.2)#
DEI AS % GEI	91.9 (75.2 – 96.2)	96.6 (93.2 – 98.1)*
ESTIMATED METABOLISABLE ENERGY (MEI) - MJ/d	10.3 (6.9 – 15.2)	8.3 (6.5 –11.5)#
MEI AS %GEI	86.9 (70.2 – 91.2)	91.6 (88.2 –93.1)*

Apparent Digestible Energy Intake = Gross energy minus stool energy losses and expressed as a percentage of gross energy intake.

Estimated Metabolisable Energy Intake = Gross energy minus stool energy losses minus estimated urinary energy losses (5%) and expressed as a percentage of gross energy intake

FIGURE 3.2 NSP INTAKE FOR CF AND REFERENCE GROUPS

Values are presented for each individual. The bars represent the median. The CF group was receiving a NSP-free feed prior to the trial so NSP intake represented food only.

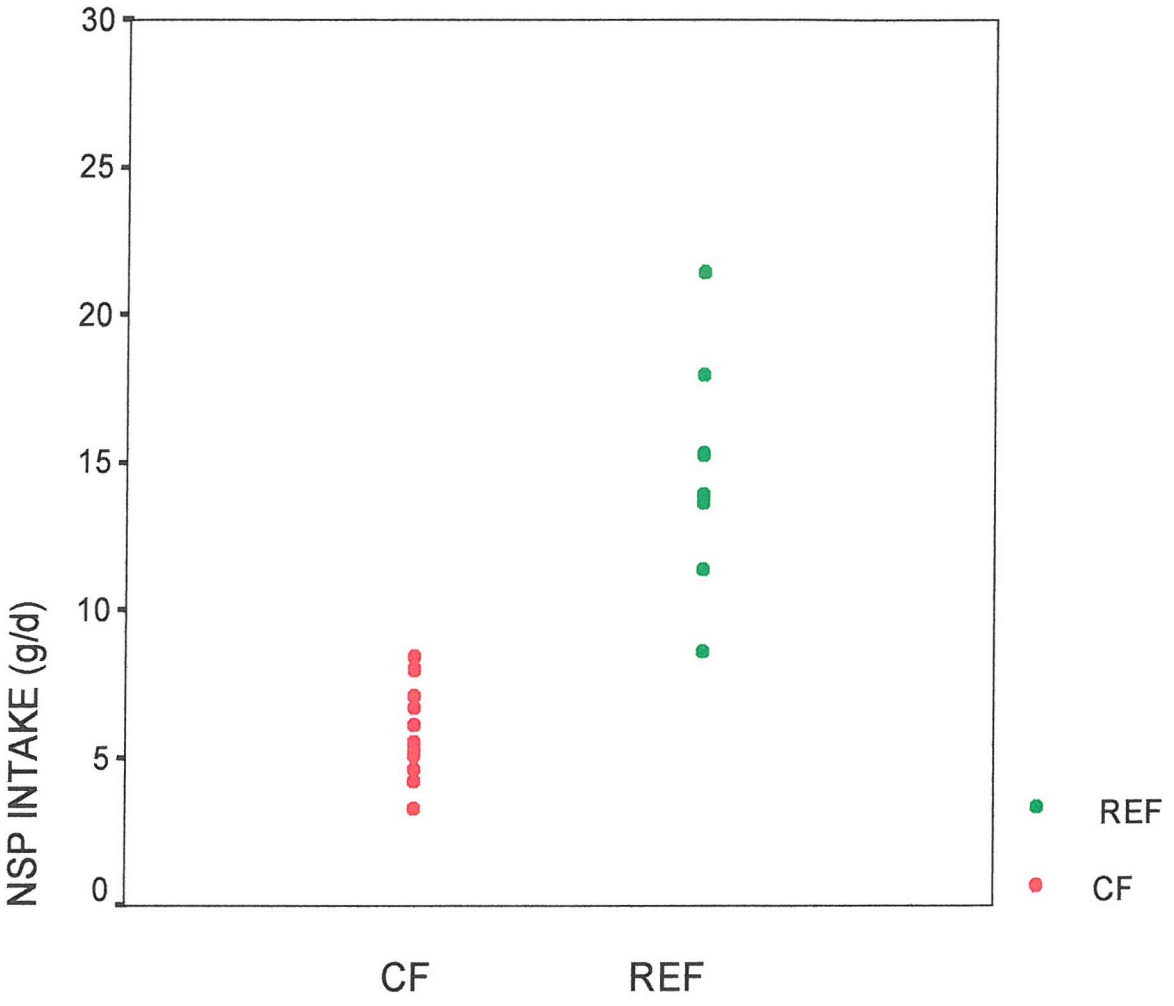


TABLE 3.4 ENERGY AND NUTRIENT INTAKES FOR CF AND REFERENCE GROUPS

The first column of significance (*P*) values compared food intake between CF patients and healthy subjects. The second column of *P* values compared food and feed intake for the CF patients with food intake only for the healthy subjects.

NUTRIENT	CF GROUP FOOD	CF GROUP FOOD AND FEED	REFERENCE GROUP FOOD	*P* VALUE FOOD	*P* VALUE FOOD & FEED
GROSS ENERGY (MJ/d)	6.6 (4.9 – 10.7)	11.7 (8.3– 17.6)	9.0 (7.1 – 12.4)	$P < 0.05$	$P < 0.01$
FAT (g/d)	53.1 (35.5 – 108.8)	102.1 (69.4 – 157.8)	68.4 (54.2 – 109.9)	$P < 0.05$	$P < 0.01$
PROTEIN (g/d)	44.3 (27.4 – 73.8)	93.9 (51.4 – 133.8)	62.8 (51.1 – 92.5)	$P < 0.01$	$P < 0.05$
FIBRE (g/d)	5.8 (3.3 – 8.4)	5.8 (3.3 – 8.4)	14.6 (8.6 – 21.5)	$P < 0.01$	$P < 0.01$

SUMMARY

Median energy, protein and fat intake from food and feed for the CF group was statistically significantly greater than intake for the reference group. Median NSP intake from food and feed for the CF group was however only half of the NSP intake from food for the reference group.

3.1.3 GASTROINTESTINAL SYMPTOMS FOR CF AND REFERENCE GROUPS

Gastrointestinal symptoms were categorised as 1 = no symptoms: 2 = mild / moderate symptoms: 3 = severe / frequent symptoms from the retrospective bowel habit questionnaire (BHQ), and the bowel habit diary (BHD) which was completed over the stool collection period. The results are summarised in Table 3.5

TABLE 3.5 SYMPTOMS AND STOOL COLLECTION DATA FROM THE BOWEL HABIT QUESTIONNAIRE AND DIARY: PHASE 1

SYMPTOMS AND STOOL RESULTS	BOWEL HABIT QUESTIONNAIRE	BOWEL HABIT DIARY
COMPLAINING OF ABDOMINAL PAIN	50% (6/12) CF 0% REFERENCE	8% (1/12) CF 0% REFERENCE
STOOL FREQUENCY (1/DAY)	50% (6/12) CF 50% (4/8) REFERENCE	75% (9/12) CF 50% (4/8) REFERENCE
STOOL CONSISTENCY (NORMAL STOOL PASSED)	42% (5/12) CF 100% REFERENCE	92% (11/12) CF 100% REFERENCE
STOOL COLOUR (BROWN)	83% (10/12) CF 100% REFERENCE	100% CF 100% REFERENCE

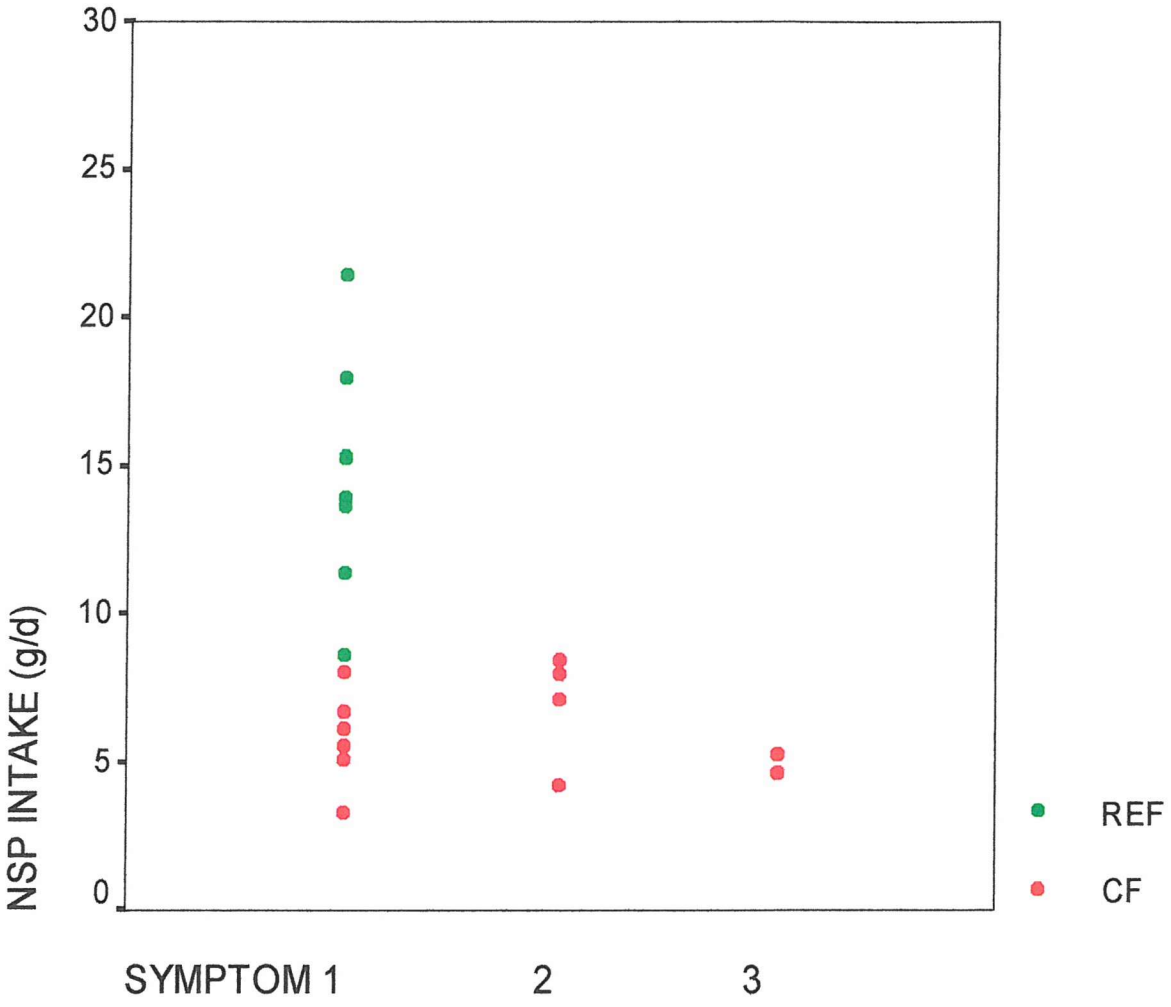
The bowel habit questionnaire (BHQ) was cross–referenced against the bowel habit diary (BHD) to identify whether symptoms were under or over reported. 17% (2/12) CF patients reported daily / severe abdominal pain in the BHQ (Figure 3.3). Only 8% (l/12) CF patients however reported daily / severe

abdominal pain during the BHD period. 33% (4/12) CF patients reported occasional abdominal pain in the BHQ (Figure 3.3) but none of these four patients reported abdominal pain during the BHD period. There was no association between NSP intake and the occurrence of abdominal pain for the CF group ($r = 0.04$; NS). The reference group rarely complained of abdominal pain in the BHQ and BHD.

33% (4/12) CF patients reported 2 - 4 stools daily and 17% (2/12) reported less than 1 stool per day in the BHQ. During the BHD however 75% (9/12) of the CF group reported 1 stool daily. 50% (4/8) of the reference group reported 1 stool daily in the BHQ and BHD. Only 42% (5/12) CF patients reported passing a "smooth soft" stool in the BHQ but 92% (11/12) CF patients reported passing a "smooth soft" stool during the BHD period. All healthy subjects reported passing a " smooth soft" stool in the BHQ and BHD. 17% (2/12) CF patients reported pale stools indicative of maldigestion / malabsorption in the BHQ but all CF patients reported brown stools during the BHD period. All healthy subjects reported brown stools in the BHQ and BHD. There is obvious disparity between the results from the bowel habit questionnaires and diaries. The reasons for this disparity are discussed in Chapter 4.

FIGURE 3.3 NSP INTAKE VERSUS OCCURRENCE OF ABDOMINAL PAIN FOR CF AND REFERENCE GROUPS (BOWEL HABIT QUESTIONNAIRE)

The symptom categories are as follows; 1 = no pain;
2 = mild / moderate and occasional pain; 3 = severe / frequent pain.



3.1.4 STOOL OUTPUT FOR CF AND REFERENCE GROUPS

Despite a substantially lower NSP intake, stool output variables were markedly greater for the CF group compared with the reference group. Stool wet weight (Figure 3.4) and dry weight (Figure 3.4) were greater for the CF group than reference group (112.0 g/d; 74.4 – 306.5 g/d) compared with (85.4 g/d; 41.0 – 170.4 g/d; $P < 0.05$) and (41.4 g/d; 21.7 – 95.0 g/d) compared with (25.7 g/d; 12.8 – 39.3; $P < 0.01$) respectively. The median percentage water in stool was 64.1% (56.8% to 81.2%) for CF patients and 72.8% (49.3% to 76.9%; NS) for the reference group. Fractionation of the dry stool results (Figure 3.5) for the CF group were: Fraction A: 3.9 g/d (0.5 -13.1 g/d); Fraction B: 3.5 g/d (1.2 - 7.3 g/d); Fraction C: 11.7 g/d (3.0 -19.0 g/d); Water soluble fraction: 17.3 g/d (8.7 - 32.2g/d). Fractionation of the dry stool results (Figure 3.5) for the reference group were: Fraction A: 2.9 g/d (0.6 - 7.1 g/d; NS); Fraction B: 3.7 g/d (1.9 - 6.5 g/d; NS); Fraction C: 7.4 (3.4 -10.9 g/d; NS); Water soluble fraction: 10.4 g/d (5.0 -18.5 g/d; NS).

Stool bacteria (Figure 3.4) was greater for the CF group (10.6 g/d; 3.2 -19.0 g/d) than the reference group (6.4 g/d; 2.1 – 8.4 g/d; $P < 0.01$). The median value for bacteria as a percentage of stool dry weight was 23.5% (14.8% to 37.0%) for the CF group and 22.0% (5.8% to 40.7%; NS) for the reference group. There was a strong relationship between stool bacteria and stool dry weight for the CF group only, as bacteria represented a larger percentage of stool dry weight for this group (CF: $r = 0.7$; $P < 0.01$: Reference: $r = 0.2$; NS) (Figure 3.6). Pooled samples of stool bacteria in the CF group had approximately twice the lipid content of the reference group (0.5 g/g bacteria compared with 0.3 g/g bacteria) equivalent to (5.1 g/d; 1.6 - 9.2g/d) compared with (1.7 g/d; 0.5 - 2.2; $P < 0.01$) respectively. The relationship between stool lipid and stool bacterial mass (Figure 3.7) was therefore strong for the CF group ($r = 0.8$; $P < 0.01$) but weak for the reference group ($r = 0.2$). Bacterial lipid as a percentage of stool lipid was similar for both groups 24.1% (15.9%

to 42.4%) and 24.2% (16.9% to 45.5%; NS) respectively. Bacterial energy for the CF group was twice the reference group (0.4 MJ/d; 0.1 - 0.7 MJ/d) compared with (0.2 MJ/d; 0.1 - 0.2 MJ/d; $P < 0.01$) and represented (30%; 24 - 47%) and (23%; 6% - 43%; $P < 0.05$) of stool energy respectively. SCFA production from bacterial fermentation was estimated using the McNeil (1984) calculation (Table 3.6). SCFA energy was greater for the CF group (0.3 MJ/d; 0.1 - 0.4 MJ/d) compared with (0.2 MJ/d; 0.1 - 0.3 MJ/d; $P < 0.05$) for the reference group and represented 1.9% (0.7% to 4.3%) GEI compared with 2.1% (0.8% to 2.8%; $P < 0.01$) GEI respectively.

Stool energy losses (Figure 3.8) for the CF group were approximately twice the reference group (1.1 MJ/d; 0.4 – 2.6 MJ/d) compared with (0.7 MJ/d; 0.4 – 1.0 MJ/d; $P < 0.05$) equivalent to 8.1% (3.9 to 24.8%) of GEI for the CF group and 8.1% (4.1% to 13.5%; NS) of GEI for the reference group. Stool energy per gram wet weight of stool was (CF: 9.2 kJ/g (5.4 -12.3 kJ/g);Reference: 7.2 kJ/g (5.8 -13.1 kJ/g) and dry weight was (CF: 24.4 kJ/g (19.9 - 28.9 kJ/g); Reference: 26.1 kJ/g; 23.4 - 28.8 kJ/g) respectively. Stool lipid (Figure 3.9) for the CF group was approximately twice the reference group (16.9 g/d; 7.0 - 58.1 g/d compared to 6.6 g/d; 3.6 - 8.9; $P < 0.01$). Stool lipid as a percentage of stool dry weight for the CF group was twice the reference group (43.6%; 32.4% to 61.1%) compared with (23.6%; 18.0% to 30.3%; $P < 0.01$). Assuming that stool lipid energy has the same heat of combustion value as dietary fat, stool lipid energy for the CF group was twice the reference group (0.7 MJ/d; 0.3 - 2.3 MJ/d) compared with (0.3 MJ/d; 0.1 - 0.4 MJ/d; $P < 0.01$). This represented 66.3% (58.5% to 89.0%) and 36.4% (27.4% to 41.2%; $P < 0.01$) of stool energy respectively. Stool lipid energy as a percentage of total GEI for the CF group was almost twice the reference group (5.5%; 2.4% to 22.1% compared with 3.1%; 1.5% to 3.8%; $P < 0.01$).

3.1.5 RELATIONSHIP BETWEEN ENERGY AND NSP INTAKE AND STOOL COMPOSITION VARIABLES FOR CF AND REFERENCE GROUPS

The relationship between stool wet weight and GEI or NSP intake was weak for both CF and reference groups (less than r = 0.1). The relationship between NSP and stool dry weight was weak (CF: r = 0.3: Reference: r = 0.3) and for GEI (CF: r = 0.2: Reference: r = 0.2). There was a moderate relationship between GEI and stool bacteria for the CF group (r = 0.5) but a weak relationship for the reference group (r = 0.1) and similar weak relationships with NSP. Stool energy was only weakly associated with GEI (CF: r = 0.5; Reference: r = 0.1) and NSP (CF: r = 0.1; Reference: r = 0.1). In conclusion there were no strong relationships between nutrient intake and stool composition for either group.

FIGURE 3.4 STOOL OUTPUT VARIABLES FOR THE CF AND REFERENCE GROUPS

The median and individual values are presented

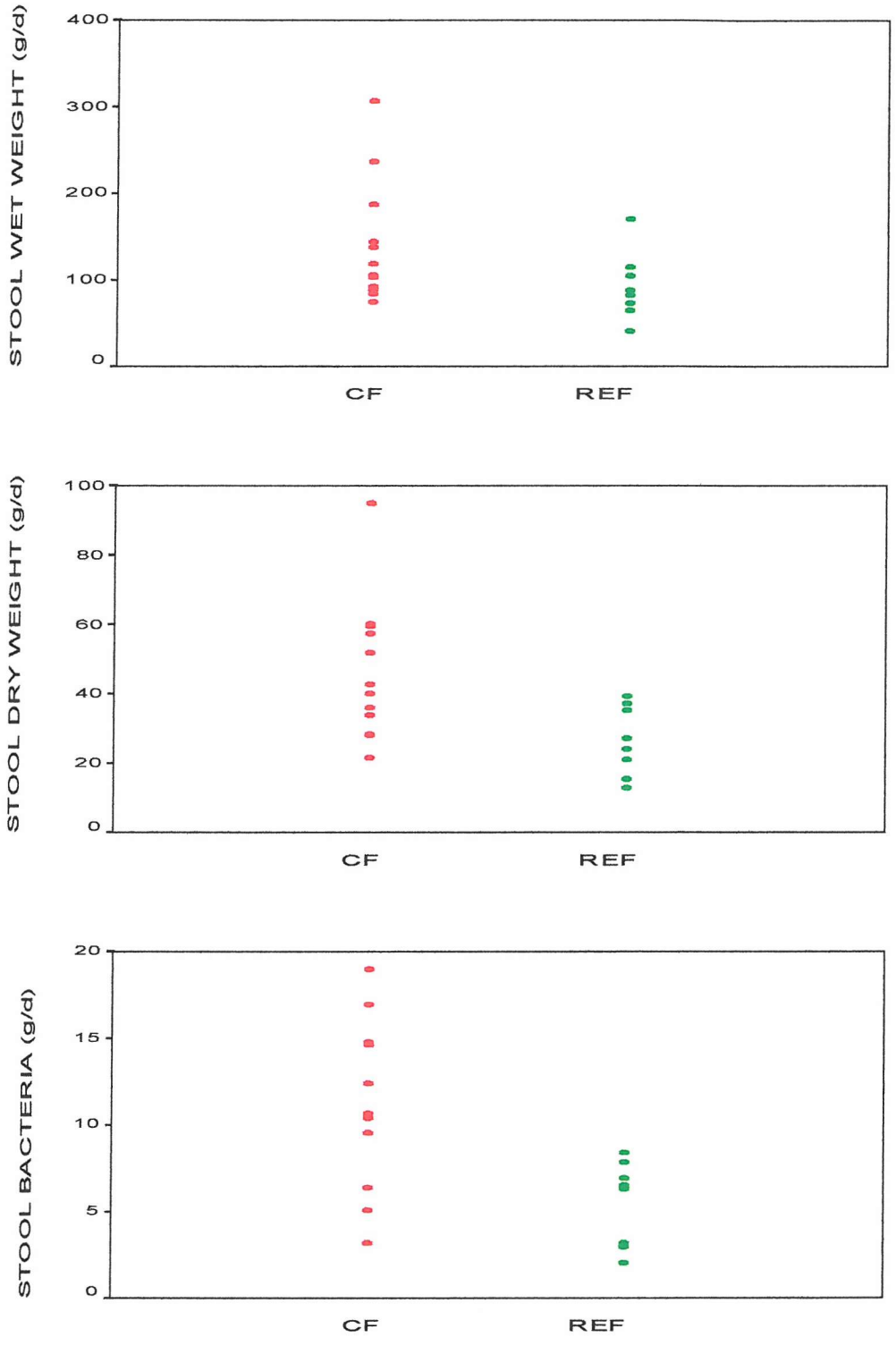


FIGURE 3.5 DAILY FRACTION COMPONENTS OF DRY STOOL WEIGHT FOR CF AND REFERENCE GROUPS

Fraction A represents large plant material, Fraction B small plant material and Fraction C stool bacteria.

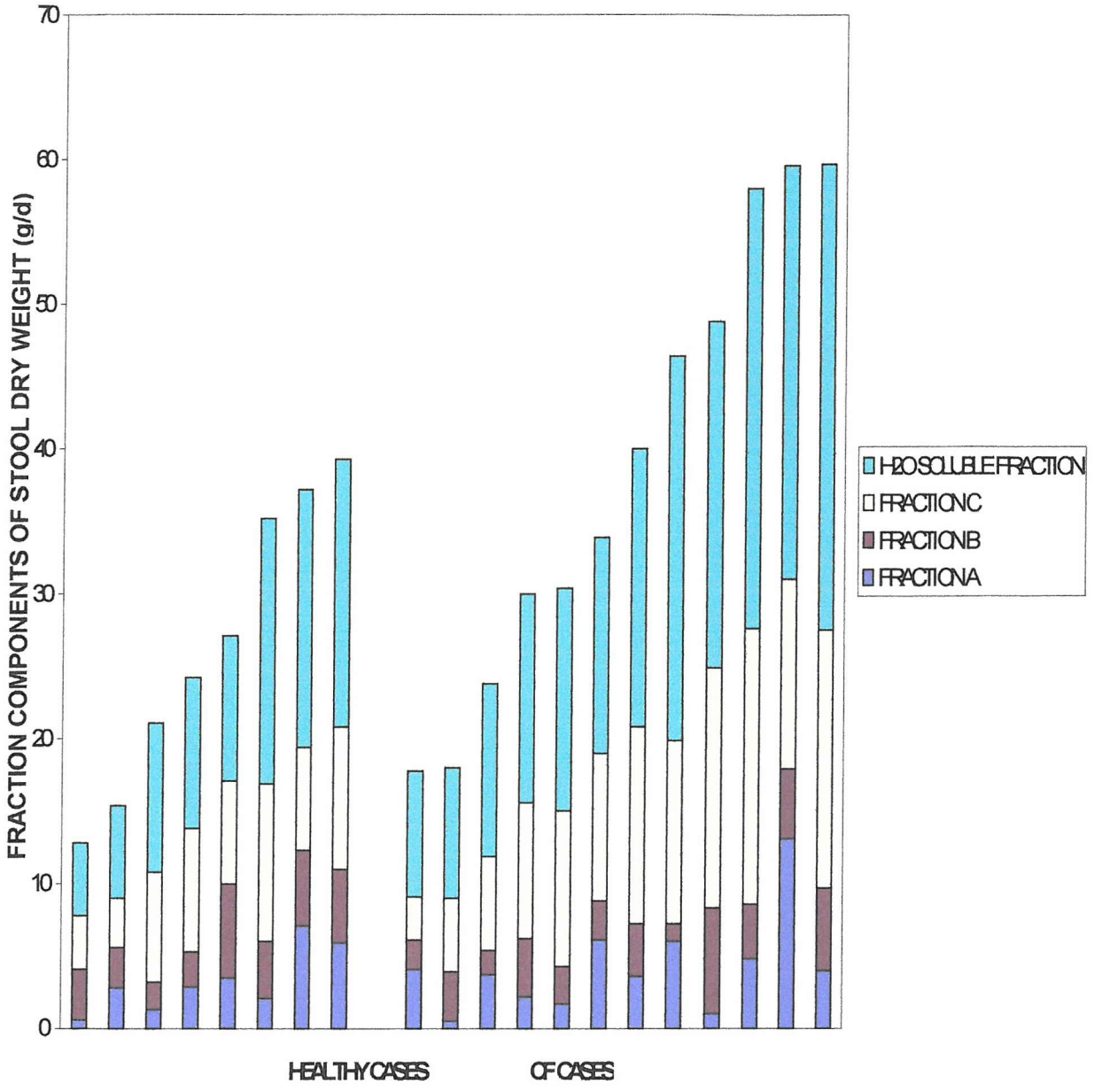


FIGURE 3.6 STOOL BACTERIAL MASS VERSUS STOOL DRY WEIGHT FOR CF AND REFERENCE GROUPS

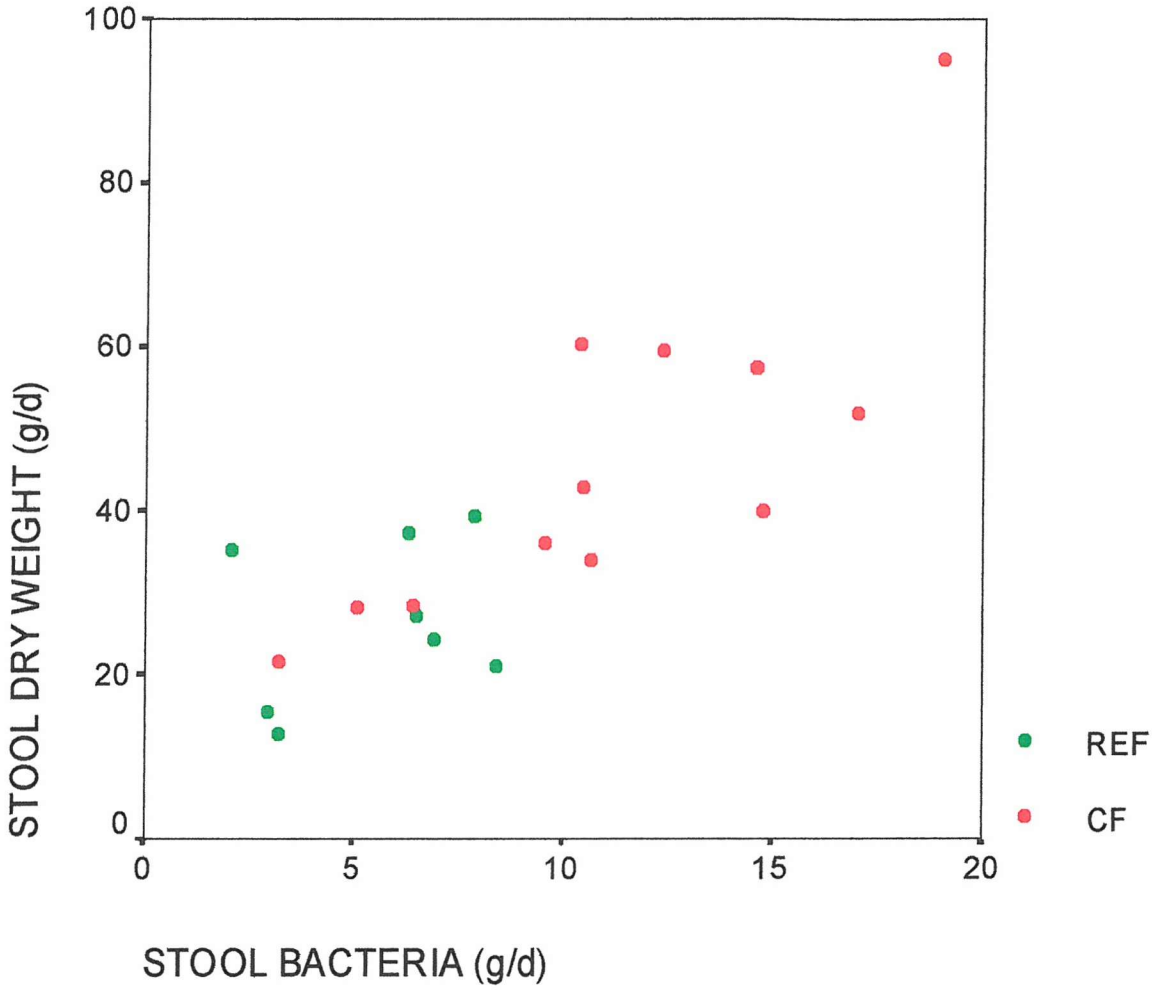


FIGURE 3.7 STOOL LIPID VERSUS STOOL BACTERIAL MASS FOR CF AND REFERENCE GROUPS

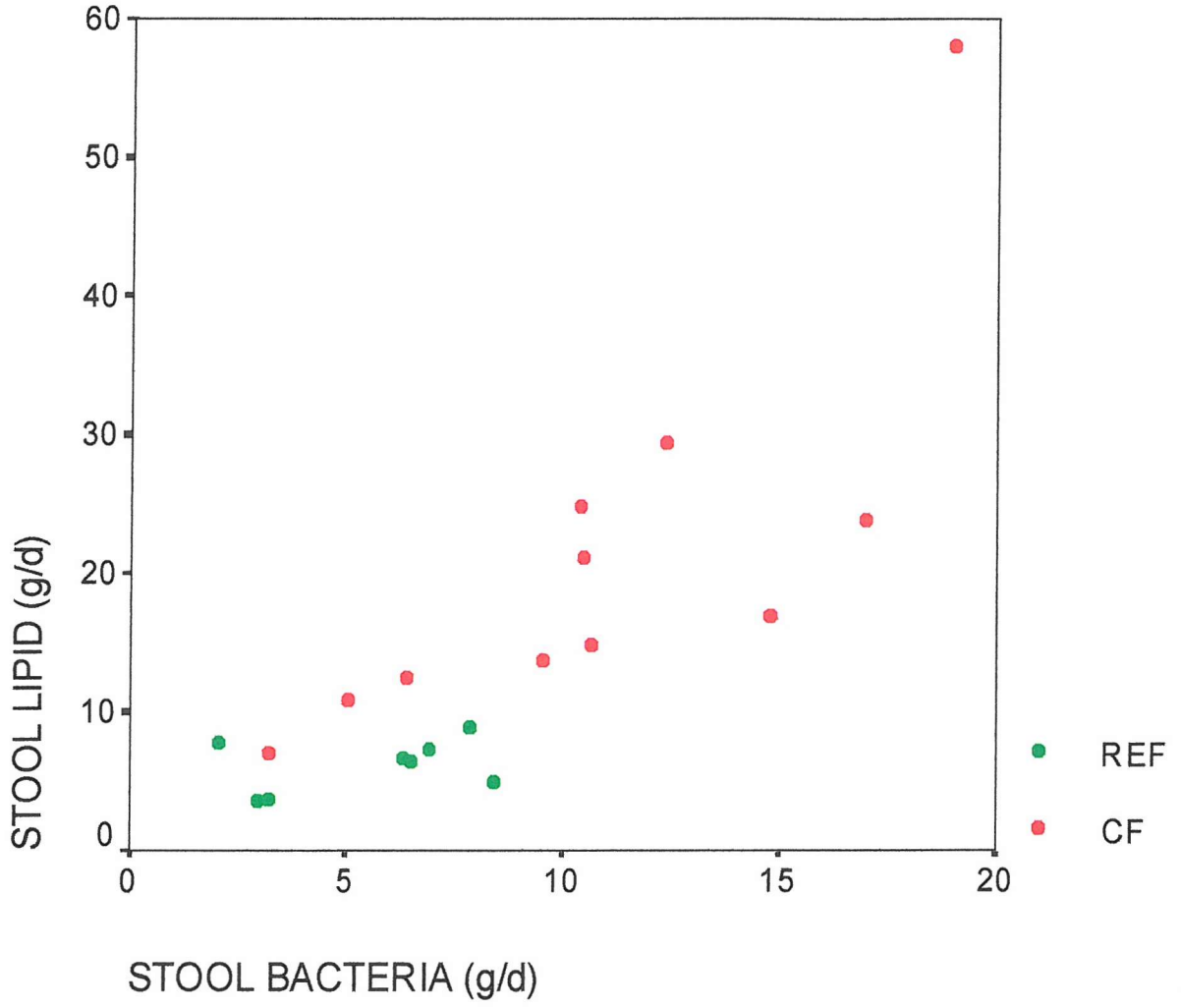


FIGURE 3.8 STOOL ENERGY FOR CF AND REFERENCE GROUPS

Values are presented in increasing rank order.

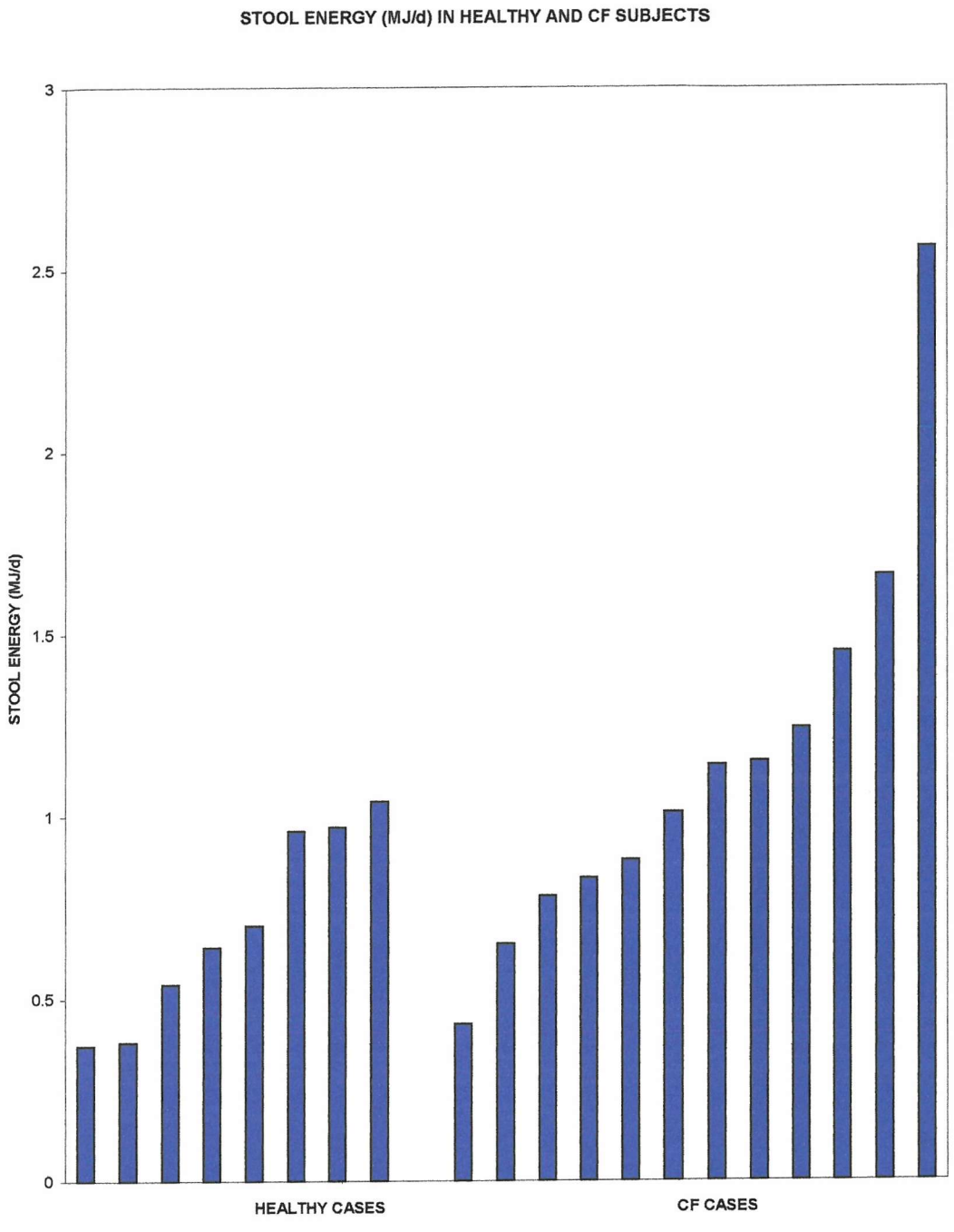


FIGURE 3.9 STOOL LIPID FOR CF AND REFERENCE GROUPS

Values are presented in increasing rank order. Only eleven of the CF patients participated in this analysis.

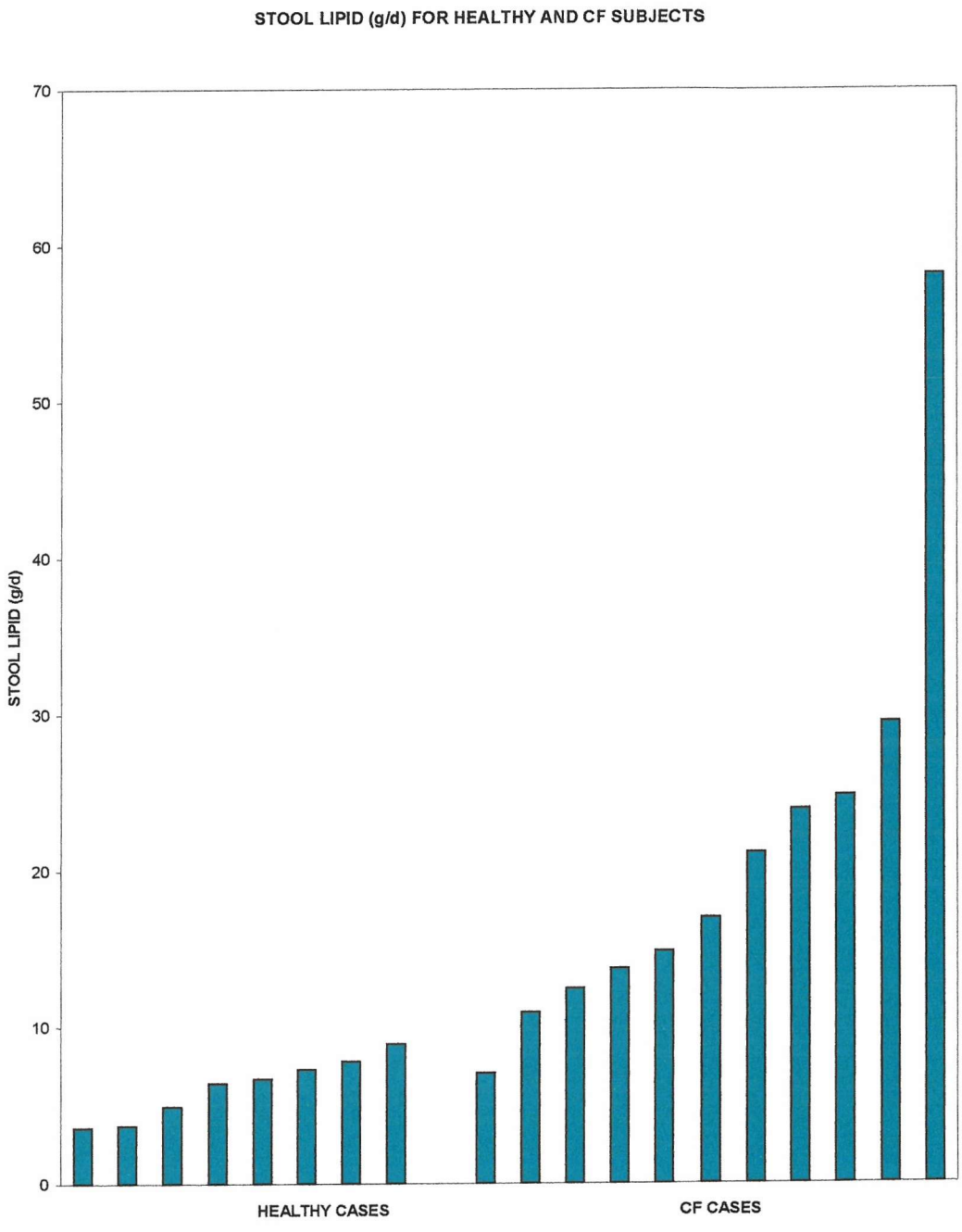


TABLE 3.6 ADDITIONAL POTENTIAL ENERGY THAT MAY BE PROVIDED FROM COLONIC FERMENTATION (MCNEIL CALCULATION 1984) FOR CF AND REFERENCE GROUPS

The asterix denotes a significance level of $P < 0.01$ and # denotes a significance level of $P < 0.05$. Median values are presented with range in parenthesis.

STOOL INDICES	CF GROUP (N = 12)	REFERENCE GROUP (N = 8)
BACTERIAL ENERGY – MJ/d	0.4 (0.1 – 0.7)	0.2 (0.1 – 0.2)*
HEXOSE REQUIRED TO SYNTHESISE FAECAL BACTERIAL MASS (g/d)	34.9 (10.6 – 62.7)	21.2 (6.8 – 27.7)*
TOTAL ENERGY YIELD (MJ/d)	0.7 (0.2 – 1.1)	0.4 (0.1 – 0.5)*
ENERGY AVAILABLE AS SCFA (MJ/d)[1]	0.3 (0.1 – 0.4)	0.2 (0.1 – 0.3)#

[1]Total Energy Yield – Stool Bacterial Energy

SUMMARY

Total energy yield for the CF group was approximately twice the reference group. As bacterial energy for the CF group was also double energy available as SCFA was relatively similar between the groups.

3.2 RESULTS FOR PLACEBO AND NSP SUPPLEMENTED GROUPS: PHASE 2

3.2.1 POPULATION CHARACTERISTICS

The CF patients were assigned to treatment groups by an independent researcher blinded to treatment. The three CF children in the placebo group had a median age of 8.4 years. Their weights ranged from 22.5 to 46.6 kg and their heights from 119.7 to 154.6 cm. Median weight for height was 100% and median fat free mass was 23.0 kg. Weights for the two CF adults were 41.5 kg and 59.2 kg and heights were 150.5 cm and 169.0 cm. BMI was 18.3 kg/m^2 and 20.9 kg/m^2 and fat free mass was 30.2 kg and 41.2 kg. As in the first phase the CF adults did not have larger stools or nutrient intake compared with the CF children in the placebo group therefore the results for adults and children were combined.

The seven CF children in the NSP supplemented group had a median age of 14.2 years. Their weights ranged from 21.7 to 47.8 kg and their heights from 118.0 to 154.6 cm. Median weight for height was 104% and median fat free mass was 23.6 kg. The characteristics of the population studied in Phase 2 are presented in detail in Table 3.7

TABLE 3.7 CHARACTERISTICS OF THE CF GROUP AS PLACEBO AND NSP SUPPLEMENTED GROUPS.

Values are given as medians with range in parenthesis

None of the differences in anthropometric measures between the groups attained statistical significance. As there were only two adults in the placebo group both values are given for each variable.

VARIABLES	PLACEBO CHILDREN (N=3)	PLACEBO ADULTS (N=2)	NSP SUPPLEMENTED CHILDREN (N=7)
DECIMAL AGE (YEARS)	8.4 (7.3-10.5)	26.3, 31.1	14.2 (7.1 – 16.5)
WEIGHT (kg)	27.0 (22.5 – 46.6)	41.5, 59.2	34.8 (21.7 – 47.8)
HEIGHT (cm)	129.0 (119.7 – 154.6)	150.5, 169.0	137.0 (118.0 – 154.6)
TRICEPS SKINFOLD THICKNESS (mm)	15.1 (8.9 – 15.4)	11.6, 26.7	15.7 (10.4 – 21.8)
MID ARM CIRCUMFERENCE (mm)	192.0 (182.0 – 222.0)	207.0, 264.0	228.0 (170.0 – 230.0)
MID ARM MUSCLE CIRCUMFERENCE (mm)	164.0 (133.6 – 174.6)	170.6, 180.1	168.1 (137.3 – 193.4)
WEIGHT FOR HEIGHT (%) / BMI (kg/m^2)	100 (100-104)	18.3, 20.9	104 (94-144)
FAT FREE MASS (kg)	23.0 (17.5 – 33.3)	30.2, 41.2	23.6 (17.8 – 34.1)

3.2.2 ENERGY, NSP AND FAT INTAKES FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Median gross energy intake (GEI) was 28.0% greater for the placebo group compared with the NSP supplemented group (Figure 3.10). Estimated metabolisable energy intake (MEI) was calculated as GEI minus stool and estimated urinary energy losses. MEI as a percentage of GEI was 85.2% (84.0% to 90.1%) for the placebo group and 86.1% (71.8% to 89.9%) for the NSP supplemented group (Table 3.8).

Median NSP intake (Figure 3.11) for the NSP supplemented group was twice the placebo group (12.7 g/d; 6.9 - 17.3 g/d) compared with (4.9 g/d; 3.6 - 9.9 g/d; $P < 0.01$). Applying the assumed heat of combustion factor for NSP this provided 0.2 MJ/d (0.1 - 0.3 MJ/d) and 0.1 MJ/d (0.1 - 0.2 MJ/d; $P < 0.01$) respectively. NSP energy as a percentage of GEI was three times greater for the NSP supplemented group (2.0%; 1.6% – 2.3%) compared with (0.7%; 0.5% - 1.3 %; $P < 0.01$) for the placebo group.

Median dietary fat intake was greater for the placebo group than the NSP supplemented group (114.7 g/d; 96.6 -131.7 g/d) compared with (86.1 g/d; 61.8 - 105.8 g/d; $P < 0.05$) (Table 3.9). Applying the assumed heat of combustion factor for fat this provided 4.5 MJ/d (3.8 - 5.2 MJ/d) compared with 3.4 MJ/d (2.4 - 4.2 MJ/d; $P < 0.05$) respectively, equivalent to (35.3%; 32.3 - 37.4%) of GEI and (30.9%; 27.9 - 36.2%: NS) of GEI. Median protein intake was greater for the placebo group than the NSP supplemented group (99.1 g/d; 96.6 – 131.7 g/d) compared with (64.9 g/d; 60.5 – 113.4 g/d; NS) (Table 3.9). Applying the heat of combustion factor for protein this provided 2.3 MJ/d (2.1 – 2.8 MJ/d) compared with 1.5 MJ/d (1.4-2.7 MJ/d; NS) respectively, equivalent to 19.9% (14.5% - 20.4%) of GEI and 17.2% (15.5 – 20.0%; NS) of GEI.

FIGURE 3.10 GROSS ENERGY INTAKE FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Values are presented in increasing rank order.

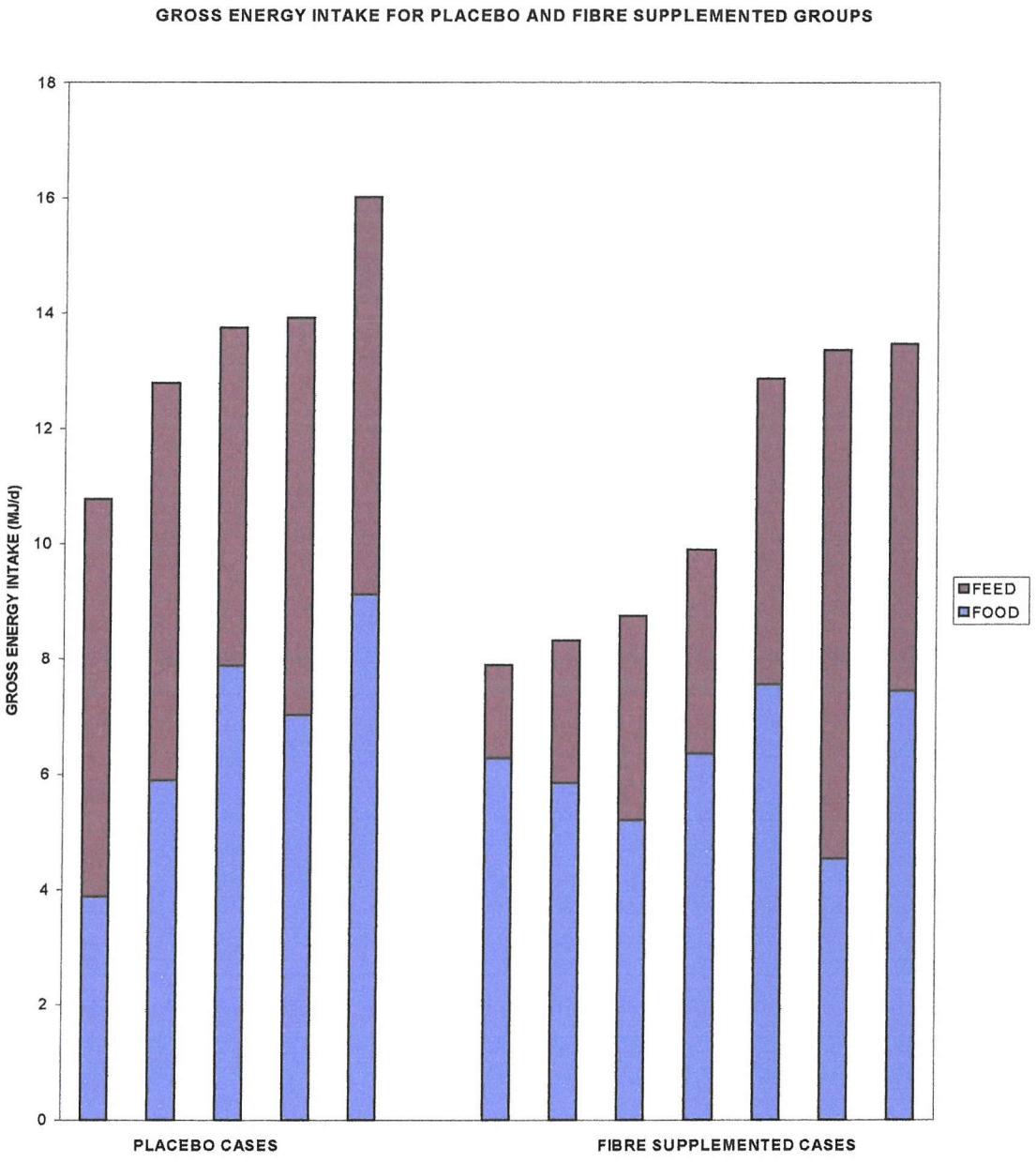


TABLE 3.8 DEFINITIONS OF ENERGY PRESENTED FOR PLACEBO AND NSP SUPPLEMENTED GROUPS .

Values are presented as medians with the range in parenthesis. A # denotes statistical significance at $P < 0.05$. All other differences in energy intake between the groups did not attain statistical significance.

ENERGY INDICES	PLACEBO GROUP (N=5)	NSP SUPPLEMENTED GROUP (N=7)
GROSS ENERGY INTAKE (GEI) (MJ/d)	13.7 (10.8 – 16.0)	9.9 (7.9 – 13.5
APPARENT DIGESTIBLE ENERGY INTAKE (DEI) (MJ/d)	13.1 (9.6 – 14.4)	8.4 (7.1– 12.4)#
DEI as % GEI TOTAL	89.0 (89.0 – 95.1)	91.1 (76.8 – 94.9)
ESTIMATED METABOLISABLE ENERGY (MEI) (MJ/d)	12.4 (9.1 – 13.6)	7.9 (6.6 – 11.7)#
MEI as % GEI TOTAL	85.2 (84.0 – 90.1)	86.1 (71.8 – 89.9)

Apparent Digestible Energy Intake = Gross Energy minus Stool Energy losses and expressed as a percentage of Gross Energy Intake

Estimated Metabolisable Energy Intake = Gross Energy minus Stool Energy losses minus Estimated Urinary Energy Losses (5%) and expressed as a percentage of Gross Energy Intake

FIGURE 3.11 NSP INTAKE FOR PLACEBO AND NSP SUPPLEMENTED GROUPS.

Values are presented for each patient in increasing rank order. The placebo group received a NSP-free feed.

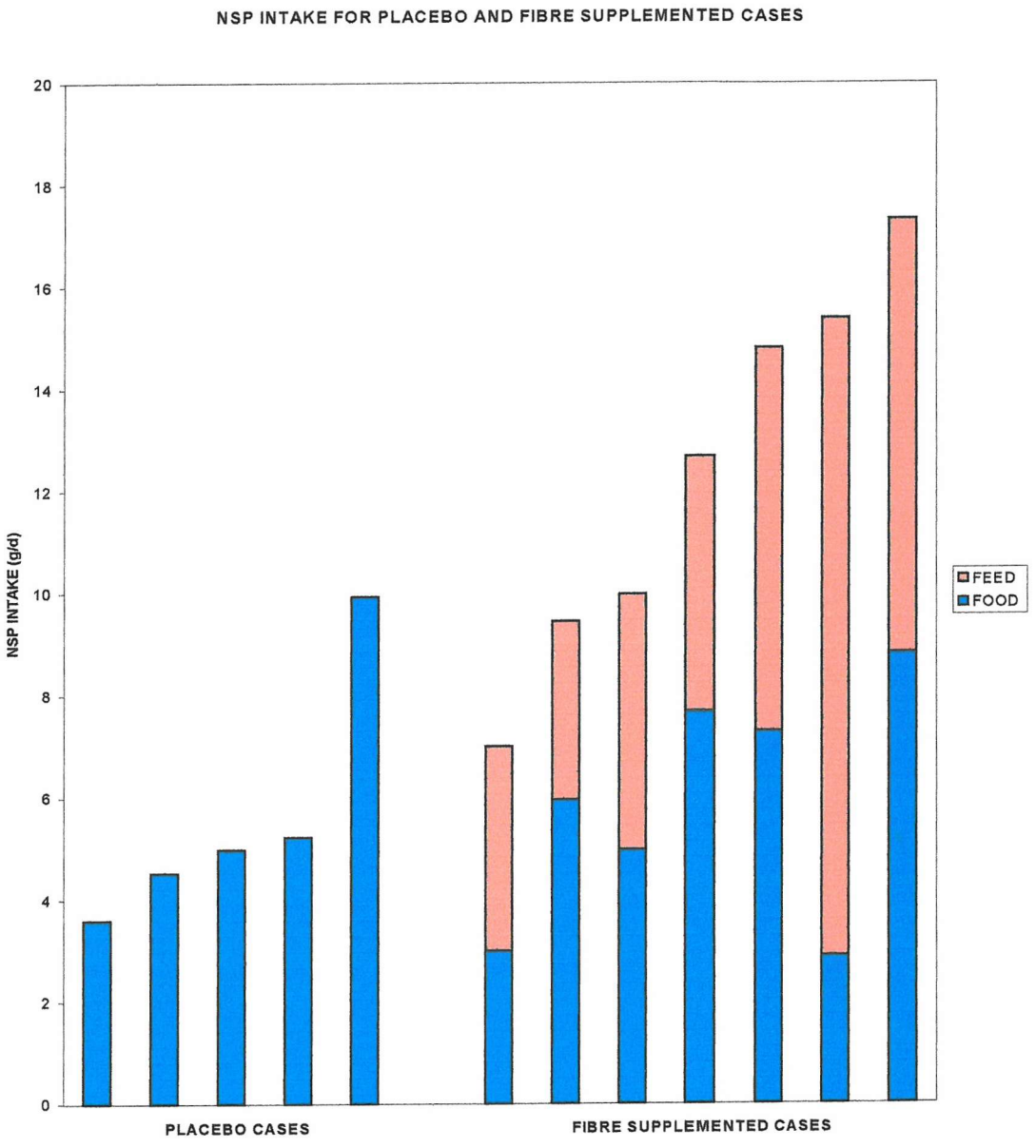


TABLE 3.9 ENERGY AND NUTRIENT INTAKE FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Median values are given with ranges in parenthesis. The asterix denotes a significance level of $P < 0.01$. A # denotes a significance level of $P < 0.05$. All other differences in nutrient intake did not attain statistical significance

NUTRIENT	FOOD	FEED	TOTAL
GROSS ENERGY (MJ/d)			
PLACEBO GROUP	7.0 (3.9 – 9.1)	6.9 (5.9 – 6.9)	13.7 (10.8 – 16.0)
NSP GROUP	6.3 (4.5 – 7.6)	3.53 (1.6 – 8.9)	9.9 (7.9 – 13.5)
FAT (g/d)			
PLACEBO GROUP	56.7 (38.6 – 81.6)	58.0 (49.3 – 58.0)	114.7(96.6 – 131.7)
NSP GROUP	49.4 (22.2 – 57.1)	29.0 (20.3 – 72.5)#	86.1 (61.8 – 105.8)#
PROTEIN (g/d)			
PLACEBO GROUP	43.1 (34.6 – 69.9)	56.0 (47.6 – 56.0)	99.1 (96.6 – 131.7)
NSP GROUP	40.9 (33.6 – 55.3)	28.0 (19.6 – 70.0)#	64.9 (60.5 – 113.4)
NSP (g/d)			
PLACEBO GROUP	4.9 (3.6 – 9.9)	0.0	4.9 (3.6– 9.9)
NSP GROUP	5.9 (2.9 – 8.8)	5.0 (3.5 – 12.5)*	12.7 (6.9-17.3)*

SUMMARY

The differences between total gross energy intake, protein and fat intake for the placebo and NSP supplemented groups were largely created by the variability in feed volumes which were calculated independently of this study and based on the estimated energy requirement of the patient and their appetite for food. Ideally feed volumes should be comparable between the patient groups but unfortunately this was not the case due to the large variability in the requirements of the patients that were recruited to the study especially in the NSP supplemented group.

3.2.3 GASTROINTESTINAL SYMPTOMS FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Symptoms were categorised as 1 = no symptoms: 2 = mild / moderate symptoms: 3 = severe / frequent symptoms from the retrospective bowel habit questionnaire (BHQ), and the bowel habit diary (BHD) which was completed over the stool collection period. The results are summarised in Table 3.10

TABLE 3.10 SYMPTOMS AND STOOL COLLECTION DATA FROM THE BOWEL HABIT QUESTIONNAIRE AND DIARY: PHASE 2

SYMPTOMS AND STOOL RESULTS	BOWEL HABIT QUESTIONNAIRE	BOWEL HABIT DIARY
COMPLAINING OF ABDOMINAL PAIN	72% (5/7) NSP 40% (2/5) PLACEBO	0% NSP 40% (2/5) PLACEBO
STOOL FREQUENCY (1/DAY)	72% (5/7) NSP 80% (4/5) PLACEBO	100% NSP 100% PLACEBO
STOOL CONSISTENCY (NORMAL STOOL PASSED)	57% (4/7) NSP 0% PLACEBO	43% (3/7) NSP 0% PLACEBO
STOOL COLOUR (BROWN)	86% (6/7) NSP 80% (4/5) PLACEBO	86% (6/7) NSP 80% (4/5) PLACEBO

As in Phase 1 the bowel habit questionnaire was cross-referenced against the bowel habit diary to identify whether symptoms were under or over reported. 29% (2/7) of the NSP supplemented group reported daily / severe abdominal pain in the BHQ (Figure 3.12) but no pain during the BHD period. 43% (3/7) of the NSP supplemented group reported occasional abdominal pain in the BHQ (Figure 3.12) but no pain during the BHD period. 40% (2/5) of the placebo group reported only occasional symptoms in the BHQ (Figure 3.12)

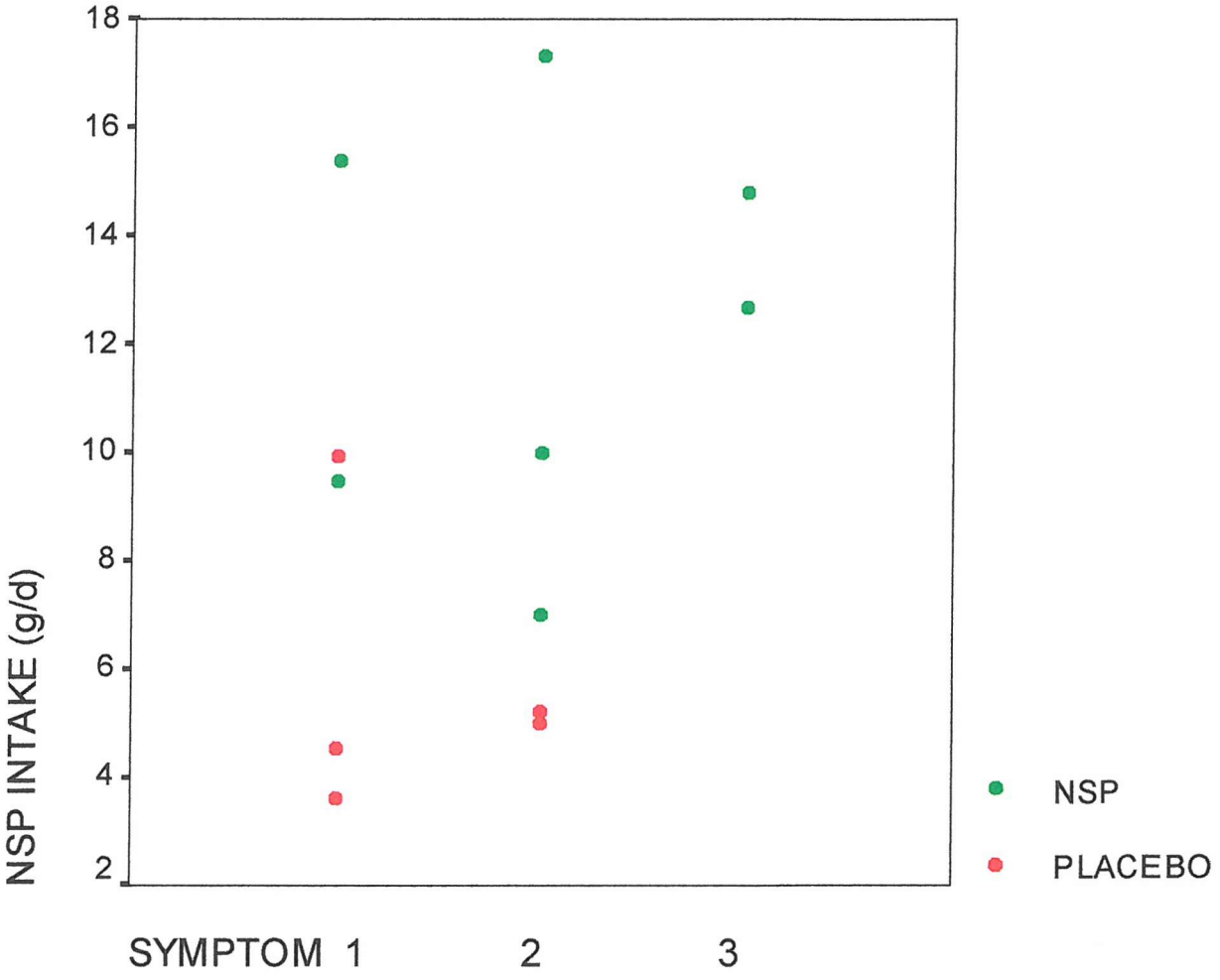
and BHD. NSP intake was only weakly associated with abdominal pain for both placebo (r = 0.3) and NSP supplemented groups (r = 0.1).

14% (1/7) of the NSP supplemented group reported 2 - 4 stools daily and another 14% (1/7) of the same group reported less than 1 stool per day in the BHQ but during the BHD period both these patients passed 1 stool daily. 20% (1/5) of the placebo group reported less than 1 stool per day in the BHQ but all patients passed 1 stool daily during the BHD. 57% (4/7) of the NSP supplemented group reported passing "smooth, soft" stools in the BHQ and 43% (3/7) of the same patients reported " smooth soft" stools during the BHD period. All the patients in the placebo group reported stools that were indicative of maldigestion / malabsorption in the BHQ and BHD.

14% (1/7) of the NSP supplemented group and 20% (1/5) of the placebo group reported pale stools in the BHQ. The same patients from both groups reported pale stools during the BHD period. All the patients in the NSP supplemented and placebo groups reported a problem with "wind" as rare. As in the first phase there was a disparity between symptoms recorded in the BHQ and the BHD. The reasons for this are discussed in Chapter 4.

FIGURE 3.12 NSP INTAKE VERSUS OCCURRENCE OF ABDOMINAL PAIN FOR PLACEBO AND NSP SUPPLEMENTED GROUPS (BOWEL HABIT QUESTIONNAIRE)

The symptom categories are as follows; 1 = no pain;
2 = mild / moderate and occasional pain; 3 = severe / frequent pain.



3.2.4 STOOL OUTPUT FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Despite increasing NSP intake, stool output variables were comparable between the groups except for two patients with consistently high results. Median stool wet weight (Figure 3.13) and dry weight (Figure 3.13) were greater for the NSP supplemented group than the placebo group (159.0 g/d; 31.3 – 333.3 g/d) compared with (96.5 g/d; 66.3 – 149.7 g/d; NS) and (46.8 g/d; 15.7 – 120.1 g/d) compared with (30.5 g/d; 20.2 – 48.7; NS) respectively. If studied on an individual basis however, the majority of stool weights were comparable between the groups. The median percentage water in stool was 61.0% (49.8% to 80.3%) for NSP supplemented group and 68.6% (49.5% to 77.6%; NS) for the placebo group. Fractionation of dry stool results were (Figure 3.14) Placebo group: Fraction A: 1.5 g/d (6.0 - 3.4 g/d); Fraction B: 2.4 g/d (1.3 - 6.3 g/d); Fraction C: 7.7 g/d (5.9 - 15.6 g/d); Water soluble fraction: 13.1 g/d (9.3 - 24.4 g/d). Fractionation of dry stool results were (Figure 3.14) NSP supplemented group: Fraction A: 4.1 g/d (2.0 - 9.6 g/d; $P < 0.05$); Fraction B: 4.7 g/d (0.4 - 10.8 g/d; NS); Fraction C: 14.0 g/d (3.3 - 22.8 g/d; NS); Water soluble fraction: 19.9 g/d (6.3 -38.4 g/d; NS).

Median stool bacteria (Figure 3.13) was greater for the NSP supplemented group (13.1 g/d; 3.1 – 23.5 g/d) compared with the placebo group (7.4 g/d; 6.6 – 15.1 g/d; NS). The median value for bacteria as a percentage of stool dry weight was 20.0% (15.4% to 32.1%) for the NSP supplemented group and 31.0% (22.0% to 34.0%; $P < 0.05$) for the placebo group. There was a strong relationship between stool bacteria and stool dry weight for both groups; Placebo group ($r = 0.9$; $P < 0.05$) and NSP supplemented group ($r = 0.9$; $P < 0.01$) (Figure 3.15). Bacterial lipid for the NSP supplemented group was greater (0.6 g lipid/g bacteria) compared with (0.5 g lipid/g bacteria) for the placebo group. This represented (5.3 g/d; 1.7 -12.4 g/d) bacterial lipid for the NSP supplemented group and (3.4 g/d; 3.1 - 7.0 g/d; NS) for the placebo group equivalent to 24.3% (16.1% to 28.8%) of stool lipid and 31.5% (20.7%

to 33.6%; $P < 0.05$) respectively. The relationship between stool lipid and stool bacterial mass (Figure 3.16) was strong for the NSP supplemented group (r = 1.0; $P < 0.01$) and moderate for the placebo group (r = 0.7; NS). Bacterial energy for the NSP supplemented group (0.5 MJ/d; 0.1 - 0.8 MJ/d) was twice the placebo group (0.2 MJ/d; 0.2 - 0.5 MJ/d; NS) representing 29% (22.5% to 43%) and 28.5% (19% to 30%; NS) of stool energy respectively. SCFA production from bacterial fermentation was estimated using the McNeil (1984) calculation. SCFA energy (Table 3.11) was comparable between the groups 0.2 MJ/d (0.2 - 0.4 MJ/d) for the placebo group and 0.3 MJ/d (0.1 - 0.5 MJ/d; NS) for the NSP supplemented group and represented (1.9%; 0.3% to 2.6%) of GEI and 2.1% (0.9% to 4.0%; NS) of GEI respectively.

Median stool energy losses (Figure 3.17) were similar for both groups (1.2 MJ/d; 0.7 – 1.6 MJ/d) for the placebo group compared with (1.2 MJ/d; 0.4 – 3.0 MJ/d; NS) for the NSP supplemented group. This represented 9.8% (4.9% to 11.0%) GEI and 8.9% (5.1% to 23.2%; NS) GEI respectively. Stool energy per gram wet weight and dry weight were Placebo group: 10.7 kJ/g (7.9 - 16.3 kJ/g); NSP supplemented group: 9.9 kJ/g (5.3 -12.8 kJ/g; NS) and Placebo group: 33.2 kJ/g (26.7 - 44.8 kJ/g); NSP supplemented group: 24.8 kJ/g (24.2 - 27.1 kJ/g; $P < 0.01$) respectively.

Median stool lipid (Figure 3.18) losses were greater for the NSP supplemented group than placebo group (23.7 g/d; 8.8 - 50.2 g/d compared to 15.4 g/d; 9.5 -20.9 g/d; NS). Stool lipid as a percentage of stool dry weight was similar for both groups (46.9%; 41.8% to 56.0%) for the NSP supplemented group and (47.2%; 35.0% to 56.2%; NS) for the placebo group. Assuming that stool lipid energy has the same heat of combustion as dietary fat, stool lipid energy was greater for the NSP supplemented group than the placebo group (0.9 MJ/d; 0.4 – 2.0 MJ/d) compared with (0.6 MJ/d; 0.4 - 0.8 MJ/d; NS). This represented 75.3% (63.5% to 86.6%) and 52.1% (38.9% to 82.6%; $P < 0.05$) of stool energy respectively. Stool lipid energy as a

percentage of total GEI was greater for the NSP supplemented group (6.9%; 4.4% to 15.4%) and (4.5%; 2.7% to 5.1%; $P < 0.05$) for the placebo group.

3.2.5 RELATIONSHIP BETWEEN ENERGY AND NSP INTAKE AND STOOL COMPOSITION VARIABLES FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

There were moderate relationships between stool wet weight and GEI (Placebo group: r = 0.6; NSP supplemented group: r = 0.4) and NSP (Placebo group: r = 0.3; NSP supplemented group: r = 0.4). There were weak relationships between stool dry weight and GEI for the placebo group (r = 0.1) but moderate for the NSP supplemented group (r = 0.4) and moderate for NSP (Placebo group: r = 0.3; NSP supplemented group: r = 0.4). There was also a moderate relationship between GEI and stool bacteria for the NSP supplemented group (r = 0.5) but a weak relationship for the placebo group (r = 0.2) and similar relationships for NSP intake (NSP supplemented group: r = 0.5; Placebo group: r = 0.1). There was a moderate relationship for the NSP supplemented group between stool energy and GEI (r = 0.4) and NSP (r = 0.4) compared to the weak relationship for the placebo group: GEI (r = 0.1) and NSP (r = 0.3). In conclusion there were no strong relationships between nutrient intake and stool output for either group.

FIGURE 3.13 STOOL OUTPUT VARIABLES FOR THE PLACEBO AND NSP SUPPLEMENTED GROUP

The median and individuals values are presented

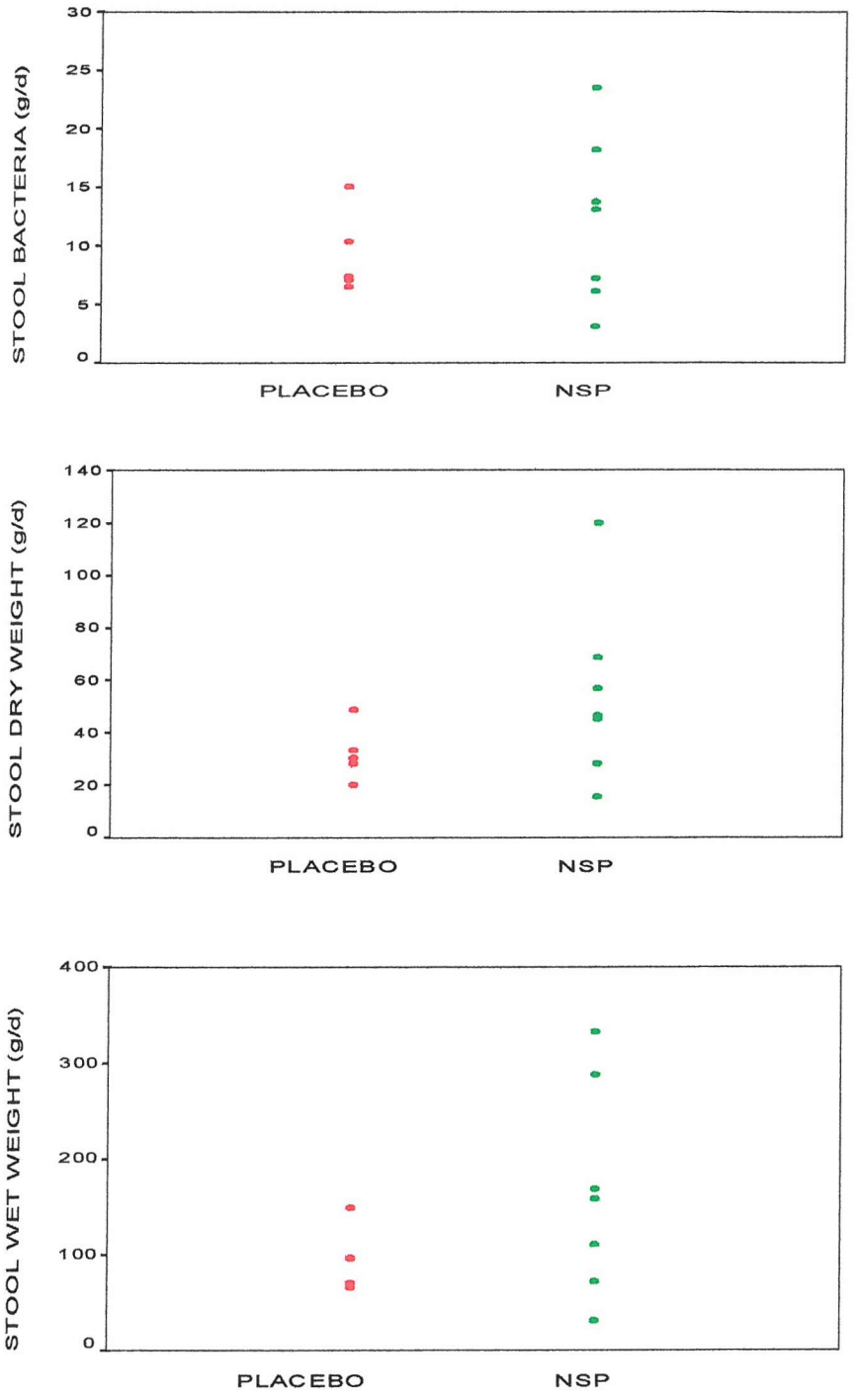


FIGURE 3.14 DAILY FRACTION COMPONENTS OF DRIED STOOL FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Fraction A represents large plant material, Fraction B small plant material and Fraction C stool bacteria.

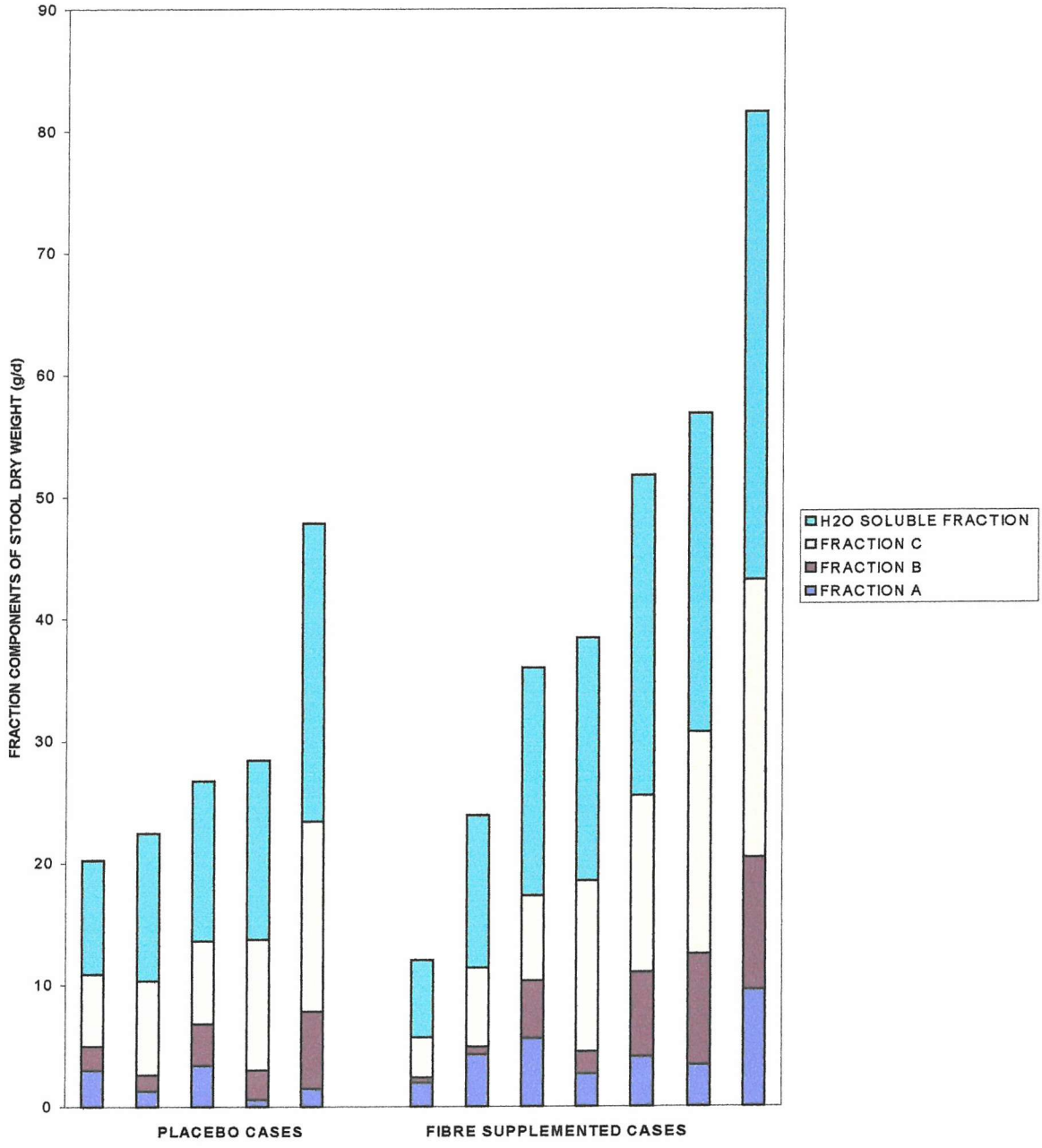


FIGURE 3.15 STOOL BACTERIAL MASS VERSUS STOOL DRY WEIGHT FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

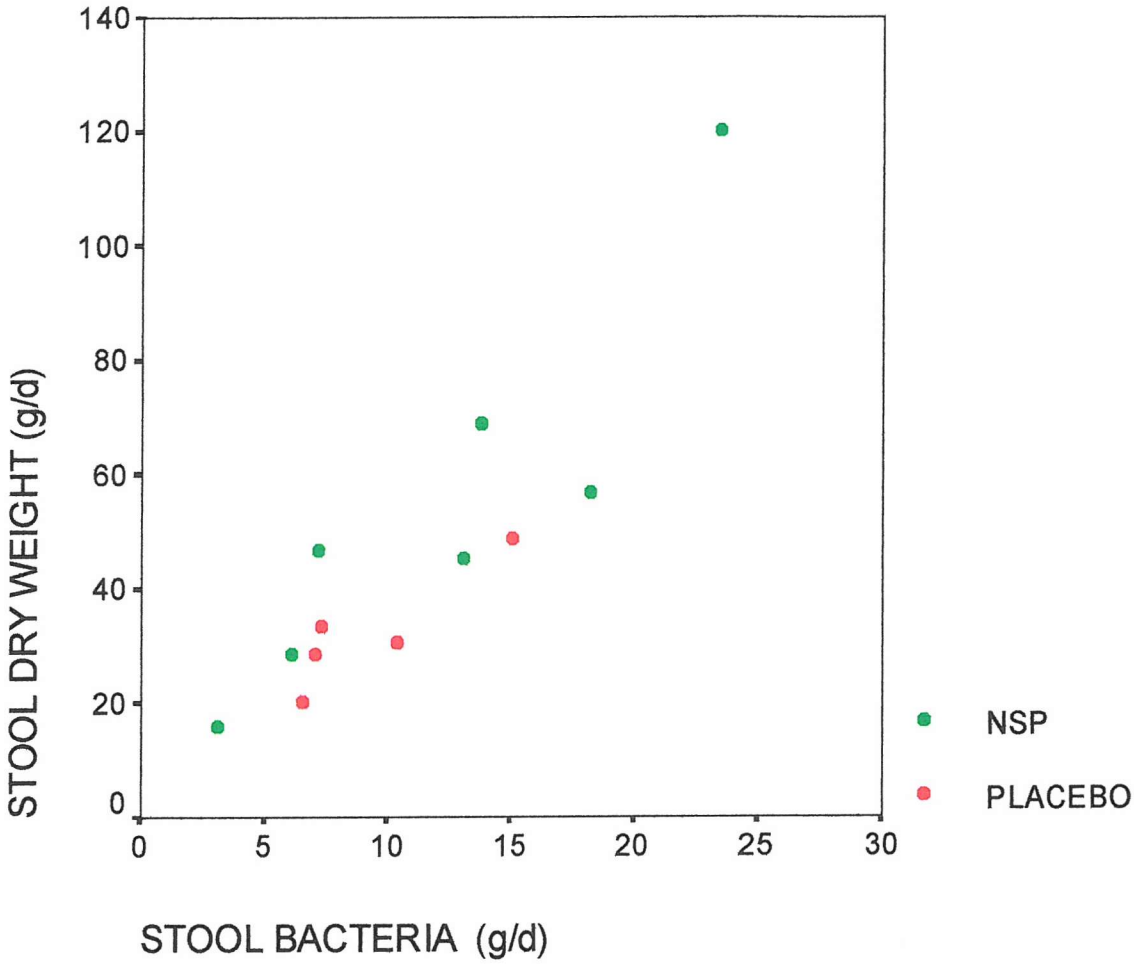


FIGURE 3.16 STOOL LIPID VERSUS STOOL BACTERIAL MASS FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

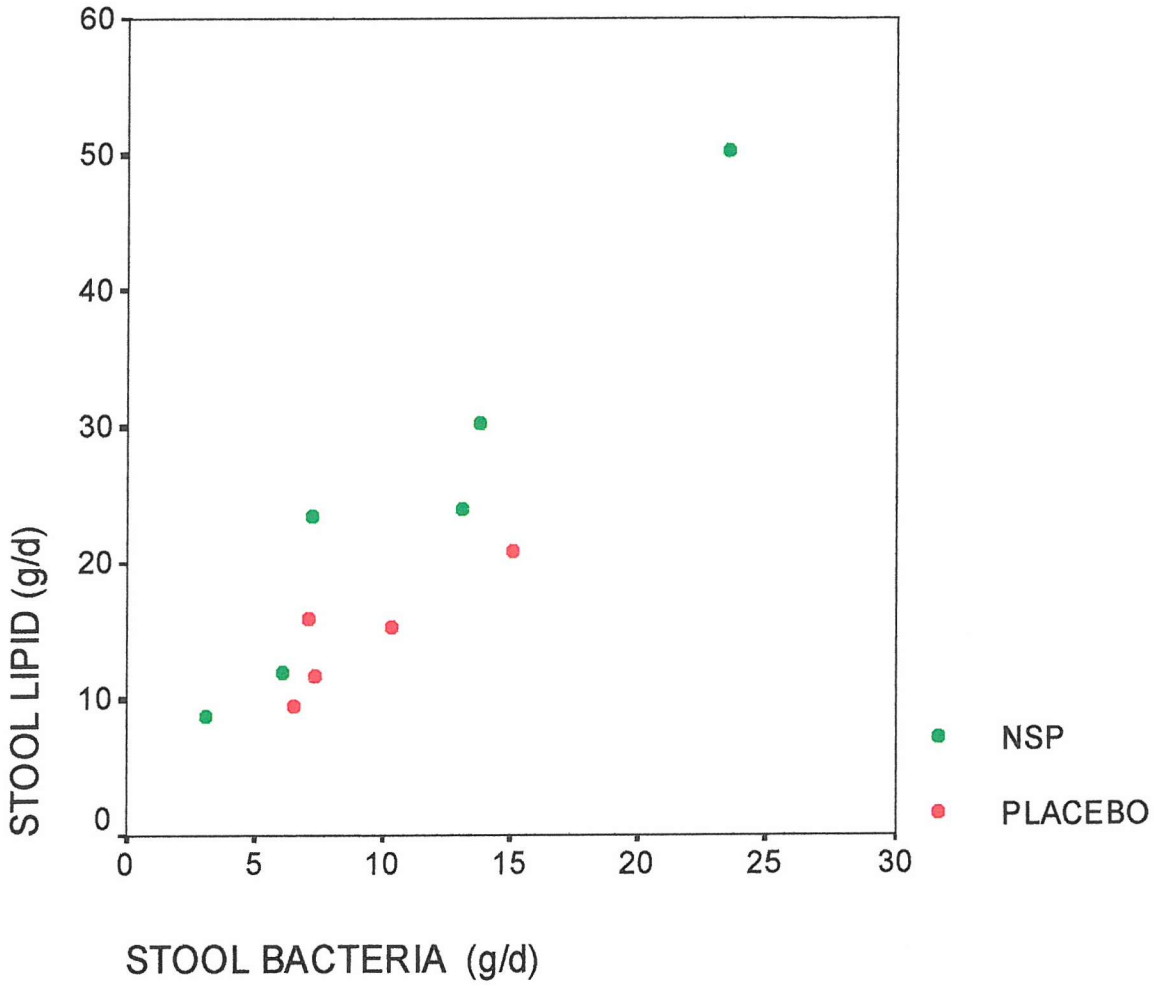


FIGURE 3.17 STOOL ENERGY FOR PLACEBO AND NSP SUPPLEMENTED GROUPS.

The values are presented for each patient in increasing rank order.

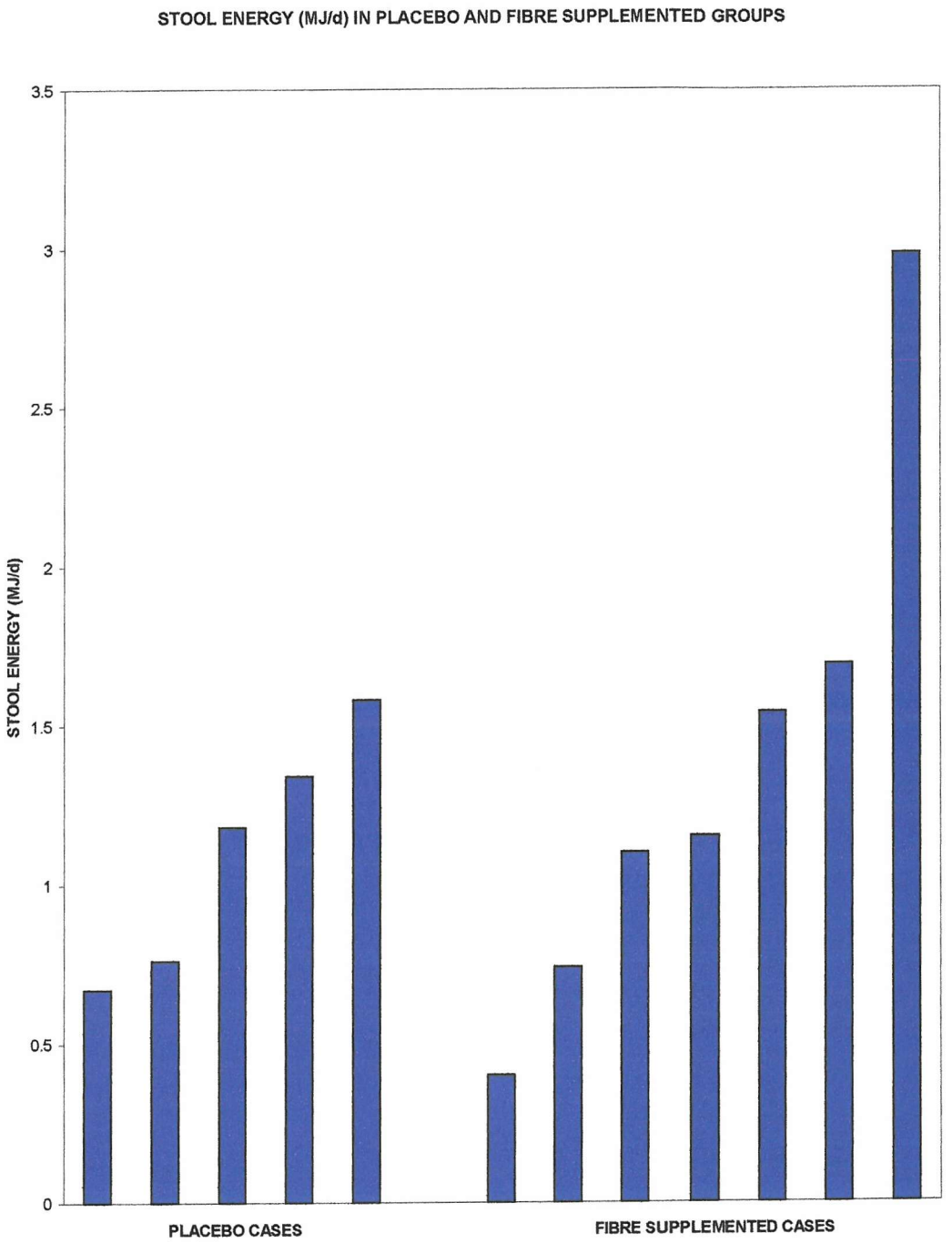


FIGURE 3.18 STOOL LIPID FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Stool lipid is presented in increasing rank order. Only eleven of the CF patients participated in this analysis.

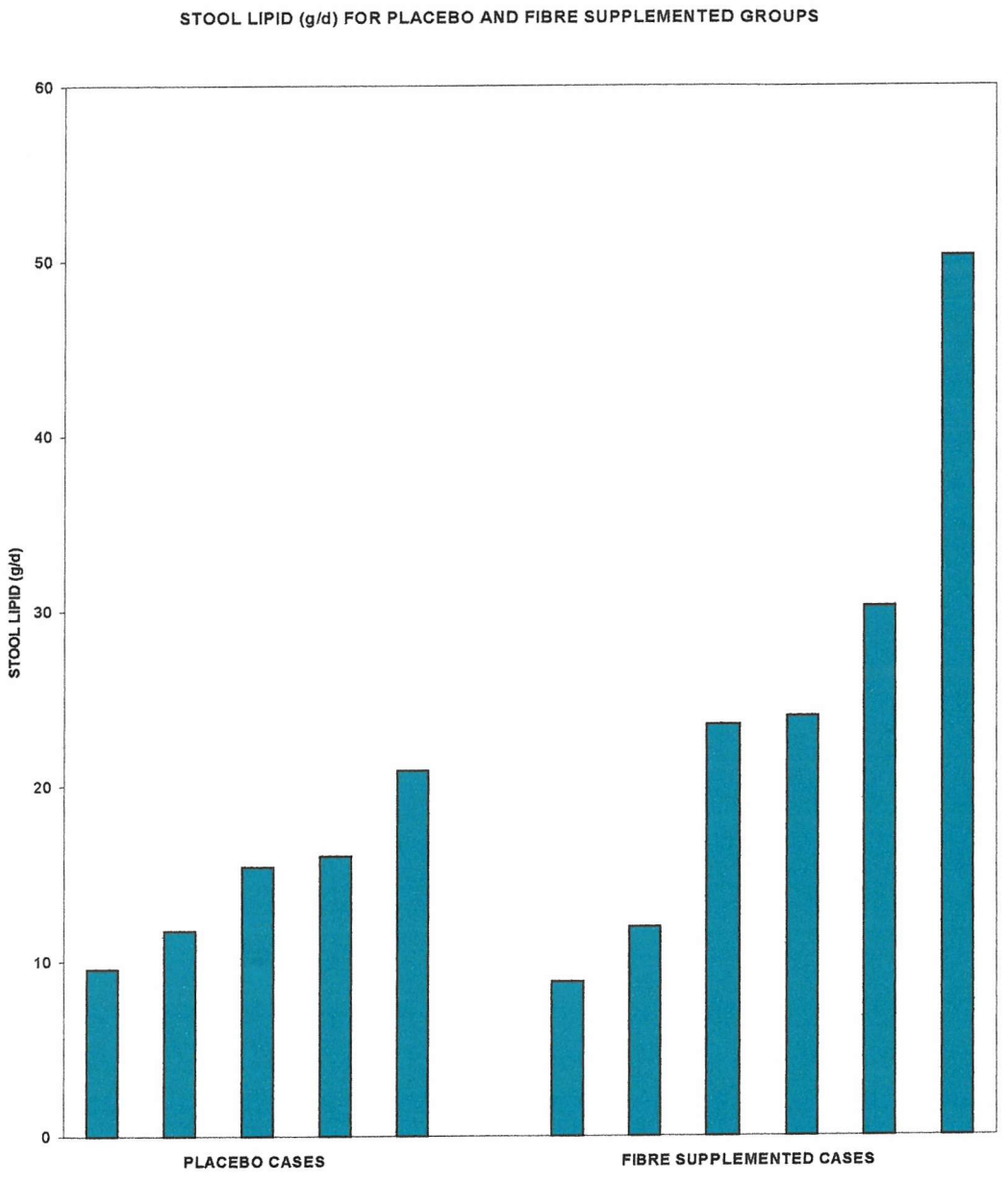


TABLE 3.11 ADDITIONAL POTENTIAL ENERGY THAT MAY BE PROVIDED FROM COLONIC FERMENTATION (MCNEIL CALCULATION 1984) FOR PLACEBO AND NSP SUPPLEMENTED GROUPS

Median values are given with range in parenthesis. None of the differences between the groups attained statistical significance.

STOOL INDICES	PLACEBO GROUP (N=5)	NSP SUPPLEMENTED GROUP (N=7)
BACTERIAL ENERGY (MJ/g)	0.2 (0.2 – 0.5)	0.5 (0.1 – 0.9)
HEXOSE REQUIRED TO SYNTHESISE FAECAL BACTERIAL MASS (g/d)	24.3 (21.6 – 49.8)	43.2 (10.4 – 77.7)
TOTAL ENERGY YIELD (MJ/d)	0.4 (0.4 – 0.9)	0.8 (0.2 – 1.4)
ENERGY AVAILABLE AS SCFA (MJ/d)[1]	0.2 (0.2 – 0.4)	0.3 (0.1 – 0.5)

[1]Total Energy Yield – Stool Bacterial Energy

SUMMARY

The total energy yield for the NSP supplemented group was approximately twice the placebo group. As bacterial energy for the NSP supplemented group was also double the energy available as SCFA was relatively similar.

CHAPTER 4 DISCUSSION

4.1 INTRODUCTION

The literature review described the role of NSP in gastrointestinal function in the healthy individual and hypothesised how a low NSP intake might alter function in CF. To briefly summarise, NSP is responsible for decreasing colonic transit time, stimulating bacterial mass proliferation, increasing stool weight and preventing the occurrence of gastrointestinal symptoms in the healthy individual (Cummings 1984). The UK recommendations for NSP intake for healthy adults are actually based on the quantity of NSP required to maintain stool weight above 100 g (Dept of Health 1991) as symptoms are reported to develop with stool weights of 30 – 60 g (Cummings 1986). In addition, persistently low stool weights have been associated with an increased risk of bowel cancer (Burkitt 1971b).

CF patients are reported to consume a low NSP intake compared with age-matched controls (Gavin *et al* 1997). If the role of NSP in the CF colon was similar to that reported in the healthy colon, then a lower quantity of NSP reaching the colon (especially in enterally fed patients) would reduce bacterial mass and stool weight, increase colonic transit time and precipitate gastrointestinal symptoms. An increasing incidence of large bowel cancer has been reported in the CF colon (Lloyd-Still 1990; Neglia *et al* 1995) and, as is the case in a healthy colon, low stool weights in CF patients might be a contributing factor to the increased risk.

In concluding the literature review, several questions were identified to determine whether the simple association between a low NSP intake and gastrointestinal symptoms in orally fed CF patients as demonstrated by Gavin *et al* (1997) could be reproduced in the enterally fed CF population, and whether the relationship was causally linked to colonic bacterial metabolism in the CF colon:

1. Does energy and NSP intake for a group of enterally fed CF patients differ from healthy individuals?

2. Do gastrointestinal symptoms and stool composition relate to NSP intake in a group of healthy individuals and enterally fed CF patients?

3. Does the administration of a NSP feed to a group of enterally fed CF patients affect stool composition and energy availability?

4. Does the administration of a NSP feed to a group of enterally fed CF patients relieve gastrointestinal symptoms?

The study hypothesis proposed that a low NSP intake would result in a reduced stool output and be the causal factor of abdominal symptoms for the enterally fed CF patients. Addition of a NSP supplemented feed would therefore increase stool output and relieve symptoms for this group. The study was designed as two phases of work with the following aims:

Phase 1: To investigate the differences in energy intake, NSP intake, stool losses, and the frequency of bowel habit, in a group of CF patients consuming their habitual NSP-free feed and oral diet, and a reference group of healthy individuals consuming habitual diet (Questions 1 & 2)

Phase 2: To investigate the differences in energy and NSP intake, stool losses, and the frequency of bowel habit in a CF group consuming a habitual diet and a NSP supplemented feed, and a CF group consuming a habitual diet and a placebo feed (Questions 3 & 4)

This discussion chapter is divided into two sections reflecting these phases of work.

4.2 DISCUSSION: PHASE 1

4.2.1 DO ENERGY AND NSP INTAKES DIFFER BETWEEN ENTERALLY FED CF PATIENTS AND HEALTHY INDIVIDUALS?

Despite a greater energy intake, the CF group consumed a NSP intake only half that of the healthy group supporting previous findings (Gavin *et al* 1997). All CF patients consumed an NSP intake less than their "age in grams". Only 50% of healthy subjects achieved the American recommendation for NSP intake of "age + 5 grams" (Williams *et al* 1995) and the remaining 50% consumed a NSP intake equivalent to "age in grams". Their NSP intakes were however comparable with previous data on NSP intake in healthy children of this age group in the UK (Murphy 1991; Ellis *et al* 1992; Gavin *et al* 1997).

4.2.2 DO GASTROINTESTINAL SYMPTOMS RELATE TO NSP INTAKE IN A GROUP OF ENTERALLY FED CF PATIENTS?

Half the CF patients complained of gastrointestinal symptoms and two patients with the lowest NSP intakes had severe / frequent abdominal pain. If no further analysis on stool composition were undertaken, the conclusion would be similar to the study by Gavin *et al* (1997) in that a low NSP intake was a causal factor in the pathogenesis of gastrointestinal symptoms for this enterally fed CF group. Correlations between NSP intake and stool variables however showed a weak association only therefore a low NSP intake could not predict the severity of abdominal pain as previously reported in healthy individuals (Cummings 1986). An alternative hypothesis is that the presence of other fermentable carbohydrates e.g. maldigested / malabsorbed nutrients and endogenous losses exerted an osmotic effect on the CF colon precipitating gastrointestinal symptoms.

The bowel habit questionnaire was devised previously by another investigator to assess abdominal symptoms in a CF population (Lyyra 1997) and was used as a retrospective assessment of bowel habit for this study. The daily bowel habit diary was completed during the 3 day stool collection so represented a much shorter period of time than the questionnaire. It would therefore be possible that symptoms mentioned in the questionnaire might not be reproduced in the diary. This occurred in both Phases 1 and 2 and may indicate that symptoms are consistent in very few CF patients. To support this theory of irregular and infrequent symptoms, there were two patients with a known medical diagnosis of constipation in the study but only one was identified from the questionnaire and neither from the diary. Alternatively the questionnaire and diary may not have been sensitive enough to identify the frequency of symptoms, or patients might be more likely to report symptoms to their doctor than registering them on a research questionnaire. In addition, CF patients might have mild symptoms daily that they perceive to be "normal" therefore remain unmentioned.

4.2.3 DOES STOOL COMPOSITION DIFFER BETWEEN A GROUP OF ENTERALLY FED CF PATIENTS AND HEALTHY INDIVIDUALS AND DOES IT RELATE TO NSP INTAKE?

Before discussing the results of the CF stool data in comparison with data from healthy subjects, it could be questioned whether the stool results from the healthy children were suitable as a reference. There are little previous data on stool composition in healthy children for comparison. The results for the healthy group were similar to stool data from a study of 16 healthy children (Murphy 1991) so were assumed to be a suitable reference group.

Median stool wet and dry weight were statistically significantly greater for the CF group compared with the healthy group despite a lower NSP intake. This supported previous findings of stool weight for CF patients (Murphy 1991). Stool fractionation of dry weight showed that a similar quantity of large and

small plant material (NSP) was excreted for both groups. This was an unexpected result as NSP intake was significantly lower for the CF group. The result implied that NSP was "spared" from fermentation in the CF colon (Cummings *et al* 1996) due to the presence of preferentially fermentable substrate e.g. maldigested / malabsorbed nutrients and hexose-rich CF mucus. In contrast, NSP was partially fermented in the healthy colon and only a portion excreted. Despite similar quantities of NSP in the stool for both groups, dry weight was greater for the CF group implying that there were additional components affecting stool weight in CF. To support this theory the CF group had a larger water soluble stool fraction indicating a greater presence of polysaccharides such as mucopolysaccharides or maldigested / malabsorbed starches in the stool responsible for increasing stool weight.

Stool bacterial mass was statistically significantly greater for the CF group than the healthy group despite a lower NSP intake. Murphy (1991) reported a similar bacterial mass for CF patients. Bacterial mass was therefore maintained by the preferentially fermented maldigested / malabsorbed nutrients and endogenous products in the CF colon rather than NSP. Previous studies in the healthy individual have demonstrated the ability of endogenous products to maintain bacterial mass in the absence of NSP. Bacterial mass as a percentage of stool dry weight on a NSP-free diet was similar to the percentage on a habitual diet for healthy subjects (Murphy 1991; Costello 1993). In CF patients where extra endogenous products e.g. hexose-rich mucus are presented to the colon there is even more potential for fermentation and proliferation of a larger bacterial mass in the absence of NSP.

Median stool lipid for the CF group was twice the stool lipid for the healthy group despite dietary fat intake being less than double. There are previous data available on stool lipid losses of healthy children. Wollaeger *et al* (1947) and Shmerling *et al* (1970) presented data of stool lipid losses for healthy children, adolescents and adults, which were similar to the stool lipid losses of the healthy subjects in Phase 1. Only one CF patient had a stool lipid excretion similar to the healthy subjects. This result was of clinical interest as pancreatic enzyme therapy is perceived to "normalise" lipid losses by patients but lipid losses remained elevated in the majority of patients even when consuming an "adequate" dose as defined by a non-greasy stool appearance. A further study to identify what proportion of stool lipid was maldigested or malabsorbed fat could have provided a more accurate assessment of whether enzyme dose was appropriate. A study by Murphy (1991) reported lower lipid losses in both CF patients and healthy subjects. The method used in the current study was different to that used in the study by Murphy (1991) and may explain the disparity.

Stool lipid is composed of bacterial, dietary and endogenous lipid. The role of endogenous lipid in the CF colon has previously been highlighted in a study of eight CF patients on an elemental diet (Leroy *et al* 1986). Stool lipid losses on an elemental diet were only moderately lower than lipid losses in the same patients on a habitual diet. Lipid losses were therefore maintained by an endogenous component in the absence of dietary lipid. The contribution of endogenous lipid to stool lipid can also be seen in the clinical situation where patients with large stool lipid losses are still capable of maintaining weight gain and growth. Consequently, a clinical measurement of a large stool lipid may be inappropriately interpreted as excessive maldigestion / malabsorption of dietary fat. Readjustment of pancreatic enzyme therapy may therefore prove unnecessary especially if the patient is demonstrating adequate growth.

Stool lipid energy for the CF group was double the healthy group. The assumption used in the calculation of stool lipid energy was that stool lipid had the same heat of combustion as dietary lipid but the process of maldigestion and malabsorption might alter this figure. For example malabsorbed starch in the colon is estimated at 3 kcal/g compared with starch in the small intestine at 4.2 kcal/g (Wisker & Feldheim 1988). Stool lipid might be equally altered but unfortunately there are no figures available for the energy content of maldigested / malabsorbed lipid at the present time therefore the calculation of stool lipid energy may be overestimated.

Median stool energy for the CF group was statistically greater for the CF group compared with the healthy group supporting previous work (Murphy 1991). Despite this disparity, stool energy losses represented a similar proportion of gross energy intake in both groups due to the statistically greater energy intake in the CF group. Large stool energy losses can compromise weight gain and growth but only in those individuals with marginal energy intakes. Sources of energy within the stool were maldigested / malabsorbed residue, NSP and endogenous products e.g. mucus and bacteria.

Median bacterial energy and bacterial lipid from pooled bacterial samples of the CF group were twice the healthy group. An explanation for this may be that Bacteroides is the predominant bacteria in the CF colon. This is because the cell wall of Bacteroides (gram negative bacteria) has a higher lipid content than gram positive bacteria (Mandelstam *et al* 1982) and it also possesses the extracellular enzymes required for fermenting mucin (Salyers *et al* 1977) especially important in the CF colon. Further studies on bacterial type could identify the percentage of Bacteroides and other bacterial species present in the CF colon. An alternative explanation for a high bacterial lipid for the CF group is that a surplus of maldigested / malabsorbed carbohydrate in the colon was synthesised to lipid by bacteria and stored in the cell wall (Mandelstam *et al* 1982). As lipid is the most energy dense nutrient, a higher bacterial lipid explains the larger stool bacterial energy for the CF group.

Bacterial fermentation of carbohydrate produces short chain fatty acids (SCFA) that maintain bacterial proliferation. The carbohydrate can be NSP (Williams & Olsted 1936) resistant starch (Englyst & MacFarlane 1986; Stephen *et al* 1983) or endogenous losses such as mucus (Miller & Hoskins 1981). NSP provided 77% of carbohydrate to maintain the bacterial mass for the healthy group but provided only 19% of carbohydrate for the CF group. Supplementary carbohydrate was therefore provided in the form of maldigested / malabsorbed nutrients and hexose-rich mucus (Wesley *et al* 1983) for the CF group to maintain the bacterial mass.

McNeil (1984) developed a calculation to estimate the amount of energy recovered by the host through SCFA absorption as it is impossible to measure the exact quantity of fermentable substrate that reaches the colon in living subjects. SCFA production may be underestimated by this calculation as stool bacteria represent only a proportion of the bacterial turnover in the colon. Murphy (1991) used this method to estimate SCFA absorption to represent approximately 2% of gross energy intake (GEI) in healthy subjects and 6 - 14% GEI in CF patients. In the current study, SCFA production was significantly greater for the CF group due to the larger bacterial mass but represented a similar percentage of GEI for both groups due to the larger energy intake of the CF group. SCFA production would represent a greater proportion of GEI if intakes were marginal. The theory of "colonic malnutrition" resulting from inadequate fermentable substrate and low levels of SCFA production (Gavin *et al* 1997) can therefore be eliminated as a possible cause of gastrointestinal complications e.g. Fibrosing Colonopathy and associated abdominal symptoms in CF.

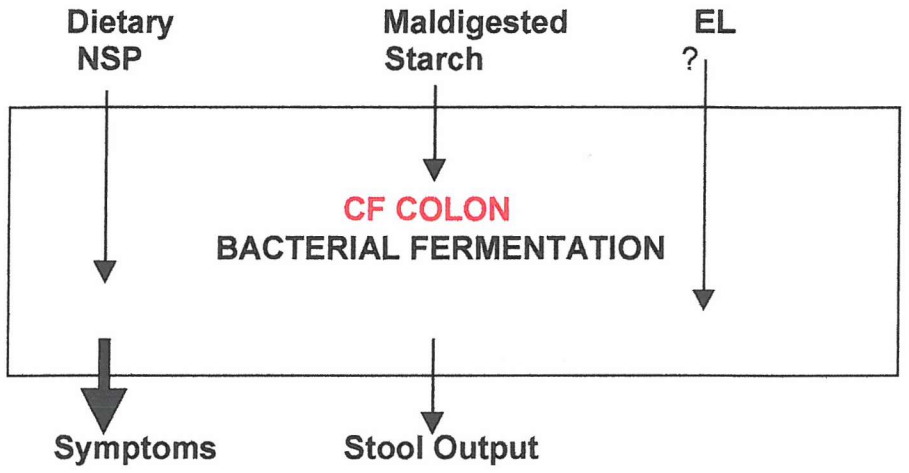
4.2.5 SUMMARY: PHASE 1

Despite a NSP intake half that of the healthy group, the CF group had a stool output twice the size. The CF colon was therefore receiving additional fermentable carbohydrate from other sources to maintain stool weight and bacterial mass. The hypothesis that a low NSP intake would compromise colonic function and reduce stool weight can therefore not be supported. As stool weights were larger for the CF group, so too were stool energy and lipid losses and this compromised energy availability. GEI from feed and food was however more than adequate to meet energy requirement for the majority of patients.

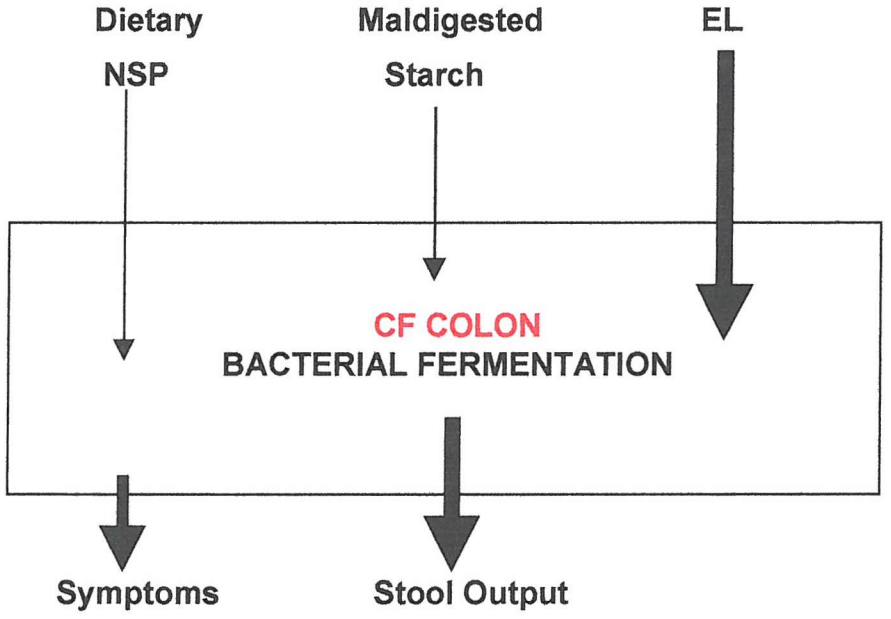
Those CF patients with the lowest NSP intakes had the most frequent abdominal symptoms, which initially appeared to support the previous study by Gavin *et al* (1997). As NSP intake did not however correlate with any stool variables, a low NSP intake could not be implicated as the cause of gastrointestinal symptoms. Another colonic substrate such as maldigested / malabsorbed fat, protein or endogenous losses was therefore the potential causal factor. The proposed pathway for the metabolism of dietary carbohydrates in the CF colon was therefore modified (Figure 4.1). Figure A illustrates the original hypothesis that reduced NSP reaching the CF colon would compromise bacterial fermentation and stool output resulting in gastrointestinal symptoms. Figure B illustrates the results from Phase 1 that despite a reduced quantity of NSP reaching the CF colon there was sufficient fermentable substrate e.g. hexose rich mucus already present to maintain bacterial mass and stool output. Despite a larger stool output, gastrointestinal symptoms still occurred therefore were not related to NSP intake.

FIGURE 4.1 PATHWAY OF METABOLISM OF DIETARY CARBOHYDRATE IN CF PATIENTS

A) HYPOTHETICAL PATHWAY



B) MODIFIED PATHWAY



EL = Endogenous losses

4.3. DISCUSSION: PHASE 2

4.3.1 DO ENERGY AND NSP INTAKES DIFFER BETWEEN PLACEBO AND NSP SUPPLEMENTED GROUPS

The NSP supplemented feed increased NSP intakes by a median of 5 g/d (3.5 - 12.5 g/d), an increase of 50% (19% -63%) above NSP intake from food. The recommendation for NSP intake (Williams *et al* 1995) is "age + 5 grams" for children and "age + 10 grams" for adolescents. 86% (6/7) CF patients receiving the NSP supplemented feeds consumed "their age" in grams of NSP and 14% (1/7) child consumed "age + 5 grams". To assess whether NSP intake was "normalised" by the NSP supplemented feed, these figures were compared with NSP intake from healthy subjects in Phase 1. As 50% of healthy subjects consumed a NSP intake equivalent to "age + 5 grams" and the remainder consumed "age in grams" all the CF patients in the NSP supplemented group in Phase 2 "normalised" their NSP intake. Increasing NSP further was practically restricted by the variation in volumes of feed the patients were consuming, which was dependent on their energy requirement and controlled independently of the study. The results showed that despite "normalising" NSP intake, NSP intake was only weakly associated with stool indices. This implied that the recommendations for NSP intake for healthy individuals (Dept of Health 1991; Williams *et al* 1995) do not apply to the enterally fed CF group as maldigested / malabsorbed nutrients and endogenous products are exerting a similar physiological effect to NSP in the CF colon.

4.3.2 DOES THE ADDITION OF A NSP SUPPLEMENTED FEED TO A GROUP OF ENTERALLY FED CF PATIENTS RELIEVE GASTROINTESTINAL SYMPTOMS

The NSP supplemented feed was expected to reduce the incidence of gastrointestinal symptoms for the NSP supplemented group but they actually complained of more symptoms than the placebo group. The hypothesis that increasing NSP intake in the enterally fed CF group would relieve abdominal symptoms can therefore not be supported. Two patients in the placebo group also complained of symptoms supporting the conclusion from Phase 1 that another colonic substrate was responsible for the occurrence of gastrointestinal symptoms in this CF group.

4.3.3 DOES THE ADDITION OF A NSP SUPPLEMENTED FEED TO A GROUP OF ENTERALLY FED CF PATIENTS AFFECT STOOL COMPOSITION AND ENERGY AVAILABILITY

Stool wet and dry weights were comparable between the groups except for 2 patients in the NSP supplemented group. With the addition of 5 g NSP (3.5 - 12.5 g/d) to the feed, of which approximately 70% was soluble and approximately 30% insoluble NSP, it was possible to quantify the bacteria that might be generated from the increased hexose available (McNeil 1984). Stool bacteria was estimated to increase by approximately 1 g (0.7 - 2.7 g/d), and the remaining 1.5 g (1 - 3.8 g/d) of insoluble NSP would be excreted in the stool although a portion may also be fermented in the descending colon (Cummings *et al* 1987; McIntyre *et al* 1991). The bacterial mass for the NSP supplemented group however remained comparable with the placebo group except for two patients who had consistently high stool output results. Stool fractionation results showed that the large and small plant fractions containing the insoluble NSP component of food and feed were larger for the NSP supplemented group. Considering that NSP intake from food was similar

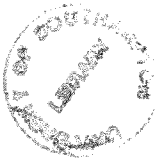
between the groups, an increase in these fractions was assumed to represent the additional NSP from the feed. The increase in the large and small plant fractions were however greater than expected from calculated values. The presence of preferentially fermented substrates in the colon had therefore created a "sparing effect" on NSP fermentation (Cummings *et al* 1996) and all the additional insoluble NSP was excreted unfermented into the stool. The water soluble fraction was larger for the NSP supplemented group indicating that the inulin and resistant starch in the feed were also "spared" from fermentation and excreted in the stool. Further studies on the nature of the polysaccharides present in the water soluble fraction could confirm whether they are representative of the inulin and resistant starch present in the feed. Inulin may have remained unfermented because the specific bacterial enzymes required to hydrolyse it might not have been synthesised within the four week feeding period. This process is reported to take a minimum of fourteen days but the synthesis may be gradual (Brunsgaard *et al* 1995; Key & Mathers 1995; Gibson *et al* 1995). More simply, their synthesis may not have been required due to the large amount of hexose already present in the form of maldigested / malabsorbed nutrients and mucus maintaining bacterial mass.

Median stool lipid was greater for the NSP supplemented group despite a significantly smaller dietary fat intake. One explanation for this result is that the NSP supplemented feed reduced lipid digestibility by impairing small intestinal digestion and absorption (Lairon *et al* 1985; Eastwood & Morris1992; Verbeek *et al* 1995; Spiller 1996). Inulin (representing 55% of the NSP in the feed) is reported to be less viscous than other types of NSP (Roberfroid *et al* 1993) therefore lipid digestion might be more affected than lipid absorption. Further studies on the nature of the stool lipid present could clarify whether digestion or absorption of lipid was principally influenced. An alternative explanation for higher lipid losses from the NSP supplemented group is that the biological process of digestion and absorption might have been more naturally impaired in those patients in the NSP supplemented

group. Due to the confines of this study it was not however possible to measure baseline stool output of all the patients receiving the placebo feed prior to intervention, which would have identified the source of increased lipid losses in Phase 2. Phase 1 was restricted to measurement of baseline stool output when receiving a variety of habitual feeds. A more plausible explanation for the larger stool lipid loss is that the bacterial mass for the NSP supplemented group contained a higher percentage of lipid. This difference in bacterial lipid between the groups might have been created by the pooled sample analysis as there was more variability in the stool bacterial mass for the NSP supplemented group compared with the placebo group. In addition, the larger bacterial lipid for the NSP supplemented group explains the larger bacterial energy content, as lipid is the most energy dense nutrient.

Median stool energy losses were similar for both groups despite a statistically greater NSP intake in the NSP supplemented group. This result does not support previous studies that suggest average stool energy losses are greater with higher intakes of NSP due to increased bacterial growth stimulated by additional fermentable NSP (Southgate & Durnin 1970; Kelsay *et al* 1978; Stephen *et al* 1983). Stool energy losses were however comparable between the groups therefore energy availability was not compromised. This was because the additional NSP was "spared" from fermentation and excreted in the stool creating insignificant additional energy losses.

To minimise the effect of antibiotics on bacterial mass for those patients who received them during the feeding period, the trial was continued for a further two weeks after antibiotics were discontinued in five patients (three patients in the NSP supplemented group and two patients in the placebo group). Previous studies report that it takes approximately two weeks to alter bacterial flora (Vince *et al* 1990; Gibson *et al* 1995) therefore it was deemed a sufficient period of time to continue the trial if antibiotics were given. It would however appear that two weeks might have been an insufficient period, as bacterial mass was distinctly lower for the five patients compared with those who did



not receive antibiotics. A specific quantity of NSP given daily in addition to habitual intake does not therefore maintain a stable bacterial mass in the presence of antibiotics as originally hypothesised

The amount of energy recovered by the host through bacterial fermentation and SCFA absorption was estimated (McNeil 1984). Additional fermentable substrate in the form of the NSP supplemented feed was originally hypothesised to enhance bacterial fermentation resulting in greater SCFA production. As additional NSP was largely excreted rather than fermented, it was not capable of enhancing SCFA production. An adequate fermentable substrate was already present in the form of maldigested / malabsorbed nutrients and endogenous products to maintain a similar bacterial mass between the groups so SCFA production was comparable.

Throughout both phases of the study stool composition for the CF groups demonstrated a large inter - individual variability. This was most notable in the second phase when smaller numbers of patients were involved. The NSP supplemented group contained two patients who had a consistently larger stool output than the rest of the group. One of these patients had uncontrolled malabsorption, but the largest stool losses were from a patient who had received a NSP supplemented feed as treatment for constipation for two years prior to the study. Without evidence of colonic function and stool composition for this patient prior to commencing a NSP supplemented feed, it is difficult to comment on whether the large stool losses are representative of habitual stool composition or as a result of long term use of a NSP feed. If they are the latter, this might imply that long term intake of additional NSP may be utilised more efficiently with time rather than excreted. Daily exposure to a specific quantity of NSP may initiate gradual synthesis of extra enzymes required to metabolise the increased NSP load (Brunsgaard *et al* 1995; Key & Mathers 1995). If so, stool output would be expected to slowly increase but plateau after a period of time. This patient had substantial stool energy losses

and effective dietetic management of this patient was crucial in preventing weight loss. Further investigation of stool output from this patient on a NSP-free feed for one to two months might identify whether these large stool losses were actually due to the long term effect of a NSP supplemented feed. If so, this may imply that a NSP supplemented feed is an inappropriate feed for use in enterally fed CF patients.

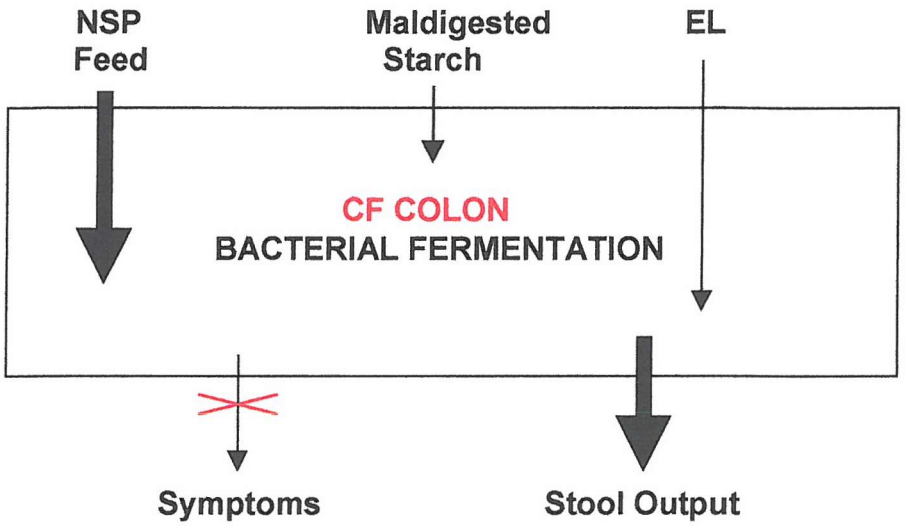
4.3.2 SUMMARY: PHASE 2

The NSP contribution from the enteral feed "normalised" NSP intakes for the NSP supplemented group, but the additional NSP did not increase stool weight or bacterial mass significantly compared with results from the placebo group. More patients in the NSP supplemented group complained of abdominal symptoms than the placebo group. The hypothesis that an increased NSP intake would increase stool weight, bacterial mass and reduce the incidence of gastrointestinal symptoms can therefore not be supported. Due to the large quantity of preferentially fermentable substrate already present in the colon, the additional NSP was surplus to requirements of the bacterial mass and was excreted rather than fermented. The length of the feeding period may however have been inadequate to observe an increased utilisation of NSP with time, but large stool losses seen for one patient who received a NSP supplemented feed over two years might indicate that long term use of the feed could considerably compromise energy availability.

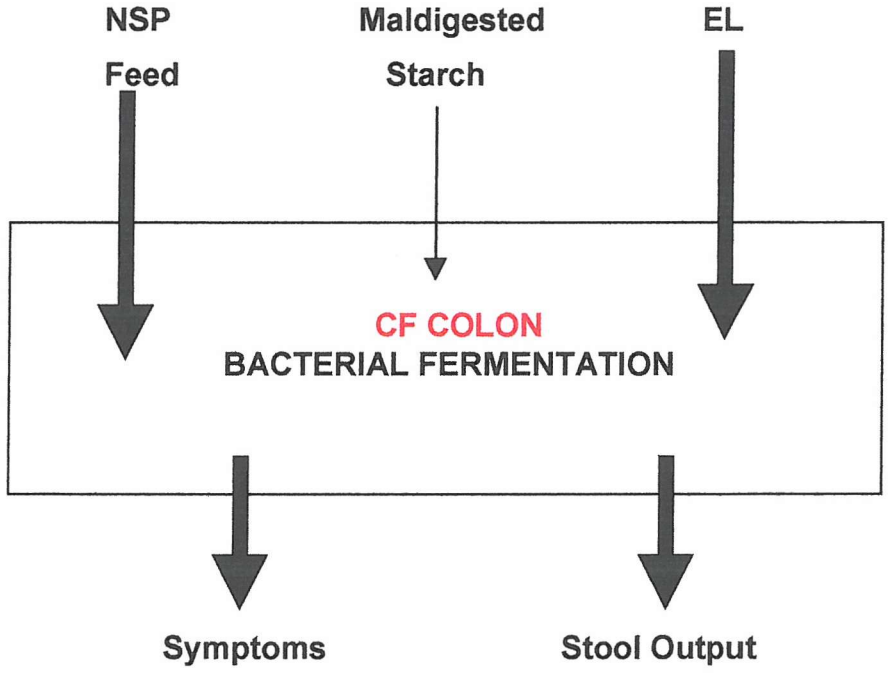
The hypothetical pathway for metabolism of a NSP supplemented feed in the CF colon has therefore been modified (Figure 4.2). Figure A illustrates the original hypothesis that a NSP supplemented feed would increase stool output and prevent the occurrence of abdominal symptoms. Figure B illustrates the conclusion from Phase 2 that as preferentially fermented substrates e.g. hexose rich CF mucus maintained stool output, additional NSP was incapable of increasing stool indices further. Consequently it was also incapable of reducing the incidence of gastrointestinal symptoms.

FIGURE 4.2 PATHWAY OF METABOLISM OF A NSP SUPPLEMENTED FEED IN CF PATIENTS

A) HYPOTHETICAL PATHWAY



B) MODIFIED PATHWAY



EL = Endogenous losses

4.4 FUTURE STUDIES

Due to the large inter-individual variability between CF patients demonstrated in this study, future studies into the role of NSP in the CF colon should recruit a larger number of patients in an attempt to obtain a more accurate measure of the typical response. For example, rather than select the enterally fed group of patients, an alternative approach could be to specifically recruit those CF patients with gastrointestinal symptoms using a more specific bowel habit questionnaire and medical assessment of colonic function. Once recruited, NSP intake, stool weight and bacterial mass can be measured using the methods employed in the present study. For those patients where a causal relationship is demonstrated between a low NSP intake and low bacterial mass, NSP could be added to the diet for at least a month, but a longer period of time would allow for adaptation of colonic bacterial enzymes. For those patients who do not demonstrate a causal relationship between NSP intake and gastrointestinal symptoms i.e. a low NSP intake but a large bacterial mass, then alternative causes of symptoms could be explored such as uncontrolled maldigestion / malabsorption or the frequent use of antibiotics.

As bulk laxatives are currently used to treat abdominal pain related to faecal loading in the CF colon in the clinical situation, a future study recruiting similar patients to those described above, could also determine the effect of laxatives on colonic bacterial metabolism using the methods described. The mechanism of effect of laxatives in the CF colon is assumed to be the same as that in the healthy colon as the clinical results are similar but the effect on stool composition in CF has not yet been identified.

4.5 SUMMARY

A summary of the principle findings is as follows:

Firstly, long term enterally fed CF patients consumed an oral NSP intake half that of healthy subjects. Despite this, CF patients had a stool output twice the size, therefore there were additional fermentable substrates reaching the colon in the form of maldigested / malabsorbed nutrients and endogenous losses to maintain colonic function. Half of the CF group had no gastrointestinal symptoms despite consuming a low NSP intake. A low NSP intake was also not strongly associated with any of the stool variables and therefore cannot be the cause of abdominal symptoms of the enterally fed CF group.

Secondly, the addition of NSP for four weeks did not increase stool output significantly or reduce the incidence of gastrointestinal symptoms of the enterally fed CF patients due to the greater role of maldigested / malabsorbed nutrients and endogenous losses on colonic function and stool output.

4.6 CONCLUSION

Gavin *et al* (1997) proposed that a low NSP intake was a causal factor in the pathogenesis of abdominal symptoms in CF patients. The objective of this study was to investigate the role of NSP in preventing symptoms in an enterally fed CF group by providing additional NSP to their NSP-free feeds. The primary outcome variables included stool weight, bacterial mass, lipid and energy losses and frequency of abdominal symptoms.

In comparison with the healthy group, the enterally fed CF group complained more frequently of abdominal symptoms but the symptoms were not due to a low NSP intake or stool weight as stool output was adequately maintained by maldigested / malabsorbed nutrients and endogenous losses. Additional NSP was spared from fermentation and excreted in the stool and therefore did not increase stool weight significantly or reduce the incidence of symptoms.

In light of these observations, it would appear that the reported relationship between a low NSP intake and symptoms (Gavin *et al* 1997) was not directly linked to colonic bacterial metabolism in CF. The proposal that NSP supplemented feeds have a role in the dietetic management of abdominal symptoms in the enterally fed group can therefore not be supported until further studies are undertaken.

APPENDIX 1

BOWEL HABIT DIARY

Name:_____

Date of starting the diary:_____

Please mark the occurrence of symptoms and bowel movements in each day by circling your answer. Please look at the *example* below.

Date:	EXAMPLE DAY
Upper stomach ache	(None) Mild Moderate Severe
Lower stomach ache	None Mild (Moderate) Severe
'Wind' or bloating	(None) Mild Moderate Severe
Nausea (feeling sick)	(None) Mild Moderate Severe
Vomiting	(None) Mild Moderate Severe
Heartburn	None (Mild) Moderate Severe
Pain when passing stools	None Mild Moderate (Severe)
Number of stools passed per day	0 1 2 (3) 4 5 6 7 8
Consistency of stools	Normal Hard (Loose) Diarrhoea
Colour of stools	Brown Pale (Black) Red (blood in stool)

Date:	DAY 5
Upper stomach ache	None Mild Moderate Severe
Lower stomach ache	None Mild Moderate Severe
'Wind' or bloating	None Mild Moderate Severe
Nausea (feeling sick)	None Mild Moderate Severe
Vomiting	None Mild Moderate Severe
Heartburn	None Mild Moderate Severe
Pain when passing stools	None Mild Moderate Severe
Number of stools passed per day	0 1 2 3 4 5 6 7 8
Consistency of stools	Normal Hard Loose Diarrhoea
Colour of stools	Brown Pale Black Red (blood in stool)

BOWEL HABIT

ASSESSMENT

Your name_____

Date of
Birth_____

Contact Telephone
Number_____

1 = none 2 = mild/moderate

This is an assessment about your CURRENT bowel habits. 3 = severe

Please answer the questions by ticking (✓) only one tick per question unless otherwise stated.
Please answer as thoroughly as you can.

1. How often do you have tummy ache?

- ☐ 3 Every day
- ☐ 2 Almost every day
- ☐ 2 1-2 times per month
- ☐ 1 Very seldom
- ☐ 1-2 times per week
- ☐ Never (if never, move to question 4)

2. What part of your tummy aches?

- ☐ Upper part
- ☐ Middle part
- ☐ Lower part

3. When you have tummy ache, how long does it last?

- ☐ 0-15 min
- ☐ 15-30 min
- ☐ 30-60 min } 2
- ☐ 2 1-2 hours
- ☐ 3 3-4 hours
- Other .....

4. How often do you have a bowel motion?

- ☐ Less than once a week 3
- ☐ Once a week 2
- ☐ 2-3 times a week 2
- ☐ 8 or more times a day 3
- ☐ 1 Once a day
- ☐ 2 2-4 times per day
- ☐ 3 5-7 times a day

5. What size is your usual bowel motion?

- ☐ Only a little bit = the size of several pebbles 2
- ☐ A moderate amount = the size of 1-2 sausages 1
- ☐ A lot = the size of 3-4 sausages 2

6. Does the bowel motion ever vary in quantity?

- ☐ No
- ☐ Yes. Please explain (e.g. depending on)

.....

.....

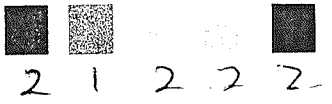
7. Do you pass a bowel motion after every meal?

- ☐ No 1
- ☐ Yes. How long after a meal? 3
  - ☐ 0-15 min
  - ☐ 15-30 min
  - ☐ 30-60 min
  - ☐ 2 1-2 hours
  - ☐ 2 3-4 hours

8. What does your bowel motion usually look like? (You can tick more than one category if needed).

- ☐ 3 Separate hard lumps, like nuts
- ☐ 2 Sausage shaped but lumpy
- ☐ 1 Like a sausage or snake but with cracks on its surface
- ☐ 1 Like a sausage or snake, smooth and soft
- ☐ 2 Soft blobs with clear cut edges
- ☐ 2 Fluffy pieces with ragged edges, a mushy stool
- ☐ 3 Very watery stool / diarrhoea
- ☐ 3 Greasy diarrhoea

9. Which of the colours below does your bowel motion usually look like? Please circle the most appropriate colour



2 1 2 2 2

10. Do you feel pain when passing a bowel motion?

- 1 ☐ Never
- 2 ☐ Sometimes
- ☐ Usually 2
- ☐ Always 3

If you feel pain, please describe it more. Where, when and what kind of pain do you feel?

_____

_____

11. Does your bowel motion smell more offensive if enzymes are not taken with a meal?

☐ No (1) ☐ Much worse (3)

12. Is your bottom itchy or sore after passing a bowel motion?

☐ No (1) ☐ Yes (2)

13. Have you recently passed blood in your bowel motion (e.g. seen as red or black stool)

1 ☐ No ☐ Yes
☐ Don't know 2

14. How often do you have problems with "wind"?

☐ 3 Every day 2 ☐ 1-2 times a month
☐ 2 2-3 times a week 1 ☐ Very seldom
☐ 1 Never

15. How often do you vomit?

☐ 3 Every day ☐ 2 1-2 times a month
☐ 2 2-3 times per week ☐ 1 Very seldom
☐ 1 Never

16. How often do you have heartburn?

☐ 3 Every day ☐ 2 1-2 times a month
☐ 2 2-3 times per week ☐ 1 Very seldom
☐ 1 Never

Please mark by ticking (✓) foods that cause you symptoms. If the food causes symptoms, mark what kind of symptoms they cause. You can tick more than one symptom, if needed. At the end of the table you can add other foods that cause you symptoms, but are not mentioned in the table.

Food	I do not eat	Does not cause symptoms	Causes symptoms	Tummy ache	Bloating and wind	Nausea	Abnormal stool (diarrhoea, bulky stool)	Other symptoms, what
DAIRY								
milk, ice cream								
butter, margarine								
cream								
cheese								
FRUIT/VEG								
onions								
cabbage								
oranges, orange juice								
bananas								
apples								
sweetcorn								
FRIED FOOD								
roast dinners								
fried chips,								
fried breakfast								
meat, bacon, sausages								
CEREAL PRODUCTS								
pasta/rice								
bread								
breakfast cereals								
CONFECTIONERY								
chocolate								
nuts								
cakes/puddings								

APPENDIX 2

CF CONSENSUS EQUATION

The following formula is used to calculate the Daily Energy Requirement (DER).

1. Calculate the basal metabolic rate (BMR) using the following World Health Organisation Equations for predicting BMR (in Calories) from body weight (in kg).

Age range	Females	Males
0 - 3y	61.0wt - 51	60.9wt - 54
3 - 10y	22.5wt + 499	22.7wt + 495
10 - 18y	12.2wt + 746	17.5wt + 651
18 - 30y	14.7wt + 496	15.3wt + 679
30 - 60y	8.7wt + 829	11.6wt + 879

2. Calculate daily energy expenditure (DEE) by multiplying the BMR by activity coefficient plus disease coefficient:

Activity Coefficients (AC)

Confined to bed	(BMR x 1.3)
Sedentary	(BMR x 1.5)
Active	(BMR x 1.7)

Disease Coefficients (DC)

Normal lung function (FEV_1>80% predicted)	[BMRx(AC+0)]
Moderate long disease ($FEV_1$140-70% predicted)	[BMRx(AC+0.2)]
Severe lung disease (FEV_1<40% predicted)	[BMRx(AC+0.3*)]

*May range up to 0.5 with very severe lung disease.

3. Calculate DERs from DEE, taking into account the present of steatorrhoea.

   For pancreatic sufficient patients (including patients on enzymes with a coefficient of fat absorption > 93% of intake): DER = DEE.

   For pancreatic insufficient patients the coefficient of fat absorption (CFA) must be determined as a fraction of fat intake: DER + DEE (0.93/CFA). If a stool fat collection is not available to determine the fraction of fat intake, an approximate value of 0.85 may be used in the calculation.

Reference

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APPENDIX 3

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Faculty of Medicine
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Information sheets

Dear Patient

We would like *you* to take part in a study to look at whether we can improve bowel function in cystic fibrosis. Feeds given via a tube are low in fibre. As fibre is needed to keep the bowel working a low fibre tube feed may worsen normal bowel function. This sometimes results in pain or constipation, which you can regard as part of having cystic fibrosis. We would like to see if we can improve bowel function by changing your tube feed for a total of 5 weeks. You will be selected to receive either a feed with fibre or a feed without fibre. Neither you nor your doctor or nurse will know which feed contains fibre and which does not. The feeds will have the same calories as those you are already taking and therefore have no side effects. We will invite you to have metabolic rate measured prior to the study to calculate the exact number of calories you need daily.

We will also look at how fats in your diet are handled in the body. This will happen by using fat which is marked (non-radioactive label, ^{13}C) and is ingested by mouth as a chocolate flavoured drink. By measuring the marker in stool, in breath (carbon dioxide) and in blood (if you are 18 years or older) we can see how much of the fat is digested, absorbed and stored. As you may know, in cystic fibrosis fat losses in stool are common despite enzymes. This test will help us to understand why fat is poorly absorbed in cystic fibrosis.

The plan of the study is explained overleaf.

If you need antibiotics at any stage, the study will be discontinued and can be restarted a minimum of a month later.

If you do not wish to take part in this study this is not a problem and will not influence your treatment in any way.

If you need any more information please do not hesitate to contact us.

Thank you for taking the time to read this.

Yours sincerely

Dr. Steve Wootton
Senior lecturer in human nutrition
Tel: 01703-796317

Joan Gavin
Paediatric dietitian
Tel: 01703-796072

Kirsi Laiho
PhD student
Tel: 01703-796317

Professor A A Jackson
Director
Professor D J P Barker
Associate Director

University of Southampton

Description of procedures

Resting metabolic rate

Resting metabolic rate will be measured by indirect calorimetry, where you will lie down on a bed and a clear, ventilated hood is placed over your head. The measurement will take approximately 1 hour. We will ask you not to exercise and not to eat or drink anything except water on the morning of the test or on the previous evening from 10pm.

Test drink

A chocolate flavoured test drink contains the ^{13}C marked fat.

Breath samples

In this test you will be asked to blow into a small bag.

Blood samples

A cannula will be inserted into forearm and 5 ml blood samples will be collected before test drink and then hourly for 6 hours.

Body composition measurement

Your body composition will be measured using bioelectrical impedance. This measurement involves positioning of four electrodes, to hand, wrist, foot and ankle, and passing a small electric current between the electrodes and from this we can measure how much fat and muscle there is in your body.

Food diary

Over a 5 day period you will be asked to weigh everything you eat or drink, except water, using scales and the diary that we provide for you. It is helpful if you can give as much detailed information as possible e.g. whole fat milk, semi-skimmed milk or skimmed milk. The purpose of the food diary is to record your habitual food and nutrient intake, therefore we ask you not to change your normal eating habits during the study.

Stool collection

You are asked to collect all stools over a 3 day period into containers provided. When collecting stools, please do not urinate into the sample bag, do not include toilet paper in the sample bag and make sure that all samples are labelled with:

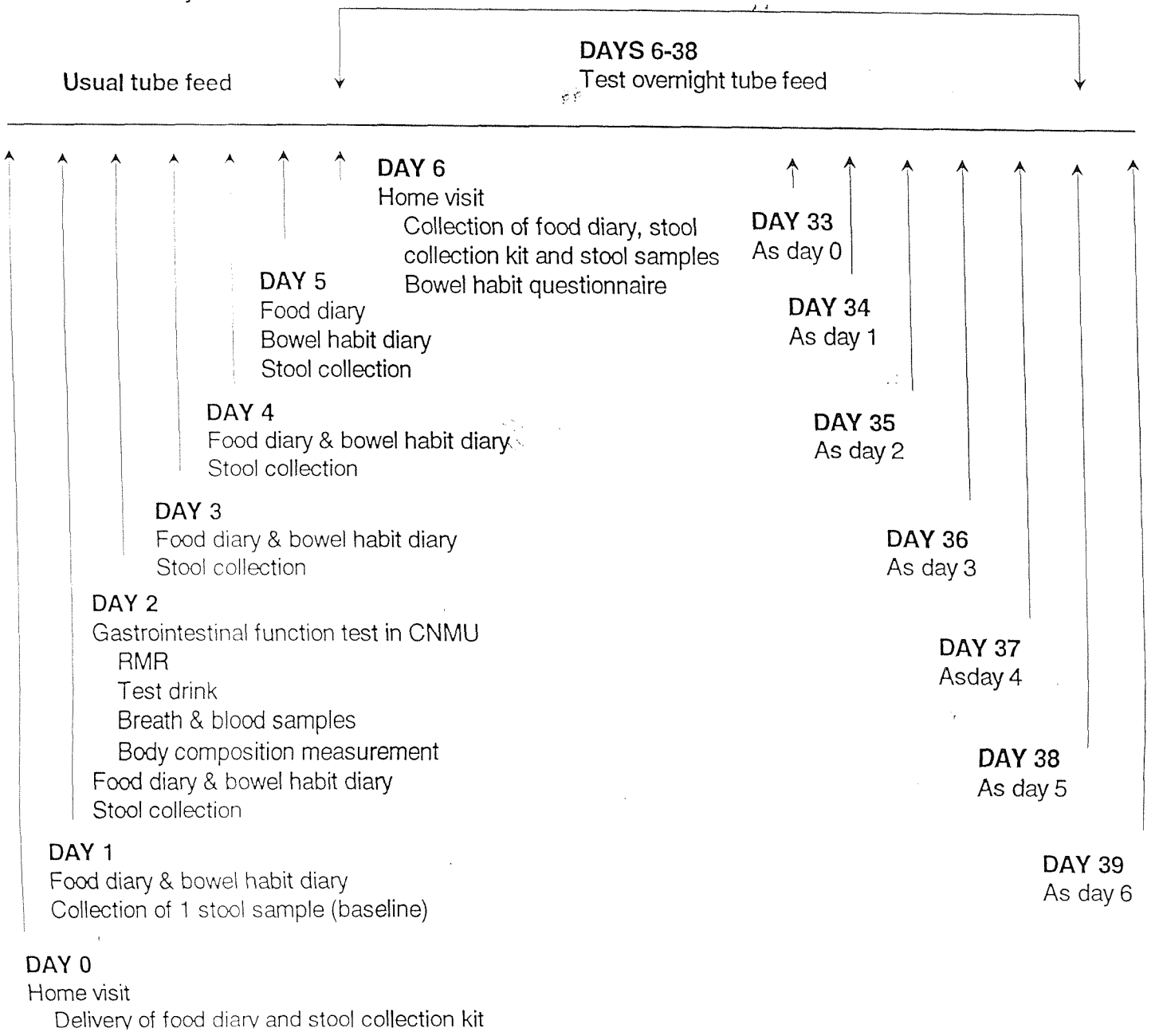
your name
the date
the time

Avoiding foods naturally containing ^{13}C label

Three days before ingestion of the test drink and during stool collection please avoid the following foods:

Cornflakes, Frosties, Crunchy nut cornflakes or any other breakfast cereal containing corn, sweetcorn, corn on the cob, popcorn, tortilla chips and corn based crisps such as Wotsits and Monster Munch, custard powder and ready made custard, cane sugar (beet sugar is allowed), pineapple, mango or any tropical fruits.
Please use beet sugar provided
If you are unsure about these foods or any other foods please ask us first

Protocol of the study



APPENDIX 4

INDIVIDUAL CHARACTERISTICS OF CF GROUP

Patient	Decimal age	Weight (kg)	Height (cm)	TST (mm)	MAC (mm)	MAMC (mm)	FFM (kg)
1	7.0	20.5	119.2	12.8	175	134.8	15.6
2	8.3	25.7	126.9	9.0	178	149.7	22.2
3	30.9	43.5	150.5	13.9	208	164.3	30.8
4	26.2	58.1	169.0	23.5	258	184.2	42.0
5	14.1	47.1	154.4	15.1	225	177.6	36.0
6	10.3	33.0	135.0	21.3	225	158.1	23.1
7	16.3	43.5	153.5	14.4	220	174.8	32.3
8	15.1	48.0	152.6	21.2	233	166.4	33.0
9	11.5	31.2	124.8	12.8	220	179.8	22.2
10	10.0	24.0	126.2	10.3	190	157.6	19.4
11	7.0	21.4	116.0	13.3	170	128.2	17.4
12	14.1	37.4	151.5	8.0	209	183.9	29.3

INDIVIDUAL CHARACTERISTICS OF THE HEALTHY GROUP

Patient	Decimal age	Weight (kg)	Height (cm)	TST (mm)	MAC (mm)	MAMC (mm)	FFM (kg)
1	9.5	29.8	135.6	10.4	206	173.3	24.4
2	11.1	43.2	151.0	11.7	229	192.2	36.2
3	11.5	40.2	150.8	18.7	231	172.3	30.4
4	13.4	51.2	151.7	24.6	197	219.7	34.0
5	15.7	68.4	171.0	9.3	288	258.8	57.3
6	16.5	70.0	173.4	19.8	298	235.8	49.1
7	7.8	30.7	133.4	10.3	191	158.6	23.6
8	14.3	51.5	159.0	24.6	245	217.0	44.1

KEY CODE

TST = Triceps Skinfold Thickness (mm)
MAC = Mid Arm Circumference (mm)
MAMC = Mid Arm Muscle Circumference (mm)
FFM = Fat Free Mass (kg)

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