

UNIVERSITY OF SOUTHAMPTON

**INFLUENCE OF DIET AND DISEASE ON HUMAN INTESTINAL
MICROFLORA, COLONIC FUNCTION AND FAECAL ENERGY**

**A THESIS SUBMITTED FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY**

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UNIVERSITY OF SOUTHAMPTON
ABSTRACT
FACULTY OF MEDICINE
DEPARTMENT OF HUMAN NUTRITION
Doctor of Philosophy
INFLUENCE OF DIET AND DISEASE ON HUMAN INTESTINAL
MICROFLORA, COLONIC FUNCTION AND FAECAL ENERGY.
By Jane Louise Murphy.

The availability of energy from the diet is determined by the extent of digestion and absorption and the metabolic activity of the colonic microflora. Attempts to measure the energy available to the body through digestion and absorption presume that all of faecal material is derived solely from dietary residue. Two other components may also contribute to the material present in faeces; endogenous secretions and cellular debris and colonic bacterial microflora.

The studies presented in this thesis have demonstrated the normal limits of faecal energy in healthy adults and during growth and development in childhood. The magnitude of faecal energy could be modified with alterations in dietary non-starch polysaccharides (NSP) intakes and with maldigestion and/or malabsorption in the disease cystic fibrosis (CF). It has been possible to isolate faecal bacteria and demonstrate that a major component of the energy, lipid and nitrogen within the stool may be derived from the colonic bacteria.

The metabolic activity of the colonic microflora could be modified with alterations in dietary NSP intakes and the availability of maldigested and/or malabsorbed dietary residue and endogenous losses in CF. Colonic fermentation may determine the extent to which energy may be salvaged and made available to the host in the form of short chain fatty acids.

These findings demonstrate the importance of determining the origins of energy within the stool and the impact of colonic bacterial function in relation to the maintenance of energy balance with diets rich in fermentable substrate and in extensive maldigestion and/or malabsorption.

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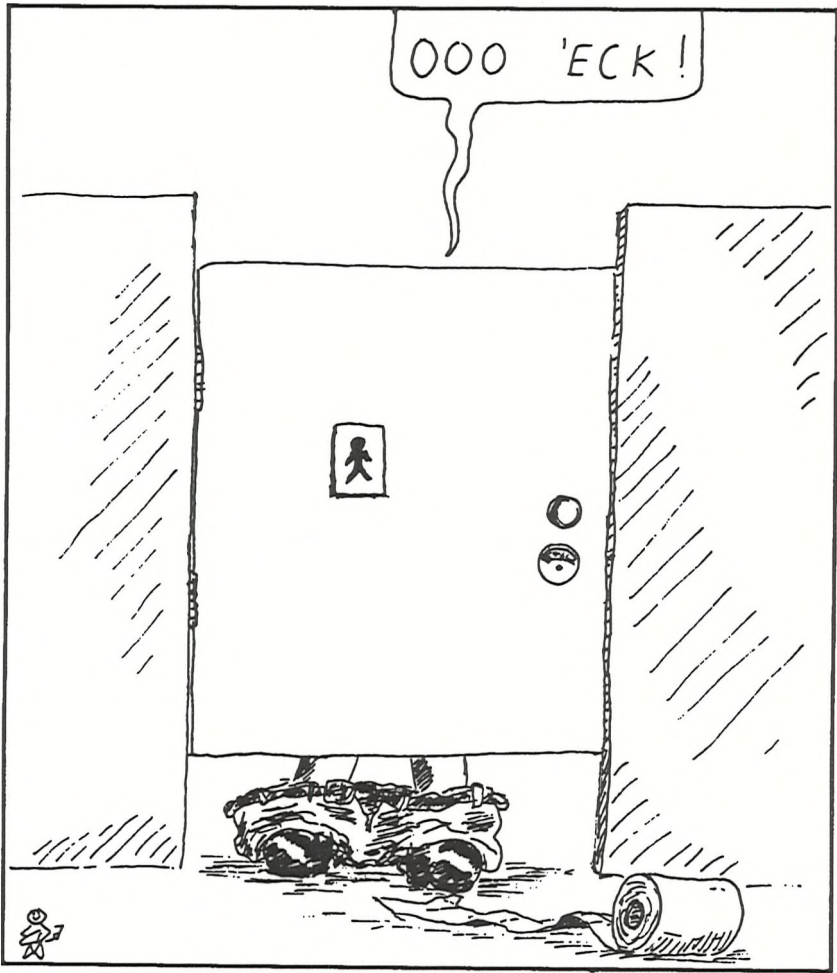
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CARICATURE.



PUBLICATIONS.

Unless otherwise indicated by acknowledgements or references to published literature, the work contained in this thesis is the work of the author. The findings presented in this thesis have been reported, in part, in the following publications.

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MAIN ABBREVIATIONS USED IN THE TEXT

CF	Cystic Fibrosis
DE	Digestible energy
FE	Faecal energy
E coli	<i>Escherichia coli</i>
GEI	Gross energy intake
LPM	Large plant material
MCT	Medium chain triglycerides
MEI	Metabolisable energy intake
NSP	Non-starch polysaccharides
SCFA	Short chain fatty acids
SPM	Small plant material

CHAPTER 1. INTRODUCTION.

The inter-relationship between the availability of energy from food in growth and in the maintenance of health is an important fundamental concept in human nutrition. This information is essential to provide an understanding of the extent to which the food within our diet meets the body's requirement for energy under different conditions. Firstly, in normal individuals and during periods of ill health, and secondly during growth and development in childhood. We also need to know body energy requirements during the therapeutic management of pathological states. The 'availability of energy' is a term that is widely used to describe the amount of energy present in food as lipid, protein and carbohydrate which can be utilised by the body in normal metabolism. The energy potentially available to the body depends upon the nature and characteristics of food and the ability of the body to utilise this energy. This is thought to be determined by the extent of digestion and absorption and through the oxidation by a number of complex metabolic processes.

Attention has focused upon the upper gastro-intestinal tract because this represents the portion of the human gut where the processes of digestion and absorption take place. The proportion of energy from food which could be digested and absorbed under normal conditions has been given the term 'digestibility'. Attempts to measure digestibility have expressed this as the difference between dietary energy intake and the energy content of faeces. This approach assumes however that all of the energy within faeces is derived directly from the diet which is a presumption that is incorrect.

Faeces represent the final product of gastro-intestinal activity which is dependent upon the extent of digestion and absorption and the activity of the extensive microflora which have the capacity to ferment carbohydrate from dietary residue and endogenous losses. It is known that apart from non-digested dietary residue other components make up faecal energy including endogenous losses in the form of digestive secretions, mucus and sloughed cells in addition to bacteria from the large intestine. Although this information was developed from studies at the turn of the century it would seem that there has been little attention directed towards the origins of faecal energy in more recent years. As it has not been possible to separate each of the components of faecal energy, the term 'apparent digestibility' was introduced to make an allowance for this discrepancy. It is recognised in the report of the WHO/FAO/UNU [1985] on energy and

protein requirements that the available energy of food, determined as intake minus losses is not the same as true digestibility. It would seem however, that more recent energy balance studies which have examined the digestibility of dietary substrates have ignored the potential contribution made by endogenously derived sources of faecal energy or the interchangeable balance of specific nutrients across the gut.

It is clear that without knowledge of both the magnitude and origins of energy within the faeces, it would seem impossible to have a clear understanding of the extent to which energy is made available or how factors such as disease may alter this. Consequently our understanding of the extent to which the availability of energy could be limited under different circumstances is unclear. This information is of fundamental importance in the consideration of energy requirements associated with growth and if we are to understand the complex mechanisms associated with pathological conditions and provide appropriate therapeutic management.

The influence of changes in diet on the availability of energy from lipid, protein and carbohydrate remains unresolved. In particular the amount of non-starch polysaccharides (NSP), usually referred to as 'dietary fibre' is thought to reduce the availability of energy from lipid and protein within food. Whilst several hypotheses have attempted to explain this effect of dietary NSP on energy availability, attention has focused upon an effect of dietary NSP on small intestinal function. The mode of action of dietary NSP at the level of the large intestine indicates that human colonic function could have an important influence on the amount of energy available to the body. Whilst the relevance of the colonic metabolism has been largely ignored in man, it would seem the role of colonic function in relation to body energy metabolism and the availability of energy from the diet could have important implications both in health and disease.

The aim of the studies reported in this thesis was to determine the magnitude and composition of faecal energy in health and during growth in childhood and how this may alter with changes in diet or under pathological states associated with maldigestion and/or malabsorption. This information would develop our understanding of the impact of human colonic function in relation to the amount of energy available to the body.

The studies reported in this thesis are presented in four experimental chapters. Before examining the magnitude and composition of faecal energy, it was necessary to examine the variability in measurements of daily faecal energy content from stool collections performed over a period of time. The three day stool collection has been the most common approach to assess daily faecal energy. Attention was centred upon the reliability in measuring representative daily faecal energy each day from three day stool collections as it was the intention to employ this procedure in further studies.

The second experimental chapter examined the magnitude and composition of faecal energy in a group of normal healthy children. This information would also provide a basis for comparison against diseases associated with maldigestion and/or malabsorption where there could be imbalances in the availability of energy from the diet.

This possibility was examined in chapter 6 within the disease cystic fibrosis. Cystic fibrosis is a condition where there may be pancreatic exocrine insufficiency which leads to the maldigestion of lipid, protein and carbohydrate resulting in increased faecal losses. Uncorrected this may severely limit the amount of energy available from the diet and in turn will contribute to an energy deficit sufficient to limit growth. It is not clear whether this may occur through maldigestion alone or whether this could be accompanied with malabsorption. Maldigestion occurs through insufficient enzymic activity to allow the hydrolysis of lipid, protein and carbohydrate. Thereby dietary substrate is presented in a form which cannot be absorbed by the body. Alternatively or in addition to maldigestion, digested residue could be malabsorbed through a limitation or failure of the absorptive process itself.

The extent to which the magnitude and composition of faecal energy could be altered with changes in the dietary NSP content of the diet in normal healthy adults is reported in chapter 7. The principle aim of this study was to re-evaluate this effect of dietary NSP on the reduction in apparent digestibility of energy in relation to the impact of human colonic function.

CHAPTER 2. REVIEW OF THE LITERATURE.

2.1 INTRODUCTION.

The energy yielded from individual foods or mixed diets could be determined with knowledge of the complete chemical energy content of foods and its metabolic products within the body. As this information is not available, 'metabolisable energy' values are assigned to foods and mixed diets by a variety of systems. This introduces a number of assumptions concerning the composition of foods and their metabolic products as well as the extent to which food is digested and absorbed. Moreover these energy values are used to represent average values relating to healthy individuals consuming 'normal' diets thereby presuming that the metabolisable energy in different diets which may be complex, is uniform [USDA, 1983]. At present there are a number of different methods which have been developed in an attempt to calculate the metabolisable energy content of foods. In essence these methods have centred upon the work of Atwater around the turn of the century [Atwater & Bryant, 1900]. In basic terms, Atwater determined the total energy yield referred to as 'heats of combustion' of lipid, protein and carbohydrate of various food products. These values were corrected by what was called a 'coefficient of availability' from measurements of the energy content of faeces and urine. Whilst it is agreed that there is the opportunity to introduce errors through the methodology used and the assumptions made in deriving such factors [Atwater & Bryant, 1900], in particular it was assumed that all of the energy within faeces and urine is dietary in origin. This approach makes no allowance for the potential role of colonic function or the contribution made by endogenous losses and faecal bacteria towards the energy content of stools.

In this review, attention is initially centred upon what is currently understood to represent the digestibility and available energy of food to provide a background for the experimental studies presented in this thesis. In relation to the concept of availability of energy, consideration is then given towards the magnitude and composition of faecal energy and how this may be influenced by changes in diet, under pathological conditions and as a result of therapeutic management.

2.2 AVAILABILITY OF ENERGY.

The energy value of food and the ability of the body to utilize food energy has been expressed in different ways. Since the time of the classical studies of Atwater, the meanings of some of the terms necessary in any discussion of food energy values have changed which has led to some confusion. The historical background and the current uses of such terms have been the subject of a number of reviews [Merrill & Watt, 1955; Widdowson, 1955]. The following sections provides a summary of some of the most important terms and attempts to distinguish between past and present meanings. To assist the reader, figure 2.1 accompanies the following sections to illustrate the relationship between some of these expressions.

2.2.1 GROSS ENERGY.

Gross energy content of food or heat of combustion is the amount of energy contained in the energy-yielding nutrients of food (carbohydrate, lipid, protein and in alcohol) [FAO/WHO/UNU, 1985]. This is the energy released when a particular food is completely combusted in a bomb calorimeter [Miller & Payne, 1959]. The gross energy content of foods within the diet can be calculated if the chemical composition of food is known by applying the gross energy value of carbohydrate (4.15 kcal; 17.5 kJ/g), lipid (9.4 kcal; 39.3 kJ/g), protein (5.65 kcal; 23.6 kJ/g) and alcohol (7.1 kcal; 29.7 kJ/g) to the amounts of these nutrients in food [Merrill & Watt, 1955]. Whilst there are marked variations between the heats of combustion actually determined and those calculated, Atwater and Bryant [1900] showed that this difference could be 5-6% of the former value. These values indicated are average heats of combustion values derived from the heats of combustion of a large number of food materials and can be used to calculate the gross energy intake for a mixed diet. It should be clearly recognised however that there are differences for the heat of combustion values for proteins, lipids and carbohydrates found in different foods. For example the heat of combustion value for vegetable protein is 5.0 kcal/g; 20.9 kJ/g, fruit protein 5.20 kcal/g; 21.8 kJ/g, cereal protein 5.80 kcal/g; 24.3 kJ/g [Atwater & Bryant, 1900].

2.2.2 DIGESTIBLE ENERGY.

As it has not been possible to distinguish between metabolic and endogenous products in the faeces and undigested residue from food, the digestibility of energy in practice is referred to as 'apparent digestibility' [Widdowson, 1955]. This is measured by subtracting the energy content of faeces from the amount of energy measured from food intake ($DE = GE - FE$). It should be noted however that Atwater preferred to use the term 'availability' to mean apparent digestibility which has thereby introduced some confusion to this area.

2.2.3 METABOLISABLE ENERGY.

The expressions 'metabolisable energy' (ME) and 'available energy' are often used synonymously to represent the energy that is available to the body. In simple terms the metabolisable energy value of food is an expression of the amount of energy available for metabolism and is measured as the difference between the energy in food and energy both in faeces and urine ($ME = GE - FE - UE$). The measured energy values reported for foods within the diet are referred to as metabolisable energy [USDA, 1983]. Theoretically it is not the same as the actual food-derived energy available to the body. For example it does not allow for the possible recovery of energy through colonic short chain fatty acid absorption, or the contribution made by products of metabolism towards the energy content of urine such as the energy used to convert ammonia to urea [Blaxter, 1971] nor the contribution made by endogenous losses or bacteria to the energy content of faeces. However this distinction is not commonly recognised.

2.2.4 CALCULATION OF THE METABOLISABLE ENERGY OF HUMAN MIXED DIETS.

Whilst it is not possible to calculate the exact energy yield for individual foods and mixed diets, metabolisable energy values are given to foods by various systems. Food tables published in Britain (Paul & Southgate, 1978) and in the United States (USDA, 1976-1988) both multiply the protein, lipid and carbohydrate and alcohol content of foods by energy conversion factors to determine a food's metabolisable energy value. However the energy conversion factors used in the two systems are slightly different.

The factors developed by Atwater & Bryant [1900], so called 'Atwater factors' are currently used to determine metabolisable energy value of foods in the United States. Merrill & Watt [1955] provide a review of how these factors for calculating the energy value of foods were derived by Atwater, and they have subsequently modified these factors to be used as general factors to calculate the energy value of mixed diets. On the basis of their work they stated that if these factors are used, the deviation between the true and calculated metabolisable energy was no greater than 5% of the true value for most diets. The energy conversion factors derived for carbohydrate, lipid, protein and alcohol of a mixed diet currently used are: 4 kcal;17 kJ/g, 9 kcal;37 kJ/g and 4 kcal;17 KJ/g, 7 kcal;29 kJ/g respectively.

In the UK a slightly different approach has been used to calculate the metabolisable energy content of the diet [Paul & Southgate, 1978]. The major difference is the way in which the metabolisable energy of carbohydrate is calculated. Whilst the US method determines the carbohydrate in foods by difference, available carbohydrate is determined analytically for the UK food tables, that is the sum of free sugars (glucose, fructose, sucrose, lactose, maltose and higher maltose analogues), dextrans, starch and glycogen expressed as monosaccharide which are believed to be completely digested and absorbed in the gut. This analytically determined carbohydrate content of a food is multiplied by an energy conversion factor of 3.75 kcal;16 kJ/g to determine the energy available [Paul & Southgate, 1978]. In contrast to the Atwater system, this method assumes that there is no contribution from dietary NSP to the metabolisable energy content of a diet.

2.2.5 ENERGY VALUE OF DIETARY NON-STARCH POLYSACCHARIDES.

Apart from the concern over the extent to which energy conversion factors may give an accurate indication of the metabolisable energy of the diet at the present time, there are conflicting opinions over the extent to which energy may be made available from diets with increased amounts of NSP. In addition the use of appropriate factors which may be applied to calculate the metabolisable energy content of the diet remains unclear. The concept that dietary NSP may contribute to human energy metabolism is based on the knowledge that carbohydrate of NSP are fermented by bacteria in the large intestine and that the short chain fatty acids produced are absorbed within the colon [Cummings & Englyst, 1987].

It has been indicated that both the approaches applied in the United States and Britain to calculate the energy content of foods show bias if the diet contains increased amounts of unavailable complex carbohydrate, as NSP and resistant starches [FAO/WHO/UNU, 1985]. Consequently a number of systems to calculate the metabolisable energy of the diet have included the NSP content of the diet [Southgate, 1975; Miller & Judd, 1984]. Atwater factors overestimate the metabolisable energy of the diet by applying a factor of 17 kJ/g for complex carbohydrate and the approach of Paul & Southgate [1978] excludes dietary NSP and thereby underestimates the metabolisable energy content of the diet [Livesey, 1990]. By estimating the amount of substrate available for fermentation, it is thought that dietary NSP could contribute approximately 13 kJ/g (3 kcal/g) to human metabolism [Cummings, 1981a]. This is supported by the calculations of Goranzon & Forsum [1987] where 2.5 kcal/g (10 kJ/g) could be derived from cereals and 3.1 kcal/g (13 kJ/g) from beans, vegetables and fruits. Whilst it is not known how much energy may be made available from short chain fatty acid absorption or the factors governing their metabolic fate, it is assumed from these calculations that up to 70-75% of the gross energy of dietary NSP may be available for metabolism [Cummings, 1981a; Livesey, 1990]. With this information, it has been proposed that a metabolisable energy value of (8.4 kJ/g; 2 kcal/g) could be applied to unavailable complex carbohydrates [British Nutrition Foundation, 1990]. Thus it would seem that this could constitute a significant proportion of the total energy intake for individuals on high NSP intakes. However the use of such a factor introduces a number of assumptions. For example, it assumes that only unavailable complex carbohydrate are used as a substrate for fermentation by colonic bacteria, that there are no changes in urinary energy losses, and that all of the energy within faeces is dietary in origin. Therefore it would seem that there is the potential of introducing significant errors and its limitation in its application to mixed diets eaten at the present time would require further evaluation.

Whilst it would seem that the NSP content of the diet should not be overlooked in the calculation of metabolisable energy of mixed diets, further exploration of the possible errors and factors which may influence the empirical relationship between gross and metabolisable energy is required.

2.2.6 SUMMARY.

Despite various modifications to the energy values assigned to protein, lipid and carbohydrate, it appears that the present approach to calculate the metabolisable energy content of human mixed diets remains based upon the classical studies of Atwater at the turn of the century. However, it is important that the limitations and errors involved by such an approach is recognised and that they are based upon a number of assumptions. In addition the extent to which dietary NSP may influence the metabolisable energy of a diet and its implications both in health and disease or where there may be alterations in diet require further evaluation.

2.3 ORIGINS OF FAECAL ENERGY.

The amount of energy within the faeces each day in healthy adults is generally believed to be equivalent to approximately 3-5% of the gross energy intake [Southgate & Durnin, 1970]. As faecal energy appears to be relatively small with respect to intake and the collection and chemical analysis of faecal material is both time consuming and unpleasant, the potential contribution that faecal energy may make in limiting the availability of energy from the diet is often overlooked in energy balance studies. From the literature, it appears that the amount of energy within faeces could range between 500-700 kJ/d in normal healthy adults [Southgate & Durnin, 1970; Miles, Brooks, Barnes, Marcus, Prather, Bodwell, 1984; Stephen, Wiggins, Englyst, Cole, Waymen, Cummings, 1986]. Early studies by Rubner at the turn of the century measured the heat value of each gram of dry human faeces in healthy adults on different diets [Rubner, 1902]. The energy value of each gram of human faeces remained relatively constant at approximately 6.2 kcal/g (25.9 kJ/g) of dry faeces and that this changes only significantly when there is 'poor utilisation' of food.

The energy within faeces is derived from lipid, protein and carbohydrate. The composition of faeces is largely dependent upon the characteristics and amount of food taken within the diet. Faecal energy may also include a component of endogenous origin such as endogenous secretions and cellular debris and colonic bacteria apart from dietary residue. The potential contribution made by each of these components to lipid, protein and carbohydrate within faeces is not clear.

Attention in the literature has been previously directed towards the measurement of nitrogen and lipid within faeces. The following sections present an overview of our current knowledge of the lipid and nitrogen contents of stools in normal healthy individuals. In addition any evidence to suggest that there are endogenous components which may contribute to faecal energy is considered.

2.3.1 FAECAL LIPID.

Normal healthy adults are reported to excrete less than 5 or 6g of lipid within the faeces each day on a normal dietary intake [Wollaeger, Comfort, Osterberg, 1947; Southgate & Durnin, 1970; Wrong, Edmonds, Chadwick, 1981]. More than 90% of the total lipid in faeces consist of saturated and unsaturated fatty acids of different chain lengths, particularly C16 and C18 fatty acids. The remaining 10% of faecal lipids are sterols comprising mainly of a mixture of cholesterol and its metabolites (neutral sterols) and bile acids and its metabolites (acidic sterols) [Wrong *et al.* 1981].

Previous studies have shown that neither the quantity of dietary lipid nor the type of lipid produce significant changes in total faecal lipid [Annaegers, Boutwell, Ivy, 1948] and particularly for diets containing up to 150 g/d lipid [Cooke, Thomas, Mangall, Cross, 1953]. However Wollaeger and colleagues [1947] found considerable variation in faecal lipid output from 2-7 g/d in normal individuals when dietary lipid intake varied from 100-200 g/d. From these studies it was not clear whether differences in the type of dietary lipid or if total energy intake could explain these findings. When faecal lipid is expressed as a percentage of dietary lipid intake, it appears that the percentage lipid absorbed remains relatively constant [Walker, Kelleher, Davies, Smith, 1973]. Wollaegers and coworkers [1947] demonstrated that faecal lipid could be equivalent to 4% of dietary lipid intake. However as stated by Cooke & Holmes [1984], this expression did not take into account of the possibility that faecal lipid may have an endogenous component.

2.3.2 ENDOGENOUS ORIGIN FOR FAECAL LIPID.

Whilst the presence of lipid in faeces has been known since the late 19th century, the source of this lipid has not been completely resolved. Lipids derived from unabsorbed dietary lipid, bacteria, and from endogenous sources such as bile and the breakdown of desquamated cells have all been suggested as a major

source of faecal lipid by various workers [Sperry, 1929; Blomstrand, 1955; Wiggins, Howell, Kellock, Stadler, 1969].

The work of Hill & Bloor [1922] in dogs and cats has shown conclusively that the lipid in faeces is very different to the lipid in the diet. Faecal lipid appears to contain a higher proportion of saturated fatty acids than the diet [Holmes & Kerr, 1923]. The major fatty acids in faeces are thought to be 10-hydroxy stearic acid together with 6-, 7-, 8-, 9-hydroxy stearic acid [James, Webb, Kellock, 1961]. This difference led many workers to believe that little of the lipid in faeces could originate from the diet [Sperry & Bloor, 1924]. In man the presence of endogenous faecal lipid following the consumption of lipid free diets has been confirmed by more recent investigations. Lewis & Partin [1954] showed that approximately 1-2g/d lipid remains in the faeces even if there is no lipid in the diet. Other recent evidence that faecal lipid maybe endogenous in origin has also been provided from studies with labelled lipids. Bergstrom & Blomstrand [1956] have shown that only 50% of faecal lipid was dietary in origin in a study of one normal subject and it could be estimated that 1g of non-dietary lipid may be excreted per day in the faeces.

The differences in saturation between dietary and faecal lipids has been explained by either a differential absorption of different fatty acids in the small intestine, or a significant contribution from endogenous fatty acids to faecal lipids and bacterial alterations of maldigested and/or malabsorbed dietary lipid by bacterial action in the large intestine [Wiggins *et al.* 1969]. If fatty acid absorption is determined by factors such as chain length and the degree of saturation as suggested by Fernandes and coworkers [1962], the composition of dietary lipid would be different to faecal lipid. Studies of the fatty acid composition of ileostomy dejecta have shown that the fatty acids match the fatty acid pattern of dietary lipid [Wiggins *et al.* 1969]. This would indicate that differences in the faecal and dietary fatty acid composition in normal subjects can not be explained by events occurring in the small intestine.

If the amount of lipid in ileostomy fluid is similar to that in faeces but the composition is different, it is suggested that qualitative changes in the lipid composition may occur in the large intestine [Wrong *et al.* 1981]. This may be due to the absorption of dietary lipid and the addition of endogenous lipid in an equivalent amount or as the result of bacterial transformations of lipid within the lumen of the gut. However it has been suggested that major alterations in the

composition of both endogenous and dietary lipid occur through the saturation of unsaturated fatty acids by the bacteria of the large intestine without any metabolism of the basic carbon skeleton [Wiggins *et al.* 1969].

The contribution made by each of the components of faecal lipid is less understood. No studies have measured the contribution made by endogenous lipid. Endogenous lipid in the intestinal lumen is thought to originate from the desquamation of intestinal epithelial cells as lipid has been found in the mucosa from biopsies from defunctioned colons [Binder & Van Noorden, 1969]. Analysis of chloroform/ methanol extracts of mucus derived from the alimentary tract show that lipids may constitute 40% of intestinal mucus in rats [Slomiany, Galicki, Kojima, Slomiany, 1980]. This suggests that the lipid within faeces can also be derived from this source. However part of the lipid within faeces may also be contributed by the large intestinal bacterial microflora. There has been only one study using a fractionation procedure to isolate faecal bacteria which has demonstrated that a substantial part (between 27-55%) of faecal lipid may be accounted by the bacteria in dogs [Sperry, 1929]. Fatty acids between the 12-20 carbon chain length account for the majority of bacterial fatty acids [Gunstone, Harwood, Padley, 1986]. Bacteria may also contain odd-chain and branched fatty acids as well as 3-hydroxy and cyclopropane derivatives. The total lipids present in *Escherichia coli* are believed to represent 10-15% of total cell carbon [Luria, 1960]. Lipids are located not only within the cell wall but also in the cytoplasmic membrane which is a lipoprotein layer. In *Bacilli*, lipids are stored as granules of poly- β hydroxybutyrate which may constitute 10-25% of the dry weight. Other studies have measured the total amount of lipid within bacteria from the large intestine from the ruminant [Merry & McAllen, 1983; Bauchart, Legay-Charmier, Doreau, Gaillard, 1990]. From these studies, the amount of lipid ranged between 0.1-0.3g per gram of bacteria.

2.3.3 FAECAL NITROGEN.

Daily faecal nitrogen values in normal individuals have been reported to average between 1-2 g/d [Wollaeger *et al.* 1947; Cooke *et al.* 1953] equivalent to 10% of intake [Cooke & Holmes, 1984]. Almost all faecal nitrogen is thought to be derived from endogenous sources [Cooke & Holmes, 1984] which could explain the observation where faecal nitrogen was unaffected by variation in dietary protein content [Reifenstein, Albright, Wells, 1945]. Although in a study of ileostomy patients, a decrease in mean daily losses of ileostomy nitrogen was

observed with reduced dietary intakes of protein [Gibson, Sladen, Dawson, 1976]. This would suggest that dietary intake may determine ileostomy nitrogen losses but in normal healthy individuals other factors determine the nitrogen content of faeces.

2.3.4 ENDOGENOUS ORIGIN FOR FAECAL NITROGEN.

Whilst there has been little recent attention directed towards the origin of nitrogen within faeces, early studies in animals have shown that faecal nitrogen is never reduced to zero. Values ranging between 0.1-0.8 g/d have been reported under such conditions [Mendel & Fine, 1912; Lusk, 1906]. This would suggest that a substantial part of the nitrogen within faeces must be derived from a source other than diet. Studies using ^{15}N labelled protein and protein hydrolysate indicate that the majority of faecal protein is derived from endogenous sources such as intestinal enzymes, urea and possibly even plasma proteins which have leaked from the systemic circulation through the gut wall [Crane & Neuberger, 1960]. The amount of each of these components which may contribute towards faecal nitrogen remains unclear. Whilst it is not known how much nitrogen from endogenous losses may enter the large intestine, the amount of protein represented by intestinal cells has been estimated. It has been calculated that the protein which may pass into the lumen from the intestinal mucosa could be 75-90g [Nasset, 1965], and by measuring DNA loss, da Costa and coworkers [1971] calculated that 12% of this protein could be attributed to intestinal cells.

In addition the colonic bacterial microflora may also account for part of the nitrogen within faeces. It is believed that nitrogen may represent 6-7% of bacterial cells from mixed populations of bacteria [Smith & McAllen, 1974] and in human faecal bacteria [Stephen & Cummings, 1980c]. Although the nitrogen content of bacteria is thought to vary not only due to experimental procedure but also because of variations in the environment and growth conditions [Stephen, 1980].

Studies of the contribution made by bacteria to nitrogen within the faeces is limited. As early as 1883, Rubner presented data to suggest that between 7-11% of faecal nitrogen can be accounted by bacteria. More recently, using a fractionation procedure to isolate the bacteria within faeces, it has been shown that approximately 60% of the total faecal nitrogen could be accounted by bacteria [Stephen & Cummings, 1980c]. This would suggest that the bacteria within

faeces may represent a major proportion of total faecal nitrogen under normal circumstances.

2.3.5 FAECAL CARBOHYDRATE.

Owing to the digestion and absorption of free sugars under normal circumstances, there are only trace amounts of free sugars which are thought to originate from the diet in normal human faeces [Gryboski, Zillis, Ma, 1964]. However this does not mean that most of the carbohydrate is absorbed in the small intestine as carbohydrate provides substrate for fermentation by the intestinal microflora. This is discussed in more detail in section 2.4. Faecal carbohydrate has been previously determined by an indirect approach from total faecal energy content using bomb calorimetry and then subtracting the energy derived from total protein (assuming that most of faecal nitrogen is protein) and lipid [Heymsfield, Smith, Kasriel, Barlow, Lynn, Nixon, Lawson, 1981; Kien, Cordano, Cook, Young, 1982]. Another method by Ameen & Powell [1985] attempted to determine the faecal hexose carbohydrate by spectrophotometry present as monosaccharide, oligosaccharide and polysaccharide but not of pentoses. They have shown that in children aged 1 month to 14 years faecal carbohydrate excretion ranged from 0.04-0.85 g/d. Alternatively total carbohydrate within faeces has been determined by gas liquid chromatography which measures the amount of individual sugars and cellulose [Stephen *et al.* 1986].

At present it can only be speculated whether there is any carbohydrate in the faeces which may be endogenous in origin. It is known that endogenous supplies of carbohydrate from mucus as glycoproteins and sloughed epithelial cells may be fermented by colonic bacteria to supply their energy requirements for growth in the colon [Hoskins & Boulding, 1982]. This may suggest that little, if any endogenous carbohydrate may be lost within faeces [Allen, 1983]. In addition the amount of faecal carbohydrate attributed to bacteria has not been examined. It is known that carbohydrates are found in bacterial cells not only in nucleic acids but also as oligosaccharides and in a variety of polysaccharides [Luria, 1960]. Whilst some polysaccharides are present as reserve material often as discrete granules of starch or glycogen, other polysaccharides are present in the cell wall. Whilst it is clear that the colonic bacteria may contribute to faecal mass [Stephen & Cummings, 1980a], it could be that bacteria account for part of the carbohydrate within faeces.

It is commonly believed that a major component of human faeces is undigested plant material or NSP. Nevertheless only 3-5 g/d of dietary NSP remains in the faeces in normal individuals [Southgate & Durnin, 1970]. Total carbohydrate excretion in healthy subjects has been reported to be approximately 7 g/d of which the majority was composed of NSP residue as pentoses, arabinose and xylose [Stephen, Wiggins, Cummings, 1987]. It was also suggested that some of the remaining sugars particularly ribose and glucose could be bacterial in origin.

2.3.6 SUMMARY.

There would appear to be little information from the present literature indicating what proportion of the energy content of stool could be attributed to endogenous components and colonic bacteria in addition to dietary residue. Whilst it has not been possible to measure each of the components of faecal energy, the amount of bacteria within faeces can be determined by a technique described by Stephen & Cummings [1980a]. This has also enabled the characterisation of the proportion of faecal nitrogen which could be attributed to bacteria under normal circumstances [Stephen & Cummings, 1980c].

2.4 DIETARY NON-STARCH POLYSACCHARIDES.

The effect of dietary NSP on bowel function has long been recognised with bacterial fermentation of carbohydrate being first described in animals in the 1890s. The digestion of insoluble NSP in the gastro-intestinal tract from a wide variety of plant sources in man was first recognised around 50 years ago in adults [Williams & Olmstead, 1936] and in children [Hummel, Shepherd, Macy, 1943]. Over the last two decades there have been a number of studies which have investigated how dietary NSP may influence physiological processes in the human large intestine and its functional significance is becoming more apparent.

In other parts of this thesis particularly in relation to the energy value of foods (section 2.1.5), a number of terms have been introduced which may be related to the 'unavailable' carbohydrate content of the diet. The following section initially considers some of the terminology used to describe different fractions of complex carbohydrates. Secondly consideration is given towards some of the current evidence with reference to the properties of dietary NSP, its function and metabolic responses in the human large intestine.

2.4.1 WHAT ARE DIETARY NON-STARCH POLYSACCHARIDES?

Recently there has been much debate over the definition of dietary 'fibre'. The term 'dietary fibre' was probably first used by Hipsley in 1953 with regard to a protective effect against toxæmia of pregnancy. However its more common use is thought to originate from a paper by Trowell [1972]. He proposed that 'fibre is the polysaccharide, lignin material that provides the skeleton structure of the plant cell wall and interstices which resist digestion by the enzymes of the human gut'. However it is apparent that the term 'fibre' has been frequently used incorrectly and assumed to represent crude fibre or cellulose. This confusion has stimulated other workers to suggest other terms. Dreher [1987] lists over twenty terms applied to 'dietary fibre' including roughage, non nutritive fibre and plant fibre. Spiller & Fasset-Cornelius [1976] have suggested the use of the term 'plantix'. Trowell & coworkers [1976] subsequently extended his original definition proposing that 'dietary fibre is the sum of lignin and the polysaccharides that are not digested by the endogenous secretions of the human digestive tract'. This fraction of the diet was thought to be equivalent to 'unavailable carbohydrates' a term possibly first introduced by McCance & Lawrence [1929].

Cummings [1981b] criticised the definition proposed by Trowell [1972] on the basis that this definition of 'fibre' should not include any assumptions over its physiological role in man. This is because both the precise effects of the breakdown of 'fibre' in the human digestive tract is unclear and different types of 'fibre' can be degraded to different extents with considerable variation among individuals. Chemical analysis of fibre shows that the main component is polysaccharide but as there are other polysaccharides in the diet such as starch, the use of the term 'fibre' would be inappropriate. A different approach [Englyst, Wiggins, Cummings, 1982] measures dietary 'fibre' as a chemically defined fraction which was given the term non-starch polysaccharides (NSP) by Englyst and colleagues [1987]. Non-starch polysaccharides are principally the polysaccharides of the plant cell wall and the major identifiable constituent of dietary 'fibre' including all non- α glucan polymers in the diet such as cellulose, pectin, hemicellulose, gums and gels [Cummings & Englyst, 1987] (Figure 2.2). As the human intestinal enzymes are unable to hydrolyse these polymers, they pass into the large intestine where they may be fermented by the colonic bacteria. On further analysis, NSP can be divided into soluble and insoluble fractions to distinguish between cellulose and non-cellulosic polysaccharides (NCP) [Englyst, Trowell, Southgate, Cummings, 1987]. Non-cellulosic polysaccharides

include polysaccharides such as hemicellulose, pectin, storage polysaccharides (inulin and guar) and gums and mucilages. Lignin is not a carbohydrate and is not included as part of NSP. Chemically, lignin represents a group of polyphenolic compounds of different molecular weight and is a minor contributor to the diet with the exception of wholegrain cereals.

A recent report by the British Nutrition Foundation [1990] has given a detailed review of complex carbohydrates in foods. In their opinion, 'complex carbohydrates' which may be synonymous with polysaccharides can be divided into 'available complex carbohydrates', which are virtually only starches, and 'unavailable complex carbohydrates' which are NSP and resistant starches.

The principles for the measurement of NSP and dietary 'fibre' have been the subject of considerable debate. Whilst the gravimetric procedure to determine dietary 'fibre' includes all NSP, as well as lignin and resistant starch [Prosky, Asp, Furda, De Vries, Schweizer, Harland, 1984], the Englyst enzymatic method using gas liquid chromatography measures only NSP and excludes lignin and resistant starch [Englyst *et al.* 1982].

In the light of this information, the term non-starch polysaccharides (NSP) will be used in this thesis previously referred to as dietary 'fibre,' to describe a group of substances generally of plant origin which are not digested by human intestinal enzymes.

2.4.2 PROPERTIES OF DIETARY NON-STARCH POLYSACCHARIDES.

Various properties of NSP are important in relation to the mode of action of NSP in the human digestive tract [Cummings, 1982]. The composition and properties of plant NSP vary greatly depending on the source, species of plant and physiological stage of growth. The majority of complex carbohydrates in the human diet are derived from foods of plant origin as storage polysaccharides for example starch, galactomannans, cell wall polysaccharides for example cellulose, hemicellulose, and isolated polysaccharides in foods such as gums and mucilages and pectin. A range of polysaccharides are present in animal tissues which contribute a small amount of complex carbohydrate to the diet [British Nutrition Foundation, 1990]. The quantities of dietary NSP consumed in man vary greatly. In the British diet, normal healthy adults consume between 20-25 g/d [OPCS, 1990] (measured as dietary 'fibre'). The average daily intake

of dietary 'fibre' measured as NSP by the Englyst technique in normal healthy adults was approximately 12g [Bingham, Pett, Day, 1990] . Whereas in rural sub-African areas, estimates of dietary NSP intakes can be 5-8 times higher [Bingham, Cummings, McNeil, 1979]. The physical properties of NSP which appear to be of most nutritional significance are considered in the following sections including the influence of dietary NSP on stool weight and human intestinal transit.

2.4.3 THE INFLUENCE OF DIETARY NON-STARCH POLYSACCHARIDES ON STOOL WEIGHT.

There have been many studies in man in which dietary NSP from a wide variety of sources have resulted in an increase in faecal weight. This has been the subject of an extensive review by Cummings [1986]. In contrast studies where the diets of normal subjects were completely depleted in NSP using chemically defined liquid diets, a reduction in faecal volume and decreased frequency of stool excretion were observed when compared to free choice diets [Winitz, Adams, Seedman, Davis, Jayko, Hamilton, 1970]. The effect of dietary NSP on stool weight is thought to be attributed to the ability of NSP to hold water [Eastwood, 1973; Kelsay, Behall, Prather, 1978]. Cereal NSP is commonly assumed to be the best example of NSP through its water holding capacity. The greater the water holding capacity, the greater the effect on stool weight [Eastwood, 1973; Cummings, Hill, Jenkins, Pearson, Wiggins, 1976]. Alternatively, Wyman and coworkers [1978] have suggested that through cereal manufacturer's processing, the properties of wheat bran are altered since no change in wet stool weight was observed when bran was added to a low NSP diet. Moreover in another study, whilst faecal excretion more than doubled when NSP was added to the diet, the amount of water within faeces did not change [Fuchs, Dorfman, Flock, 1976].

There have been relatively few studies comparing the *in vivo* and *in vitro* water holding effects of different types of NSP. Stephen & Cummings [1979] studied the effects of a range of NSP containing foodstuffs on stool weight and measured their *in vitro* water holding capacity. Their data suggested that materials that hold more water *in vitro* such as gums and pectin are likely to be the least effective in increasing faecal bulk. This was in contrast to the effect of cereal NSP bran or bagasse. A more recent study has also confirmed the findings of this study where the water holding capacity of bran was found to be lower than that of

pectin [Forsum, Eriksson, Goranzon, Sohlstrom, 1990]. Despite the conflicting evidence, it is apparent that the water holding capacity of NSP may be one but not the only mechanism by which NSP can increase stool output.

Particle size preparations of NSP have a more effective influence on increased stool output [Brodrigg & Groves, 1978]. The water holding capacity of coarser bran has been shown to be greater than that of fine bran. However the digestibilities of these two preparations also differ significantly. Large particles are more slowly degraded and are more likely to survive passage through the gut. They may well exert a physical effect on colonic function by providing a greater bulk and surface area for more efficient metabolism [Cummings, 1986]. Thus it would seem that both the water holding capacity and particle size of NSP can not account for the changes observed in faecal weight.

Non-starch polysaccharides, in particular the so-called 'soluble' NSP such as pectin, gum, carrot cabbage that promote bacterial metabolism have an indirect effect on faecal weight. The fermentability of these fibres can result in increased bacterial proliferation with the possibility of increased bacterial mass in faeces and an increase in faecal weight [Stephen & Cummings, 1980b]. In addition it has also been reported that there could be a possible decrease in faecal weight over a period of between 4-12 weeks [Spiller & Fassett-Cornelius, 1980]. There is evidence to suggest that there is a bacterial effect from studies in rats where a stabilisation of faecal weight and bacteria were observed after consuming a high NSP diet for 4 weeks [Walter, Eastwood, Brydon, Elton, 1986]. In the study by Mason and coworkers [1990] the reduction in faecal weight over a 12 week period in normal subjects with raised intakes of NSP was thought to be attributable to an adaptation in the activity of the colonic bacteria with prolonged feeding of NSP.

Given that increased stool weights have been found to be greater with increased intakes of dietary NSP, one study has failed to show this effect when baked beans were added to the diets of South Indian subjects living in the tropics [Kurpad, Holmes, Shetty, 1988]. It is known that individuals from this region have large faecal outputs and shorter transit times. Further increases in dietary NSP may well have had little effect on bacteria due an adaptive response to the availability of increased amounts of NSP habitually present in the colon. Alternatively, Cummings [1988] has suggested that the small number of subjects and

the possibility that the subjects could have changed their diets to accommodate the extra NSP may also explain this observation.

There have been other mechanisms suggested which may explain this apparent bulking effect of different kinds of NSP. Cummings [1978] has suggested that the chemical composition of NSP and possibly the pentose fraction of non-cellulosic polysaccharide accounts for the increase in faecal weight. This association is however unexplained and further evidence to support this is limited. Finally, a novel idea has been proposed in the recent study by Forsum and colleagues [1990]. They suggest that soluble compounds derived from dietary NSP which are solubilized in the water phase of intestinal contents are less likely to be fermented by the colonic bacteria. Consequently such molecules may form gels and contribute towards faecal bulk or they may contribute towards the osmotic properties of the fluid in the large intestine and cause water retention in the large intestine.

2.4.4 THE INFLUENCE OF DIETARY NON-STARCH POLYSACCHARIDES ON HUMAN INTESTINAL TRANSIT.

Transit time is the time taken for a substance to pass through the gastro-intestinal tract. On average it takes 24-72 hours for most materials to travel from the mouth-to-anus. Of this time, 18-64 hours is spent in the large intestine which would suggest that transit is mainly a colonic event [Bond & Levitt, 1976]. From epidemiological studies, slow transit time has been associated with the high prevalence of large bowel disorders such as colon cancer and diverticular disease [Burkitt, Walker, Painter, 1972]. As a consequence it is believed that populations with fast transit times which have a low prevalence of colonic disorders are thought to have an increased dietary NSP intake.

Dietary NSP in particular cereal NSP has received a lot of attention with regard to its effect on transit. Many studies have demonstrated that a raised intake of NSP can increase transit times [Kirwan, Smith, McConnell, Mitchell, Eastwood, 1974]. It would appear that NSP is the most important dietary component altering transit times. However there is evidence emerging for an independent role of transit in determining colonic function rather than the result of a dietary or environmental factor. Evidence to support this is provided by a study whereby the variability in transit time and stool output could not be attributed simply to

dietary intake in subjects taking identical diets [Cummings, Southgate, Branch, Houston, Jenkins, James, 1978].

The weight of bacteria within faeces has been found to vary with transit time for a group of individuals on identical diets [Stephen, 1980]. When transit time was altered pharmacologically by using drugs which effect colonic motility, daily output of bacteria increased with more rapid transit, and vice versa despite a constant dietary intake [Stephen & Cummings, 1980d; Stephen, Wiggins, Cummings, 1987]. With a faster turnover rate, the growth of bacteria is believed to be more efficient resulting in increased bacterial mass as the requirement for energy for the maintenance of bacterial cells is reduced and more of the available energy supply can be used for bacterial cell growth. Therefore it is thought that any component of the diet which may alter transit time will affect colonic bacteria through a change in the growth of bacteria independent of any other nutrient effects [Stephen, 1985].

2.4.5 SUMMARY.

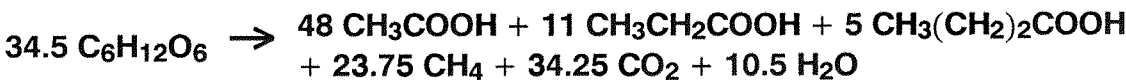
In summary, it would appear that the effects of NSP on colonic function can be altered by not only the quantity of NSP but also the physical and chemical state of the NSP in question. Moreover it should be recognised that a number of other factors independent of diet could also influence colonic function including bowel habit, exercise, stress, hormones, small-intestinal function and other intraluminal events such as salt and water absorption, and bacterial metabolism [Cummings, 1979].

2.5 COLONIC BACTERIAL FERMENTATION.

Whilst dietary NSP may have an effect on practically all areas of the gastro-intestinal tract, its main site of action is at the level of the colon. This would suggest that its action must involve an effect on colonic function and/or on the metabolic activity of the colonic microflora. Whilst the fermentation or anaerobic breakdown of carbohydrate has been extensively studied in animals and particularly in the rumen [Wolin, 1981], its functional significance in man is less well understood.

Fermentation is defined as an anaerobic process whereby bacteria break down dietary and other carbohydrate substrate to obtain energy for growth and maintenance of cellular function [Cummings & Englyst, 1987]. Whilst the condi-

tions within the large intestine and the presence of an extensive microbial flora are believed to be very similar to those in the rumen, fermentation in the rumen is understood well enough to derive a generalised equation for fermentation in the human colon [Miller & Wolin, 1979]:



Whilst the products of fermentation may vary between species the three main end products of anaerobic carbohydrate breakdown are short-chain fatty acids (SCFA: acetic, propionic and butyric acids), gases (hydrogen, carbon dioxide and methane) and energy. A variety of potentially fermentable carbohydrates may enter the large intestine. Whilst attention has been particularly focused upon dietary NSP, other components may provide the intestinal bacteria with carbohydrate to supply their energy requirements for growth [McNeil, 1988]. These substrates include the undigested dietary residues such as starches and sugars [Flourie, Florent, Jouany, Thivend, Etanchaud, Rambaud, 1986; Bond & Levitt, 1976], endogenous secretions and cellular debris (intestinal glycoproteins and mucopolysaccharides)[Hoskins & Boulding, 1982].

Whilst it is not known how much carbohydrate may be delivered to the colon, the relative proportions of each of these substrates however will vary with the dietary intake, the extent of maldigestion and malabsorption in disease such as in cystic fibrosis and the contribution made by endogenous components. It is believed that there are marked differences in the activity of the colonic bacteria in different parts of the colon [Cummings & MacFarlane, 1991]. As figure 2.3 illustrates, bacterial activity is greatest in the right colon where substrate availability is thought to be increased. The following section presents a brief overview of some of the substrates for colonic bacterial fermentation.

a. Non-starch polysaccharides.

The plant cell wall polysaccharides can be divided analytically into two classes: cellulose and non-cellulosic polysaccharides [NCP]. However it is clear that the extent of NSP breakdown is variable.

i. Cellulose.

There have been a number of studies reporting the digestion of cellulose over the last 50 years. It would appear that about 50% of cellulose is degraded in almost every case [Williams & Olmsted, 1936; Southgate & Durnin, 1970; Prynne & Southgate, 1979]. Different sources of cellulose are fermented to different degrees. Prynne and Southgate [1979] have shown that the cellulose present in fruit and vegetables is approximately 75% digested. In contrast, cellulose breakdown is reduced when present in wheat bran or if fed in a purified form [Heller, Hackler, Rivers, Van Soest, Roe, Lewis, Robertson, 1980]. Studies of digestion in the rumen have shown that cellulose is dependent on its crystallinity and on the degree of association with substances like lignin, cutin and silica [Van Soest, 1975]. As cellulose is an insoluble material, its digestion can only occur when bacterial enzymes can gain access to its surface. In contrast to the cellulose present in fruit and vegetable cell walls, pure cellulose is highly crystalline and less susceptible to enzymic break down. More recently, some studies measuring the extent of NSP breakdown in patients with ileostomy have shown that NSP from wheat, oats, maize, banana and potatoes resist digestion almost completely [Englyst & Cummings, 1985; 1986; 1987], with complete recovery of NSP from wheat bran and pectin [Sandberg, Andersson, Hallgren, Hasselblad, Isaksson, 1981]. From these studies using the ileostomist as a model, all the NSP in the human diet reaches the large intestine [Cohen, Wolever, Thompson, Jenkins, 1983; Englyst & Cummings, 1985]. However the suitability of the ileostomy model to study starch absorption and fermentation in man should be questioned on the grounds that the gut of these patients is not normal. One important difference is the increased number of bacteria in the terminal ileum compared to the normal ileum [Drasar & Hill, 1974].

ii. Non-cellulose polysaccharides (NCP).

Non-cellulose polysaccharides represent the major component of the polysaccharide in the plant cell wall [Cummings, 1984]. Non-cellulosic polysaccharides are a more diverse group of compounds both physically and chemically than cellulose. The water soluble NCP may be amongst the most digestible components of NSP. Some water soluble polysaccharides such as pectin are completely fermented in the human gut (about 80% on average) [Cummings, Southgate, Branch, Wiggins, Houston, Jenkins, Jivraj, Hill, 1979], although no

generalised response to the ingestion of a NSP supplement could be demonstrated in all individuals [Prynne & Southgate, 1979].

iii. Lignin.

It is believed that lignin is not digested to any significant extent in the human gut [Cummings, 1981b]. However lignin itself may influence the fermentation of the carbohydrate components of plant cell walls. There is evidence to suggest that wheat bran-NSP are digested much less than the NSP in the less lignified cell walls such as cabbage, carrot [Williams & Olmstead, 1936; Hoppert & Clark, 1945]. The mechanism by which lignin may inhibit enzyme hydrolysis of NSP is unknown. Prevention of access to the carbohydrates by bacteria is thought to be unlikely although lignin may have specific inhibitory or 'toxic' properties that inhibit bacterial action [Cummings, 1981b].

b. **Unabsorbed carbohydrate; sugars and starches.**

The passage of unabsorbed carbohydrate into the large intestine has been studied by various techniques. It appears that most of the attention in the literature has been directed towards investigations in ileostomy patients which are assumed to represent effects under normal circumstances. Intubation studies allow the study of the passage of carbohydrates into the large intestine in healthy subjects.

Oligosaccharides are almost completely lost in passage from the ileum to the anus [McNeil, 1988]. In the large intestine of both the rat and man, Bond and Levitt [1976] demonstrated that the metabolism of ^{14}C -glucose and ^{14}C -sucrose resulted in the production of breath and faecal $^{14}\text{CO}_2$. One sixth of the radioactivity was recovered in the faeces with the remainder metabolised and fermented by colonic bacteria to short chain fatty acids. Similar observations have been demonstrated in patients with jejuno-ileal bypasses [Bond, Currier, Buchwald, Levitt, 1980]. Lactulose, a synthetic disaccharide which is resistant to human digestive enzymes and other poorly absorbed oligosaccharides such as stachyose and raffinose may pass unchanged into the colon [Fleming & Floch, 1986].

It has also long been thought that starch is completely digested and absorbed in the gut. However as the result of a number of studies in animals and in man, human colonic bacteria appear to be capable of fermenting some forms of starch from *in vitro* incubations, resulting in an increase in the bacterial cell mass and production of SCFA [Englyst & MacFarlane, 1986]. Starch does not have a constant composition but is a mixture of varying proportions of amylose and amylopectin in microscopic grains of various sizes and shapes. Therefore it is thought that its susceptibility to salivary and pancreatic amylases and hence completeness of digestion varies with its source [Wrong *et al.* 1981]. McNeil and coworkers [1982] reported in ileostomy patients that 1-2 gms of starch were excreted per day following the consumption of their normal dietary intake. After specially prepared meals losses of starch varied from 1-3% in white bread [Englyst & Cummings, 1985], 4% from cornflakes [Englyst & Cummings, 1985] and up to 90% of that in bananas [Englyst & Cummings, 1986]. The extent and manner of cooking is also important as Englyst & Cummings [1987] found that starch malabsorption of potato can range between 3-12%. As a result of these studies of starch digestion, a nutritional classification has been proposed by these workers on the basis of a physical form and susceptibility of starch to pancreatic amylase. Starch may be classified into readily digestible starch (RDS), partially resistant starch (PRS) and resistant starch. Starch malabsorption has been also measured by a breath hydrogen technique where 10-20% of the carbohydrate escaped digestion [Anderson, Levine, Levitt, 1981] and up to 20% of starch from cooked potatoes [Thornton, Dryden, Kellehar, Losowsky, 1986]. Tomlin & Read [1990] did not support the view that increased intakes of resistant starch influence colonic function in health as no difference in stool weight, frequency or transit time were observed when diets were supplemented different amounts of resistant starch. From breath hydrogen tests they suggest that resistant starch is completely fermented to end-products including hydrogen gas which is excreted via the lungs. In contrast, an attempt to obtain more direct estimates of starch malabsorption under normal circumstances has been reported from intubation studies. Stephen & coworkers [1983] have shown that following the consumption of either a diet containing 20g of starch from banana and rice or 60g from banana, rice and potato in healthy subjects, 9.3% of the starch from the 20g starch meal and 6.0% from the 60g starch meal could be recovered. From this information it was suggested that this may represent a substantial source of substrate for the colonic microflora and could provide much of the carbohydrate required to support the known bacterial content of faeces. Flourie & coworkers [1988] detected much less malabsorption using

similar techniques to Stephen when diets containing 100g or 300g starch from white bread, potato and noodles were fed to healthy subjects where recoveries in the terminal ileum were 4.1% and 3.2% for the low and high starch diets. The difference between the two studies is thought to be attributed to differences in the nature of the starches studied [Cummings & Englyst, 1991].

c. Endogenous carbohydrate.

Whilst it appears that endogenous carbohydrate can be fermented by bacteria in the large intestine, the quantity and nature of endogenous material is unclear. The most likely source of endogenous carbohydrate for bacterial fermentation is the glycoprotein component of mucus apart from other sources such as desquamated cells. Glycoproteins are basically a peptide core with side groups of oligosaccharides and terminal molecules of sialic acid or fucose. They contain considerable amounts of the amino sugars galactosamine and glucosamine [Allen, 1983]. Colonic mucus is therefore believed to have a high carbohydrate content of between 65% [Allen, 1979] to 85% [Smith & Podolsky, 1986] of dry weight. Mucus degradation by the colonic bacteria is well documented [Salysers, Vercellotti, West, Wilkins, 1977]. Hoskins & Boulding [1982] have specifically identified sub-populations of bacteria that produce extracellular glycosidases which can degrade oligosaccharides of mucin in the gut lumen. The quantities of mucus secreted into the colon are not known. However, a small amount of mucus may enter the colon from the stomach and small intestine. This was estimated by measuring the hexosamine content of mucus which comprises both the amino sugars glucosamine and galactosamine of mucins [Stephen, Haddad, Phillips, 1983]. It was estimated that the carbohydrate contribution of mucins was of the order of 3-4 g/d. However it was borne in mind from this study that no allowance was made for any mucus or other sources of carbohydrate within the colon itself. However there are difficulties in isolating pure mucins from secretions and the knowledge of the mechanisms of mucus secretion and their control is limited. These factors therefore make the identification of the amount of mucus secreted into the human digestive tract difficult [Forstner, 1978].

d. **Other substrates.**

With the increased use by food companies of manufactured carbohydrates to improve the texture and other properties of foodstuffs, the fate of these modified celluloses and other polysaccharides in the human gut remains unresolved [Cummings & Englyst, 1987].

In summary, there is much evidence to demonstrate that bacterial metabolism of carbohydrate may occur within the anaerobic environment of the large intestine. In contrast to the aerobic processes in the rest of the body, it would seem that the products of anaerobic fermentation are particularly unique to the large intestine.

2.5.1 COLONIC NITROGEN METABOLISM.

Colonic nitrogen metabolism in the large intestine is poorly understood and much of our knowledge of colonic nitrogen metabolism has been derived from ruminant studies. Whilst the amount of nitrogen entering the human large intestine is not clear, the potential sources of nitrogen, originate from ileal discharge, urea breakdown, mucus desquamated cells and intestinal enzymes.

For many years, the major source of nitrogen for the intestinal microflora was thought to be ammonia derived from circulating urea and hydrolysed to ammonia by ureases of bacteria [Salyer & O'Brien, 1980]. Recent studies using ¹⁵N labelled urea suggest that very little hydrolysis of urea occurs in the colonic lumen [Wrong, Vince, Waterlow, 1985]. However ruminant studies have shown that the conversion from urea to ammonia may occur at the site of the mucosa where a stable population of facultative anaerobes has been identified which are capable of ureolysis [Wallac, Cheng, Dinsdale, Orskov, 1979]. In addition non urea sources of nitrogen may also provide ammonia for the microflora in the form of amine groups of protein, peptides and amino acids derived from the diet, mucus and sloughed and epithelial cells or other bacteria [Hespell & Smith, 1983; MacFarlane, Cummings, Allison, 1986; Vince, 1986]. It is also thought that amino acids may be metabolised by bacteria for protein synthesis [Wrong *et al.* 1981]. Any ammonia not required by the microflora can be readily absorbed from the large intestine [Castell *et al.* 1971] and reabsorbed in to the portal circulation, detoxified into urea in the liver and excreted in urine.

Colonic nitrogen metabolism is complex. The importance of human colonic microflora as its role in nitrogen metabolism and protein turnover in man has been demonstrated [Moran & Jackson, 1990] which implied that much of what we know about colonic function would require re-evaluation. It is thought that the extent to which carbohydrate is available for fermentation will influence colonic metabolism. It is suggested that when carbohydrate is abundant, the colonic microflora may be able to use various nitrogen compounds for protein synthesis. However if the availability of carbohydrate is reduced, the bacteria adapt and the breakdown of nitrogenous products may occur in order to supply their energy requirements for growth and maintenance [McNeil, 1988]. Recent work supports this where it was reported that the fermentation of mucus and protein [MacFarlane *et al.* 1986] may continue even after an overnight fast under normal circumstances [Scheppach, Pomare, Elia, Cummings, 1991].

2.5.2 THE IMPORTANCE OF FERMENTATION.

Recent studies have considered the importance of fermentation as a major function of the human colon and that this fermentation has an effect not only on other functions of the colon, but also on whole body metabolism [Cummings, 1984]. The evidence to support this is considered in the following sections.

2.5.3 SHORT CHAIN FATTY ACIDS.

It has been established that SCFA may be absorbed from the human colon and may contribute to the host's energy supply [McNeil, 1984; Ruppin, Bar-Meir, Soegel, Schmitt, 1980]. It has been shown that the output of SCFA in faeces is relatively small with respect to the magnitude of faecal loss [Rubenstein, Howard, Wrong, 1969; Hoverstad & Bjornekleit, 1984]. As no specific patterns of SCFA have been observed which may relate to colonic bacterial activity, it is thought that faecal SCFA excretion is not a sensitive representation of SCFA metabolism within the colon unless there are changes in the production and absorption of SCFA induced by dietary means or through antibiotic therapy [Cummings, 1985]. Changes in the excretion of SCFA have been demonstrated with alterations in diet [Fleming & Rodriguez, 1983; Midvedt, Johannsson, Carlsdedt-Duke, Norin, Gustaffson, 1990] with a positive association observed between faecal wet weight and SCFA excretion [Scheppach, Fabian, Sachs, Kasper, 1988].

Absorption of SCFA stimulates sodium and water absorption from the colon and the secretion of bicarbonate, thereby being an important contributor to salt and water homeostasis in the colon [Crump, Argenzio, Whipp, 1980; Roediger, Hetworth, Willoughby, Pins, Moore, Truelove, 1982]. Following absorption into the mucosal cell SCFA provide an important energy source for the colonic mucosa, in particular butyrate [Roediger, 1980]. This is supported by recent work of Kripke and coworkers [1989] who found that short chain fatty acids (SCFA) infused into the rat colon have trophic effects throughout the intestinal tract. Similarly, the products of hind gut fermentation rather than NSP previously indicated by Jacobs & Lupton [1984], stimulate intestinal epithelial cell proliferation in the colon and small intestine [Goodlad, Ratcliffe, Fordham, Wright, 1989]. Acetate and propionate pass directly to the liver where they may provide an additional energy source for metabolism. Acetate is thought to pass to peripheral tissues for metabolism by muscle [Knowles, Jarrett, Filsell, Ballard, 1974]. Whilst the metabolic fate of SCFA has been demonstrated, it has not been possible to provide a quantitative estimate of the contribution of fermentation in the human colon to energy metabolism in human tissues.

2.5.4 ENERGY FROM SHORT CHAIN FATTY ACIDS.

Whilst the production of SCFA has been well documented, the amount of SCFA produced and absorbed in the human colon is less clear. It is widely appreciated that SCFA are an important energy source in ruminants which contribute to between 60-90% of energy requirements [Allo, Oh, Longhurst, Connolly, 1973]. The importance of this energy yielding process in relation to energy requirements in man is often underestimated or even ignored in energy balance studies. Whilst the contribution of fermentation in the human colon to energy metabolism is not known, how much carbohydrate may pass the terminal ileum on a habitual intake and the amount of mucus and other sources of carbohydrate secreted by the human colon remains unclear. Some idea of the colonic contribution has been made by estimating the amount of substrate available for fermentation.

As the British adult diet contains between 20-25g/d NSP [OPCS, 1990] (measured as dietary 'fibre') with approximately 5g/d present within faeces [Southgate & Durnin, 1970], it is assumed that around 15g of NSP may be fermented each day. Of the 150-200g starch consumed each day [Bingham *et al.* 1979], 15-20g could be provided for fermentation assuming that starch malabsorption may be at a maximum of 20%. At around 10% this would produce

15-20g more for fermentation. Also with some mucus glycoprotein, around 30-40g of carbohydrate may be fermented in the large intestine. Using the equation shown in section 2.4, approximately 3-400 mmol of SCFA could be generated. Whilst faecal losses of SCFA are minimal, SCFA absorption could represent between 6-9% of the daily energy requirements [McNeil, 1988].

Based upon the growth requirements of intestinal bacteria, and the assumption that daily faecal bacterial content to be 16g dry weight, Wolin [1981] calculated that 650-700 mmol of SCFA would be produced, equivalent to 12-14% of energy requirements. Using a variation of this approach and from the measurement of faecal bacteria [Stephen & Cummings [1980a], it was suggested that 60g of carbohydrate were fermented daily yielding between 500-600 mmol of SCFA which was equivalent to up to between 5-10% of daily energy requirements [Cummings, 1981a, McNeil, 1984].

The implications of this 'energy salvage' within the colon is discussed by Grosslaus [1983] in relation to diets with high intakes of NSP and in diseases of the small intestine. Whilst it is not known how much energy may be recovered through SCFA absorption in such circumstances, further studies are required to consider these questions in relation to energy requirements in both health and disease.

2.5.5 GASEOUS PRODUCTION.

Whilst the main gases produced as a result of fermentation are hydrogen and carbon dioxide, the role of gaseous production in health is not understood. It is thought that normal individuals produce different proportions of hydrogen and carbon dioxide [Wolin, 1981]. Some bacteria, the so called end-chain metabolisers may be able to use end products of fermentation in further energy-yielding reactions. Methanogenic bacteria are believed to utilise carbon dioxide and hydrogen to produce methane, whilst sulphate reducing bacteria utilise colonic hydrogen to produce hydrogen sulphide [Gibson, MacFarlane, Cummings, 1988]. The two groups thereby compete for colonic hydrogen, with some individuals carrying the sulphate reducing bacteria tending not to produce large amounts of methane in the colon and vice versa. The consequences of this bacterial competition and significance of between individual differences in these groups of bacteria requires further exploration.

2.5.6 SUMMARY.

In summary, the activity of the colonic bacteria appear to have an important influence on metabolic events at the level of the large intestine and on body tissues. Given that there is the potential to recover energy through SCFA absorption, it would seem that colonic function has an important role in both digestion and metabolism in man.

2.6 THE COLONIC BACTERIAL MICROFLORA.

Studies over the last twenty years have demonstrated the complexity of the normal colonic microflora. The normal bacterial count within the faeces is on average at least 10^{11} colonies per gram [Finegold, Sutter, Mathisen, 1983], and there may be over 400 separate species of anaerobic bacteria [Moore & Holdeman, 1974]. Five genera account for the majority of bacteria within faeces: *Bacteroides*, *Eubacteria*, *Bifidobacteria*, *peptostreptococcus* and *fusobacteria*. The first four are saccharolytic indicating that they can utilise various complex carbohydrates present in the intestine [Woods & Gorbach, 1986]. Various facultative and aerobic micro-organisms also exist in the colonic flora. Post mortem studies applying the method of Stephen & Cummings [1980a] suggest that bacteria can account for more than 61% of the dry weight and 81% of the wet weight of intestinal contents [Banwell, Branch, Cummings, 1981].

Zubrzycki & Spaulding [1962] demonstrated that the faecal flora remains constant within an individual although the major groups of bacteria may vary between individuals [Gorbach, Nahas, Lerner, Weinstein, 1967]. Little difference between the faecal flora of infants and adults has also been reported [Ellis-Pegler, Crabtree, Lambert, 1975]. Changes in the delivery of substrate may alter the metabolic activity of bacteria [Florent, Flourie, LeBlond, Rautureau, Bernier, Rambaud, 1985; Wyatt, Bayliss, Holcroft, 1986] or may alter the balance of different species of bacteria [Edwards, 1988].

There are difficulties in documenting colonic bacterial metabolism in man. The human colon, particularly the right colon is almost inaccessible to direct observation. Apart from intubation techniques to study the terminal ileum, attention has been directed towards the passage of material through the gut and its products with faeces. Most studies of human faecal microflora have used an *in vitro* model system by classical bacteriologic techniques of counting and ident-

ifying bacteria in faeces to reflect the mechanisms of *in vivo* interactions. However by such an approach it is clear that there are a number of assumptions introduced [Hill & Drasar, 1975; Moore & Holdeman, 1974].

The normal faecal flora are assumed to represent the colonic flora. Moore and coworkers [1978] examined the microflora of the colonic contents within individuals following sudden death and confirmed that the colon contain a representative population of bacteria present in the faeces. However it is not known whether this observation would be apparent in living people. It may well be that the isolation of faecal bacteria may represent a portion of the bacteria within the colon as there are differences in bacterial activity in different regions of the colon. In addition faecal bacteria is assumed to represent only the luminal flora and that associated with the mucosal epithelial surfaces may also differ [Bornside, 1978]. However Croucher and coworkers [1983] have shown that the flora associated with the mucosal surface do not appear to be significantly different from those in the lumen of the bowel.

The approach of isolating bacteria within faeces by filtration and centrifugation was probably first described by Strasburger in 1902 [Sperry, 1929]. Sperry [1929] indicated that by using this approach, a number of early studies showed that the contribution made by bacteria within faeces could range between 3-46% of the faecal dry weight in normal subjects consuming their habitual food intake. It was suggested that this wide variation could be attributed to differing centrifuging conditions [Ehrenfordt, 1910]. In addition it was not clear whether there was complete separation of bacteria from other faecal components [Sperry, 1929].

More recently direct microscopic counting has been used to estimate bacteria numbers [Finegold, Attebury, Sutter, 1974]. Whilst direct microscopic counting take account of both dead and living bacteria, Rall and coworkers [1970] have suggested that total numbers may be underestimated as bacteria tend to stick together and form clumps. Some bacteria may also be too small to be seen using light microscopy at the high magnifications used for counting. Whilst there are many different species of bacteria within human stools, many of the bacteria have not been identified such that an extrapolation from the bacterial count to a weight of bacteria is difficult to estimate [Stephen & Cummings, 1980a]. In addition bacterial size varies with growth during the period of exponential growth and are larger than resting cells.

Another approach to the estimation of bacterial mass in the ruminant has been the use of chemical markers which are believed to characterise bacterial components. The markers most commonly used have been diaminopimelic acid (DAP), ribonucleic acid (RNA) and isotopes ^{35}S , ^{15}N or ^{32}P which are incorporated into bacterial protein. However the DAP content of different species varies widely and assumptions must be made when the weight is converted to the bacterial population. RNA content can also be easily affected by diet and measurements can be contaminated by mucus [Stephen & Cummings, 1980a].

The method of complete separation of bacteria by fractional centrifugation has been used in ruminant studies [Smith & McAllan, 1974]. With the development of ultracentrifuges, a more recent study using a method based on a technique for isolating bacteria from the ruminant has enabled the measurement of the contribution made by bacteria within faeces in humans [Stephen & Cummings, 1980a]. This study demonstrated that approximately 55% of the dry weight and 70% of the wet weight of faeces can be accounted by bacteria in individuals following typical 'western' diets [Stephen & Cummings, 1980a]. As bacteria can account for approximately 61% of dry weight and 81% of wet weight of intestinal contents in post mortems [Banwell *et al.* 1981], it would appear that bacteria can account for a similar proportion of both faecal weight and the weight of intestinal contents. Bacteria are 80% water [Luria, 1960] and as they appear to comprise a major fraction of faecal solids they are an important water holding component of the material moving through the gut.

2.6.1 SUMMARY.

Attempts to study colonic bacterial metabolism in man have focused upon the composition of faecal bacteria. This information however is of limited value with respect to the study of colonic function. Further studies are required to investigate the colon directly which could only be achieved by intubation studies.

2.7 EFFECT OF ALTERATIONS IN DIET ON THE BACTERIAL CONTRIBUTION TO HUMAN FAECAL MASS.

2.7.1 DIETARY NON-STARCH POLYSACCHARIDES.

Several investigations have been made of the effect of dietary NSP on the types of faecal bacterial species by counting and identifying microorganisms in faeces, with few positive results. No change in the concentration of bacteria nor in the distribution of species have been found in healthy subjects given bran [Drasar, Jenkins, Cummings, 1976; Fuchs *et al.* 1976], sugar cane bagasse [Walters, Baird, Davies, Hill, Drasar, Southgate, Green, Morgan, 1975], guar gum [Drasar & Jenkins, 1976], and pectin [Doyle, Wolfman, Varso, Floch, 1981] and with the addition of bananas and plantain [Drasar & Jenkins, 1976].

Using a different approach however it could be demonstrated that both the nature and amount of dietary NSP could influence the extent of its fermentation by the colonic microflora [Stephen & Cummings, 1980b]. When subjects were given dietary NSP from cabbage which is thought to be extensively broken down in the large intestine, a 60% increase in stool output was observed as a result of microbial growth [Stephen & Cummings, 1980b]. The much less digestible wheat NSP produced only a small change in bacterial mass within the stool which suggested that wheat NSP remained largely undigested and retained water in the gut. Applying the method of Stephen & Cummings [1980a] a recent study showed that between 45 to 50% of faeces could be attributed to bacteria following the consumption of constant diets supplemented by 30g wheat bran in healthy subjects [Cabotaje, Lopez-Guisa, Marlett, 1990].

The findings of Stephen & Cummings [1980b] however could not be supported by another study which also applied their technique to isolate faecal bacteria within normal subjects consuming diets containing different sources of dietary NSP such as cereals, pulses, fruit and vegetables [Forsum *et al.* 1990]. The bacterial content of faeces appeared to be increased following the consumption of diets containing dietary NSP from whole grain cereals which contain NSP of 'low digestibility'. However bacteria accounted for a greater proportion of faecal weight with a diet containing pulses, fruits and vegetables which are thought to increase faecal output by the stimulation of bacterial growth in the large intestine than a diet containing NSP from cereals.

A number of studies have investigated the effects of isolated polysaccharides on faecal weight. An increase in faecal weight was demonstrated when diets were supplemented with ispaghula husk in contrast to the effect of guar gum and xanthan gum [Tomlin & Read, 1988]. However, they suggest that the major mechanism by which the soluble polysaccharide ispaghula increased stool mass was through water holding rather than by the stimulation of bacterial growth. Nevertheless, bacterial mass was not determined in this study. It has also been shown that supplementing the diet with corn starch can increase faecal stool mass [Shetty & Kurpad, 1986]. Although the contribution made by bacteria to stool mass was not measured, it was suggested that the increased faecal weight could be attributed to the stimulation of colonic bacterial growth. This study however has other implications as it demonstrates that starch alone may influence colonic function.

It may well be that bacteria contribute to the increased faecal weight observed when diets have been supplemented with isolated polysaccharides or some types of starch. Whilst many studies have been directed towards the influence of the amounts of NSP on the faecal weight, alterations in the amount of bacteria within faeces remain unclear.

2.7.2 RESIDUE-FREE OR ELEMENTAL DIETS.

Without dietary NSP it is thought that the activity of bacteria still continues, but to a limited extent. The magnitude of stool production and stool frequency has been reported to be reduced in individuals fed NSP-free diets [Winitz, *et al* 1970; Kien, Cordano, Cook, Young, 1981; McCamman, Beyer, Rhodes, 1977]. In the absence of dietary residue in the stool, much of the faecal output must be attributed to bacteria and endogenous losses such as intestinal mucus and other secretions. These would provide bacteria with carbohydrate to meet their energy requirements for growth and maintenance. However this remains at the level of speculation as there have been no studies which have measured the contribution made by bacteria towards faecal weight following the consumption of such diets.

Of the few studies which have investigated the types of bacteria within faeces of individuals on low residue diets, only Winitz and coworkers [1970] has been able to report significant reductions in many species of faecal bacteria. Whilst subsequent studies have shown a reduction in faecal bulk, they have failed to

demonstrate any profound reduction of faecal bacterial microflora [Attebury, Sutter, Gold, 1972; Bornside & Cohn, 1975].

2.7.3 EVIDENCE FROM ILEOSTOMY PATIENTS.

In contrast to the results of faecal bacteria, the studies investigating the effects of dietary alterations on bacteria of ileostomy effluent have demonstrated major changes in bacterial species [Fernandez, Kennedy, Hill, Truelove, 1985; Berghouse, Hori, Hill, Hudson, Lennard-Jones, Rogers, 1984]. This difference is thought to be attributed to the higher concentration of carbohydrate within the proximal colon than the rectosigmoid region where the fermentable material is almost exhausted.

2.7.4 SUMMARY.

In summary, studies which have investigated the effect of diet on bacterial species within faeces appear to be inconclusive and least informative. The quantitative approach developed by Stephen & Cummings [1980a] would appear to have been the most useful investigative tool in assessing the influence of diet on faecal bacteria. This information has also provided some understanding of the mechanism of action of certain types of NSP on human metabolism.

2.8 EFFECT OF DIETARY NON-STARCH POLYSACCHARIDES ON ENERGY, LIPID AND NITROGEN WITHIN THE STOOL: THE CONTRIBUTION MADE BY COLONIC MICROFLORA.

Whilst the nature and the amount of dietary NSP could influence colonic bacterial activity and result in raised stool outputs as discussed in section 2.6, increases in the energy, lipid and nitrogen content of faeces have also been reported. The following section considers the extent to which bacteria may contribute towards the observed increase in the energy, lipid and nitrogen content of faeces with raised intakes of dietary NSP.

2.8.1 ENERGY CONTENT OF FAECES.

There have been a number of studies which have measured the energy content of stools following the consumption of diets with different amounts of dietary NSP [Cummings, 1986]. Studies have demonstrated that the amount of energy within faeces was greater with raised intakes of dietary NSP in comparison to low or normal intakes [McCance & Walsham, 1948; Kelsay *et al.* 1978; Calloway & Kretsch, 1978; Stephen *et al.* 1983].

There have been some reports which have shown that a raised intake of dietary NSP from a variety of sources may also result in an increased loss of energy, nitrogen and lipid within the stool and a reduction in the apparent digestibility of energy, lipid and nitrogen [Southgate & Durnin, 1970; Kelsay *et al.* 1978; Goranzon, Forsum, Thilen, 1983; Goranzon & Forsum, 1987, Miles, Kelsay, Wong, 1988; Wisker, Maltz, Feldheim, 1988].

Conversely, a study in which the diet was totally depleted in dietary NSP showed a significant increase in the apparent digestibility of lipid within the stool in comparison to a free choice diet (the digestibility of energy and nitrogen were not compared) [Kien *et al.* 1981]. In the studies of Southgate & Durnin [1970] and Wisker and coworkers [1988], the total increase in the energy lost within the stool associated with the increased intake of NSP, exceeded the gross energy contained within NSP.

A number of workers have attempted to explain this observation. It has been suggested that much of the increase in energy within the faeces could be due to undigested NSP [McCance & Walsham, 1948; Calloway & Kretsch, 1978]. It has also been thought that there is an effect of dietary NSP on the digestion and absorption of protein and lipid within the small intestine which may account for the reduction in the apparent digestibility of energy, lipid and protein [Southgate & Durnin, 1970]. It has been demonstrated however that stool mass may increase as the result of the stimulation of colonic microfloral activity with increased intakes of some types of dietary NSP [Stephen & Cummings, 1980b]. There is no information of the extent to which bacteria may contribute towards increased faecal energy losses by modifying the intake of NSP. It can be postulated whether the reduced apparent digestibility of energy could reflect colonic bacterial growth resulting in an increase in the contribution made by bacteria to faecal energy.

2.8.2 FAECAL NITROGEN.

From the literature, it would seem that raised dietary intakes of NSP result in an increased loss of nitrogen within faeces, whether the source of NSP is from wheat [Cummings *et al.* 1976; Stephen *et al.* 1986], oats [Calloway & Kretsch, 1978], fruit and vegetables [Kelsay *et al.* 1978; Miles *et al.* 1988]. This is supported from studies where the diets were supplemented with isolated polysaccharides [Cummings *et al.* 1979; Slavin & Marlett, 1980]. However in another study a generalised response to the consumption of a NSP supplement could not be demonstrated [Prynne & Southgate, 1979]. Several mechanisms have been suggested that may explain how dietary NSP increase faecal nitrogen. From a study of the apparent digestibility of nitrogen during the consumption of large amounts of whole grain or high extraction wheat and oat products [McCance & Widdowson, 1947], it was suggested that the nitrogen in faeces was virtually of 'metabolic and microbiological origin'. Similar conclusions were presented by Walker [1975]. As an alternative explanation, Saunders & Betchart [1980] suggested that the nitrogen loss with raised dietary NSP intakes was the result of protein associated with the cell wall material which was thought to be less digestible than most dietary proteins. Alternatively, protein metabolism could be impaired by dietary NSP because it reduces the activity of proteolytic enzymes trypsin and chymotrypsin [Scheeman, 1978] or that nitrogen from digestive secretions could be less well absorbed due to the increased colonic volume following the consumption of increased dietary NSP [Bender, Mohammadiha, Alams, 1979]. However it would seem that the form in which the nitrogen occurs within the stool is of considerable importance. At the turn of the century Lissauer [1906] reported that around 11.0% of the total faecal nitrogen could be accounted by bacteria in normal subjects consuming mixed diets by isolating faecal bacteria by fractional centrifugation. More recently, Stephen & Cummings [1980c] have shown that bacteria could be attributable for 60% of the total nitrogen within the faeces from normal adults consuming an average UK diet. Subsequently in studies measuring the effect of NSP from cabbage and bran on microbial growth, 63% and 34% respectively of the increase in nitrogen within the stool could be accounted by bacterial nitrogen [Stephen & Cummings, 1980b].

Thus it is clear the potential contribution bacteria could make to the increased nitrogen loss within the stool with increased NSP intakes. However, as it is not possible to measure the amount of nitrogen within intestinal secretions and

cellular debris, the contribution made by each of these endogenous components remain unresolved.

2.8.3 FAECAL LIPID.

A number of studies in which the dietary NSP intake has been raised show an increased loss of lipid within the stool. This is apparent where the diet has been qualitatively changed [Southgate & Durnin, 1970; Kelsay *et al.* 1978; Stephen *et al.* 1986; Miles *et al.* 1988] or where the increased intake of dietary NSP has been produced by the addition of isolated polysaccharides [Cummings *et al.* 1979; Slavin & Marlett, 1980]. The concept that lipids associated with dietary NSP are intrinsically less digestible has been suggested as the cause for the increased faecal lipid on bran-enriched diets [Southgate, 1982]. However it has been shown that extracted lipid of cereals is well digested and the digestion of vegetable protein and lipid *in vitro* can occur without the rupture of the cellulose walls [Heupke, 1932]. More recently, Walker [1975] has identified relatively few 'aleurone cells' from cereal NSP having lipid within the cellular structure and suggests that faecal lipid is composed of endogenous products. It was shown 60 years ago by isolating faecal bacteria through fractional centrifugation that 40% of the total excretion of lipid within the faeces could be accounted by bacteria in normal subjects [Sperry, 1929]. There would appear to have been no studies which have examined the proportion of lipid within the stool which may be attributed to bacteria with raised intakes of dietary NSP.

2.8.4 SUMMARY.

It would seem that attention has been directed towards the influence of dietary NSP on the absolute energy content of faeces with the assumption that all of this energy is attributed to dietary residue. Whilst a major proportion of human faecal mass can be attributed to bacteria, it could be that part of the energy content of faeces could be bacterial in origin as well as possibly other endogenous components. In the consideration of apparent digestibility the potential contribution made by components other than dietary residue should not be ignored.

2.9 THE AVAILABILITY OF DIETARY ENERGY IN MALDIGESTION AND/OR MALABSORPTION AND THE CONTRIBUTION MADE BY INCREASED FAECAL ENERGY LOSSES AND COLONIC BACTERIAL MICROFLORA.

Gastro-intestinal dysfunction with maldigestion and/or malabsorption of lipid, protein and carbohydrate is frequently associated with unbalanced losses of nutrients within the stool. Therefore raised faecal energy losses could contribute towards a limitation in the availability of energy from the diet and thereby restrict growth or cause weight loss. It is commonly assumed that increased faecal energy losses are derived directly from the diet presuming that faeces is composed of dietary residue which has not been absorbed. The possibility that this dietary residue could be undigested and thereby in a form which could not be absorbed by the body is often overlooked.

The following section considers the magnitude and composition of faecal energy in maldigestion and/or malabsorption and the evidence to support the view that not all of stool energy consists simply of dietary residue is examined. Table 2.1 provides a summary of reported values for lipid and nitrogen losses within the stool in diseases associated with gastro-intestinal dysfunction.

2.9.1 FAECAL ENERGY.

There have been relatively few studies which have considered the measurement and nutritional consequences of raised faecal energy losses in disease. One notable study is that of Heymsfield and coworkers [1981] who measured faecal energy within a group of individuals with malabsorptive problems such as pancreatic carcinoma and inflammatory bowel disease. In patients with malabsorption, faecal energy losses were between 2.0-4.0 MJ/d which were 3-6 times greater than a group of subjects without malabsorption. There have been other studies which have measured faecal energy losses in lactose-malabsorbing children [Brown, Khatun, Parry, Ahmed, 1980], short bowel syndrome [Rodrigues, Lennard-Jones, Thompson, Farthing, 1990], preterm infants [Bijleveld, Vonk, Dankert-Roelse, Okten, Fernandes, 1986]. There have been two previous studies which have measured the energy contents of stools in the disease cystic fibrosis [Bijleveld *et al.* 1986; Koletzko, Corey, Ellis, Spino, Stringer, Durie, 1990]. Attention was specifically directed towards the use of wet stool weight as a parameter for the lipid and energy content of stools in the study reported by

Bijleveld and coworkers [1986]. Whereas the energy content of stools was determined in the study reported by Koletzko and coworkers [1990] to calculate the percentage of malabsorbed energy with the drug cisapride in cystic fibrosis patients.

Much attention in the literature has been primarily directed towards measurements of lipid and nitrogen within faeces. In particular the amount of faecal lipid would appear to be the most frequently used index of maldigestion and malabsorption.

2.9.2 FAECAL LIPID.

The term 'steatorrhoea' was introduced by Kuntzmann in 1824 to describe the globules of lipid visible to the naked eye within faeces [Hendry, 1960]. It is generally accepted that steatorrhoea by definition implies an excess of lipid in the faeces with a consequent loss of energy from the body [Losowsky, Walker, Kellehar, 1974]. As lipid is the most energy dense substrate it has been suggested that steatorrhoea is the main cause of faecal energy loss [Fernandes, Kneepens, Bijleveld, Vonk, 1985]. This would assume that high stool lipid losses would also represent a large amount of energy lost within the stool. Thus if steatorrhoea is improved, the presumption is made that the amount of energy available from the diet would increase. By applying these assumptions this may partly explain why faecal lipid rather than faecal energy measurements have been used as an index of maldigestion and/or malabsorption in many studies.

It is considered that a daily lipid of excretion less than 5g/d indicates the absence of steatorrhoea [Hendry, 1960; Skala, Kronl, Vulterinova, Stastna, Vavrinkova, Horackova, Parikova, 1968; Pinter & Mclean, 1968]. Table 2.1 shows that there are a wide range of faecal lipid losses within each group of patients. The lipid content of stools is believed to be proportional to lipid intake [Losowsky, Walker, Kellehar, 1974]. This has been demonstrated in a number of diseases associated with gastro-intestinal dysfunction. These include coeliac disease [Bassett, Keutmann, Van Zile Hyde, Van Alstine, Russ, 1939; Chung, Morales, Snyderman, Lewis, Emmett Holt, 1951], tropical sprue [Black, Fouramn Trinder, 1946], pancreatic insufficiency [Wollaeger *et al.* 1947] and in cystic fibrosis (CF) [Forstner, 1986].

2.9.3 FAECAL NITROGEN.

In comparison to faecal lipid measurements, faecal nitrogen losses have been measured less widely in the study of diseases associated with maldigestion and/or malabsorption [Dornberger, Comfort, Wollaeger, Power, 1948; Schmerling, Forrer, Prader, 1970]. It is thought that raised lipid losses within the stool do not necessarily accompany increased faecal nitrogen losses [Dornberger *et al.* 1948]. In coeliac disease, variable increases in faecal nitrogen were observed and particularly in patients with high faecal lipid losses [Green & Wollaeger, 1960]. Consequently, Cooke & Holmes [1984] have suggested that disorders of exocrine pancreatic function may be more common in coeliac patients.

2.9.4 FAECAL CARBOHYDRATE.

Whilst the lipid and nitrogen content of stools have been investigated in many diseases, the amount of carbohydrate as simple sugars or any form of complex carbohydrate, NSP or starch is not clear. Faecal carbohydrate has been estimated in diseases associated with malabsorption by subtracting the energy derived from total protein (by multiplying the nitrogen measured in faeces by 6.25 assuming that proteins contain 16% nitrogen) and lipid within faeces from the total faecal energy content [Heymsfield *et al.* 1981]. More attention has been directed towards assessing carbohydrate malabsorption by using non-absorbed sugars [Murphy, Eastham, Nelson, Pearson, Laker, 1989; Dalzell, Freestone, Billington, Heaf, 1990], or by using hydrogen breath tests [Bond, Currier, Buchwald, Levitt, 1980; Kruis, Forstmaier, Scheurlen, Stellaard, 1991].

2.9.5 ORIGINS OF ENERGY WITHIN THE STOOL.

The amount of energy from lipid, nitrogen and carbohydrate which could be attributed to endogenous secretions and cellular debris or the colonic bacterial microflora remains at the level of speculation as there would appear to be no studies which have specifically considered this possibility. Without this information the presumption is often made that the energy content of stools in disease simply consists of dietary residue.

2.9.6 MUCUS SECRETIONS.

It has been demonstrated that mucus degradation can occur by extracellular enzyme production from colonic bacteria in healthy subjects [Hoskins & Boulding, 1982]. However the evidence to suggest that mucus degradation may occur specifically in disease is less clear. In the disease cystic fibrosis where there is increased production of an abnormally thick mucus in the gut the composition of mucus has been examined. Wesley and coworkers [1983] have demonstrated a difference in the carbohydrate structure between normal mucus and mucus in CF patients in the gut. The CF mucus tended to be enriched with glucose, galactose, N-acetylglucosamine and total carbohydrate per mg protein and per oligosaccharide chain. In addition fractions from CF mucus were also found to have a higher content of fucose than normal specimens [Clamp & Gough, 1979]. Studies on lipids in submandibular saliva of patients with CF indicate that CF saliva contains 66% more lipid than that of normal subjects and exhibit an elevated content of neutral lipids, glycolipids and phospholipids [Slomiany & Slomiany, 1984]. Although no studies have specifically measured the lipid content of mucus secretions in the small intestine and large intestine, this component may well be increased. The amino acid profile and antigenic properties however of intestinal mucins from patients with CF have been shown to be similar to that in control mucins [Mantle, Forstner, Forstner, 1984].

Whilst the composition of CF mucus has been examined, it has not been possible to directly quantify mucus production and epithelial cell losses. Thus the contribution made by carbohydrate within mucus and other endogenous carbohydrates within intestinal secretions and cellular debris for colonic fermentation by bacteria remains unresolved. The possibility that energy may be salvaged through the absorption of SCFA in health has been examined in previous studies [Cummings, 1981a; McNeil, 1984]. In CF patients where mucus production is elevated, the potential capacity for energy to be salvaged may be substantial.

2.9.7 SUMMARY.

Whilst there have been relatively few studies which have specifically measured faecal energy losses in maldigestion and/or malabsorption, attention within the literature has been directed towards the measurement of the lipid and nitrogen contents of stools to provide indexes of gastro-intestinal dysfunction. The relative contribution made by each of the components of faecal energy remains unclear.

In studies which have measured faecal energy or attempted to calculate apparent absorption of energy, the contribution made by endogenous losses or the colonic bacteria has been ignored. With no discrimination between these components of faecal energy, the true digestibility of energy may well be higher than indicated.

2.10 THERAPEUTIC MANAGEMENT.

With information developed from studies which have examined the gastro-intestinal problems associated with maldigestion and/or malabsorption, a variety of drugs are available which are used as part of the therapeutic management of a number of diseases.

The standard approach to the management of the gastro-intestinal problems associated with cystic fibrosis and chronic pancreatitis (although to a reduced extent in comparison to cystic fibrosis), has been exogenous pancreatic enzyme replacement therapy (PERT). As it is commonly assumed that a reduction in lipid losses would also represent a small amount of energy lost within faeces, most studies have measured total faecal lipid and nitrogen losses to assess the effectiveness of PERT [Stead, Skypala, Hodson, Batten, 1987; Bouquet, Si-naasappel, Neijens, 1988]. Consequently, it would seem that little attention has been directed towards the magnitude of faecal energy losses during the therapeutic management of disease.

Whilst there is considerable information of the influence of PERT and other drug therapy on faecal lipid and nitrogen losses (particularly in the disease cystic fibrosis) the following section provides a brief overview of our present knowledge. Attention is also focused upon the potential influence that antibiotic therapy may make in restricting the metabolic activity of the colonic bacterial microflora.

2.10.1 PANCREATIC ENZYME REPLACEMENT THERAPY AND FAECAL LIPID LOSSES.

The aim of PERT is to deliver sufficient active enzyme within the small intestine with a pH close to neutrality [Roy, Weber, Lepage, Smith, Levy, 1988]. However within any patient, there are a number of factors which can influence the effectiveness of PERT such as potency and dosage of the enzyme preparation,

acid-secreting status of the patient, the remnant capacity to secrete bicarbonate and enzymes and the capacity to compensate for the bile acid loss associated with the maldigestion of nutrients [Roy *et al.* 1988]. Despite a relatively small reduction in the amount of lipid within faeces, the inactivation of orally ingested conventional pancreatic enzyme preparations by gastric acid is thought to be a major factor responsible for a poor response [Dutta, Rubin, Harvey, 1983]. Consequently, many attempts have been made to inhibit the destruction by neutralisation of gastric acid using bicarbonate or other antacids and the inhibition of gastric acid secretion by cimetidine [Durie, Bell, Linton, Corey, Forstner, 1980]. Despite a small reduction in the amount of lipid excreted within faeces with the use of bicarbonate and pancreatin when compared to enzyme alone, it has been still necessary to use large doses of both the enzyme and bicarbonate.

The recent introduction of enteric-coated encapsulated microspheres, which resist inactivation by acidic gastric contents, presents the enzyme in an active form to the duodenum. The coating dissolves at a pH of approximately 6.0 [Zentler-Munro, 1987]. The amount of lipid within faeces in patients with CF with enteric-coated microspheres is decreased yet the extent of this reduction does reach normal levels in CF patients [Mischler, Parrell, Farrell, Odell, 1982; Stead *et al.* 1987; George, Pinero, Miller, Toskes, Sitren, Downey, 1990; Vyas, Matthew, Milla, 1990] or in patients with chronic pancreatitis [Dimagno, Malagelada, Go, Moertel, 1977; Marotta, O'Keefe, Markes, Girdwood, Young, 1989]. Even when the dosage of the enzyme has been raised, no improvement in the amounts of lipid lost within faeces has been observed [Bouquet *et al.* 1988]. In some patients however, the use of conventional preparations of enzymes have proved to be more effective than enteric microspheres [Roy *et al.* 1988]. Attempts to improve the efficacy of enteric coated microspheres with antacids, cimetidine and sodium bicarbonate or cisapride, a novel gastro-intestinal prokinetic agent, have had limited success [Gow, Bradbear, Francis, Shepherd, 1981; Kopelman, Durie, Gaskin, Weixman, Forstner, 1985; Smith, Handy, Weller, Booth, 1989; Koletzko *et al.* 1990]. It has been suggested that a synthetic prostaglandin of the PGE₂ class (Misoprostol) could improve the degree of lipid malabsorption by stimulating the secretion of bicarbonate by the duodenum and aiding the release of exogenous pancreatic enzymes [Robinson & Sly, 1990].

Comparisons has been reported between the efficacy of the most commonly used enteric coated microspheres in the UK, Creon (Duphar laboratories, UK) and Pancrease (Cilag, UK)[Beverley, Kellehar, MacDonald, Littlewood, Robinson, Walters, 1987; Williams, MacDonald, Weller, Fields, Pandov, 1990; George *et al.* 1990]. Despite Creon containing 60% more lipase and 210% more amylase in comparison to pancrease, no difference in the efficacy of each of these two preparations were observed.

Over the last few years very high lipase containing enzymes have been introduced in the USA and Canada such as Pancrease MT-10; Pancrease MT-16 (McNeil Pharmaceutical, USA), Zymase (Organon inc, USA). These have attempted to aid patient compliance by reducing the quantity of enzyme capsules to be taken with each meal. In one study although less capsules of high lipase enzyme activity were required to produce a similar degree of lipid absorption as standard dose Pancrease, lipid losses could still equivalent to up to 30% of lipid intake [Robinson, Olinsky, Smith, Chitravanski, 1989]. This would suggest that whilst compliance may be improved with high dose enzymes, there would appear to be no impact on the reduction of faecal lipid losses. Moreover there is no information of the extent to which stool energy losses could be influenced by high dose enzymes in CF patients.

It has been shown that in patients with cystic fibrosis taking inappropriately high doses of pancreatic enzymes, a reduction in enzyme dosage may well have little or no effect on lipid malabsorption [Robinson & Sly, 1989]. In an attempt to explain the failure of enteric coated microspheres to normalise faecal lipid in patients with CF, several other factors have been indicated which may be responsible for the failure of lipid digestion and absorption with PERT in cystic fibrosis.

There would appear to have been little attention focused on differentiation between maldigested and/or malabsorbed residue within the stool. Despite adequate digestion with PERT, it could be that there is a limitation or failure in the ability of the body to absorb digested residue. Excessive amounts of mucus present in the small intestine are thought to provide an additional barrier to lipid absorption [Dodge, 1986]. In addition to the presence of small intestinal lipases, acid resistant lingual and gastric lipases has been demonstrated in patients with CF [Fredrikson & Blackberg, 1980]. Whilst some studies have shown that lipase activity is either normal or increased, lingual lipase may have a major role in the

digestion of lipids in CF because these enzymes could act in the acidic conditions found in the stomach as well as in the neutral conditions of the duodenum [Abrams, Hamosh, Dutta, Van Hubbard, Hamosh, 1987]. Its activity it thought to represent 90% of the total lipase activity in the duodenum [Roy *et al.* 1988]. It would also appear that even in the absence of PERT, 50-60% of the dietary lipid is absorbed [George & Mangos, 1988].

Furthermore as suggested by Roy and coworkers [1988], bile salts are required for micelle formation in pancreatic lipolysis. In cystic fibrosis the entero-hepatic circulation of bile acids is interrupted because bile acids are adsorbed onto undigested food particles and proteins and thus are lost within the faeces. Therefore a decrease in bile acid concentration in luminal contents of the small intestine effects the formation of micelles. This loss of bile acids in faeces also results in an abnormal dominance of glycine-conjugated bile salts over taurine-conjugated bile salts. The glycine-conjugated bile acids precipitate in the relatively acidic environment of the duodenum and are thus not available for lipid solubilisation [Roy *et al.* 1988].

In the light of this knowledge, a number of investigators have proposed new methods of treatment. These include the administration of taurine as a means of enhancing absorption of the products of lipolysis of pancreatic enzymes [Roy *et al.* 1988]. N-acetyl cysteine has been used to decrease the viscosity of mucus in the airways because of its capacity to breakdown sulphide bonds. However one study demonstrated no success in improving lipid absorption with N-acetyl cysteine as adjuvant therapy [Bouquet *et al.* 1988]. George & Mangos [1988] have suggested that PERT may be more effective with colipase. Colipase is secreted by the human pancreas which is needed to clear the lipid-water interface of bile salts and therefore provide binding sites for lipase to triglyceride droplets. At present, no studies have investigated the possible benefits of colipase with PERT.

Most of the pancreatic enzymes currently used are crude extracts of hog pancreas. The use of an acid stable fungal lipase has been introduced in a study of pancreatic steatorrhoea in pancreatectomised dogs [Griffin, Anderson, Farn-don, 1989]. Even though a marked decrease in lipid excretion was observed with fungal lipase in comparison to conventional enzymes, further studies would be necessary to investigate the possible benefits of its application in man.

2.11 PANCREATIC ENZYME REPLACEMENT THERAPY AND THE NITROGEN AND CARBOHYDRATE CONTENT OF FAECES.

From the literature it is clear that PERT has been primarily directed towards the correction of steatorrhoea in patients with CF. Whilst there is some information of the influence of PERT on faecal nitrogen losses our knowledge of faecal carbohydrate with PERT is severely limited.

Reductions in faecal nitrogen losses with PERT have been reported ranging between 1-2 g/d in CF patients with PERT [Beverley *et al.* 1987; George, Miller, Toskes, Tucker, Pinero, 1987; George *et al.* 1990] compared to approximately 6 g/d with no PERT [Mischler *et al.* 1982]. The extent of carbohydrate malabsorption has not been given much attention. In addition to the hydrogen breath test study in CF patients with PERT [Perman & Rosenstein, 1986], Hoffman and colleagues [1987] have measured the amount of hexose carbohydrate in faeces in patients with CF on PERT by applying the method of Ameen & Powell [1985]. As faecal hexose carbohydrate attained more normal levels (ranging from 0.2-1.5 g/d), they suggested that carbohydrates are absorbed well in CF with PERT and that any malabsorbed carbohydrate could have been fermented by colonic bacterial microflora. It should be noted that whilst this approach would appear to measure the hexose carbohydrate content of stools, no allowance is made for the dietary residue from NSP or starches which may make some contribution towards faecal mass or other possible sources of endogenous carbohydrate from the colon itself such as the glycoprotein component of mucus. It has also been implied however, that colonic fermentation of carbohydrate may allow the opportunity to recover at least in part some of the malabsorbed starches and sugars suggesting that there is the potential capacity for energy to be salvaged. The possibility that the colon could play an important role in carbohydrate malabsorption by salvaging carbohydrate has been demonstrated in another study of patients with jejunioileal bypass using an ileal perfusion technique [Bond *et al.* 1980]. Between 29-84% of a 50g dose of [^{14}C] sucrose failed to be absorbed in the small intestine. Less than half of the [^{14}C] sucrose not absorbed in the small intestine however, appeared in the faeces.

2.11.1 ANTIBIOTIC THERAPY AND OTHER DRUGS.

Most of the literature has concentrated upon the influence of antibiotic drugs on bacterial metabolism such as SCFA in faeces and mucin breakdown [Hoverstad, Carlstadt-Duke, Lingraas, Norin, Saxerholt, Steinbakk, Midvelt, 1986a; Carlstedt-Duke, Hoverstad, Lingaas, Norin, Saxerholt, Steinbakk, Midvelt, 1986]. However our understanding of the effect of antibiotics on the colonic microflora in relation to their contribution to faecal mass and energy is not known in both normal individuals and in disease. It is thought that under normal circumstances SCFA may be absorbed in the large intestine as a result of bacterial fermentation and may contribute towards the energy supply. However it is unclear what effect antibiotics may have on the metabolic activity of the colonic bacteria during the therapeutic management of disease. A number of antibiotics are used in the clinical management of chest infections in CF and have been one of the major developments in reducing morbidity and mortality in CF [Kohler & Rolles, 1986]. Whilst the extent to which SCFA may contribute towards energy supply in cystic fibrosis or any other diseases associated with maldigestion and/or malabsorption is not known, it can be speculated whether antibiotics may suppress colonic bacteria activity and thereby reduce the potential capacity of the recovery of energy through SCFA absorption.

Evidence from animal studies show that microbial activity in the digestive tract could be suppressed in pigs with antibiotics resulting in a reduction in the amount of lipid in faeces which could be attributed to bacteria [Eggum, Anderson, Rotenberg, 1982]. It was implied that the suppression of this bacterial activity may also reduce bacterial lipid synthesis. Thus it would seem that antibiotics may reduce the faecal excretion of lipids through their ability to reduce the contribution made by the bacteria within faeces. It is known that drugs such as sulphaguanidine and terramycin cause a decrease in faecal lipids [Hendry, 1960], yet whether they have a direct effect on bacterial lipid is unclear.

In a long term Norwegian-Swedish collaborative study, the effect of several antimicrobials upon microflora associated characteristics has been investigated in healthy subjects [Hoverstad *et al.* 1986a; Hoverstad, Carlstadt-Duke, Lingraas, Norin, Saxerholt, Steinbaak, Midvelt, 1986b]. The effect of a number of antibiotics on faecal excretion of SCFA were demonstrated such as clindamycin, ampicillin and vancomycin. It would appear that some but not all antimicrobials induce changes in faecal SCFA but this may also depend on the concentration

of the drug. The effect of an antibiotic may also depend on the 'antimicrobial spectrum' as some antibiotics are specific for certain bacteria [Hoverstad *et al.* 1986a]. In addition these workers have also shown that some of the antibiotics result in disturbances in the breakdown of intestinal mucin by colonic bacteria [Carlstedt-Duke *et al.* 1986]. Consequently it could be that there were alterations in the relative proportion of mucin and bacteria within faeces.

2.11.2 SUMMARY.

In summary, there would appear to have been relatively few studies which have specifically measured faecal energy losses in diseases associated with maldigestion and/or malabsorption and during therapeutic management. Whilst the origins of faecal energy and the contribution made by each of the components of faecal energy is poorly understood, many studies have applied the concept of apparent digestibility as dietary intake minus faecal losses to estimate the extent of lipid, nitrogen or carbohydrate malabsorption. The presumption is still made that increased faecal losses are derived directly from diet and are simply undigested residue. Further studies are clearly required if we are to understand how imbalances in the availability of energy from the diet may contribute towards a delay in normal growth or cause weight loss and how the treatment of the problems associated with gastro-intestinal dysfunction in disease could be improved.

2.12 CONCLUSIONS.

In conclusion it is clear that our knowledge of a number of issues raised in this review of the literature is limited. There are several questions which remain outstanding and require further investigation, for example,

- What are the origins of energy within the stool and the relative contribution made by maldigested and/or malabsorbed dietary residue, endogenous secretions and cellular debris, colonic bacteria to faecal energy?
- What are the proportions of faecal energy which could be attributable to lipid, protein and carbohydrate?
- How do each of the components of faecal energy alter with diet (in particular with NSP), disease (cystic fibrosis, pancreatitis, crohn's disease), drugs (in particular antibiotics), pancreatic enzyme replacement therapy?
- Does the energy within the stool originate from maldigested dietary residue (lipid, protein, carbohydrate), or dietary residue that has been digested but not absorbed?
- What is the role of colonic bacterial activity in relation to nitrogen and energy metabolism within the body? How may this alter with changes in diet, disease and during therapeutic management?

2.13 AIMS OF RESEARCH.

From our present knowledge it would appear that there are a number of specific questions which remain outstanding. In order to understand how much energy could be derived from the diet, further consideration should be directed towards determining the extent to which faecal energy losses may limit the availability of energy from the diet. In an attempt to provide answers to these questions the aim of this thesis was specifically focused upon the following:

A. In health:

- i. The magnitude and variability of daily energy content of stools.
- ii. The relative proportions of energy within the stool derived from lipid, protein and carbohydrate.
- iii. The extent to which each of the three major faecal components:
 - maldigested and/or malabsorbed dietary residue,
 - endogenous losses and cellular debris,
 - colonic bacterial microflora,may contribute towards faecal energy.

B. The extent to which normal growth in childhood, alterations in dietary non-starch polysaccharide intake and gastro-intestinal disease associated with maldigestion and/or malabsorption may influence i, ii and iii.

Table 2.1 Summary of studies which have measured faecal lipid and nitrogen losses in diseases associated with maldigestion and/or malabsorption.

DISEASE	LIPID (g/d)	NITROGEN (g/d)	REFERENCE
Chronic relapsing pancreatitis Coeliac disease	21.6 4.3 - 5.3	3.2 0.7- 1.5	[Dornberger <i>et al.</i> 1948]
Cystic Fibrosis	20.8 - 27.2	1.1 - 4.3	[Chung <i>et al.</i> 1951]
Chronic pancreatitis Cancer of the pancreas Pancreatectomy	5.0 - 125.0 17.0 - 27.0 13.0 - 28.0	-	[Raffensperger, D'Agostino Manfredo, Ramirez, Brooks, O'Neill, 1967]
Malabsorption	10.0 - 27.0	-	[Crowe & Blackburn 1956]
Pancreatic disease	11.2	-	[Pimparker, Tulskey, Kalser, Bockus, 1960]
Malabsorption	7.0 - 29.0	1.1 - 5.6	[Pinter & McLean 1968]
Exocrine pancreatic insufficiency	6.0 - > 25	0.3 - > 2.5	[Schmerling <i>et al.</i> 1970]
Chronic pancreatitis Coeliac disease Crohn's disease Pancreatic disease	68.5 64.8 74.7 167.0	-	[Bo-Linn & Fordtran 1984]
Malabsorption	1.0 - 50.0	-	[Pedersen & Halgreen 1984]

Figure 2.1 Inter-relationship between the terms used to describe the availability of dietary energy from food.

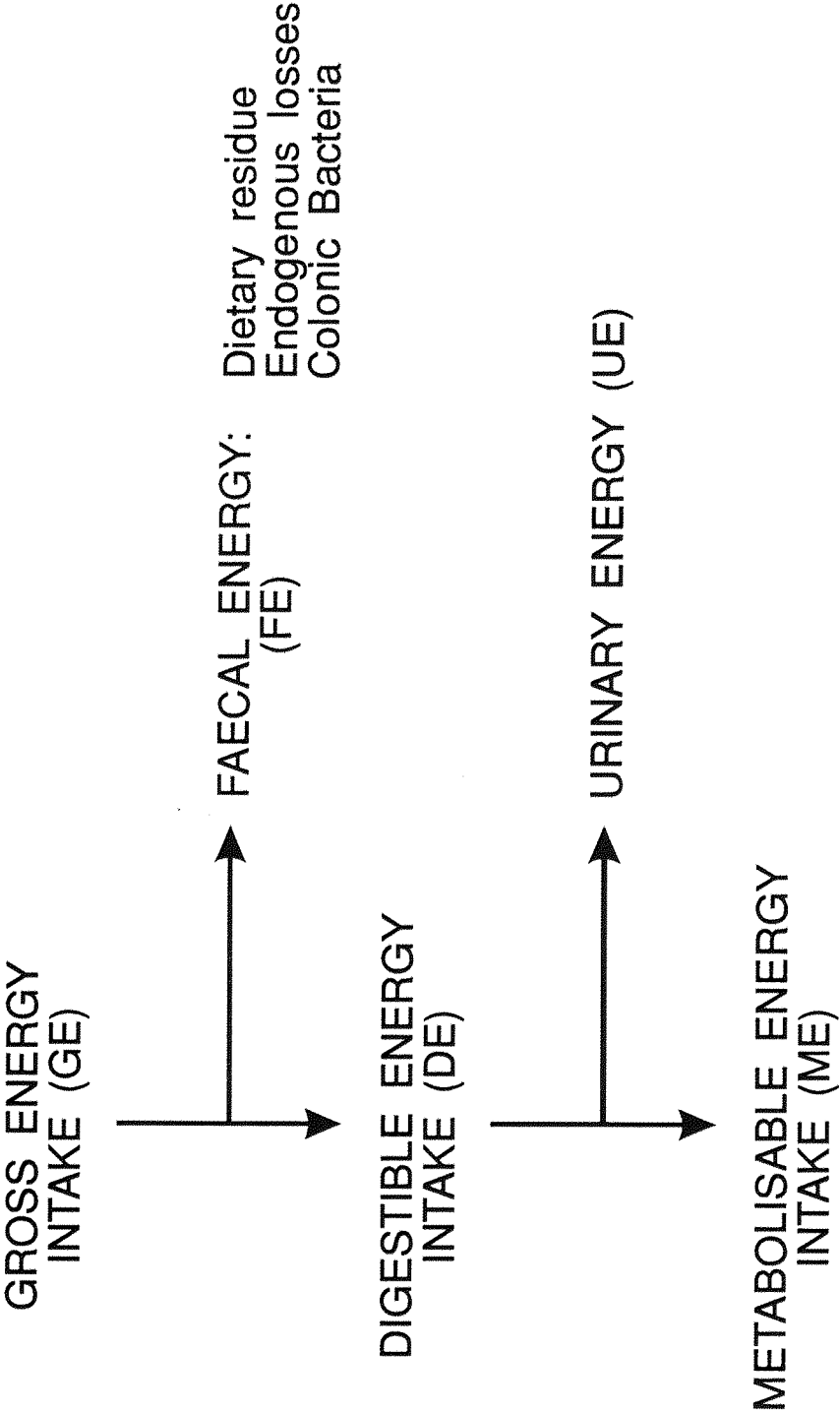
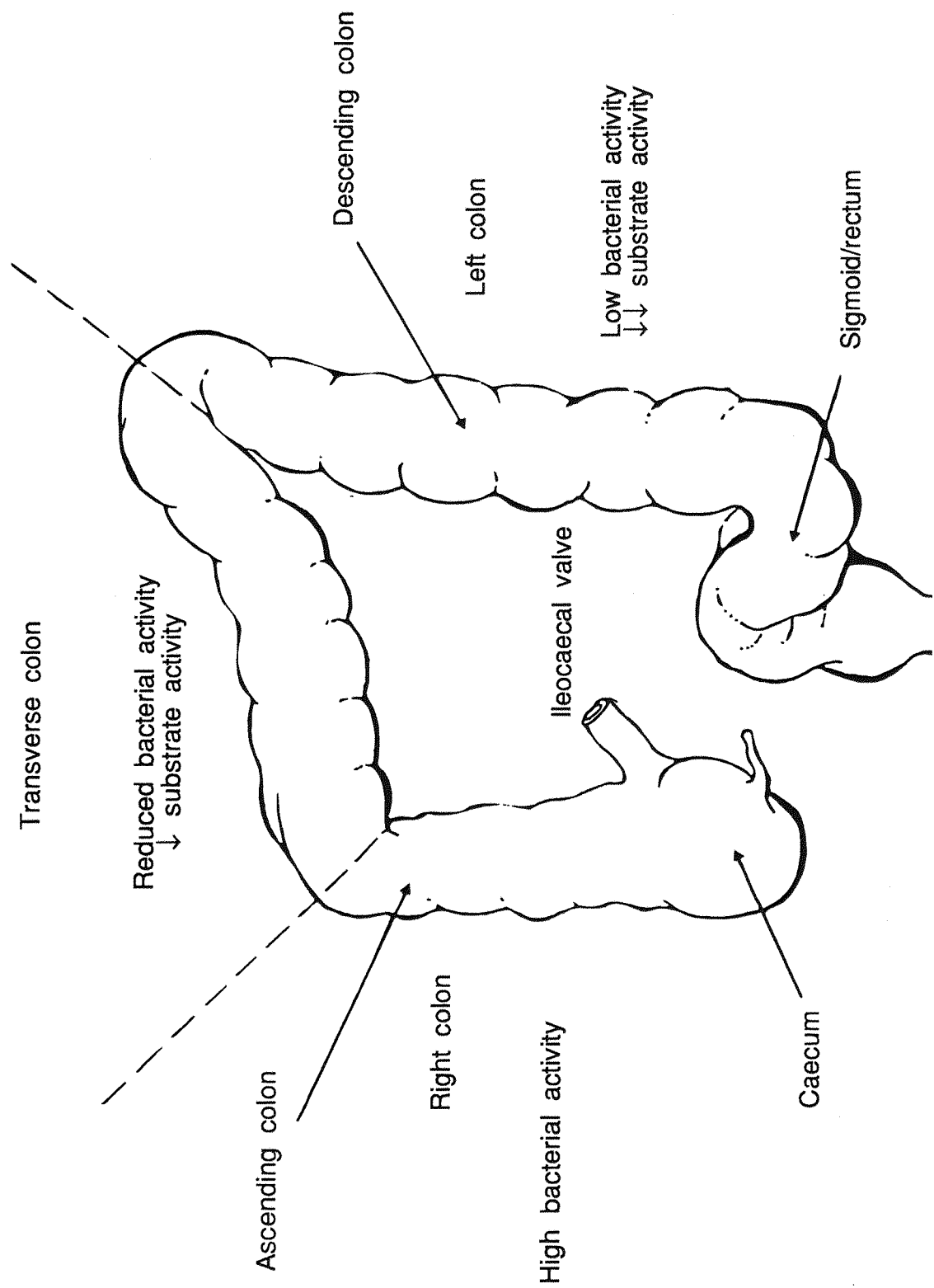


Figure 2.2 The relationship between the terms used to describe the components of dietary 'fibre' developed from different analytical procedures. Adapted from Asp & Johansson, 1984.

DIETARY FIBRE					
RESISTANT STARCH	LIGNIN	NON-STARCH POLYSACCHARIDES NSP			
		CELLULOSE	NON-CELLULOSIC POLYSACCHARIDES NCP		
			HEMI CELLULOSE	PECTIN	OTHER POLYSACCHARIDES
		INSOLUBLE COMPONENT		SOLUBLE COMPONENT	

Figure 2.3 Different activities of bacterial microflora in the large intestine.
Adapted from Cummings & MacFarlane, 1991.



CHAPTER 3. METHODOLOGY.

3.1 INTRODUCTION.

The methodology presented in this thesis is divided into two parts. The first part describes the methodology involved in the measurement of dietary intake, the collection and preparation of stools for analysis and the energy, lipid and nitrogen content of stools. The bacterial content of dried stool and the amount of energy, lipid and nitrogen within bacteria is also reported. The coefficient of variation was employed as a measure of the reproducibility of the assay given for repeated aliquots of homogenised stool samples collected from a normal healthy adult unless otherwise indicated. As these methods have been previously reported in full by other workers, only a brief outline will be presented with the inclusion of any modifications to the techniques.

The second part of this chapter presents the evidence to validate the methodology employed. Attention is directed towards the following areas:

- i. The effects of a delay in freezing and the freeze/thawing process on the measurement of faecal energy.
- ii. The measurement of the energy, lipid and nitrogen content of *Escherichia coli*.
- iii. Justification of the lipid assay employed to measure total faecal lipid in the stools of patients with cystic fibrosis.

3.2 STANDARD LABORATORY TECHNIQUES.

3.2.1 DIETARY ENERGY INTAKES.

To assess daily energy intake for each of the experimental studies, food consumption was determined by the weighed food intake technique in the standard manner described by Marr [1971]. The methods, validity used and the variability associated with this technique have been extensively reviewed [Marr, 1971; Bingham, 1987]. The weight of each food item was determined to the nearest 2g for food weighing between 0-130g, and 5g for weights of food greater than 130g on pre-calibrated electronic scales (Hanson, UK). Apart from the study

presented in chapter 4, weighed food intake was recorded over a period of seven days in each of the studies. This is in accordance with the evidence presented by several investigators where it has been demonstrated that a seven day food record would be a sufficient period of time to estimate energy and energy-yielding nutrients in children and adults [Liu, Stamler, Dyer, McKeever, McKeever, 1978; Bingham, McNeil, Cummings, 1981; Marr & Heady, 1986; Bingham, 1987; Nelson, Black, Cole, 1989; Miller, Kimes, Hui, Andon, Johnston, 1991]. All the subjects were carefully instructed in the use of scales and the recording of food intake prior to each study. It was also emphasised that normal dietary habits must be continued throughout the study period, and not to change usual menus or amounts of food whilst keeping the record. After completion of the record, subjects were interviewed to ensure there were no omissions or errors.

Obtaining accurate dietary information involves many difficulties. Dietary assessments, no matter how carefully performed, are dependent upon information provided by the patient or subject. There has been recent concern over the extent to which subjects may under- or overestimate records of weighed food intake [Bingham, 1987]. Whilst there could be doubt over the accuracy of a report from for example, an obese or anorectic patient, it is unlikely that there would have been wide under- or overestimations for weighed food intakes within each of the subject groups examined in the present studies. It should be borne in mind that all of the subjects who participated in the studies were volunteers and were closely monitored and given support throughout and following the study period. For the assessment of dietary intake within the children studied, all of the parents were willing to give assistance.

It is recognised that there are biological markers to provide independent validity checks of dietary intake such as the 24 hour urinary nitrogen excretion as an estimate of protein intake [Isaksson, 1980; Bingham & Cummings, 1985]. Within the constraints of the present studies however, it was not possible to validate dietary intake by markers.

The diaries were coded according to the McCance and Widdowson Food Tables [Paul & Southgate, 1978] with additional supplements to the food tables [Tan, Wenlock, Buss, 1985; Holland, Unwin, Buss, 1988; 1989]. Composition of foods not provided in the tables such as processed foods were derived from information provided by manufacturers. Weighed food intake was analyzed using a computerised food composition database [Microdiet, University of Salford].

Gross energy intakes were estimated by multiplying the intakes of lipid (39.3 kJ/g), protein (as N x 6.25; 23.6 kJ/g), carbohydrate (17.5 kJ/g) and dietary NSP (17.5 kJ/g) by their respective heats of combustion values [Merrill & Watt, 1955].

Similarly metabolisable energy intake was calculated by multiplying the intakes of lipid (38 kJ/g), protein (as N x 6.25; 17 kJ/g), carbohydrate (16 kJ/g) by modified Atwater factors [Paul & Southgate, 1978]. As outlined in section 2.1 dietary NSP is assumed to be not metabolised by this approach.

3.2.2 COLLECTION AND PREPARATION OF STOOLS FOR ANALYSIS.

Faeces were collected in each study (apart from the study presented in chapter 4) for a period of 3 days between carmine markers (0.8g carmine). Carmine markers were employed to improve the accuracy of collection since irregularities in bowel habits make stool collections difficult to interpret over a period of time. In principle, intermittent markers such as carmine allow a collection to be made from the appearance of one marker to the appearance of a second marker, such that the collection represents faeces derived from the period between the intake of the two markers. However this approach has a number of practical difficulties. The marker dye is not always clearly visible and is not always discretely localised with the tendency for the dye to be spread out in faeces over several days. Whilst their usefulness in delineating the period of collection is open to doubt [Rose, 1964], this could be minimised by precise timing of the intake of the markers. Whilst the accuracy of faecal collections could be improved by other faecal markers currently available such as radio-opaque pellets, carmine markers were employed at the time of the studies because they provided the most accessible approach to mark the period of faecal collection.

Stools were collected into polyethylene bags and immediately frozen. Pooled faecal samples from the three day collections were thawed, weighed and homogenised with a known quantity of distilled water for 1-2 minutes. Portions of between 30-40g of stool homogenate were freeze-dried (Genevac, UK) to a constant weight. The length of time required to freeze-dry four 30-40g portions of stool homogenate ranged between 16-20 hours depending upon the amount of water within the stool homogenate. Faecal water was calculated from faecal weight allowing for the inclusion of water added during homogenisation and loss in weight during freeze-drying. Therefore faecal dry weight was determined as faecal weight - faecal water. The coefficient of variation for the percentage water

content of faeces and dry material within faeces measured from repeated aliquots of freeze-dried stool homogenate from a given stool sample averaged 0.6% and 8.6% respectively.

3.2.3 FAECAL ENERGY.

The energy content of faeces was measured by ballistic bomb calorimetry on samples of freeze-dried faeces [Miller & Payne, 1959])(Gallenkamp, UK). Benzoic acid, thermochemical grade was the reference source for the calibration of the instrument. Samples of 0.25g freeze-dried stool were measured in triplicate. The coefficient of variation for the measurement of energy content from tablets of the benzoic acid standard (0.19g per tablet) standard averaged 4%. The coefficient of variation from repeated aliquots of freeze-dried stool from a given stool sample averaged 8%. The coefficient of variation for 10 independently freeze-dried portions of stool from the same stool sample averaged 7%.

3.2.4 FAECAL LIPID.

The Gompertz & Sammons [1963] modification of the method of Van de Kamer and colleagues [1949] was employed to measure the lipid content of wet homogenised faeces. Stool homogenate was saponified with 5% potassium hydroxide in methanol and acidified with 33% hydrochloric acid. Fatty acids were extracted three times with petroleum ether and determined by titration with N/10 alcoholic sodium hydroxide. The fatty acids were calculated as stearic acid which is a fatty acid of representative molecular weight. All stool samples in the present studies were measured in triplicate using 20g faecal homogenate from normal individuals and 10g faecal homogenate in patients with cystic fibrosis owing to the high lipid content in stool. There is no standard heat of combustion value for the energy derived from the lipid content of stool. The amount of energy derived from lipid within faeces was calculated by multiplying faecal lipid content by the standard heat of combustion value of 39.3 kJ/g that is employed in determining the gross energy value of lipid within food. A similar approach has been employed in previous studies [Cammidge, 1914; Heymsfield *et al.* 1981; Kien, Sumners, Stetina, Heimler, Grausz, 1982]. SMA Gold Cap milk (3.5 g/100g lipid; Wyeth Nutrition, UK) was employed as a reference standard for the assay. The coefficient of variation for 10 samples of milk from a single container of SMA

Gold Cap milk averaged 7%. The coefficient of variation for lipid content of stool from repeated aliquots of wet homogenised stool from a given stool sample averaged 8%.

3.2.5 FAECAL NITROGEN.

Faecal nitrogen was analysed from 1g portions of wet faecal homogenate in triplicate using Tecator Kjeldahl apparatus (Tecator Ltd, UK). As there is no standard factor to calculate the protein content of faeces from nitrogen, the amount of protein within stools was derived by multiplying the value for faecal nitrogen by 6.25. In other words this assumes that the protein content of faeces is 16% nitrogen. This average factor of 6.25 is normally applied to determine the protein content of food although the portion of nitrogen in protein compounds varies considerably [Atwater & Bryant, 1900]. There is no standard heat of combustion value for the energy derived from the protein content of stool. The amount of energy derived from faecal protein was estimated by multiplying faecal protein content by the standard heat of combustion value of 23.6 kJ/g that is employed in determining the gross energy value of protein within food. A similar approach has been employed in previous studies [Cambridge, 1914; Heymsfield *et al.* 1981; Kien *et al.* 1982]. An ammonium sulphate solution (0.7 g nitrogen/100 mls) was employed as a reference standard for the assay. The coefficient of variation for 10 aliquots of the ammonium sulphate solution averaged 2%. The coefficient of variation for nitrogen content of stool from repeated aliquots of wet homogenised stool from a given stool sample averaged 11%.

3.2.6 FAECAL BACTERIAL MASS.

The bacterial content of dried faeces was determined by repeated fractional centrifugation using a modification of the technique developed by Stephen & Cummings [1980a]. An outline of this technique for the fractionation of faeces into its main components large plant material (LPM); small plant material or crystals (SPM); bacterial fraction is given in figure 3.1. A freeze-dried faecal sample of 0.5g was resuspended in formylsaline and 10% sodium lauryl sulphate. Faecal samples were stomached and filtered three times through muslin, and faecal fractions were transferred into 100ml centrifuge tubes and centrifuged at 30,000g for 30 mins (MSE, Hispin 21, UK). The amount of water-soluble material, within freeze-dried faeces was measured using 0.5g freeze-dried faecal samples in accordance with the procedure given by Stephen & Cummings

[1980a]. The weight difference between the freeze-dried pellet and the original weight of material fractionated was equal to the weight of the water-soluble components of faeces. Each of the fractions were freeze-dried (Genevac, UK) to a constant weight. The length of time required for freeze-drying each of fractions ranged between 10-16 hours depending upon the amount of water within each fraction. The coefficient of variation for measurements of the proportion of faecal bacterial mass in repeated samples of freeze-dried stool from a given stool sample averaged 15%. The proportion of daily faecal material that was attributable to SPM, LPM, bacteria or water soluble material is given in table 3.1 for each of the study groups.

The amount of bacteria within the bacterial fraction was confirmed by phase contrast microscopy using a haemocytometer (Weber Scientific International, UK) to enable microscopic counting of the fractions obtained by this method. Within fractionated stools from each of the study groups, no less than 99% of total faecal bacteria were present within the bacterial fraction, between 0.02-0.25% of total faecal bacteria were present in the large plant material fraction and only negligible counts of bacteria were present in the small plant material fraction.

3.2.7 ENERGY, LIPID AND NITROGEN WITHIN FAECAL BACTERIA.

A portion of the dried bacterial mass from stool samples within each group of subjects were pooled to provide a representative sample of faecal bacteria. The energy per gram of bacteria from pooled samples was determined by bomb calorimetry in an attempt to estimate the proportion of faecal energy which could be attributed to faecal bacteria. The coefficient of variation for the energy within repeated samples of pooled faecal bacteria averaged 14%. Lipid per gram of pooled faecal bacteria (in duplicate) was determined using a modification of the technique of Folch and colleagues [Folch, Lees, Sloane Stanley, 1957] described by Bauchart and colleagues [1984]. Total lipids were extracted from freeze-dried samples of pooled faecal bacteria in chloroform-methanol (2:1, v/v) according to the method of Folch and coworkers [1957]. A further extraction was subsequently employed in hexane-ethanol-hydrochloric acid (25:10:10, by vol) and total lipids were determined gravimetrically. The coefficient of variation for the lipid content within repeated samples of pooled faecal bacteria averaged 16%.

The amount of nitrogen per gram of pooled faecal bacteria (in duplicate) was determined using Tecator Kjeldahl apparatus. The coefficient of variation for the nitrogen content within repeated samples of pooled faecal bacteria averaged 11%. It is noted that only for the study presented in chapter 7, the energy, lipid and nitrogen per gram of stool bacteria were determined not only within pooled samples of faecal bacteria for each diet but also freeze-dried samples of bacteria within stool samples from each subject.

3.2.8 DATA PROCESSING AND ANALYSIS.

The collection of data for analysis was performed both manually and by the use of computerised data packages. These consisted of a statistical package developed within the Department of Human Nutrition or a major statistical package (Minitab, 1982) run on a personal computer (PS2, IBM).

The results are presented as median values and ranges as it could not be assumed that the data conformed to a normal distribution owing to the small number of subjects who participated in the studies. The tests were utilised to describe, relate and compare data. The principle tests used were the Wilcoxon rank sum tests for unpaired data (two-sample test) and signed matched pairs test (two-tailed), Pearson product moment correlation coefficient and simple linear regression. Statistical significance was assumed at the 5% level. Where comparative statistical examination could not be utilised, a descriptive interpretation of the results is presented.

3.2.9 COMMENTS.

The errors associated with the methods employed were no greater than 15%. Whilst this variation appears to be high, a low coefficient of variation would not have been expected owing to the difficulties involved in the analysis of faecal constituents because of the solid consistency and heterogeneous nature of faeces. Consequently one of the major problems encountered in the analysis of faecal material is the ability to obtain a representative sample of dried or wet faeces. In an attempt to minimise this possible source of error, faeces were homogenised with distilled water prior to freeze-drying.

The techniques employed to measure the faecal energy, lipid and nitrogen content of faeces were standard methods which have been widely employed in previous studies. However it has been shown that the Kjeldahl method of nitrogen analysis does not allow for the presence of nitrates within faeces [Kurser & Calloway, 1981]. This would suggest that the measurements faecal nitrogen could be underestimated. Whilst the net synthesis of nitrate is quite variable, it has been indicated that excess nitrate excretion may increase total nitrogen excretion by 5% [Kurser & Calloway, 1981]. Therefore the potential contribution made by faecal nitrate to total faecal nitrogen should not be overlooked.

There have been relatively few studies which have applied the technique of isolating bacteria by a differential fractionation technique in comparison to the methods used to determine the energy, lipid and nitrogen content of stools. The length of time required to perform the measurements of faecal or bacterial lipid, nitrogen and energy was relatively modest in comparison to the time required to fractionate faecal samples owing to the relatively long and repeated centrifugation of samples. The time required to fractionate four faecal samples was approximately eight hours.

From the studies which have employed this technique, there has been some concern over the extent to which the addition of water may influence the measurements for bacteria counts and the gravimetric yield of each of the fractions [Cabotaje *et al.* 1990; Forsum *et al.* 1990]. No difference in bacterial counting could be demonstrated with and without the addition of water to faeces prior to freeze-drying [Forsum *et al.* 1990]. However a reduction in the size of the bacterial fraction was observed when faeces was homogenised prior to freeze-drying in comparison to faeces which were freeze-dried without prior homogenisation but this difference was only 7% [Cabotaje *et al.* 1990].

It is important that the purity of the bacterial fraction could be confirmed and that bacteria was not separated into the other fractions. Microscopic counting provided evidence to indicate that no less than 99% of faecal bacteria was obtained in the bacterial fraction. Negligible amounts of bacteria were observed in the small plant fraction and microscopic counting of the large plant fraction showed that it contained between 0.02-0.25% of total faecal bacteria. It is noted that the purity of the bacterial fraction could also be demonstrated by Gram and plant stains, scanning electron microscopy, and by sugar analysis of the fractions [Stephen & Cummings, 1980a, Cabotaje *et al.* 1990]. Whilst none of

these procedures were performed in the present study, it appears that microscopic counting alone could confirm that the bacteria fraction was a relatively complete and pure isolate of faecal bacteria.

It is shown in table 3.1 that the contribution of each of the four fractions accounted for 94.8% to 102.7% of the total faecal dry weight over the study groups. It is noted that for the NSP-free diet, a recovery above 100% was observed when the amount of total solids obtained in each of the fractions were added together. Values greater than 100% are thought to be attributed to the recovery of buffer solutes in the large plant fraction [Forsum *et al.* 1990].

The gravimetric results of size of the fractions from the present study differed from those of Stephen & Cummings [1980a] where 55% of faecal solids could be attributed to bacteria, 17% large plant material and 2.2% as small plant material. This difference could be accounted in part to the homogenisation of faeces with water prior to freeze-drying in the present study which could reduce the size of plant material. This may explain why a greater proportion of faeces could be attributed to the small plant fraction in the present study in comparison to the results of Stephen & Cummings [1980a]. Differences could also be attributed to the nature and quantity of dietary NSP intakes as some types of dietary NSP, particularly soluble NSP may stimulate colonic bacterial activity and alter the proportion of faeces which could be attributed to bacteria. The faecal samples collected in the present study also contained a substantial proportion of soluble material. It is thought that this component consists of water-soluble unfermented NSP [Forsum *et al.* 1990] and leached bacterial nitrogen [Stephen & Cummings, 1980a]. Other leached bacterial components such as lipid and carbohydrate in addition to other material of endogenous origin such as mucus secretions and cellular debris may also be present within the water soluble fraction.

Whilst the size of fractions in the present study were different to those reported by Stephen & Cummings [1980a], they were of a similar magnitude to those reported by Forsum and coworkers [1990]. In the present study and that reported by Forsum and coworkers [1990], faecal samples were homogenised prior to freeze-drying which could account for the similar findings.

Recently it has been thought that differences in the yield of the bacterial fraction could also be attributed to the ability of different sizes of smaller mesh screens to separate small plant material from bacteria [Cabotaje *et al.* 1990]. Whilst the aperture size of the muslin used in the present study during the initial step of the procedure to fractionate faeces was not measured, evidence from the bacterial counting of each of the major fractions would suggest that differences in the size of the bacterial fraction was not attributed to the presence of bacteria within other fractions.

The coefficient of variation observed for bacterial counting observed in the present study of bacterial counts from portions of the same faecal bacterial sample was 25%. This apparently high coefficient of variation of bacterial counts is supported in the studies of Stephen & Cummings [1980a] and Forsum and coworkers [1990] which were 26.9% and between 24-34% respectively. In the present study a simple relationship between bacterial numbers and faecal bacterial mass each day could not be demonstrated in healthy children and patients with cystic fibrosis. As shown in Figure 3.2, for a given value for bacterial mass, for example 5g, there is a 50 fold range in faecal bacterial counts in healthy children. It was suggested by Stephen & Cummings [1980a] that the variation in bacterial counts could be attributed to difficulties in taking a representative sample for bacterial counting. The amount of bacteria within dry faeces each day in the present studies, as estimated by microscopic counting were of the order of 10^{13} bacteria/day dry stool (see chapters 5, 6 and 7). These values were greater than those reported by Forsum and coworkers [1990] expressed as counts of bacteria in faeces per day which were of the order of 10^{12} bacteria/day of dry stool. The freeze-drying procedure and detergent used in the fractionation procedure have the potential to disrupt bacterial cells. This may result in an overestimation of bacterial counts as bacterial fragments could be assumed to represent whole bacteria and may account for the difference in the values obtained for bacterial counts between the present study and those reported by Forsum and coworkers [1990]. It would appear that the difficulties involved in counting should be borne in mind and the values obtained interpreted with care.

Given that freeze-drying and the detergent used could cause disruption of bacterial cells and leaching of bacterial contents, this is also believed to result in an underestimation of the nitrogen content of bacteria with some of the bacterial nitrogen appearing in the soluble fraction of faeces [Stephen & Cummings, 1980a]. This may account for the apparently lower values for bacterial

nitrogen content of between 3-5% in comparison to other studies. In addition the values obtained for bacterial lipid and energy content may well also be underestimated due to the problem of ruptured of bacterial cells. Bacterial nitrogen content was approximately 7-8% in mixed populations of rumen bacteria [Smith & McAllan, 1980a], and 8% in pure cultured samples of *E. coli* [Luria, 1960]. Rubner also calculated that 1g of dry bacteria from faeces contained 11% nitrogen. In the study of Stephen & Cummings [1980a] the bacteria nitrogen content was 6%. In addition an underestimation of bacterial nitrogen could be attributed to the method employed to measure bacterial nitrogen. It is thought that the Kjeldahl method of nitrogen analysis reveals only about 80% of cellular nitrogen within bacteria as this technique measures nitrogen only in the amino, imino and amide groups [Luria, 1960]. Previous estimates of the bacterial lipid content is thought to be between 12-20% for rumen bacteria [Czerkawski, 1976] and in *E. coli*, 10-15% of the cell dry weight could be attributed to lipids [Luria, 1960]. In the present study, the lipid content of bacteria ranged between 7-20%. Apart from the early study of Sperry [1929] in dogs who measured the lipid content of faecal bacteria by a similar fractionation procedure, there have been no recent reports of the lipid content of human faecal bacteria using this approach.

Even though the actual values for bacterial nitrogen or lipid could be underestimations due to disruption of bacterial cells, the conclusions of the studies presented would not be altered as comparisons were made between groups of subjects which would suggest that underestimations could be assumed to be consistent.

3.3 VALIDATION OF THE METHODOLOGY.

Having considered the methods and the sources of errors that could be introduced during the analyses, this section presents three procedures which attempted to justify some of the methods used in the present studies.

3.3.1 EFFECT OF A DELAY IN FREEZING AND FREEZE/THAWING PROCESS ON THE MEASUREMENT OF FAECAL ENERGY.

Stools were preserved by freezing at -18°C between the collection and analysis of faeces. During the collection of stools at home or in hospital by the subjects who participated in the studies, stools were kept cool in cool boxes until they

could be transferred and stored in a freezer. It is thought that once faeces are passed from the body, there is a change in the chemical composition as a result of bacterial activity [Wrong *et al.* 1981]. In addition it is believed that some energy containing faecal compounds are volatile in the pH range of normal stool and could be lost during sample preparation [Zarling, Ruchim, Makino, 1986]. In the light of this information, this study investigated the effects of a delay between the collection and analysis and the influence of freezing process and thawing on the measurement of faecal energy.

3.3.1.1 Methods.

In an attempt to investigate the effect of storage and freezing, a faecal sample was homogenised with a known amount of distilled water and samples of homogenised faeces were taken for subsequent analysis immediately (time zero), after 24 hrs, 48 hrs and 72 hrs following storage at room temperature and freezing at -18°C . Each of the stool samples were immediately freeze-dried and the energy content of stools were measured by the methods described previously. The results are presented in table 3.2.

3.3.1.2 Results.

There was no difference in the measurements of energy per gram of wet and dry faeces in frozen/thawed stool homogenate after 24, 48 and 72 hours freezing. Similarly, storage of stool homogenate left for 24, 48 and 72 hours at room temperature had no effect on the amount of energy per gram of wet and dry faeces.

3.3.1.3 Conclusion and comments.

In conclusion, it appears that in the event of a delay in freezing stools there would appear to be no measurable effect on the energy content of stools. This would suggest that faecal energy does not change despite bacterial activity which may continue on passing of faeces and even if there is some loss of volatile fatty acids or lactic acid from stools. During the freezing process, it was not surprising that the faecal energy content did not alter because any bacterial activity within faeces would be delayed at a freezing temperature of -18°C .

The freeze-drying process has been shown to have a more significant effect on the energy content of stools through the loss of volatile fatty acids and lactic acid [Zarling *et al.* 1986]. During freeze-drying the energy content of stools was reduced by approximately 9.8% and 25% were lost in normal subjects and in patients with pancreatic exocrine insufficiency respectively. In this study they recommend pretreatment of stool samples with alkali to stabilise volatile fatty acids. However there have been no subsequent studies which have repeated their study to confirm or otherwise support their observation.

3.3.2 THE MEASUREMENT OF THE ENERGY, LIPID AND NITROGEN CONTENT OF A KNOWN BACTERIAL ORGANISM, ESCHERICHIA COLI FOR COMPARISON AGAINST THE VALUES OBTAINED FOR STOOL BACTERIA.

Whilst the purity of the bacterial fraction could be confirmed from microscopic counting, a different approach was also attempted to support this finding. The energy, lipid and nitrogen content of faecal bacteria isolated by fractional centrifugation were compared against the values obtained for the energy, lipid and nitrogen contents of a pure cultured sample of *E.coli*.

3.3.2.1 Methods.

A sample of cultured *E.coli* was initially transferred to 100ml centrifuge tubes and spun at 10,000 RPM for approximately 30 mins. The supernatant was discarded and the extracted sample of *E.coli* was freeze-dried for approximately 10 hours. The energy, lipid and nitrogen content of *E.coli* was determined by applying the methods used to determine the energy, lipid and nitrogen content of isolated faecal bacteria. The amounts of energy, lipid and nitrogen per gram of bacteria were 15.0 kJ, 0.06g and 0.10g respectively.

3.3.2.2 Comments and conclusions.

Information of the lipid, nitrogen and energy content of bacterial cells is limited with much of the available data derived from pure cultures of bacteria. As described in section 3.2.9, the nitrogen and lipid content of faecal bacteria in the present studies ranged between 3-5% and 7-20% respectively. The energy content of faecal bacteria ranged between 18-24 kJ/g.

The amount of energy per gram of *E.coli* appears to be at the lower end of the values obtained for faecal bacteria. In addition the lipid content of *E. coli* was also smaller in comparison to faecal bacteria which may account for the lowered energy values observed in *E. coli*. However it appears that the nitrogen content is greater within *E. coli*. than for faecal bacteria. The use of the detergent in the fractionation process together with freeze-drying is one factor that could cause rupturing of the bacterial cells and result in an underestimation of the values for faecal bacterial nitrogen. The difference in nitrogen content between *E. coli* and faecal bacteria could be attributed to the use of detergent which had not been used during the preparation of *E. coli* for analysis. It could be that the detergent rather than freeze-drying had caused the possible rupturing of faecal bacterial cells as the freeze-drying process had been employed during the preparation for both samples of faecal bacteria and *E. coli*.

Luria [1960] has shown that the lipid and nitrogen content of *E. coli* is 10-15% and 8% respectively for pure cultures. This value for bacterial nitrogen within *E. coli* is similar to those reported for faecal bacteria in the present studies. The amount of lipid within faecal bacteria, however was at the lower end of the range for faecal lipid content reported for *E. coli* by Luria [1960]. This difference in faecal lipid content could be attributed in part to differences in the methods employed. Alternatively it could be that the conditions used to grow *E. coli* were different as it was shown that lipid may accumulate in cells with high concentrations of acetate [Luria, 1960].

The bacterium *E. coli* represents only one of many types of bacteria present within faeces [Finegold *et al.* 1983]. Therefore it should be borne in mind that the values for the energy, lipid and nitrogen contents of faecal bacteria from the present studies represent those from a range of different bacteria found in faeces. However the measurements obtained for energy, lipid and nitrogen for faecal bacteria appear to be of a similar magnitude to those for *E. coli* which would provide further confirmation that the isolated bacterial fraction consisted of bacteria and was not contaminated with other faecal components.

3.3.3 JUSTIFICATION OF THE LIPID ASSAY EMPLOYED TO MEASURE TOTAL FAECAL LIPID IN THE STOOLS OF PATIENTS WITH CYSTIC FIBROSIS.

3.3.3.1 Introduction.

The Gompertz & Sammons [1963] modification of the method developed by Van de kamer [1949] was employed to measure the lipid content of stools in normal adults and healthy children and patients with cystic fibrosis. This titrimetric method has been the most popular approach for the measurement of faecal lipid reflected by its ease and reproducibility [Gravesen, 1964]. However Van de Kamer [1953] and Braddock and coworkers [1968] have shown that this method is unsuitable for the extraction of MCT and medium chain fatty acids quantitatively from faeces which have 8-10 carbon atoms. Under most circumstances in the analysis of faecal lipid, this is of no importance since faeces of normal subjects and patients with steatorrhoea do not contain appreciable quantities of these fatty acids. However MCT supplements have been used in the dietary treatment of patients with maldigestion and/or malabsorption. This is due to their water insolubility, faster hydrolysis by pancreatic lipase and their ability to enter the mucosal cells unhydrolysed and in the subsequent transport of fatty acids which are carried in an albumin complex in the portal blood stream [Leyland, Fosbrooke, Lloyd, Segall, Tamar, Tomkins, Wolff, 1969]. The analysis of the fatty acid pattern in stools of patients with pancreatic insufficiency however revealed that MCT absorption was somewhat impaired [Leyland *et al.* 1969]. Therefore attention was focused upon the possibility that the measurements of faecal lipid within the present group of cystic fibrosis patients could be underestimated using the Gompertz & Sammons [1964] method because medium chain fatty acids and MCT would not be determined by this approach.

3.3.3.2 Does the Gompertz & Sammons method measure MCT or medium chain fatty acids?

Initially a study was undertaken to investigate whether the Van de Kamer modification of Gompertz & Sammons [1964] did not allow the measurement of MCT or medium chain fatty acids. Only 14% of a 1g sample of MCT oil (Scientific Hospital Supplies, UK) could be recovered using the Gompertz & Sammons [1964] method. In agreement with this observation, Leyland and coworkers

[1969] obtained a recovery of 22% when MCT oil was added to a faecal homogenate according to the method of Van de Kamer and using a final gravimetric procedure to estimate faecal lipid.

In conclusion from the poor recovery of MCT oil, it appears that the Gompertz & Sammons [1963] is an inappropriate method to determine MCT or medium chain fatty acids contents of stools.

3.3.3.3 Methods to determine MCT?

Despite the failure of the Gompertz & Sammons method, the possibility that other methods available could measure MCT and medium chain fatty acids was investigated. Initially a spectrophotometric method developed by Massion & McNeely [1973] was attempted but with limited success owing to the high variability in the values obtained and from the poor recovery of MCT oil.

More confidence could be demonstrated with a technique developed by Leyland and coworkers [1969]. This method involves a gravimetric estimation of faecal lipid and involves the extraction of faecal lipid with lipid solvents, separation and evaporation of the solvents, and direct weighing of the extracted lipid. Therefore total lipid including the unsaponifiable fraction is determined and avoids the necessity for assuming an average molecular weight for faecal lipid [Losowsky *et al.* 1974]. The recovery of 1g MCT oil from the Leyland and coworkers [1969] technique was 86%. The reproducibility of the assay for 10 samples of stool homogenate weighing 10g, from the same stool sample was 18%. In the light of this information, the faecal lipid content was measured within the present group of cystic fibrosis patients using this technique.

3.3.3.4 Measurement of the faecal lipid content of stools in cystic fibrosis patients using the Leyland et al. [1969] method in comparison to the Gompertz & Sammons [1963] modification of the Van de kamer method [1949].

The faecal lipid content of stools in twenty patients with cystic fibrosis as described in chapter 6 was measured by the Leyland and coworkers method [1969] using 10g stool homogenate in duplicate. The results obtained are presented in figure 3.3 against the results for faecal lipid content determined by the Gompertz & Sammons [1963] method. Overall there is general agreement

between the values from the two methods. However only four of the twenty patients were taking oral energy supplements which contained MCT. In three of the four patients, the Gompertz and Sammons [1963] gave values greater than the Leyland *et al.* [1969]. This was surprising because the values for total lipid would have been expected to be higher for the Leyland *et al.* [1969] as total lipids were measured. The poor reproducibility of the technique by Leyland and colleagues [1969] may explain in part these apparent differences between the two methods. It is also thought that non-lipid material could be extracted and some of the lipids within faecal samples may not be completely extracted by the lipid solvents [Losowsky *et al.* 1974].

Whilst the liberal consumption of lipid within the diet has been advocated in the past as part of the dietary therapy in cystic fibrosis [Kuo, Huang, Bassett, 1965], the extensive usage of MCT oil is no longer indicated due to its unpalatability and difficulties in its use in cooking. Subsequent analysis of the nature and amounts of fatty acids consumed by each of the four patients taking supplements showed that MCT represented approximately 4-6% of lipid intake. In the remaining patients who were not taking any MCT supplements, 0.01-0.6% of the lipid intake could be accounted by MCT.

In conclusion, on the basis of these results obtained for the faecal lipid content of stools in cystic fibrosis patients by each of the methods, the Gompertz & Sammons [1964] technique was elected as a more appropriate method to measure faecal lipid owing to its ease and reproducibility. Whilst this method does not measure MCT or medium chain fatty acids within stools, this was not important because MCT appear to represent only a relatively small part of the lipid intake in cystic fibrosis patients taking oral energy supplements which contain MCT unless they are otherwise consuming foods which contain increased amounts of MCT or medium chain fatty acids.

Figure 3.1 Outline of the technique to fractionate faeces into its major components. SPM; small plant material, LPM; large plant material.

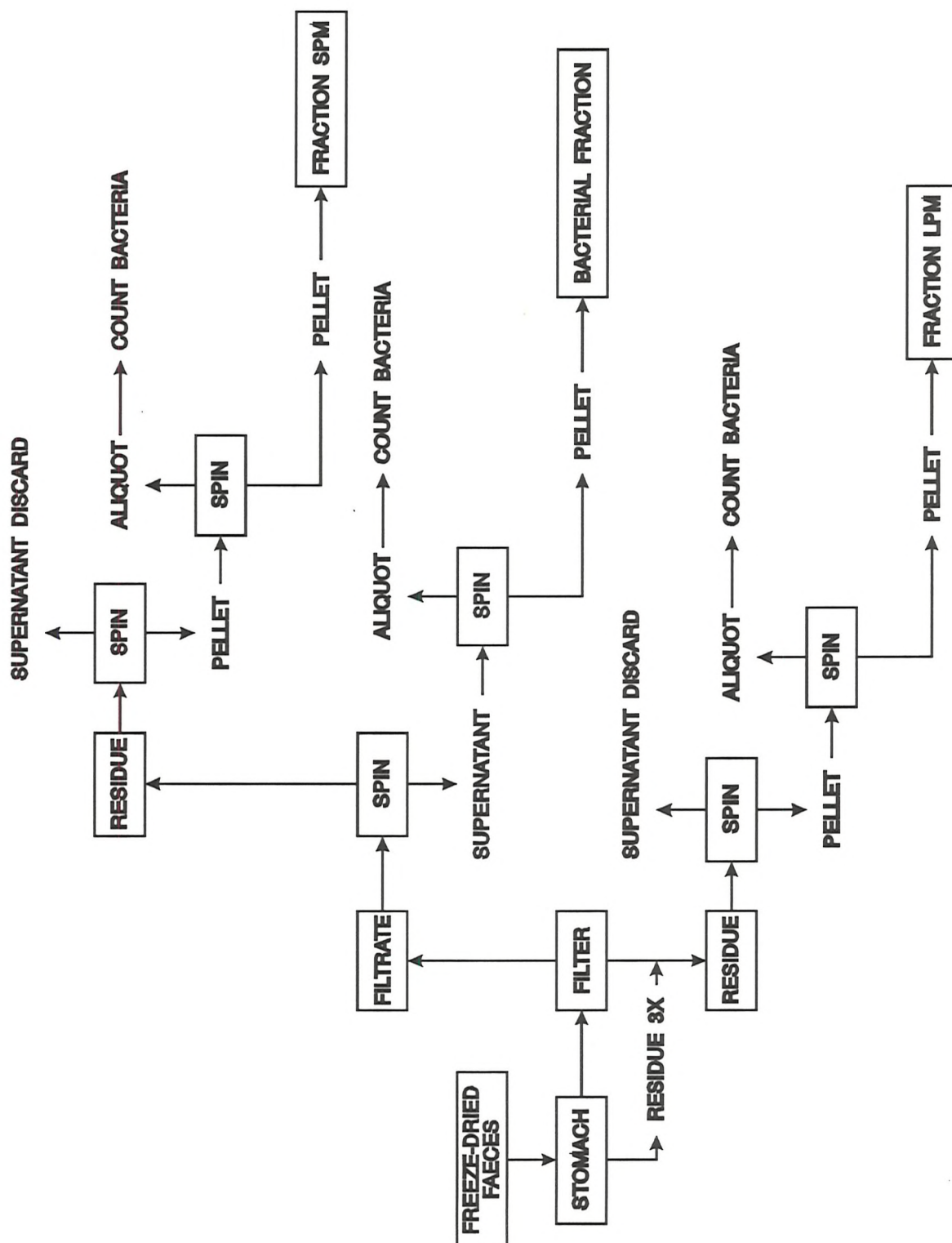


Figure 3.2 Daily faecal bacterial counts (expressed as $\times 10^{13}/d$) versus faecal bacterial mass (g/d) in healthy children and patients with cystic fibrosis.
Correlation coefficients are $R=0.17$ (NS) and $R=0.84$ ($p<0.01$).

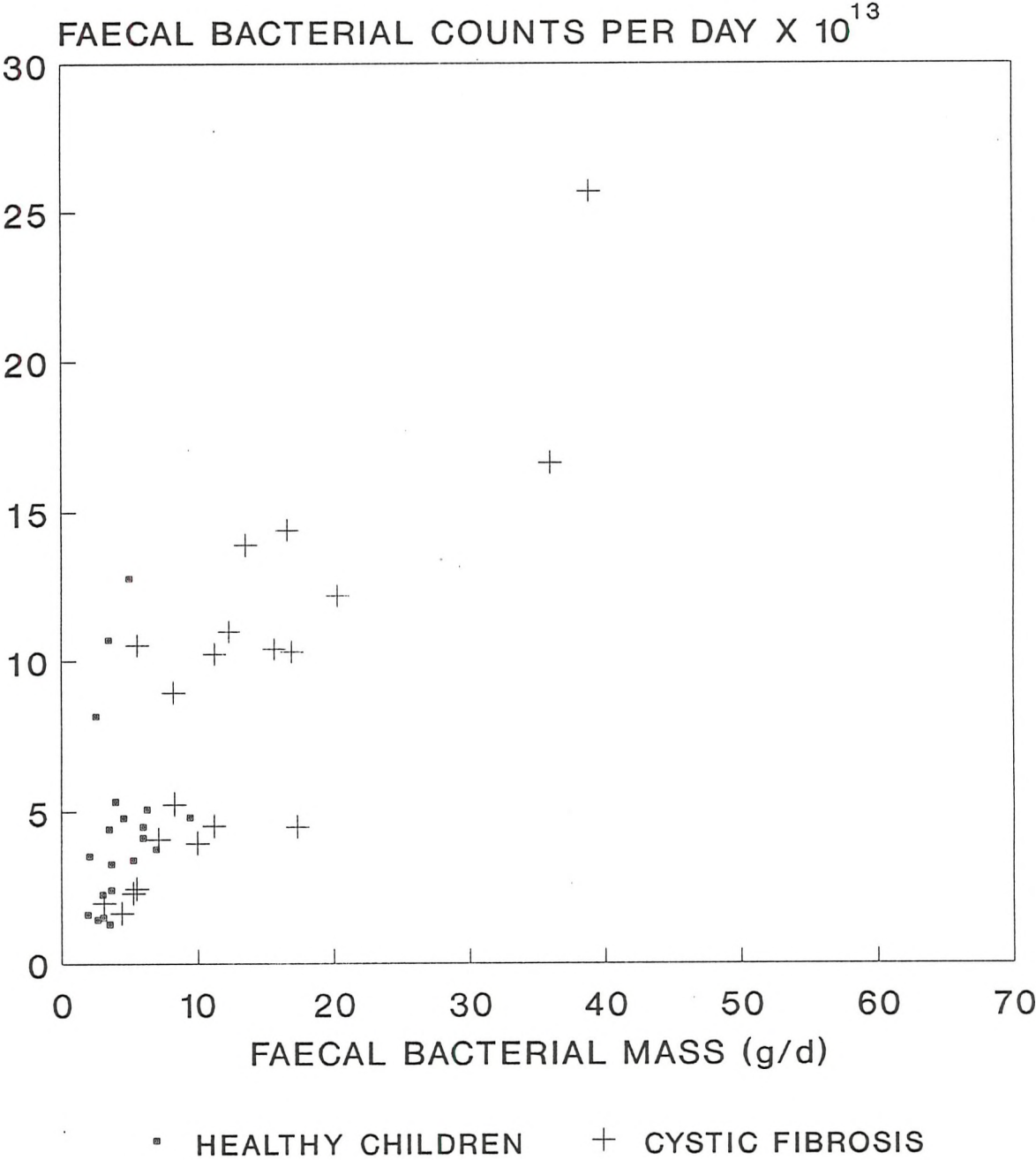


Figure 3.3 Faecal lipid content (expressed in g/d) measured by the methods according to Leyland *et al.* [1969] versus Gompertz & Sammons [1963] in patients with cystic fibrosis.

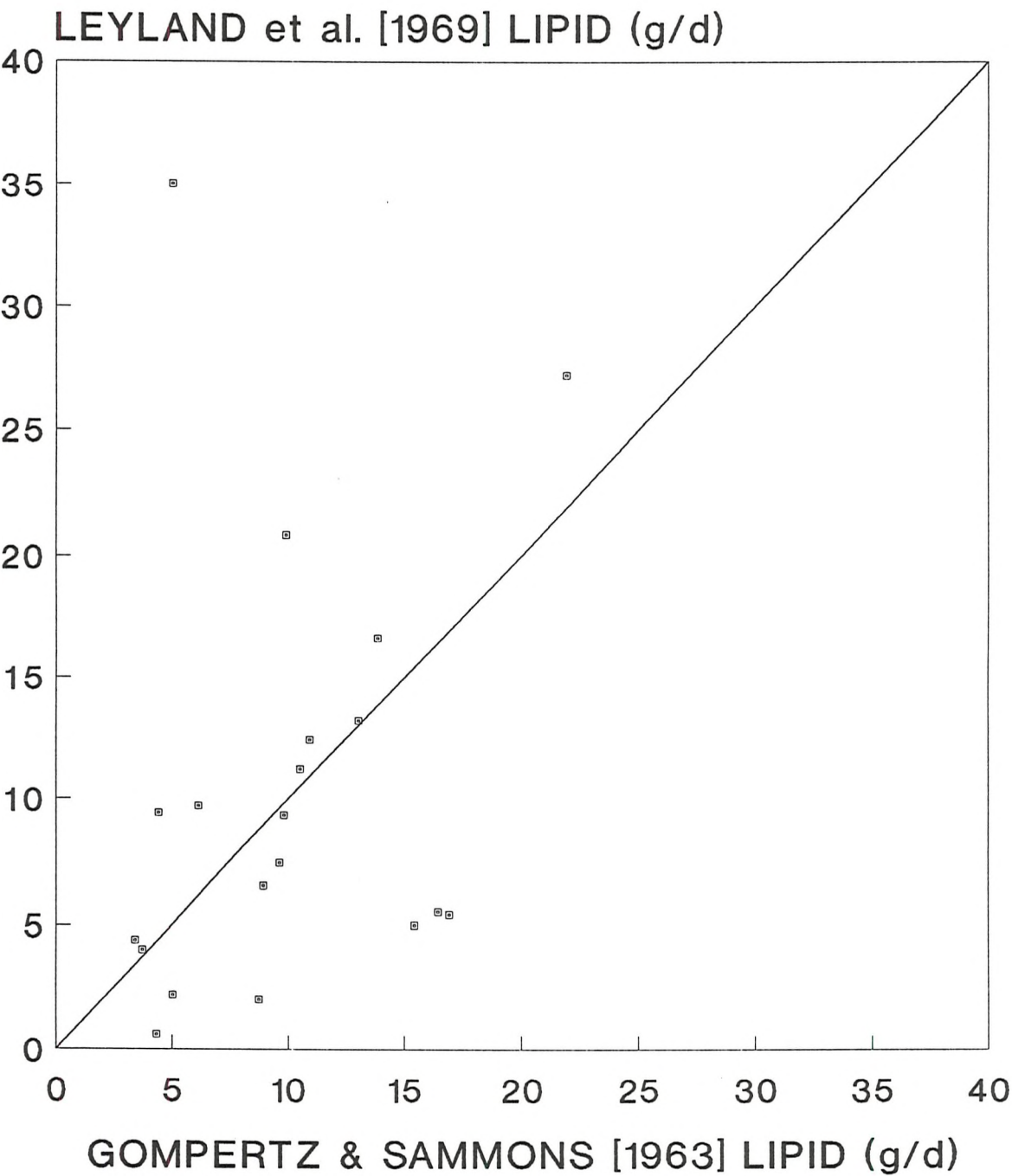


Table 3.1 The proportion of daily faecal material (expressed as %) represented by each of the major fractions, (SPM; small plant material, LPM; large plant material, bacterial fraction, water soluble material) in healthy children and adults, patients with cystic fibrosis and with alterations in dietary NSP intake in healthy adults. The values presented are medians with ranges in parentheses.

SUBJECT GROUP	SPM	LPM	BACTERIA	WATER SOLUBLE	TOTAL
HEALTHY CHILDREN	15.2 (2.9 - 36.5)	19.3 (5.7 - 36.5)	25.6 (17.0 - 41.9)	37.8 (24.5 - 51.1)	98.0
HEALTHY ADULTS	13.4 (5.7 - 18.2)	23.6 (16.8 - 33.9)	19.2 (15.2 - 29.4)	42.2 (36.7 - 45.1)	98.3
CYSTIC FIBROSIS	3.1 (0.6 - 8.0)	26.1 (5.9 - 46.2)	32.7 (19.2 - 61.4)	32.9 (13.0 - 51.6)	94.8
NSP-FREE DIET	7.5 (1.6 - 18.4)	28.1 (13.9 - 45.9)	24.0 (17.7 - 33.1)	33.2 (27.4 - 51.4)	92.8
NSP-ENRICHED DIET	9.1 (1.5 - 15.1)	17.0 (8.9 - 42.9)	42.6 (20.0 - 59.3)	34.0 (24.6 - 42.7)	102.7

Table 3.2 The effect of freezing (F) and storage at room temperature (R) after 24, 48 and 72 hours on faecal energy content per gram of dry and wet faeces.

TIME	FAECAL ENERGY (DRY FAECES kJ/g)	FAECAL ENERGY (WET FAECES kJ/g)
0	20.0	5.6
24 F	21.2	7.5
48 F	21.2	4.4
72 F	19.6	4.7
24 R	21.8	5.7
48 R	20.7	6.6
72 R	21.8	5.1

CHAPTER 4. ESTIMATION OF DAILY FAECAL ENERGY FROM THREE-DAY STOOL COLLECTIONS; INTER AND INTRA-INDIVIDUAL DIFFERENCES IN NORMAL ADULT WOMEN.

4.1 INTRODUCTION.

The studies reported in this thesis have centred upon measurements of the magnitude and composition of energy within stools each day. The measurement of daily stool output can not be simply performed from a one day stool collection as there may be variation in bowel habits between and within individuals each day [Rendtorff & Kashgarian, 1967]. In order to determine a representative daily value for stool output, a collection of stool samples must be performed over a period of days, for example three or five days. However the length of time required to provide a representative daily value for stool output remains unclear [Annaegers *et al.* 1948; Whitby & Lang, 1960]. The three-day stool collection has been generally accepted as the 'gold standard' to provide a reliable estimate of the energy, lipid and nitrogen contents of stools [Walters, Kellehar, Gilbert, Littlewood, 1990; Saaverdra & Brown, 1991]. Many studies have examined the lipid content of a representative daily value for stool output from a three-day stool collection in normal subjects [Wollaeger *et al.* 1947; Schmerling *et al.* 1970] and in gastro-intestinal dysfunction to provide an index of the extent of maldigestion and/or malabsorption [Raffensberger *et al.* 1967; Pinter & McLean, 1968; Mischler *et al.* 1982; Marotta *et al.* 1989; George *et al.* 1990]. Whilst much of the attention within the literature has been specifically concerned with the measurement of faecal lipid, no attempts have been made previously to assess the minimum number of days required to collect stools to provide a representative daily value for faecal energy. The variability in a representative daily value for faecal energy from three day stool collections has not been determined.

The aim of the study presented in this chapter was to examine the variability observed between and within individuals in representative daily values for faecal energy calculated from estimations of consecutive three-day stool collections, that is from days 1-3, 4-6, 7-9 etc. over the period of a complete menstrual cycle in a group of normal adult women. In the light of this information, the use of three-day stool collections is discussed with a view to investigate the magnitude and origins of faecal energy in further studies.

4.2 METHODS.

4.2.1 SUBJECTS.

This study formed part of a comprehensive investigation examining metabolic changes during the menstrual cycle. Six normal adult women (aged 21-28 years) participated in the study (see table 4.1). Informed consent was obtained from each of the subjects after the nature of the study had been explained. The subjects were either employees or students at the University of Southampton. They were all in good health prior and during the course of the study. None of the subjects were taking any other medication apart from oral contraceptives by three of the subjects. The study had the approval of the Southampton Hospitals and South West Hampshire Health Authority Ethical Subcommittee.

For the purpose of this investigation, each of the subjects recorded daily weighed food intake and collected stools over one complete menstrual cycle from the first day of menstruation to the beginning of the next period of menstruation. The lengths of the menstrual cycles ranged between 28-39 days. All the subjects were closely monitored and given support throughout the study period. Weighed food intake was analyzed according to the methods described in chapter 3. All stools were collected separately into polyethylene bags and immediately frozen. The date, time of collection and weight of each stool specimen was accurately recorded. All stool collections were complete for each subject.

4.2.2 ANALYSES.

Stools collected over one day were weighed, pooled and then homogenised with distilled water. The energy content of faeces was then measured following the procedure outlined previously in the methodology chapter.

4.2.3 STATISTICAL ANALYSIS.

The results are given as medians (range). Variables included under statistical analysis were wet and dry weight of stools, water content (%), stool frequency and faecal energy per gram wet and dry weights of stool. The Pearson product moment correlation coefficient was employed to compare the relationship between variables. Variability was also expressed as the coefficient of variation. The proportion of energy within the stool which could be equivalent to gross

energy intake for each subject was calculated by expressing the sum of the energy content of stools as a percentage of the total gross energy intake over the study period.

For each subject, a representative daily value for faecal energy over the study period was determined by three approaches. Firstly the energy content of stool was calculated from the total quantity of stools produced over the days when stools were passed. This approach thereby ignores the days when no stools were passed. The average daily energy contents of stools for each subject were also calculated from both three and five day consecutive periods by dividing total energy content by a factor of three or five respectively.

4.3 RESULTS.

In this group of healthy adult women stools were passed on average every 1.23 days (range 1.03-1.65 days). Daily wet and dry weights of stools are shown in figure 4.1. The overall wet and dry weights of stools measured over complete menstrual cycles were 137.8 g/d and 38.5 g/d respectively. The within subject variability for faecal wet and dry weights ranged between 33.6-67.2% and 31.6-56.8% respectively, table 4.2. The between average subject variability for both wet and dry weights of stools were 19.0% and 18.4% respectively. Stool water contents expressed as a percentage of total faecal weights are illustrated in figure 4.2. Whilst the within subject variability for each of the subjects ranged between 3.8-11.6%, the between average subject variability was 6.5%. The median water content of stools between the subjects was 71.1%.

Stool energy per gram of wet and dry stool for each subject are shown in figure 4.3. Overall the amounts of energy per gram of wet and dry stool were 6.6 kJ/g and 18.9 KJ/g. The within subject variability for stool energy per gram of wet stool ranged between 12.2-36.8% (table 4.3) and between average subject variability was 18.9%. The within subject variability for stool energy per gram of dry stool ranged between 6.0-14.5% (table 4.3) and between average subject variability was 8.2%. For all the subjects, a clear relationship could be demonstrated between faecal energy and dry weight of stool determined from stools collected for the days when stools were produced over each of the study period ($R=0.96$; $p<0.01$; figure 4.4). Whilst a more obvious difference was observed for the association between faecal energy and wet weight of stool, there was still

a clear relationship between the two variables ($R = 0.84$; $p < 0.01$; figure 4.5). Approximately 93% and 71% of the variance in stool energy could be attributed to differences in dry and wet stool weights respectively.

Daily faecal energy losses from each of the subjects calculated from one day stool collections are given in figure 4.6. The number of days when no stools were passed over the study period ranged between 1-13 days. Daily energy content of stools from one day stool collections from all subjects was 0.87 MJ/day (0.1-2.4 MJ/day). Figure 4.7 shows the values for daily faecal energy content calculated from one day stool collections and the representative daily values for faecal energy from both the three and five day consecutive periods of stool collections for each of the subjects. From one day stool collections, the within subject variability for faecal energy ranged between 30.6-58.1% (Figure 4.8) and between average subject variability was 16.3%. The median representative daily value for faecal energy content calculated from both consecutive three day and five day stool collections was 0.74 MJ/day. The within subject variability for representative daily faecal energy calculated from three day collections ranged between 19.9-55.8% (Figure 4.8) and between average subject variability was 22.4%. The within subject variability in representative daily faecal energy content derived from five day stool collections ranged between 11.8-36.7% (Figure 4.8) and between average subject variability was 22.6%.

The proportion of energy within faeces was equivalent to on average 7.5% (range 5.1-10.2%) of total gross energy intake over the study periods.

4.4 DISCUSSION.

The aim of the present investigation was to examine the minimum number of days required to collect stools to provide a representative daily value for faecal energy and the variability in measurements of representative daily values for faecal energy calculated from three-day stool collections. In addition to the main question of this study, a number of other faecal variables have been examined over the study period. From this information the extent of the variability between subjects was observed and as there may be variability within subjects, individual patterns were considered. In the present study, this variability has been described by the coefficient of variation. It is noted that the coefficient of variation statistic has been introduced previously in the methodology chapter to express the relative variability of values from a number of laboratory techniques employed

within the studies of this thesis. In the present study the coefficient of variation has been applied in a different manner to describe the variation of values both within and between a group of individuals. It is recognised that there have been a number of studies which have determined the variability in energy intake from day to day and the minimum number of daily food records required to estimate nutrient intake [Liu *et al.* 1978; Bingham *et al.* 1981; Marr & Heady, 1986; Nelson *et al.* 1989; Miller *et al.* 1991]. In the present study, the methods of Liu *et al.* [1978] and Marr & Heady [1986] were employed to determine the variability in faecal energy over the study period and the minimum days necessary to estimate daily faecal energy content. However these attempts were unsuccessful owing to the fact that subjects did not pass a stool each day and faecal markers were not employed for the purposes of this study to delineate daily collections.

In the present study the observation that stool weights may vary within individuals supported the findings of a previous study of stools patterns in healthy adult men [Rendtorff & Kashgarian, 1967]. Whilst it might be expected to observe a wide variation in faecal weights between individuals [Rendtorff & Kashgarian, 1967], in the present study faecal weights between subjects were comparable. The normal daily faecal output has been reported to range between 100-200g, of which 25-50g is solid matter [Wyman, Heaton, Manning, Wicks, 1978]. The mean daily wet and dry weights of stools over the study period were within these ranges for each of the subjects. This may imply that this group of subjects had normal bowel functions. The wide range in faecal weights for each of the individuals should be noted. The variability in stool water content was relatively small both between and within individuals. This would suggest that stool water content was relatively constant over the study period.

The results of this study challenge the general assumption that a single bowel movement daily is normal in healthy subjects. This is in agreement with the findings of Wyman and coworkers [1978]. Rendtorff & Kashgarian [1967] have suggested that stool pattern is a "biologic characteristic" peculiar to each person which may be different among individuals. The difference in stool frequency between individuals was evident in this study. This observation is of particular importance in relation to the results of stool energy content which will be considered later in this discussion.

The question should be raised as to whether there could have been an effect of the menstrual cycle on bowel function with respect to female sex hormone status. Whilst the purpose of this study was not to examine this possibility, the likelihood of such an effect on the present results should be considered from the available literature. Whilst there have been relatively few studies examining the relationship between bowel function and female sex hormones, conflicting findings have been reported. The role that menstrual cycle may have in determining gastro-intestinal transit time [Wald, Thiel, Hoechstetter, Gavalier, Egler, Verm, Scott, Lester, 1981; Davies, Crowder, Reid, Dickerson, 1986] has been challenged in a more comprehensive study by Kamm and coworkers [1989]. Also no differences in stool weight, bowel frequency during the menstrual follicular or luteal phases were observed. Furthermore Bisdée and colleagues [1989] reported a lack of a difference in transit time in addition to total faecal energy and nitrogen losses (unpublished values) over four phases of the menstrual cycle. From this information it would seem unlikely that differences in female sex hormones produced over the menstrual cycle had a major influence on the findings of the present study.

In the present investigation, attention has been particularly directed towards the examination of daily faecal energy content over the study period. Whilst the daily variation in faecal energy appeared to be large, this was not surprising as the extent of the variability in daily energy intake can be as much as 20-30% within an individual and from 4 to 45% between individuals [Bingham, 1987]. The close relationship between faecal energy and weight of stools with an apparent constancy of energy per gram dry and wet weight of stool would imply that the composition of stools must remain relatively similar between this group of individuals. It was also noted the relatively small variation in the amount of energy per gram of wet and dry weights of stool both within and between individuals. Hence this would indicate that of none of the subjects were in diarrhoeal states during part or all of the study period.

The determination of the energy content of stool each day could only be achieved by complete stool collections over more than one day because the subjects in this study did not have a daily stool output. Whilst the mean representative daily value for faecal energy calculated from both three day and five day stool collections were similar, the mean faecal energy content calculated from one day over each of the study periods was increased by approximately 14.5%. This difference in faecal energy content would account for the days when

there was no stools produced. As representative daily faecal energy contents calculated from three and five day stool collections were comparable, this would imply that measurements of representative daily stool energy content derived from a five day stool collection would be of no advantage over a three day stool collection. There appeared to be a wide variability both within and between individuals for daily faecal energy. However the results would suggest that the variability in faecal energy was greater from a one day stool collection than representative values for daily faecal energy contents calculated from both three and five day stool collections.

It has been indicated in section 2.2 that approximately 4-5% of the energy within faeces could be equivalent to the gross energy intake [Southgate & Durnin, 1970]. In the present study an attempt was made to express faecal losses of energy in relation to gross energy intake from total faecal energy losses and the overall gross intake of energy for each of the subjects. It would appear that the amount of energy within stools which could be equivalent to gross energy intake was not consistent between the subjects. However the variance of these results may be expected as this approach assumes that all of the material within faeces consists simply of dietary residue and makes no allowance for the potential contribution made by other components of faecal energy such as the colonic bacterial microflora.

4.5 SUMMARY AND CONCLUSIONS.

- i. The variability in representative daily values for faecal energy content from consecutive three-day stool collections has been demonstrated in six normal adult women over the period of a complete menstrual cycle. Whilst the mean representative daily faecal energy values calculated from both three day and five day stool collections were similar, the mean faecal energy content calculated from the days when stools were passed over each of the study periods was increased by approximately 14.5%.
- ii. The subjects tended to exhibit distinctive stool patterns and it was clear that stools were not produced every day. The number of days where no stools were passed ranged between 1-13 days.
- iii. A relatively wide variation could be demonstrated for faecal weights and energy content of stools each day both within and between individuals.

- iv. Strong relationships could be demonstrated between faecal energy and weight of stool calculated from one day stool collections over each of the study periods.

In conclusion, it would appear from the data of this study that it would be reasonable to employ a three-day stool collection to measure the representative daily value for the energy content of faeces. Whilst no attempt was made to determine the precise length of the three-day stool collection, a more accurate representation of the 72 hour period could be achieved by the use of faecal markers in further studies. A relative constancy in energy per gram wet and dry weight of stool of approximately 7 kJ/g and 19 kJ/g respectively could be demonstrated in this group of subjects. If the energy content of stool could be estimated from the weight of stool passed each day, this may avoid the necessity to collect stools over a period of time which is both time-consuming and unpleasant. This possibility will be explored in further studies presented in this thesis.

Table 4.1 The characteristics of the healthy adult women who participated in the study are given.

SUBJECT	AGE Yrs	HEIGHT m	WEIGHT kg	Body Mass Index kg/m ²
A	23	1.60	50.2	20
B	24	1.66	59.1	21
C	22	1.68	54.2	19
D	28	1.65	60.1	22
E	22	1.73	64.4	22
F	24	1.66	56.6	21

Table 4.2 **Coefficients of variation (%) for daily faecal wet and dry weights and water content of faeces measured over a complete menstrual cycle within each of the six healthy adult women.**

	A	B	C	D	E	F
Faecal wet weight	33.6	53.2	40.8	51.4	59.6	67.2
Faecal dry weight	31.6	52.7	43.4	44.8	56.8	51.9
Faecal water content	5.5	7.4	3.8	11.2	11.6	7.4

Table 4.3 **Coefficients of variation (%) for faecal energy per gram of wet and dry stool measured in faecal samples collected over a complete menstrual cycle from each of the six healthy adult women.**

	A	B	C	D	E	F
Faecal energy per gram of wet stool	17.6	19.7	12.2	20.2	36.8	25.8
Faecal energy per gram of dry stool	11.8	9.1	6.0	8.9	14.5	9.2

Figure 4.1 Daily faecal wet and dry weights for each of the six healthy adult women (A-F) measured over a complete menstrual cycle. Median values represented by the bars and ranges are given.

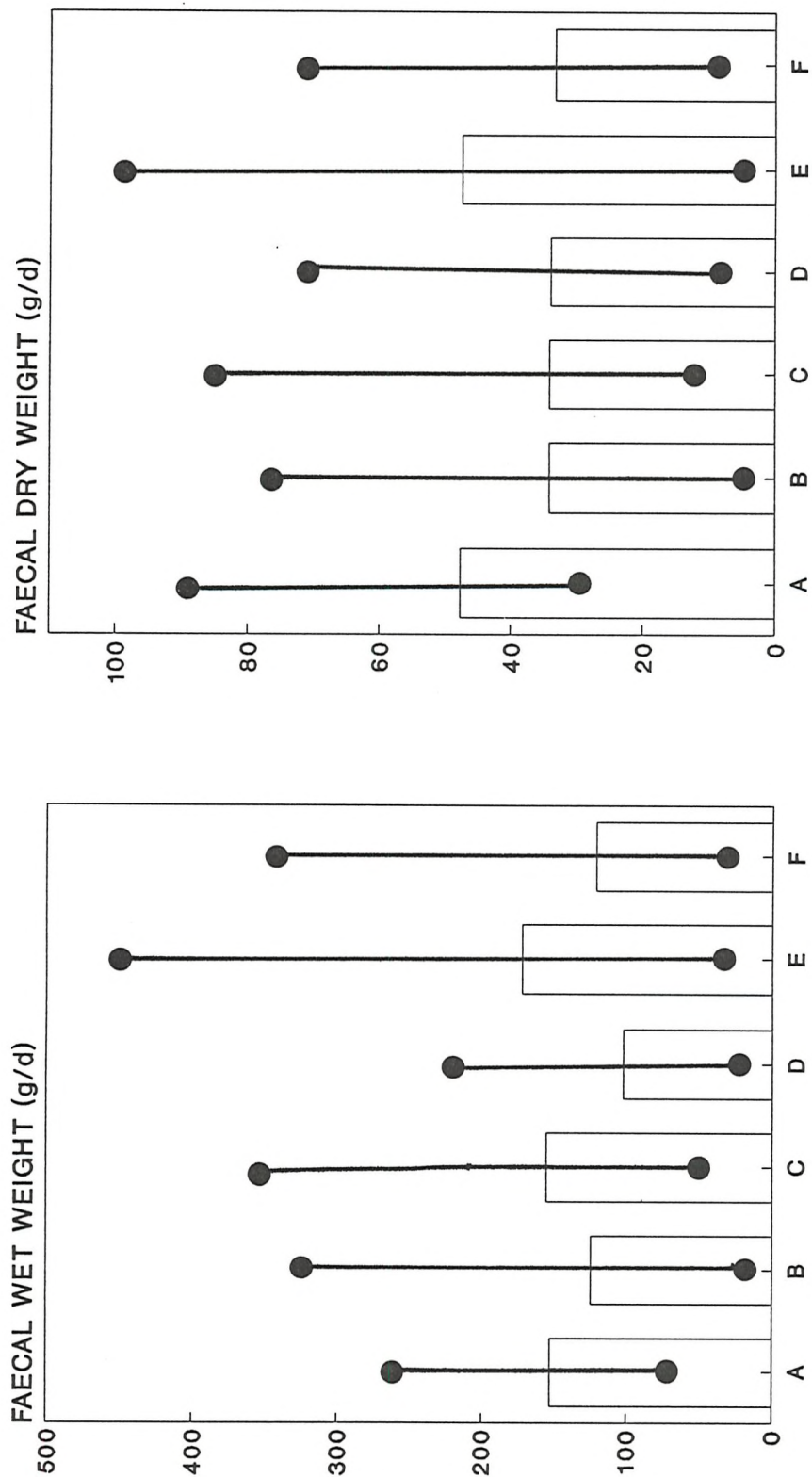


Figure 4.2 Daily water content of faeces expressed as a percentage of stool weight for each of the six healthy adult women (A-F) measured over a complete menstrual cycle. Median values represented by the bars and ranges are given.

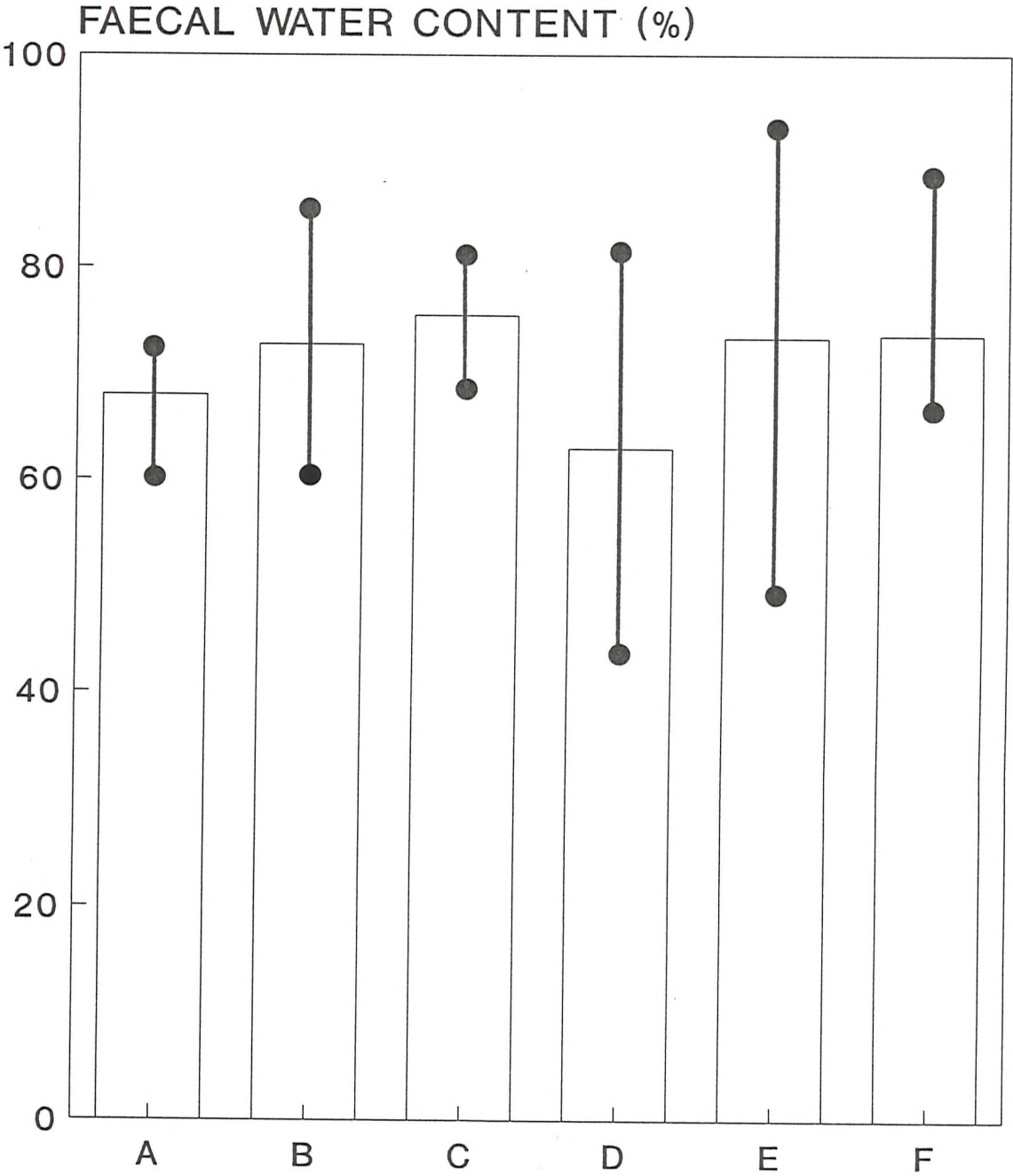


Figure 4.3 Daily faecal energy per gram of wet and dry stool for each of the six healthy adult women (A-F) measured over a complete menstrual cycle. Median values represented by the bars and ranges are given.

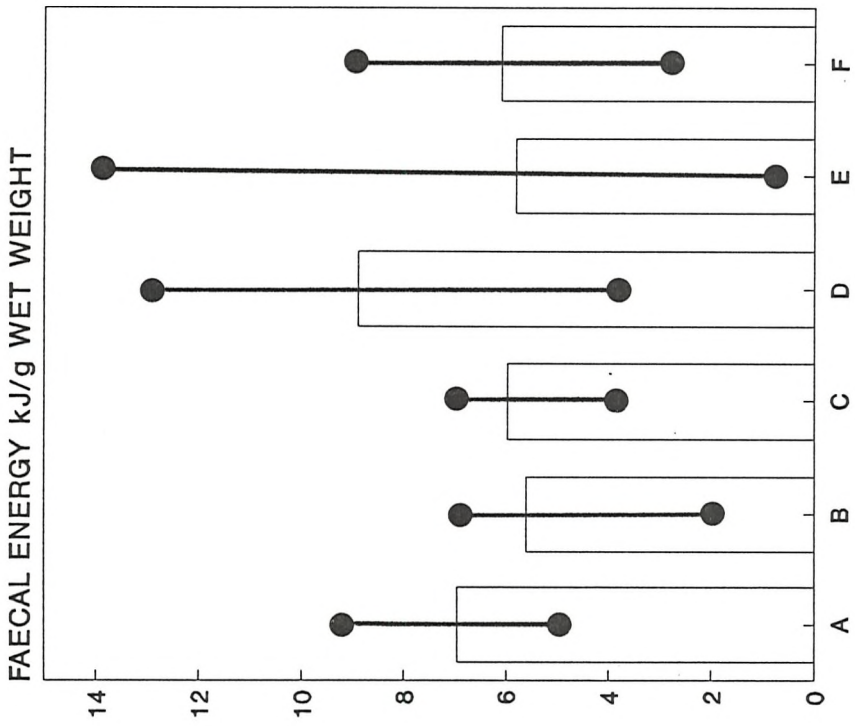
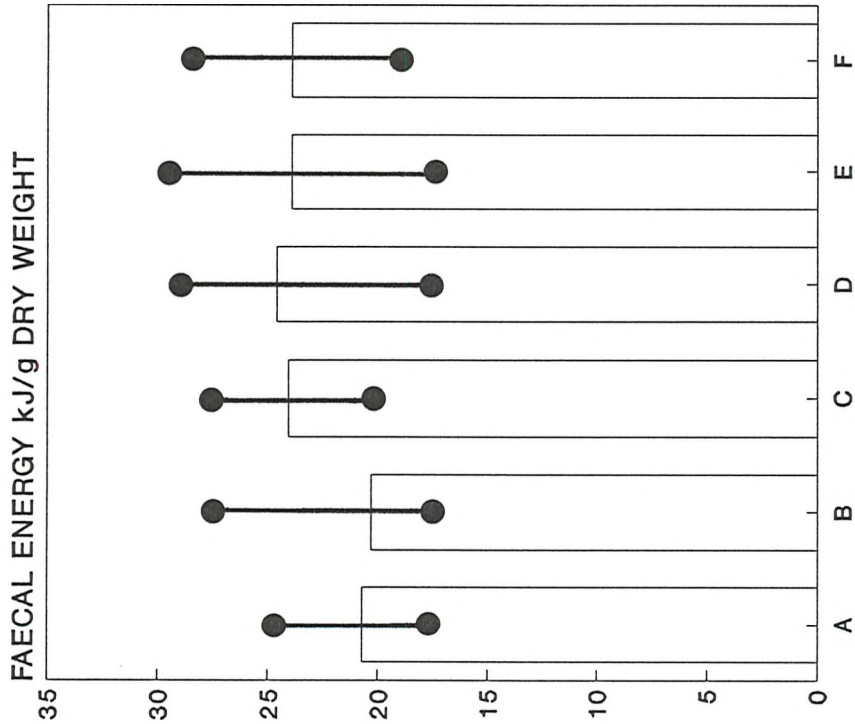


Figure 4.4 Daily faecal energy content of faeces versus faecal dry weight from the data collected over a complete menstrual cycle for six healthy adult women (A-F).

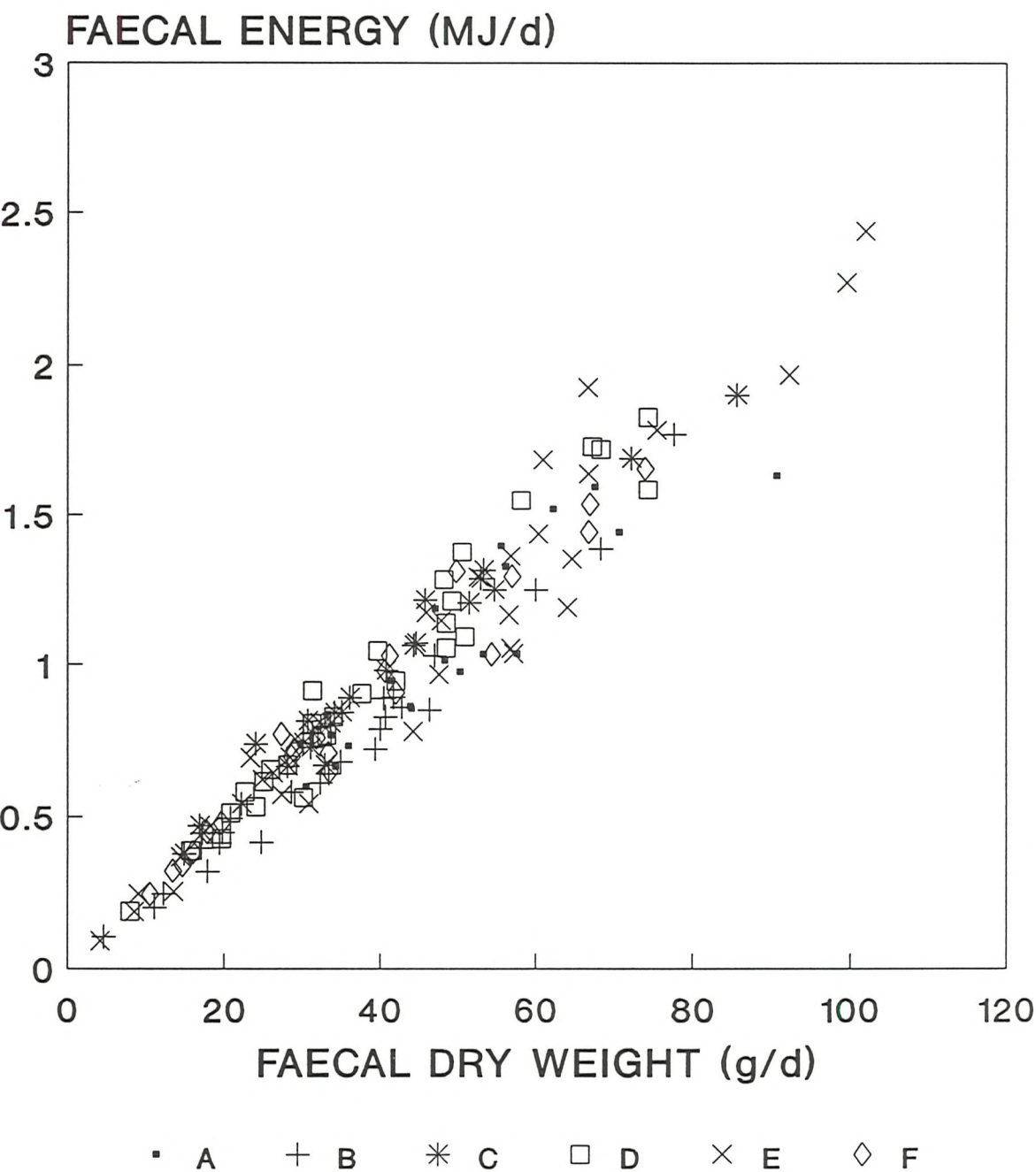


Figure 4.5 Daily faecal energy content of faeces versus faecal wet weight from the data collected over a complete menstrual cycle for six healthy adult women (A-F).

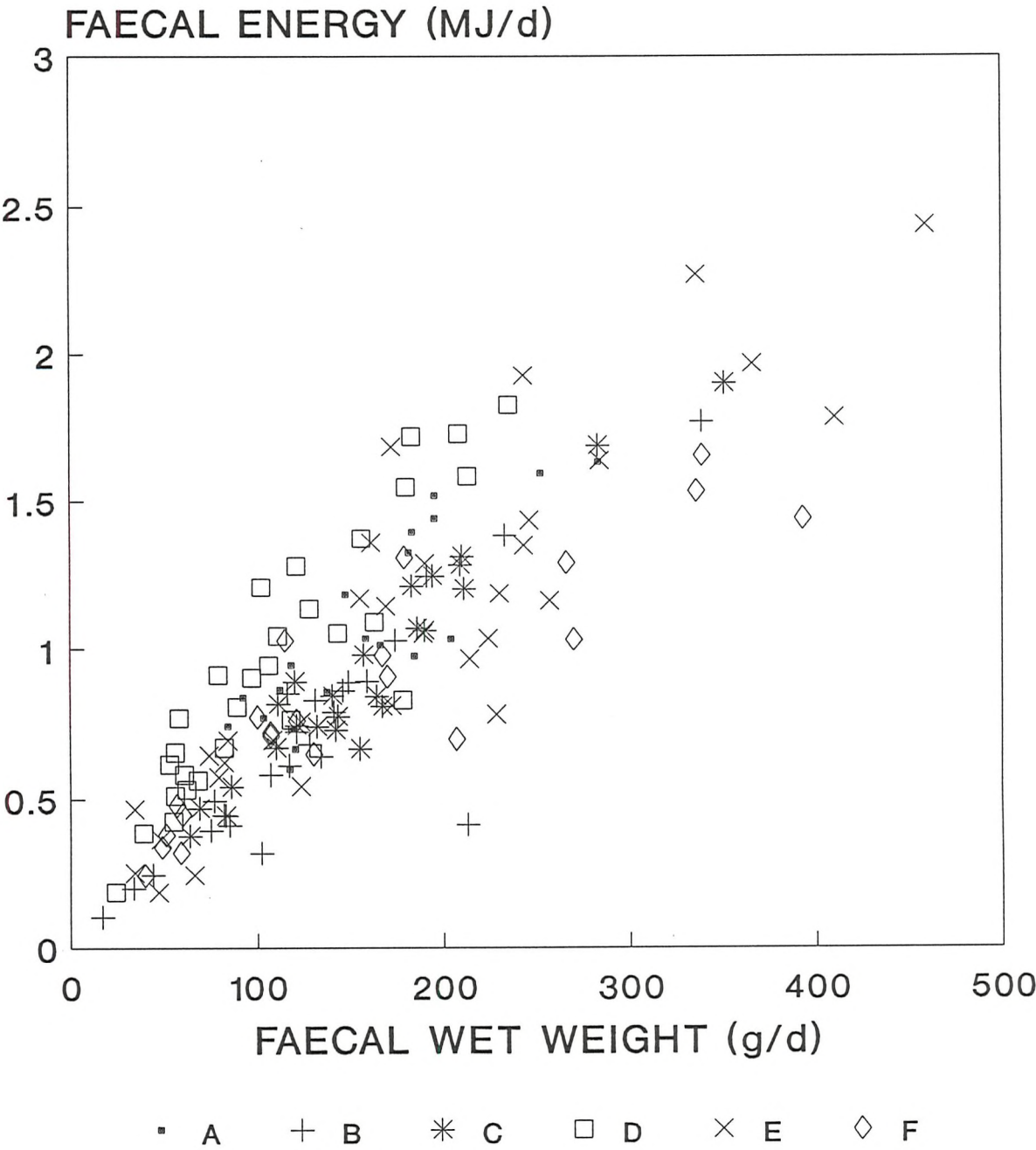


Figure 4.6 Daily faecal energy for each of the six healthy adult women (A-F) measured over a complete menstrual cycle.

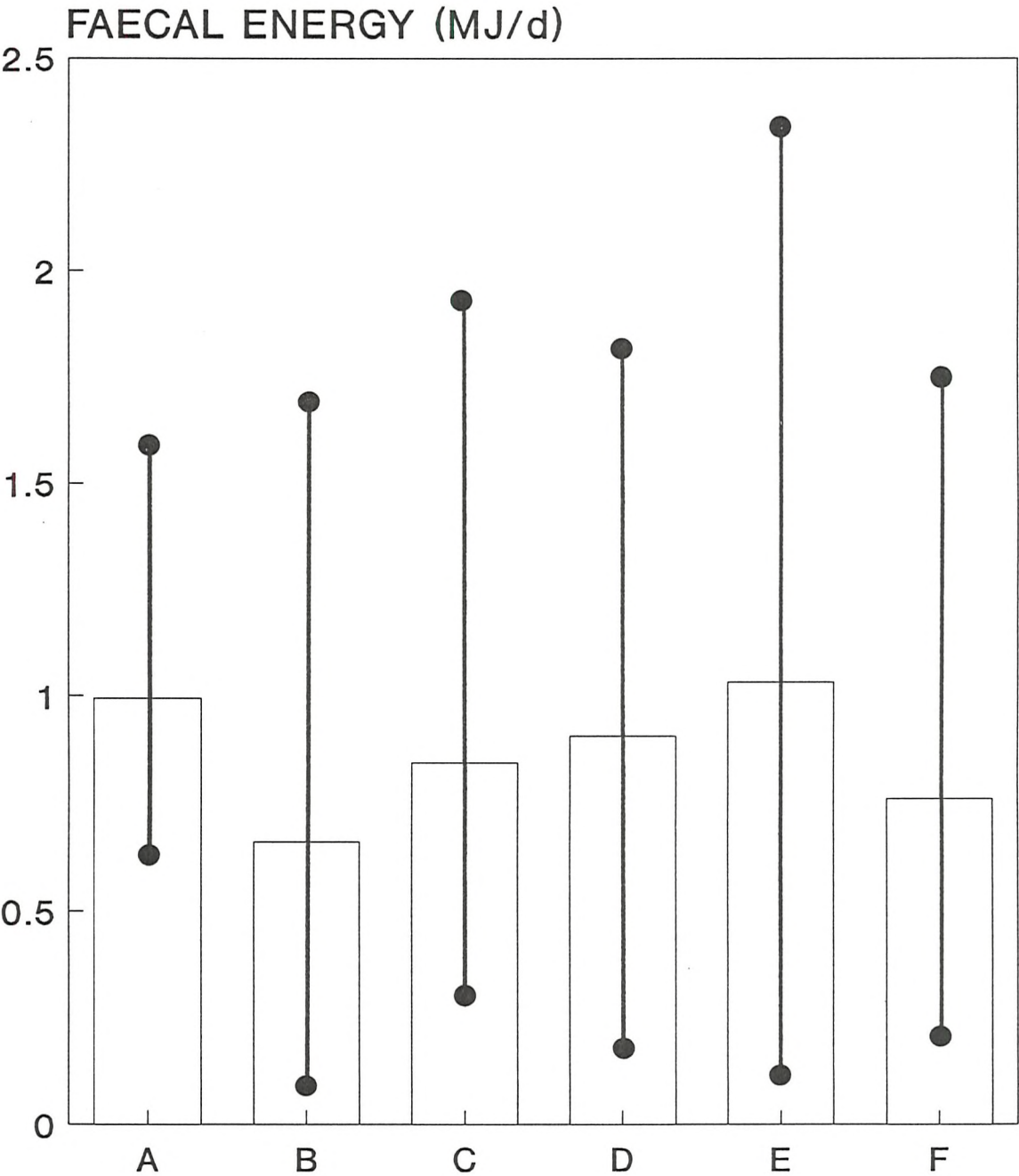


Figure 4.7 Energy content of stools measured each day and the representative daily faecal energy value calculated from consecutive three day and five day stool collections measured over a complete menstrual cycle in six healthy adult women. The values are expressed as medians.

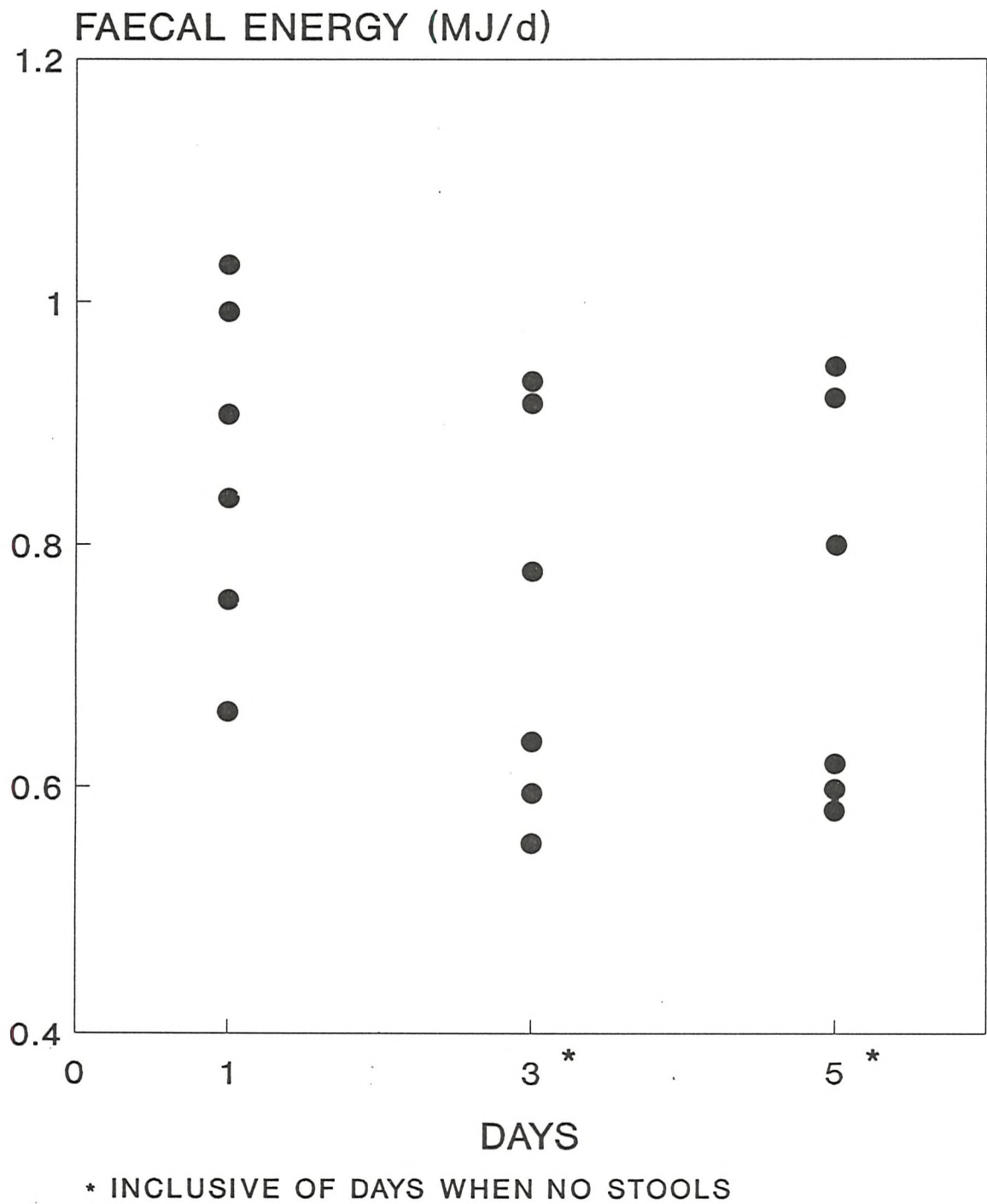
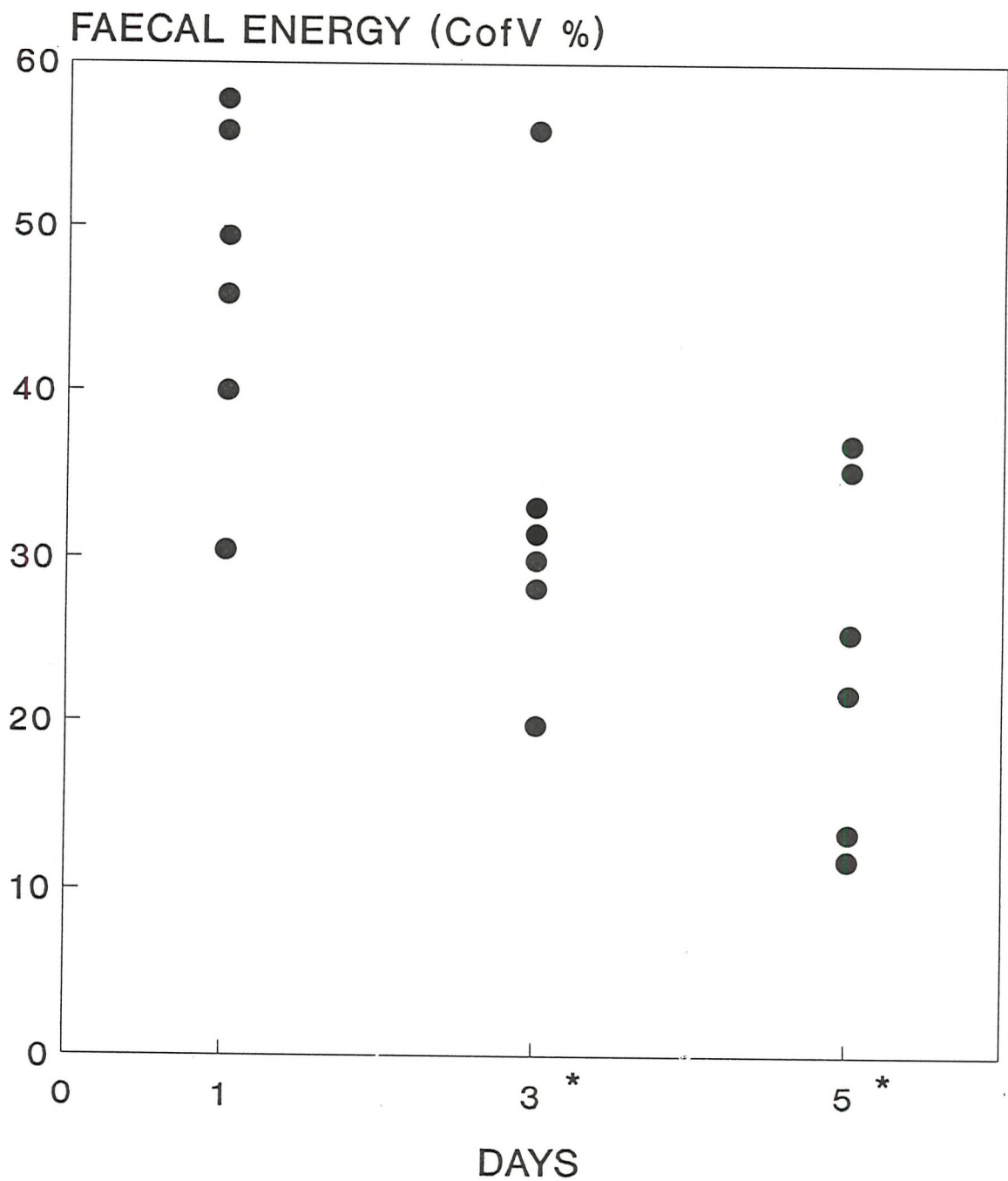


Figure 4.8 The coefficient of variation (CofV %) for faecal energy content measured each day and the representative daily faecal energy value calculated from consecutive three day and five day stool collections measured over a complete menstrual cycle in six healthy adult women.



CHAPTER 5. THE AVAILABILITY OF DIETARY ENERGY, FAECAL ENERGY CONTENT AND HUMAN COLONIC BACTERIA IN NORMAL HEALTHY CHILDREN.

5.1 INTRODUCTION.

This study is directed towards faecal energy and the extent to which this may contribute towards a limitation in the availability of energy from diet and thereby restrict growth in normal childhood.

It appears that our base of knowledge of the magnitude and origins of faecal energy in children is severely limited and particularly between the ages of 5 years up to adulthood. Studies performed over 50 years ago have given consideration towards the energy content of faeces in an attempt to evaluate energy requirements in children [Holt & Fales, 1921; Wang, Kaucher, Wing, 1936]. It would appear that in more recent studies of energy balance however, the potential contribution that faecal energy may make in limiting the availability of energy from the diet has been overlooked.

Therefore the aim of the study reported in this chapter was to determine the amount and composition of energy within the stools of healthy children. In the light of this information the extent to which faecal energy and human colonic bacteria may impress upon our understanding of dietary energy during growth in childhood is considered.

5.2 METHODS.

Twenty healthy children aged 6-11 years (10 girls; 10 boys) from a local school in Southampton participated in the study. It could be confirmed from the weights and heights of the children that they represented a normal group of individuals. Details of the subjects are shown in table 5.1. Informed consent was obtained from all of the children and their parents after the nature of the investigation had been explained. The study had the approval by the Ethical Committee of Southampton Hospitals and South West Hampshire Health Authority.

Weighed food intake was recorded for a period of seven days using digital electronic scales using the procedure outlined in the methodology chapter. The families of the subjects were instructed in the use of scales and recording of food intake prior to commencing the study. Weighed food intake was analyzed using computerised food composition databases for gross energy and metabolisable energy intakes. Further details are given in chapter 3. All the subjects were closely monitored and given support throughout the study period. After completion of the record, the families were interviewed to ensure that there were no omissions or errors.

All stools were collected between carmine markers for the final three days of the seven day study period following the procedure described in the methodology chapter. All the subjects were cooperative and capable of following the details of the study protocol. At no time was difficulty encountered nor any need found to modify the procedure to accommodate the subjects.

The methods for preparation of stools for analysis of faecal energy, lipid and nitrogen content of stools and faecal bacterial mass are given in chapter 3. A portion of dried bacterial mass from each stool sample was pooled to provide a representative sample of faecal bacterial from each of the subjects. The energy, lipid and nitrogen contents per gram of stool bacteria from pooled samples were determined using the previously described methods in chapter 3. With this information, the proportion of faecal energy, lipid and nitrogen contents of stool that may be attributed to stool bacteria could be determined.

The results are given as medians and ranges. The association between variables were tested by the Pearson product moment correlation coefficient. It is noted that some of these associations are illustrated in chapter 6 to allow comparison between the present group of healthy children and with a group of individuals with maldigestion and/or malabsorption associated with the disease CF.

5.3 RESULTS.

Gross energy intake and energy content of stools are shown in Table 5.1 for each child. Gross energy intake (GEI) was approximately 16.7% (14.1-20.0%) greater than metabolisable energy intake (MEI) estimated using modified At-water factors. Figure 5.1 illustrates the calculated difference in MEI and GEI for

each of the subjects. The difference in GEI calculated with and without the consideration of dietary NSP was approximately 4.0% (2.5-6.2%). This difference for each subject is illustrated in Figure 5.2.

The energy content of stools was approximately 0.30 MJ/day (0.1-0.7 MJ/day) and was equivalent to 3.5% (1.3-5.8%) of GEI. Faecal dry and wet weights were 15.7 g/day (5.7-36.6 g/day) and 58.6 g/day (26.2-158.1 g/day) respectively. The water content of faeces expressed as a percentage of stool wet weight was 73.7% (54.9-84.9%). A moderate association could be demonstrated between gross energy and dry weight of stools ($R=0.60$; $p<0.01$). The apparent digestibility of energy (gross energy intake - faecal energy expressed as a percentage of gross energy intake) was 96.5% (94.2-98.7%).

The amount of lipid within faeces was 2.1 g/d (1.1-3.6 g/day), equivalent to 2.9% of lipid intake (1.3-4.4%). Faecal nitrogen in this group of healthy children was 1.0 g/day (0.4-2.4 g/day), equivalent to 11.7% (4.9-19.1%) of nitrogen intake. With the assumption that 1 gram of nitrogen is equivalent to 6.25 grams of protein, protein content of faeces was derived from measurements of faecal nitrogen. There appeared to be some relationship between faecal protein and the energy content of stools ($R=0.79$; $p<0.01$), faecal protein could account for approximately 49.0% (33.0-117.2%) of the energy within faeces. A much weaker relationship between faecal lipid and faecal energy was observed ($R=0.51$; $p<0.05$), with faecal lipid accounting for 23.1% (16.2-66.0%) of the energy within faeces. Approximately 62% of the variance in faecal energy could be attributed to differences in faecal protein. Only 26% of the variance in faecal energy could be attributed to differences in faecal lipid.

Whilst a good correlation was observed between faecal wet weight and faecal energy ($R=0.77$; $p<0.01$), an even stronger association could be demonstrated between faecal dry weight and faecal energy ($R=0.98$; $p<0.01$). Approximately 59% and 96% of the variance in faecal energy could be attributed to differences in faecal dry and wet weights. The amount of energy per gram of dry faeces and wet faeces were 20.4 kJ/g (17.6-23.7 kJ/g) and 5.3 kJ/g (2.8-8.8 kJ/g) respectively.

Stool bacterial mass was 3.6 g/day (1.9-9.4 g/day), equivalent to 25.6% (range 17.0-42.0%) of faecal dry weight. A close relationship between stool bacterial mass and faecal dry weight ($R=0.77$; $p<0.01$) could be demonstrated. The number of bacteria within faeces was 4.0×10^{13} counts/day ranging from

1×10^{13} - 13×10^{13} counts/day. It could be confirmed that over 99% of the faecal bacteria could be recovered within the bacterial fraction of faeces. The amount of bacteria per gram of dry faeces was 0.26 g/g (range 0.17-0.42 g/g).

Determination of the energy, lipid and nitrogen contents of pooled faecal bacterial samples revealed an energy content of 20.4 kJ, a lipid content of 0.2 g and nitrogen content 0.04 g per gram bacteria. The energy content of bacteria was 73.8 kJ/day (39.3-191.0 kJ/day). Stool bacterial energy accounted for 25.4% (17.1-42.8%) of the energy within the faeces. The nitrogen content of stool bacteria was 0.2 g/day (0.1-0.4 g/day). There was also some relationship between stool bacterial mass and faecal nitrogen ($R=0.74$; $p<0.01$). Bacterial nitrogen accounted for approximately 16.0% (10.2-24.6%) of the nitrogen within the faeces. The amount of lipid contained within bacteria was 0.7 g/day (range 0.4-1.9 g/day). A weak relationship between bacterial mass and faecal lipid was demonstrated ($R=0.38$; NS), faecal bacterial lipid accounted for 41.0% (18.7-87.6%) of the lipid content of stools.

5.4 DISCUSSION.

The aim of the present study was to determine the magnitude and origins of faecal energy in normal healthy children. The energy content of stools in the present group of children ranged between 0.1-0.7 MJ/day and were equivalent to between 1-6% of gross energy intake. These results would agree with early studies which have shown that stools of normal children contain less than 0.5 MJ/day, equivalent to between 3.2-6.6% of gross energy intake [Holt & Fales, 1921; Wang *et al.* 1936].

Much of our current understanding of faecal energy, and the lipid and nitrogen contents of stools in healthy children is derived from studies in adult subjects where daily energy contents of stools have been reported to be less than 1.0 MJ/day, equivalent to 5% of gross energy intake. Whilst the magnitude of faecal energy is evidently greater in adults than children as energy intake increases with age, the proportion of energy which may be equivalent to energy intake would appear to be similar in both childhood and adulthood.

A seven day weighed food record has been employed in the present study to investigate gross energy and metabolisable energy intakes for each child. In the light of the inter- and intra-individual variation in dietary intake and difficulties

involved in assessing food consumption from weights of foods [Marr, 1971; Bingham, 1987], the reliability of the data for individual intake in children from a seven day period should be considered. A recent study has demonstrated that the minimum of daily food records required to estimate energy intake in children is seven days for boys and eight for girls [Miller *et al.* 1991]. This evidence would therefore support the use of the seven day weighed food record as a tool for determining energy intake in children. The estimation of gross energy intake using heats of combustion values and metabolisable energy intake using modified Atwater factors provided information of the extent of the difference between the calculated energy available to the body and the total energy content of food ingested. This difference was 14-20%. This difference allows for the nutrients that are either not digested and/or not absorbed and nitrogenous losses of protein within urine. However it is noted that this difference could be greater than the true value as the energy conversion factors used do not allow for the energy made available to the host through short chain fatty acid absorption (SCFA) as a result of colonic fermentation. It should be also borne in mind that the metabolisable energy values of foods derived from studies in adults are assumed to represent the metabolisable energy available in children. At present however, our knowledge of the possible error that may be incurred by applying this presumption is not clear.

In the present study, the lipid and nitrogen contents of stools in children of approximately 2.0 g/day and 1.0 g/day agree with values previously reported in children [Schmerling, 1970] and in adults [Southgate & Durnin, 1970]. This would indicate that despite a smaller faecal energy content in children than adults, the magnitude of the lipid and nitrogen content of stools would appear to be maintained from childhood to adulthood.

It is commonly assumed that lipid may account for most of the energy within faeces. From this study, it would seem that in healthy children, a greater proportion of the energy with the stool could be attributed to protein than lipid which was reflected in the relationships of faecal lipid and protein content with stool energy content. Calculation of the proportion of energy within the stool from protein was overestimated in one subject. This may be accounted by the errors introduced in the calculations of energy from lipid and protein within stools by applying the same factors which are employed to determine the energy value of foods as the energy values derived for lipid and protein content of stools are not known. Firstly, it was assumed that the lipid and protein content of faeces

were represented by their heats of combustion values which are employed in determining the energy value of food. In addition the amount of protein within stools was derived by assuming 1 gram of nitrogen is equivalent to 6.25 grams of protein which is used to determine the protein content of foods assuming that most foods contain 16% nitrogen.

The present investigation has demonstrated a high degree of association between the amount of energy within faeces and faecal wet and dry weight of stools. This would indicate that the energy density of stool must remain remarkably constant in children on a wet weight basis which was even more constant on a dry weight basis. It could be that the presence of water would introduce some variance. With a constancy of energy per gram of stools, this would imply that the energy content of stools could be derived from the weight of stools as indicated by Holt & Fales [1921]. This approach may be of more practical benefit in the management of some diseases if this relationship holds.

The present study has also examined the contribution made by bacteria towards the energy content of faeces. Whilst it has not been possible to quantify the amount of energy from maldigested and/or malabsorbed dietary residue or from endogenous secretions and cellular debris, it appears that the composition of stools is variable with an important contribution made by bacteria which represented between 17-42% of stool dry weight. The contribution made by bacterial microflora towards stool weight has not been previously described in children. In adults bacteria can account for 55% of stool dry weight [Stephen & Cummings, 1980a]. In the present study, a proportion of the energy within faeces could be associated with faecal bacteria. It would appear that the bacterial microflora in children could account for between 17-43% of the energy within the stool. As part of the energy within the stool could be attributed to lipid and protein, a major proportion of the lipid could be represented by bacteria, ranging between 19-88%. In addition part of the nitrogen within stools could also be attributed to bacteria, of between 10-24%. Whilst the presumption is often made that all of the energy within stools is attributed to dietary residue, it is clear from this study that the bacterial microflora may represent a major component of the energy within the stool in healthy children.

From the information derived from the present study, the production of SCFA within the colon based on the growth requirements of intestinal bacteria could be estimated using the same approach as McNeil [1984]. It could be calculated

that bacterial fermentation of carbohydrate within the colon could yield approximately 134 mmol (70.7-350.1mmol) of SCFA providing additional energy equivalent to approximately 1.9% (0.9-4.2%) (Table 6.2). Estimates of the amount of carbohydrate required to produce the weight of bacteria within faeces could not be solely accounted by dietary NSP intakes of 19.8g/day (range 12.8-33.4 g/day). With the possibility that a proportion of the daily intake of starch could escape absorption in the small intestine [Stephen *et al.* 1983], it has been indicated that starch could represent a source of substrate for colonic bacterial fermentation [Stephen, 1985]. It could be that maldigested and/or malabsorbed starch in addition to endogenous sources of carbohydrate such as the carbohydrate provided by mucus, could also provide some of the carbohydrate required to support the daily excretion of bacteria reported in the present study.

Whilst the contribution made by the absorption of SCFA in the large intestine to energy requirements has been previously reported in adults [Cummings, 1981; McNeil, 1984], there have been no other reports in children as the contribution made by bacteria to faeces has not been previously described. What is clear from the present study is that colonic bacterial activity has the capacity to recover at least some energy through SCFA absorption in children.

5.5 SUMMARY AND CONCLUSIONS.

- i. The energy content of stools in normal healthy children aged 6-11 years was approximately 0.3 MJ/day, equivalent to between 1.3-5.8% of gross energy intake.
- ii. The difference in gross energy and metabolisable energy intake was 16.7%.
- iii. Not all of the energy within stool could be attributed to dietary residue. The composition of stools was variable with a major contribution made by the faecal bacterial microflora which could account for between 17-42% of faecal dry weight.
- iv. A proportion of energy within stools could be associated with bacteria. Faecal bacteria could account for approximately 25% of the energy within stools and 41% and 16% the lipid and nitrogen content of stools respectively.

- v. Bacterial colonic fermentation may provide additional energy equivalent to between 0.9-4.2% through SCFA absorption.

In conclusion, it appears that although the energy content of stools may be relatively small in children in relation to energy intake, it is important that we have information of normal faecal energy in children to understand how imbalances in the availability of energy may contribute towards a delay in growth and development in childhood. The proportion of energy represented by faecal bacteria would also be of great significance in relation to our understanding of apparent digestibility. This information indicates that the actual digestibility of dietary energy would be higher than the value calculated by the present approach.

Table 5.1. The characteristics of the subjects who participated in the study are given including energy content of faeces and gross energy intakes. The values are presented for each subject and the median value is also given.

SUBJECT	AGE Yrs	GENDER	WEIGHT kg	HEIGHT m	GROSS ENERGY kJ/day	FAECAL ENERGY kJ/day
1	6.4	F	25.4	1.25	8690	191
2	8.7	F	30.9	1.35	8808	363
3	9.9	M	30.3	1.44	7306	355
4	9.9	F	25.3	1.40	7412	160
5	8.1	F	25.5	1.30	8710	169
6	8.1	M	27.6	1.29	8951	186
7	11.0	F	35.0	1.39	8807	286
8	10.2	M	25.0	1.34	10621	412
9	11.1	F	36.4	1.42	9865	272
10	11.1	F	47.3	1.46	11191	647
11	7.5	M	25.4	1.35	9609	446
12	8.4	F	28.6	1.34	9152	123
13	9.7	M	33.5	1.36	10108	242
14	10.4	M	38.1	1.48	9179	525
15	7.6	M	25.8	1.32	7698	294
16	10.7	F	38.4	1.53	10438	506
17	10.3	M	35.1	1.49	10803	301
18	8.3	F	30.9	1.40	9051	288
19	11.0	M	35.4	1.39	10379	458
20	10.1	M	36.3	1.45	12492	530
MEDIAN	9.9		30.9	1.39	9166	298

Figure 5.1 Daily faecal energy content (FE) determined from three day stool collections in twenty normal healthy children. Daily gross energy intakes (GEI) applying heats of combustion values [Merrill & Watt, 1955] and metabolisable energy intake (MEI) using modified Atwater factors [Paul & Southgate, 1978] were calculated from seven day weighed food intakes.

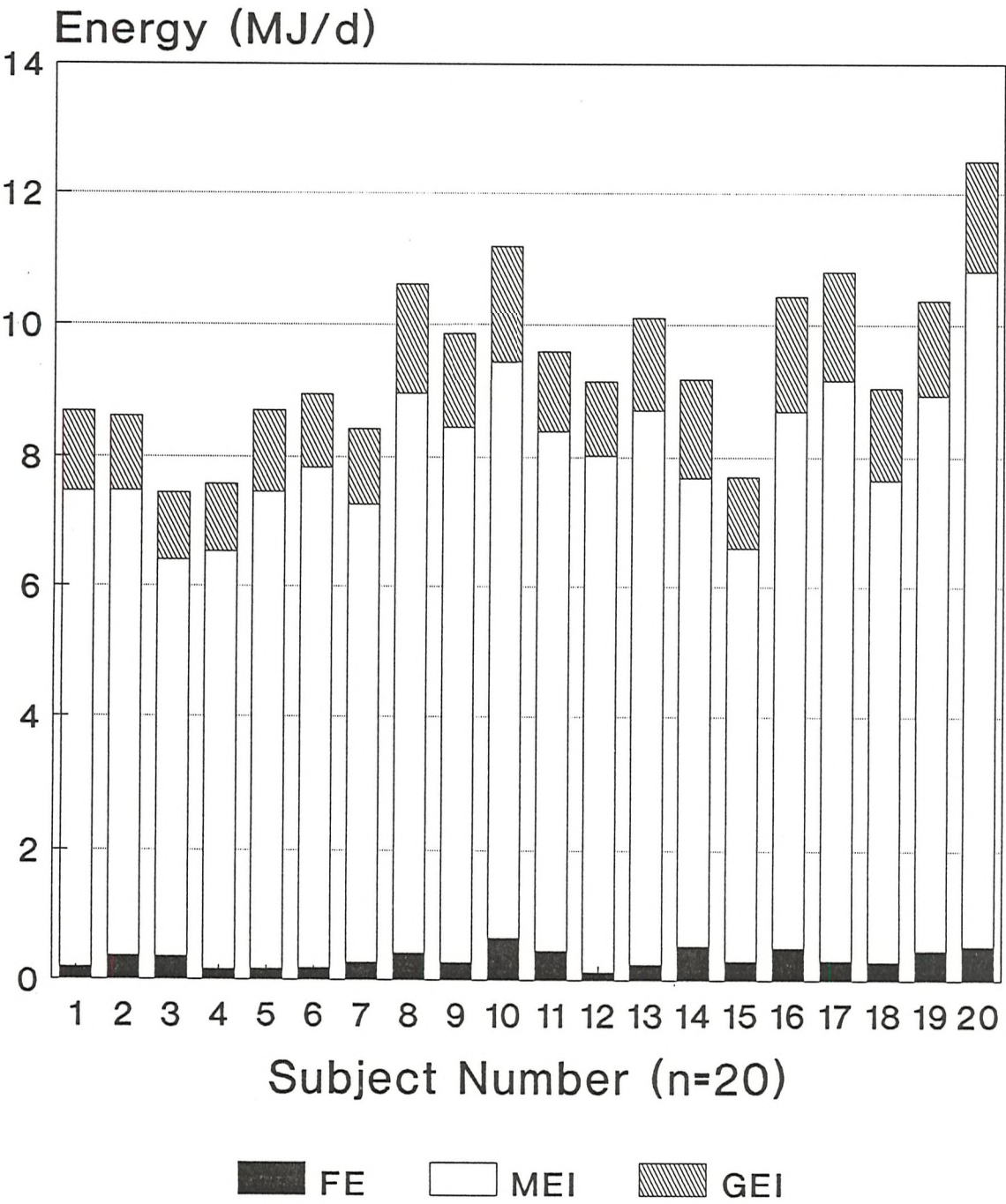
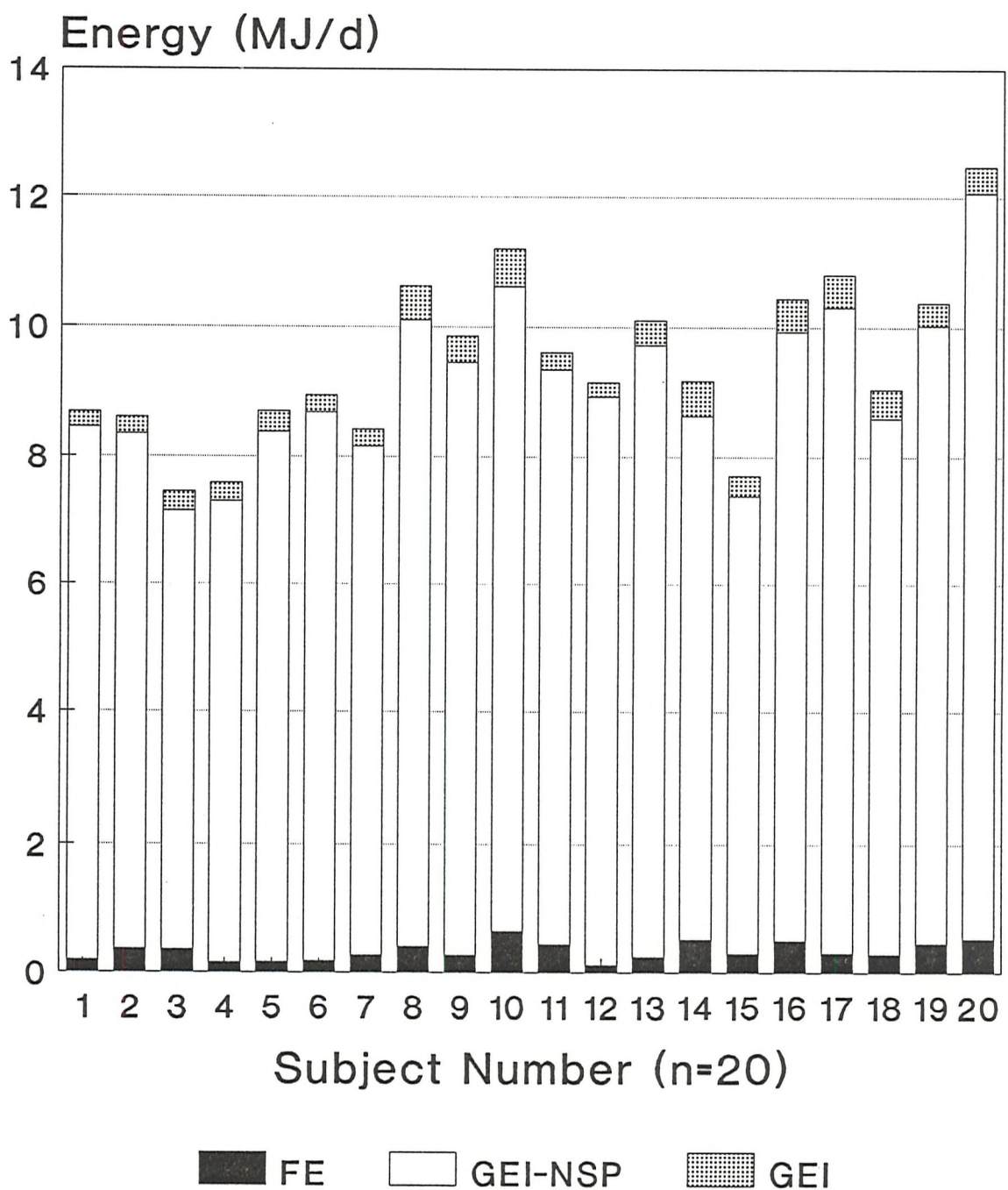


Figure 5.2 Daily faecal energy content (FE) determined from three day stool collections in twenty normal healthy children. Daily gross energy intake (GEI) and the energy from non-starch polysaccharides (NSP) were calculated by applying heats of combustion values [Merrill & Watt, 1955] from seven day weighed food intakes. Gross energy intake with and without the inclusion of energy from non-starch polysaccharides are given.



CHAPTER 6. INFLUENCE OF DISEASE ON THE AVAILABILITY OF DIETARY ENERGY, FAECAL ENERGY CONTENT AND HUMAN COLONIC BACTERIA.

6.1 INTRODUCTION.

In this study attention is focused on the extent to which pathological states may influence the availability of energy from the diet and thereby the metabolic demands for normal growth and development in childhood.

Chronic undernutrition and growth retardation in the disease cystic fibrosis (CF) has been recognised for some time [Schwachman & Kulczycki, 1958; Sproul & Huang, 1964]. This is thought to be attributed to dietary inadequacy, in particular unfavourable energy balance rather than being inherent within the disease itself [Durie & Pencharz, 1989]. Raised faecal energy losses could contribute to the development of an energy deficit in CF through maldigestion and/or malabsorption. In an attempt to limit the maldigestion in CF, pancreatic enzyme replacement therapy (PERT) has been developed. Despite these advances however, the extent to which PERT may completely normalise maldigestion in CF and the magnitude of faecal losses of CF patients on established PERT remains unresolved. Therefore without this knowledge it would seem difficult to develop a complete understanding of the aetiology of this energy deficit in CF.

The present study was performed within a group of patients with CF to illustrate a pathological condition where the maldigestion and/or malabsorption of lipid, protein and carbohydrate may limit the availability of energy from the diet. The aim of the present study was to determine the amount and origins of energy within stools each day in CF patients by applying a similar approach to the study presented in healthy children. Comparisons will be made between the two subject groups.

In addition to the main question, a secondary question was raised in this study. Some patients with CF produce large amounts of sputum especially during chest physiotherapy or during infectious exacerbation which represents a potentially important route by which energy could be lost from the body. Therefore the extent to which the energy content of sputum may contribute to an energy deficit in patients with CF was explored.

6.2 METHODS.

Twenty patients with CF aged 5-25 years (13 females; 7 males) from a CF clinic in the Wessex region were studied on their established PERT regimen. The details of the subjects are presented in table 6.1. The CF patients were taking between 11-43 capsules of Creon (Duphar, UK, 8000 BP units lipase/capsule) each day and had been using PERT in the form of enteric coated microspheres for at least one year prior to the study. All the patients self-titrated their enzyme dosage against gastro-intestinal symptoms including abdominal pain, diarrhoea, offensive bulky faeces and increased stool frequency which ranged between 1-3 times each day. No attempt was made to alter or intervene with the management of PERT. Sixteen of the twenty patients were taking antibiotics at the time of the study. Sputum samples were collected into plastic vessels over a two day period in five of the twenty CF patients. All of the subjects were in hospital at the time of the sputum collection and were taking intravenous antibiotics.

Informed consent was obtained from all of the subjects after the nature of the investigation had been explained. The study protocol had the approval of the Ethical Committee of Southampton Hospitals and South West Hampshire Health Authority.

Weighed food intake was recorded for a period of 7 days using digital electronic scales as described in the methodology chapter. The families of the subjects were instructed in the use of scales and recording of food intake prior to commencing the study. Weighed food intake was analyzed using computerised food composition databases for gross energy and metabolisable energy intakes. Further details are given in chapter 3. All the subjects were closely monitored and given support throughout the study period. After completion of the record, the families were interviewed to ensure that there were no omissions or errors such as failure to record pancreatic enzymes or supplements.

All stools were collected between carmine markers for the final three days of the seven day study period following the procedure described in the methodology chapter. All the subjects were cooperative and capable of following the details of the study protocol. At no time was difficulty encountered nor any need found to modify the procedure to accommodate the subjects.

The methods for preparation of stools for analysis of faecal energy, lipid and nitrogen content of stools and faecal bacterial mass are given in chapter 3. A portion of dried bacterial mass from each stool sample was pooled to provide a representative sample of faecal bacterial from each of the subjects. The energy, lipid and nitrogen contents per gram of stool bacteria from pooled samples were determined using the previously described methods in chapter 3. With this information, the proportion of faecal energy, lipid and nitrogen contents of stool that may be attributed to stool bacteria could be determined.

Daily sputum samples were pooled and homogenised with distilled water. Aliquots were then freeze-dried overnight (Genevac, UK) to a constant weight. The energy content of freeze-dried sputum was measured by ballistic bomb calorimetry (Gallenkamp, UK) using a similar procedure described for the freeze-dried faeces in the methodology chapter.

The values are presented as medians (ranges). Standard statistical techniques were performed to compare the relationships between variables ie. Pearson product moment correlation coefficient (R) and linear regression. Statistical comparisons between CF patients and healthy children or referred to as controls were assessed by the Wilcoxon rank sum test for unpaired data. Some of the data is presented for the healthy control group. The reader is referred to Chapter 5 for further details. A p value of less than 0.05 was considered significant.

6.3 RESULTS.

Gross energy intake (GEI) was approximately 14.9% greater (6.0-29.3%) than metabolisable energy intake (MEI) estimated using Atwater factors. Figure 6.1 illustrates the calculated difference in MEI and GEI for each subject. The difference in GE calculated with and without the consideration of dietary NSP was approximately 2.6% (1.3-5.3%)(Figure 6.2).

There was no significant difference in gross energy, metabolisable energy for both CF patients and healthy subjects (Table 6.2). Stool energy losses were three times greater in CF patients (0.99 MJ/day; 0.4-2.1 MJ/day) than controls (0.3 MJ/day; 0.1-0.7 MJ/day; $p < 0.05$), equivalent to 10.1% (4.9-19.7%) of GEI compared to 3.5% (1.3-5.8%; $p < 0.01$) of GEI in controls (Figure 6.3). Dry stool weight and wet stool weight were approximately twice as great as controls 36.7 g/day (13.7-83.2 g/day) compared with 15.7 g/day (5.7-36.6 g/day; $p < 0.01$) and

122.1 g/day (51.8-212.4 g/day) compared with 58.6 g/day (26.2-158.1 g/day; $p < 0.01$; Figure 6.4; respectively). The water content of stool expressed as a percentage of faecal weight was 71.4% (60.0-79.1%) which was not significantly different to control values (73.7%; 54.9-84.9%). Whilst a weak association between GEI and faecal dry weight ($R = 0.36$; NS) could be demonstrated in patients with CF, a moderate association between gross energy and dry weight was observed in healthy controls ($R = 0.60$; $p < 0.01$) (Figure 6.5). Apparent digestibility of energy (gross energy minus faecal energy expressed as a percentage of gross energy intake) was 89.9% (80.3-95.1%) in CF patients compared to 96.5% (94.2-98.7%; $p < 0.01$) in healthy controls.

In CF patients stool lipids were four times greater 9.7 g/day (3.4-21.9 g/day) than controls (2.1 g/day; 1.1-3.6 g/day; $p < 0.01$). Faecal lipids were equivalent to 11.7% (2.8-37.9%; $p < 0.01$) of lipid intake compared with 2.9% (1.3-4.4%) of lipid intake in controls ($p < 0.01$; Figure 6.6). Nitrogen within stools of CF patients was approximately 1.6 g/day (0.6-7.8 g/day) which was twice as great as controls (1.0 g/day; 0.4-2.4 g/day; $p < 0.01$; Figure 6.6), equivalent to 19.0% (4.0-59.9%) and 11.7% (4.9-19.1%) of nitrogen intake in CF patients and healthy controls respectively ($p < 0.01$).

There were weak associations between stool lipid and energy content of faeces for CF patients ($R = 0.43$; NS) and controls ($R = 0.51$; $p < 0.05$; Figure 6.7). Stool lipid could account for 39.8% (14.9-84.8%) of faecal lipid compared to 23.1% (16.2-66.0%; NS) in healthy controls. Only 19% and 26% of the variance in faecal energy could be attributable to differences in stool lipid for CF patients and control subjects respectively.

In CF patients there was some association between faecal protein and the energy content of stools ($R = 0.56$; $p < 0.01$; Figure 6.8) which accounted for 23.5% (10.5-94.6%) of faecal energy content. In control subjects there was a closer relationship between faecal protein and faecal energy content ($R = 0.79$; $p < 0.01$), which accounted for 49.0% (33.0-117.2%; $p < 0.05$) of the energy within stools. Only 31% of the variance in faecal energy could be attributed to differences in faecal protein in CF patients, compared to 62% in healthy controls.

Whilst stool lipid was a poor predictor of stool energy, a closer association could be demonstrated between faecal energy and either the wet or dry weight of stools in CF patients ($R = 0.86$; $R = 0.98$ respectively; $p < 0.01$) and healthy

controls ($R = 0.77$; $R = 0.98$ respectively; $p < 0.01$). These relationships are illustrated in Figure 6.9 and 6.10. Approximately 74% and 59% of the variance in stool energy could be attributed to differences in wet stool weight respectively in CF patients and healthy controls. For both groups, 96% of the variance in stool energy could be attributed to differences in dry stool weight.

The regression line describing the relationship between the energy content and dry weight of faeces were similar for the CF patients and control subjects (CF: faecal energy (kJ/day) = $78.9 + (\text{faecal dry weight (g/day)} \times 24.0)$, SEE = 91.4; controls: faecal energy (kJ/day) = $35.5 + (\text{faecal dry weight (g/day)} \times 18.0)$, SEE = 30.6). Attempts to determine whether the two lines were similar or that they represented two independent relationships using methodology described by Zerbe and coworkers (1982) revealed that the two lines were not statistically different. As it was not possible to differentiate between the two lines, a single expression to describe the relationship between faecal energy and faecal dry weight for both subjects was determined : faecal energy (kJ/day) = $-55.7 + (\text{faecal dry weight (g/day)} \times 26.1)$, SEE = 96.0, $R^2 = 95.3\%$. The amount of energy per gram dry weight of stool was significantly greater in CF patients (25.7 kJ/g; 22.7-35.9 kJ/g) than controls (20.5 kJ/g; 17.6-23.7 kJ/g; $p < 0.01$).

More obvious differences between CF patients and controls were observed for the relationship between faecal energy and faecal wet weight (CF: faecal energy (kJ/day) = $68.9 + (\text{faecal wet weight (g/day)} \times 7.6)$, SEE = 202.0; controls: faecal energy (kJ/day) = $123.0 + (\text{faecal wet weight (g/day)} \times 3.1)$, SEE = 95.3. Faecal energy per gram of wet weight of faeces was 7.6 kJ/g (5.7-11.1 kJ/g) and significantly greater than controls (5.3 kJ/g; 2.8-8.8 kJ/g; $p < 0.01$).

In patients with CF, the bacterial content of stools was 11.2 g/day (3.1-38.9 g/day) and three times greater than control subjects (3.6 g/day; 1.9-9.4 g/day; $p < 0.01$; Figure 6.11). Stool bacterial mass was equivalent to 32.7% (19.2-61.4%) of stool dry weight in CF patients compared with 25.6% (17.0-42.0%; $p < 0.05$) in healthy controls. A marked association could be demonstrated between stool bacterial mass and faecal dry weight for both CF patients ($R = 0.82$; $p < 0.01$) and healthy controls ($R = 0.77$; $p < 0.01$; Figure 6.12). The number of bacteria within faeces was 9.6×10^{13} counts/day ranging from 1.7×10^{13} - 25.7×10^{13} which was approximately twice as great as controls. It was confirmed that over 99% of the bacteria within faeces was recovered in the

bacterial fraction. The bacterial content per gram of dry stool was significantly greater in CF patients (0.33 g/g; 0.10-0.61 g/g) than controls (0.26 g/g; 0.17-0.42 g/g; $p < 0.05$).

Estimates of the energy, lipid and nitrogen content of pooled faecal bacteria from both CF patients and controls are shown in table 6.3. There was no difference between CF patients and control subjects for each of these values. Applying this data, the energy within the stool attributable to bacteria was over three times greater in CF patients (252 kJ/day; 74.6-925 kJ/day) than controls (73.8 kJ/day; 39.3-191.0; $p < 0.01$). Despite a difference in faecal energy content between CF patients and control subjects, the energy content of bacteria accounted for 29.5% (9.4-56.9%) of the energy within faeces in CF patients compared to 25.4% (17.1-42.8%) in controls (Figure 6.13). This difference was not significant. In CF patients the stool bacterial content of lipid was 1.8 g/day (0.5-6.2 g/day) which was approximately twice as great as controls (0.7 g/day; 0.4-1.9 g/day; $p < 0.01$). There were poor associations between stool bacterial mass and faecal lipid for both CF patients ($R = 0.35$; NS) and control subjects ($R = 0.38$; NS; Figure 6.14). Stool bacteria could account for 20.4% (3.3-62.8%) of faecal lipid. This was significantly less than controls (41.0%; 18.7-87.6%; $p < 0.01$; Figure 6.15).

In CF patients the amount of nitrogen within faecal bacteria was 0.3 g/day (0.1-1.2 g/day) which was approximately twice as great as controls (0.2g/day; 0.1-0.4g/day; $p < 0.01$). A moderate relationship between faecal bacterial mass and faecal protein in CF patients could be demonstrated ($R = 0.59$; $p < 0.05$) with a closer association for control subjects ($R = 0.74$; $p < 0.01$; Figure 6.16). The nitrogen content of bacteria could account for 20.6% (4.4-48.1%) of stool nitrogen which was not significantly different to controls (16.0%; 10.2-24.6%; Figure 6.15).

The amount of wet sputum and dry sputum produced each day was approximately 9.0 g/day (3.0-144.0 g/day) and 2.1 g/day (0.2-13.9 g/day). Sputum energy losses were 34.6 kJ/day (2.2-274 kJ/day), equivalent to 0.8% (0.04-4.6%) of GEI. The amounts of energy per gram of wet and dry sputum were 1.9 kJ/g (0.8-11.7 kJ/g) and 19.5 kJ/g (13.7-23.7 kJ/g) respectively.

6.4 DISCUSSION.

The present investigation would appear to be the first study that has specifically determined the magnitude and origins of energy within the stool of individuals with the disease cystic fibrosis (CF). There have been relatively few reports of the energy contents of stools in other pathological states associated with maldigestion and/or malabsorption [Brown *et al.* 1980; Heymsfield *et al.* 1981; Rodrigues *et al.* 1989]. It would appear that in this group of CF patients on PERT who believed themselves to be comparatively asymptomatic, faecal energy losses were substantially greater than healthy control children and could be equivalent to between 5-20% of the gross energy intake. This would suggest that these raised faecal energy losses may contribute in part towards an energy deficit that may be sufficient to limit growth or cause weight loss. Whilst this study did not specifically attempt to measure the efficacy of PERT, it would appear that in some patients the PERT regimen may be inappropriately regulated by self-titration in the absence of gastro-intestinal symptoms.

It has been previously described in the review of the literature (chapter 2) that studies examining the efficacy of PERT have shown that faecal lipid losses in CF patients could be as much as 12-15 g/day [Stead *et al.* 1987; Vyas *et al.* 1990], and nitrogen losses of approximately 1-2 g/day [Beverley *et al.* 1987; George *et al.* 1987; 1990] when using PERT. As the stool and nitrogen losses in patients with CF in the present study were comparable with these values from earlier investigations, this would suggest that the PERT in CF patients in this study reduced stool lipid and nitrogen to similar degrees. Despite this reduction in stool lipid and nitrogen, it appears that stool energy could still be equivalent to up to 20% of the gross energy intake in CF patients on their habitual PERT.

Whilst the magnitude of gross energy and metabolisable energy were comparable for CF patients and healthy controls, the range in the difference between gross energy and metabolisable energy in CF patients was considerable ranging between 6-20%. Whilst the factors to determine MEI and GEI were designed to be applied under normal circumstances, it would seem that the extent of the error which may be introduced in the calculation of the metabolisable energy content of the diet under pathological conditions should be given further attention.

The present approach to address the efficacy of PERT is primarily directed towards the symptomatic correction of steatorrhoea, abdominal pain relief and a decrease in stool frequency and stool mass passed each day. Whilst steatorrhoea has been frequently used as the index of maldigestion [Mischler *et al.* 1982; Stead *et al.* 1987], it is commonly assumed that when gastro-intestinal symptoms are improved, the amount of energy available from the diet would be normal. It would appear from the present study that the approach of measuring faecal lipid losses as an index of maldigestion must be questioned as energy losses could not be simply related to the amount of lipid within the stool. This would suggest that CF patients with small lipid losses could have high faecal energy losses. Whilst faecal lipid remained elevated in CF patients, equivalent to up to 38% of lipid intake less than half of the energy within the stool could be attributed to lipid. In contrast to the present finding, a previous study has been able to demonstrate an association between the lipid and nitrogen content of stool and faecal energy [Heymsfield *et al.* 1981]. However in this study faecal energy losses were examined in a heterogeneous group of patients with different pathological conditions associated with maldigestion and/or malabsorption. It could not be established whether this relationship may hold within one specific disease group.

The present data suggests that a more accurate assessment of the extent to which PERT normalises stool energy loss in CF patients could be achieved by routine assessment of faecal energy. However the facilities for the direct measurement of faecal energy are not generally available in clinical practice and the collection of faecal material is both a time consuming and unpopular procedure. In the present investigation there was a strong relationship between the energy content of stool and wet weight of stool and an even closer association between faecal energy and dry weight of stool could be demonstrated. The relationship between faecal energy and wet weight of stool has been confirmed under different pathological diseases [Heymsfield *et al.* 1981], and in lactose-malabsorbing children [Brown *et al.* 1980] and in CF patients and pre-term infants [Bijleveld *et al.* 1986]. More recently Rodrigues and coworkers [1989] have demonstrated a close association between dry stoma weight and energy absorption rather than total energy loss within patients with short bowel syndrome. From the data available in the present study, it would appear that 8kJ (2 kcal) of energy is present within each gram of wet stool in CF patients. What this information would provide is an estimation of the extent of faecal energy loss by simply weighing stools passed each day. It is suggested that the routine

measurement of faecal energy from the weight of stool may provide a more accurate assessment of the extent to which PERT reduces energy losses in CF than the measurement of faecal lipid. The apparent constancy of energy per gram of weight stool in both CF patients and healthy controls would also imply that the composition of stool must remain relatively similar over a wide range of faecal energy losses.

By analogy to normal circumstances, it is presumed that in diseases associated with maldigestion and/or malabsorption that increased faecal losses are derived directly from dietary residue that has not been absorbed. Therefore this assumes that all of the energy within stool may be attributed to maldigested and/or malabsorbed dietary residue. The failure to demonstrate a relationship between gross energy intake and weight of faeces in the present group of CF patients would suggest that the presumption that the material within faeces is derived directly from the diet is not supported. The fact that stool energy losses could be derived from endogenous secretions and cellular debris as well as colonic bacteria has been previously considered in healthy children where approximately 25% of stool energy could be attributed to bacteria.

In CF patients, it would also appear that an important component of stools is made by bacteria which could represent approximately 33% of stool dry weight, although the extent of this contribution was variable ranging between 19-61%. This variability in the contribution made by faecal bacteria could also be demonstrated from the proportions of energy, lipid and nitrogen attributed to bacteria. Although it has not been possible to quantify mucus and epithelial cell losses within the stool, this component may also represent a substantial part of stool energy losses in CF where mucus production is elevated.

Therefore it is clear that a major component of stool is not simply maldigested and/or malabsorbed dietary residue, but that a major part of energy within the stool of CF patients could be attributed to faecal bacteria. This information would also imply that despite a reduction in the calculated apparent digestibility of energy in CF patients, the contribution made by bacteria towards faecal energy content would indicate that the actual digestibility of dietary energy could be higher than indicated.

Whilst more bacteria was present within the stools of CF patients in terms of number and per gram of stool, the proportion of lipid which could be attributed to bacterial lipids was less than healthy controls. In some patients with maldigestion and/or malabsorption the lipid from dietary residue or sloughed epithelial cells may also make an important contribution towards the lipid content of faeces.

The theoretical amount of energy that may be yielded by short chain fatty acid (SCFA) absorption within the large intestine could also be estimated from the present data in this study. Details of this calculation based upon the growth requirements of intestinal bacteria [McNeil, 1984], are shown in Table 6.4 for CF patients and healthy controls. Estimates of the amount of carbohydrate suggest that the intake of NSP alone of 13.9 g/day (range 5.7-31.4) in CF patients could not produce the weight of bacteria within faeces. As outlined in control subjects (see chapter 5), carbohydrate substrate required for colonic bacterial fermentation must be derived from maldigested and/or malabsorbed starch and the carbohydrate within endogenous losses and cellular debris. Whilst the intakes of dietary NSP were comparable for CF patients and control subjects, the theoretical amount of hexose required to produce the weight of faecal bacteria was greater in CF patients than control subjects. This would suggest that the fermentable carbohydrate from maldigested and/or malabsorbed dietary residue such as starch and endogenous losses in cystic fibrosis where mucus production is raised may both form potentially important sources of fermentable substrate for bacterial fermentation within the large intestine.

Subtraction of the energy within faecal bacterial mass from this total energy yield from fermentation provides a value for the amount of energy that may be made available through SCFA absorption. From these calculations it appears that the contribution of SCFA to energy requirements is equivalent to 5.7% (1.6-14.5%) in CF patients which was significantly greater than healthy controls (1.9%; 0.9-4.2%; $p < 0.01$). This would suggest that the supply of energy in the form of SCFA from the large intestine affords the opportunity to recover at least part of the energy from either malabsorbed starches and sugars or endogenous secretions that would otherwise be lost within the stools in CF patients. This observation would support the claim made by Bond and coworkers [1980] that the colon may play an important role in malabsorption by salvaging carbohydrate and absorbed SCFA may also be an important link in explaining the 'energy gap' as discussed by Grosslaus [1983].

What is also evident for the present study is that the differences in the amount of energy recovered reflects the between individual differences in the metabolic activities of colonic bacteria. This is an important observation as it could be that the production of SCFA from both dietary and endogenous sources of carbohydrate could have a critical role in the maintenance of energy balance in CF and other pathological condition associated with maldigestion and/or malabsorption, particularly in individuals on a marginal intake of energy or in whom requirements are elevated.

The contribution made by the expectoration of sputum in relation to the potential loss of energy from the body was also investigated in the present study. It could be established that sputum has an energy content of approximately 2 kJ within each gram of wet sputum and thereby some energy could be lost within sputum. Although the volume of sputum may be large, the total sputum energy lost each day however was only equivalent to less than 1% of gross energy. The greatest sputum energy loss observed was 274 kJ/day, equivalent to approximately 5% of gross energy intake. Much of the sputum produced in CF patients is swallowed thereby allowing the opportunity to salvage the energy and nitrogen content of sputum through digestion or fermentation. However even though the amount of energy lost as sputum may be relatively small, it is important that this route of energy loss is not ignored particularly where energy intakes are marginal or during infection as sputum energy losses could contribute towards an energy deficit in CF patients.

6.5 SUMMARY AND CONCLUSIONS.

- i. Whilst the amount of energy within the diet was comparable between healthy control children and CF patients, the energy content of stool was three times greater in CF patients, equivalent to 10.6% of gross energy intake and despite their normal PERT.
- ii. Faecal lipid could account for less than half of the energy within the stool and was not simply related to faecal energy which would challenge the approach to use steatorrhoea as an index of maldigestion.
- iii. Estimation of the energy within the stool each day from stool wet weight may provide a simple way of evaluating the extent to which maldigestion and/or malabsorption in CF patients may be improved by PERT.

- iv. It should not be assumed that all of the energy within the stool is simply maldigested and/or malabsorbed dietary residue. Although the relative proportions of bacteria, dietary residue and other endogenous components may vary within the stool, the bacterial microflora represent a major component of the energy within faeces of CF patients.
- v. Colonic bacteria activity, although variable, allows the opportunity to recover some energy through SCFA from the fermentation of dietary and endogenous sources of carbohydrate which would be otherwise lost within the stool.

In conclusion, it would appear that both the extent of faecal energy losses and the metabolic activity of intestinal bacteria could both have an important influence on energy metabolism in pathological conditions associated with maldigestion and/or malabsorption. In addition the contribution made by bacteria towards faecal energy content should not be overlooked in the assessment of apparent digestibility both in health and disease.

Table 6.1. The characteristics of the cystic fibrosis patients who participated in the study are given including faecal energy losses, gross energy intakes and pancreatic enzyme replacement therapy (PERT) usage. The values are presented for each subject and the median value is also given.

SUBJECT	AGE Yrs	GENDER	WEIGHT kg	HEIGHT m	GROSS ENERGY kJ/day	FAECAL ENERGY kJ/day	PERT capsules /day
1	6.9	F	20.4	1.19	10580	2084	30
2	13.8	F	26.5	1.42	10752	1092	37
3	7.9	F	28.1	1.29	10831	1058	20
4	8.9	M	34.2	1.32	9589	850	11
5	17.4	F	38.9	1.54	5904	716	23
6	10.2	F	21.8	1.33	5652	383	20
7	22.9	M	45.3	1.59	9853	1020	12
8	14.9	M	53.2	1.67	14755	1504	30
9	9.5	M	29.5	1.33	8558	1224	9
10	9.0	F	28.0	1.31	8371	1414	19
11	18.0	F	43.3	1.55	5771	963	15
12	5.3	M	20.0	1.13	5895	493	27
13	8.5	F	24.8	1.33	13954	944	14
14	5.5	M	17.5	1.09	6632	767	16
15	5.4	F	18.3	1.13	11689	575	43
16	5.8	F	18.0	1.09	7285	539	16
17	8.1	F	34.0	1.35	11389	1152	24
18	6.3	F	20.0	1.14	7763	1134	25
19	17.4	M	58.9	1.75	15907	898	15
20	25.7	F	44.0	1.65	17543	1014	23
MEDIAN	9.0		28.1	1.33	9721	989	20

Table 6.2 Estimated gross energy intake and NSP energy intake determined by applying heats of combustion values [Merrill & Watt, 1955] and estimated metabolisable energy intake determined by applying modified Atwater factors [Paul & Southgate, 1978] from 7 day weighed food intakes in cystic fibrosis patients and healthy control subjects. The values presented are medians and ranges in parenthesis.

	CF	CONTROL	
GROSS ENERGY INTAKE (MJ/day)	9.7 (5.7 - 17.5)	9.2 (7.3 - 12.5)	NS
NSP ENERGY INTAKE (MJ/day)	0.2 (0.1 - 0.6)	0.3 (0.2 - 0.6)	NS
METABOLISABLE ENERGY INTAKE (MJ/day)	8.3 (4.9 - 15.0)	7.9 (6.3 - 10.8)	NS

Table 6.3 The energy, lipid and nitrogen per gram of bacteria measured in pooled samples of dried faecal bacteria from cystic fibrosis patients and healthy control subjects.

	CF	CONTROL
BACTERIAL ENERGY CONTENT * (kJ/g)	23.8	20.4
BACTERIAL LIPID CONTENT * (g/g)	0.16	0.20
BACTERIAL NITROGEN CONTENT * (g/g)	0.03	0.04

* ESTIMATES FROM POOLED SAMPLES

Table 6.4 Additional energy that could be provided from colonic fermentation using the calculation of McNeil (1984) calculated from the data derived from cystic fibrosis patients and healthy control subjects. The values presented are medians and ranges in parenthesis.

Significant differences are for CF patients compared with healthy controls (* p < 0.01).

	CF	CONTROL
HEXOSE REQUIRED TO SYNTHESIZE FAECAL BACTERIAL MASS (g/day) ¹	37.0 (10.3 - 128.3)	11.9 * (6.3 - 30.9)
FAECAL BACTERIAL ENERGY (kJ/day) ²	252.0 (75.0 - 925.0)	74.0 * (39.0 - 191.0)
TOTAL ENERGY YIELD (kJ/day) ³	648.0 (181.0 - 2245.0)	208.0 * (110.0 - 541.0)
ENERGY AVAILABLE AS SCFA (kJ/day) ⁴	396.0 (106.0 - 1320.0)	134.0 * (70.7 - 350.1)
ENERGY AVAILABLE FROM FERMENTATION (%)	5.7 (1.6 - 14.5)	1.9 * (0.9 - 4.2)

¹ ASSUMES 0.1 MOL ATP TO SYNTHESIZE 1g OF BACTERIAL MASS AND THAT 33g HEXOSE FROM FERMENTATION WILL GENERATE 1 MOL ATP.

² CALCULATED USING ENERGY VALUES (kJ/g) MEASURED WITHIN BACTERIAL SAMPLES FROM POOLED SAMPLES FROM EACH OF THE SUBJECTS WITHIN EACH GROUP.

³ ASSUMES 17.5 kJ/g HEXOSE.

⁴ TOTAL ENERGY YIELD - FAECAL BACTERIAL MASS.

Figure 6.1 Daily faecal energy content (FE) determined from three day stool collections in twenty patients with cystic fibrosis. Daily gross energy intakes (GEI) applying heats of combustion values [Merrill & Watt, 1955] and metabolisable energy intakes (MEI) using modified Atwater factors [Paul & Southgate, 1978] were calculated from seven day weighed food intakes.

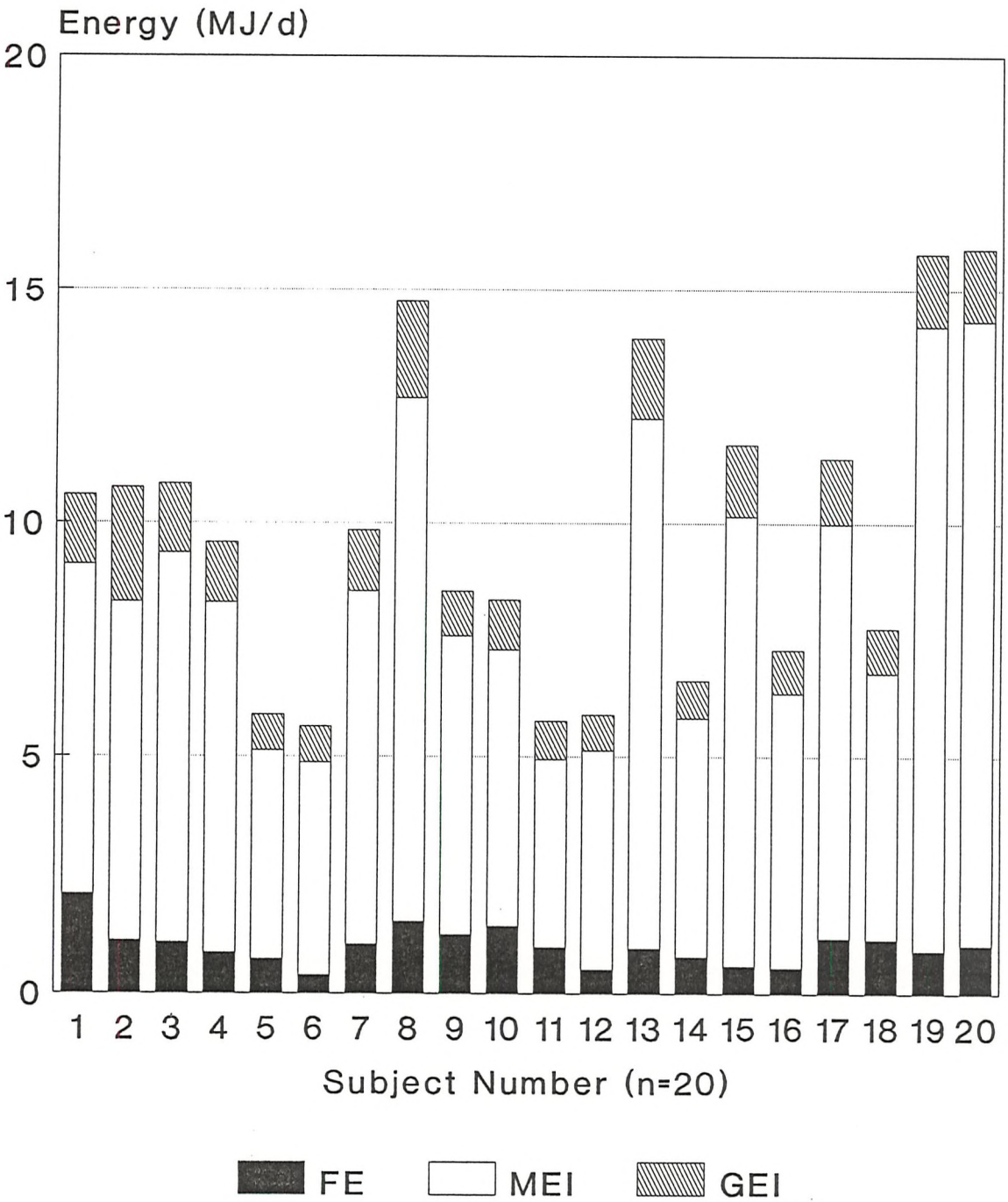


Figure 6.2 Daily faecal energy content (FE) determined from three day stool collections in twenty patients with cystic fibrosis. Daily gross energy intake (GEI) and the energy from non-starch polysaccharides (NSP) were calculated by applying heats of combustion values [Merrill & Watt, 1955] from seven day weighed food intakes. Gross energy intakes with and without the inclusion of energy from non-starch polysaccharides are given.

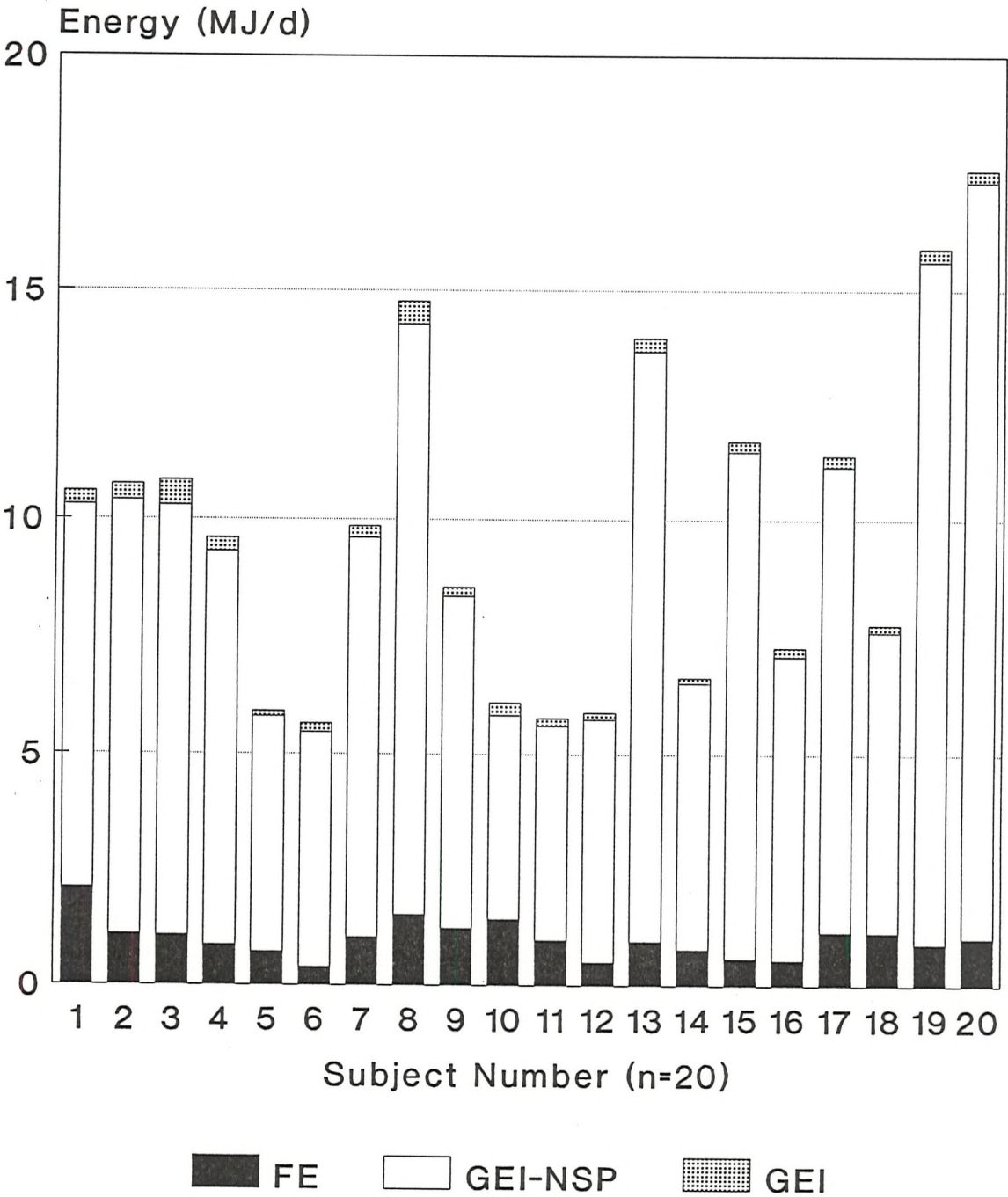


Figure 6.3 Daily faecal energy content expressed as MJ/day and as a percentage of calculated gross energy intake (%GEin) for cystic fibrosis patients and healthy control subjects. The values are presented for each subject and the bars represent the median values.

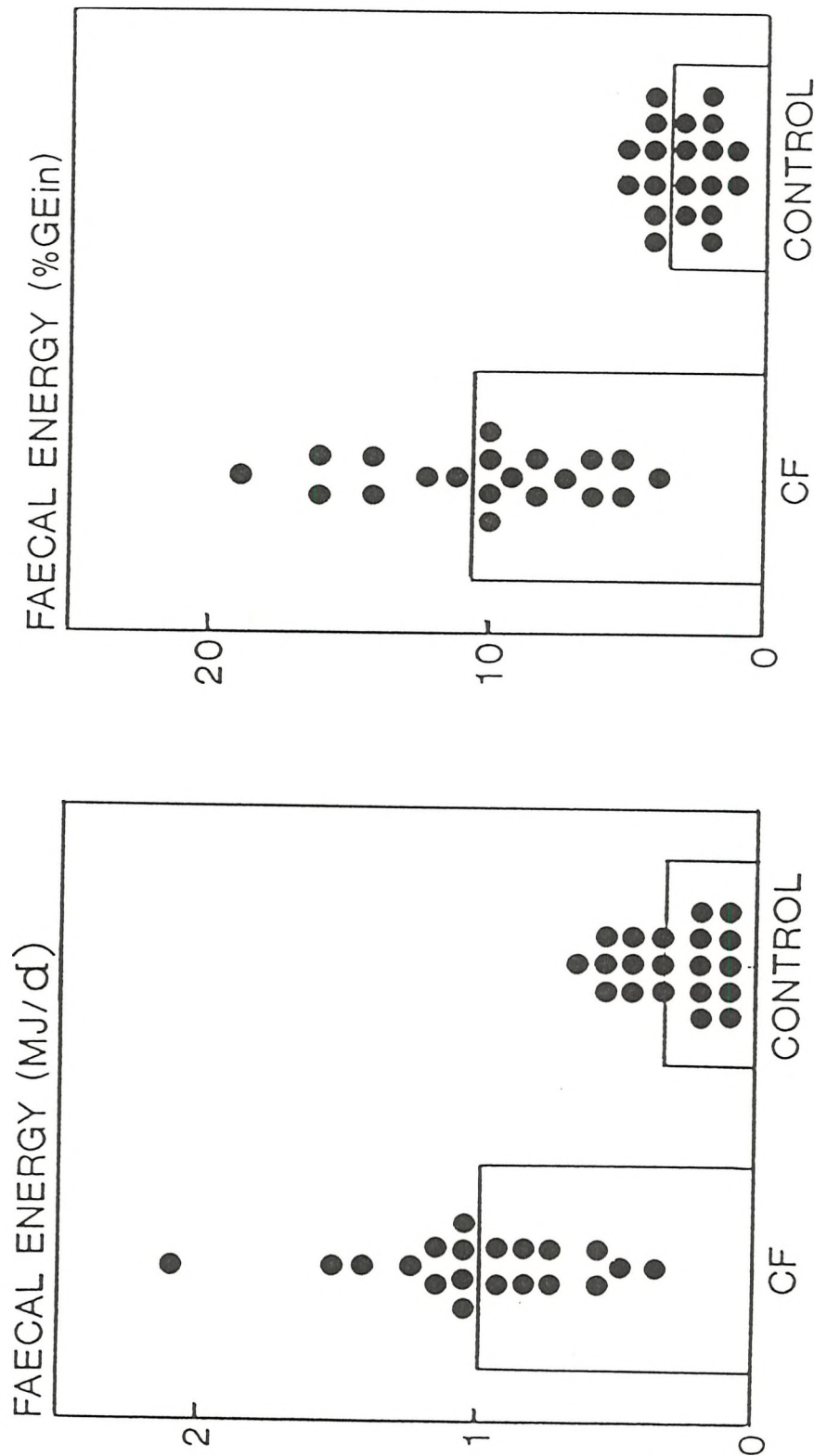
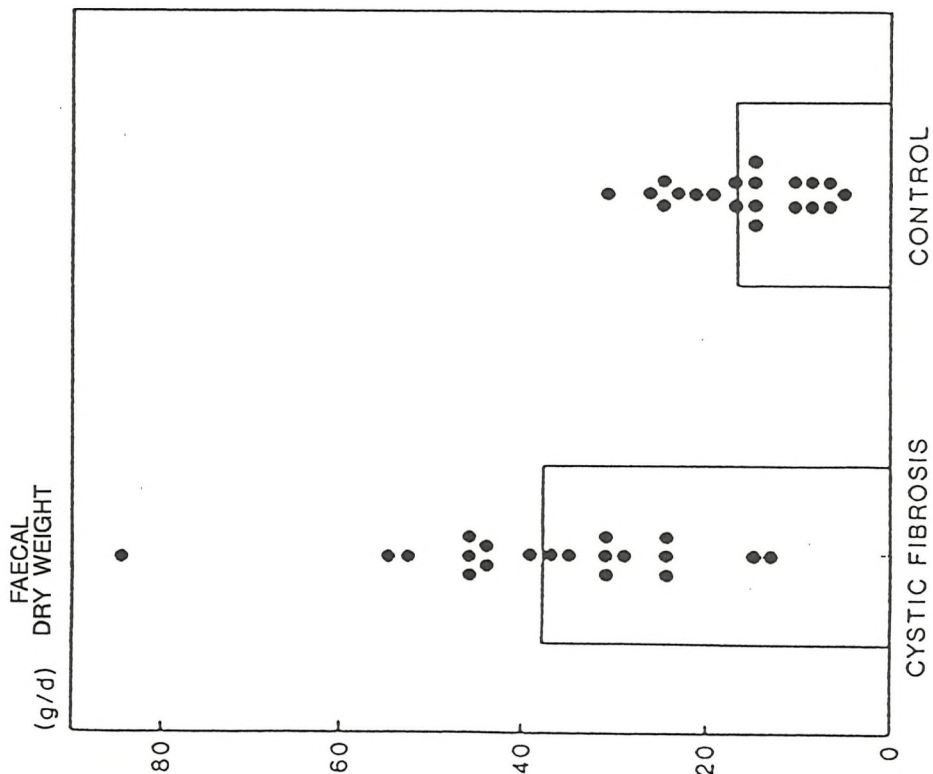
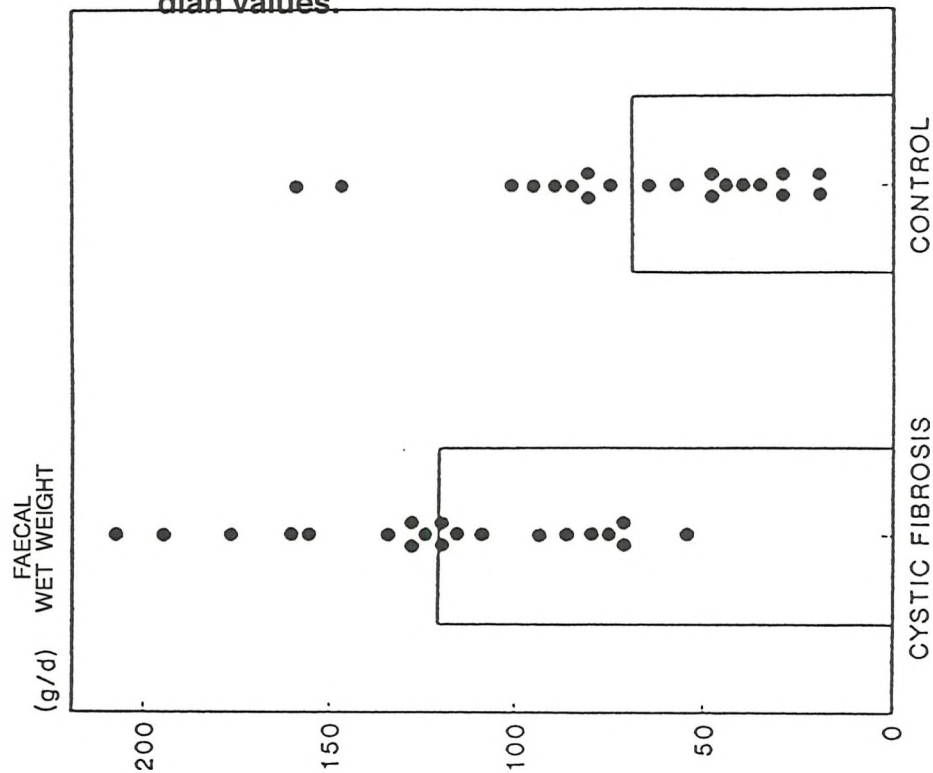


Figure 6.4 Daily faecal wet weights expressed as g/day for cystic fibrosis patients and healthy control subjects. The values are presented for each subject and the bars represent the median values.



Correlation coefficients are 0.36, (NS) and 0.60, ($p < 0.01$) respectively.



Figure 6.6 Daily nitrogen and lipid content of faeces expressed as g/day for cystic fibrosis patients and healthy control subjects. The values are presented for each subject and the bars represent the median values.

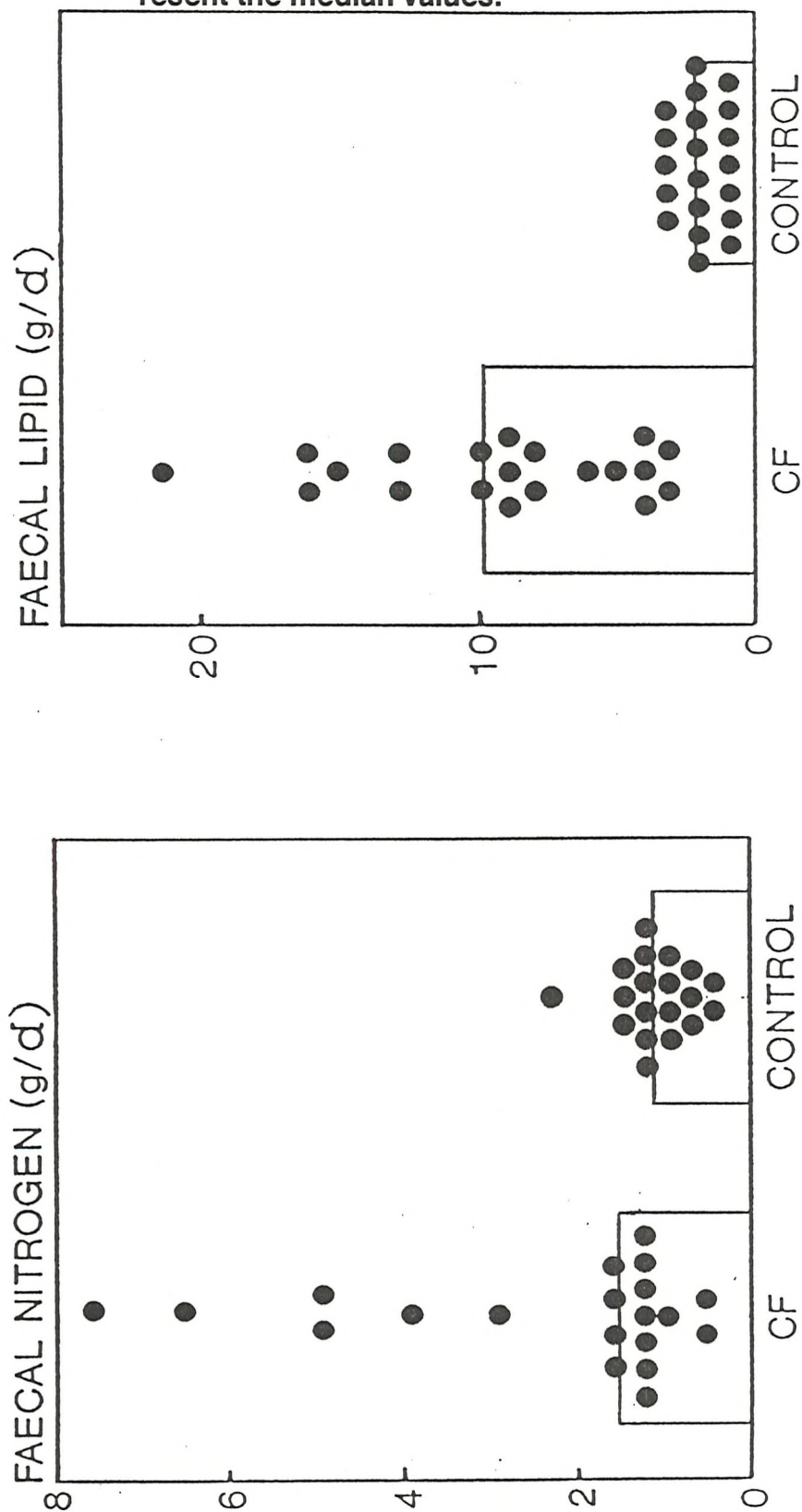


Figure 6.7 Daily faecal energy content versus lipid content of faeces for cystic fibrosis patients and healthy control subjects. Correlation coefficients are 0.43, (NS) and 0.51, ($p < 0.05$) respectively.

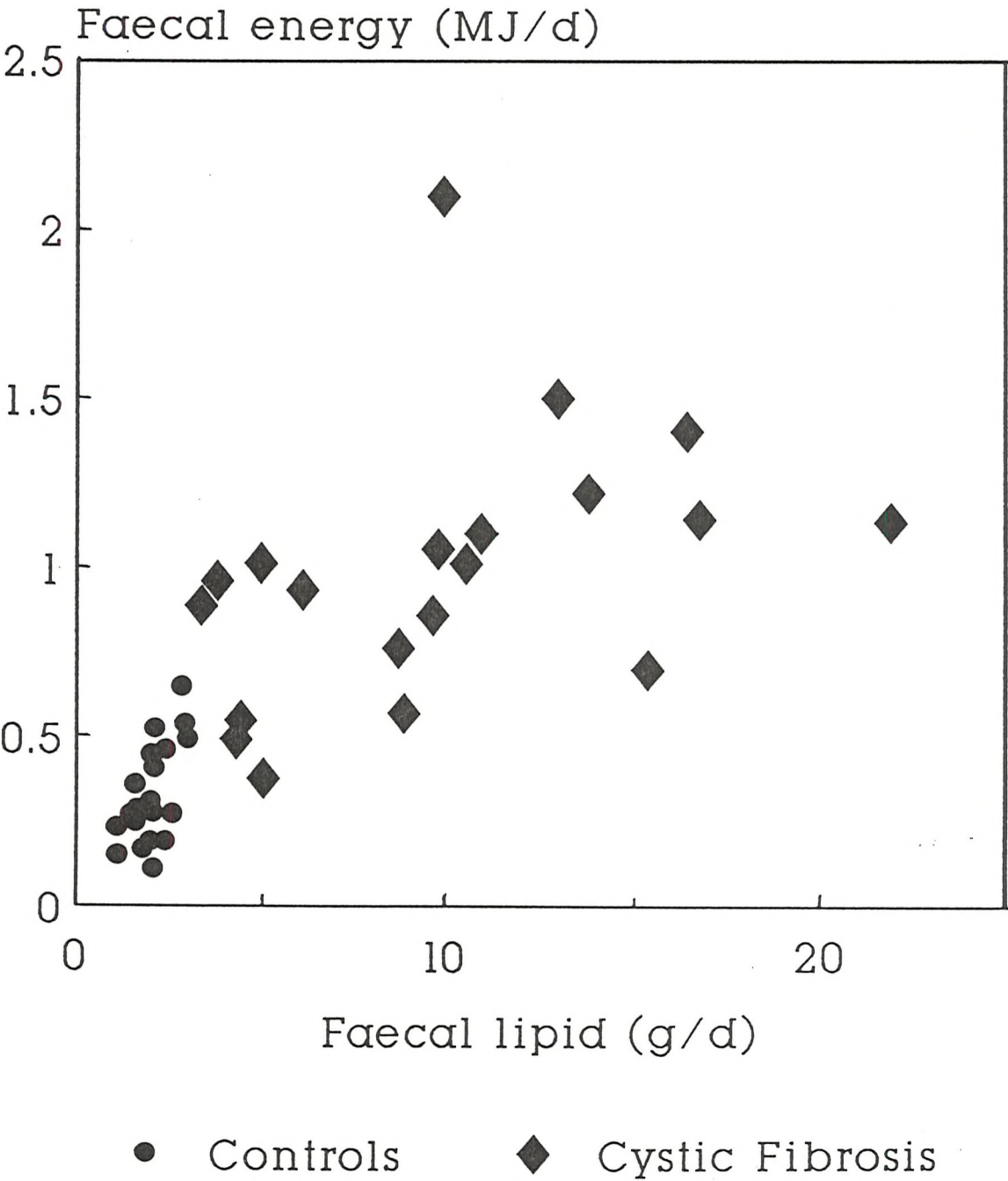


Figure 6.8 Daily faecal energy content versus protein content of faeces derived from the measurement of faecal nitrogen as $N \times 6.25$ for cystic fibrosis patients and healthy control subjects. Correlation coefficients are 0.56, ($p < 0.01$) and 0.79, ($p < 0.01$) respectively.

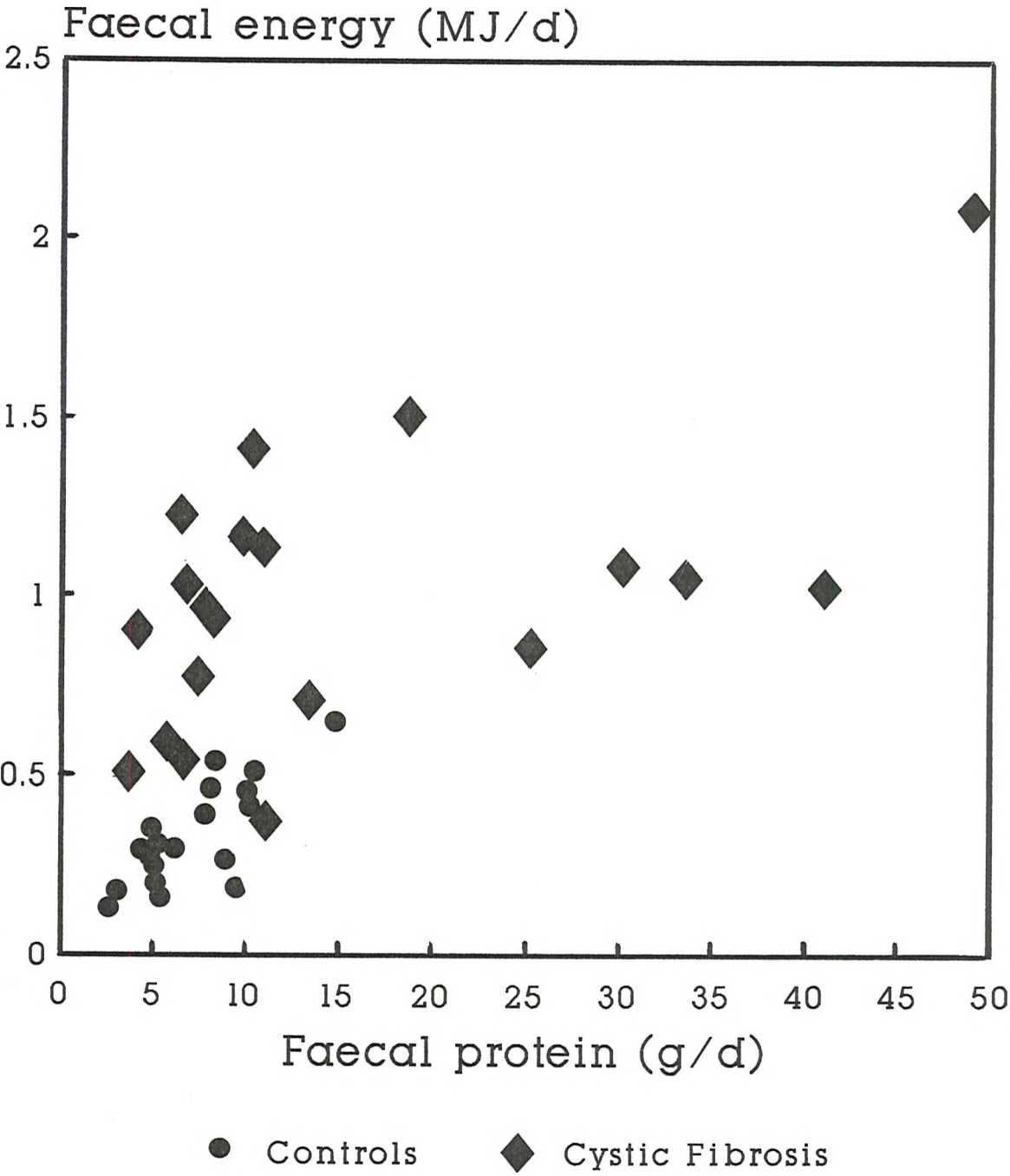


Figure 6.9 Daily faecal energy content versus wet weight of stool for cystic fibrosis patients and healthy control subjects. Correlation coefficients are 0.86, ($p < 0.01$) and 0.77, ($p < 0.01$) respectively. The regression lines are: faecal energy (kJ/day) = $68.9 + \{\text{faecal wet weight (g/day)} \times 7.6\}$; faecal energy (kJ/day) = $123.0 + \{\text{faecal wet weight (g/day)} \times 3.1\}$, respectively.

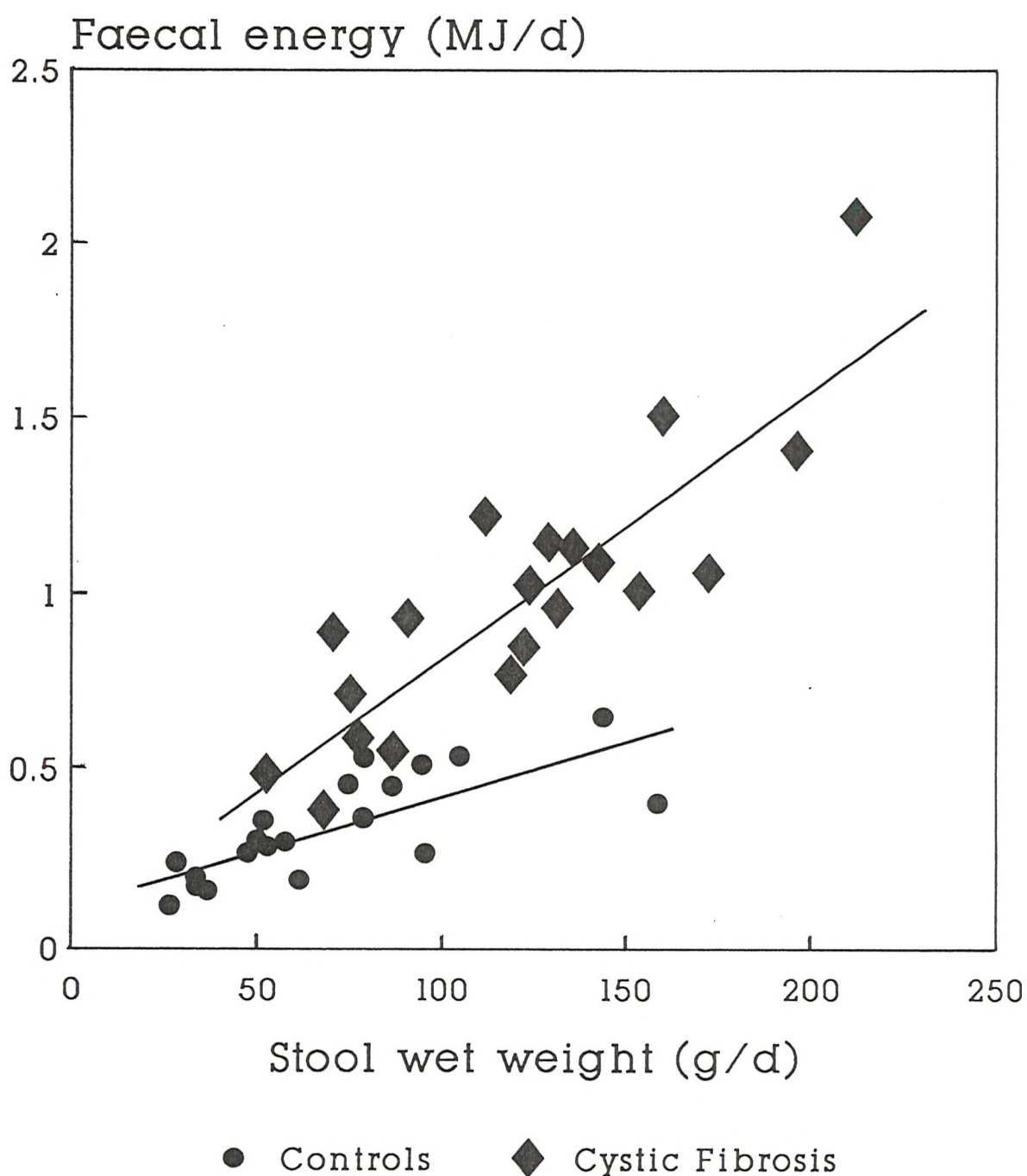


Figure 6.10 Daily faecal energy content versus dry weight of stool for cystic fibrosis patients and healthy control subjects. Correlation coefficients for both groups are 0.98, ($p < 0.01$). The regression lines are faecal energy (kJ/day) = 78.9 + {faecal dry weight (g/day) x 24.0}; faecal energy (kJ/day) = 35.5 + {faecal dry weight (g/day) x 18.0}, respectively.

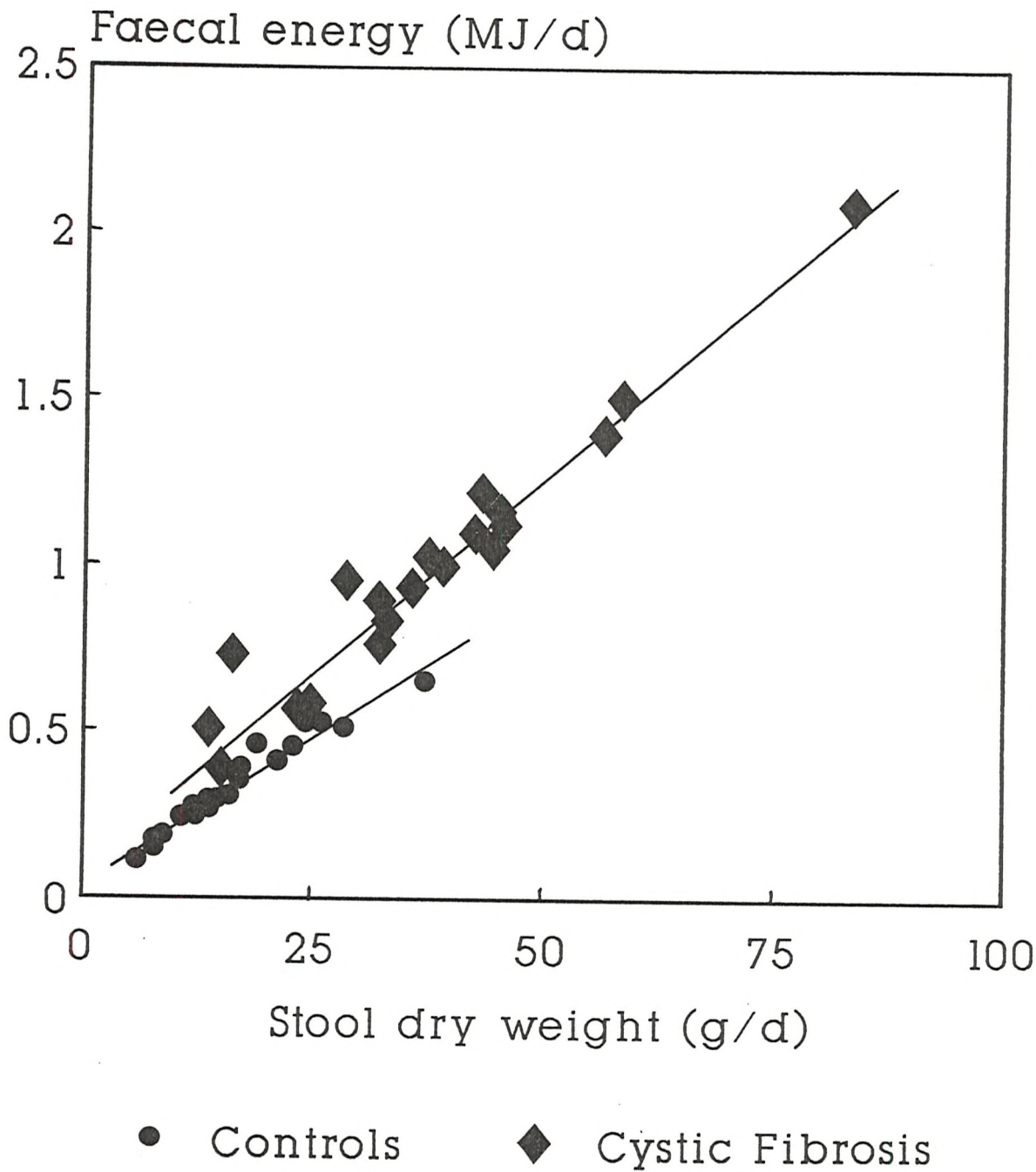


Figure 6.11 Daily bacterial content of faeces expressed as g/day for cystic fibrosis patients and healthy control subjects. The values are presented for each subject and the bars represent the median values.

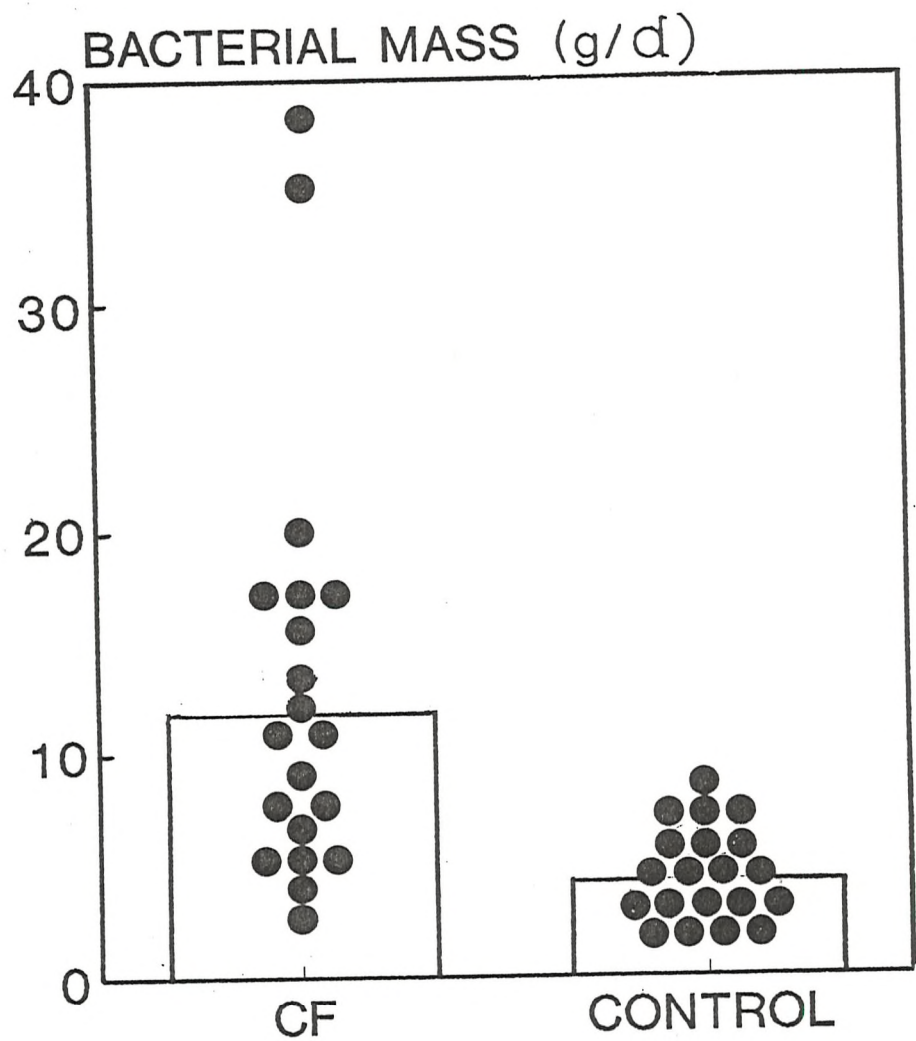


Figure 6.12 Bacterial content of faeces each day versus dry weight of stool for cystic fibrosis patients and healthy control subjects.
Correlation coefficients are 0.82, ($p < 0.01$) and 0.77, ($p < 0.01$) respectively.

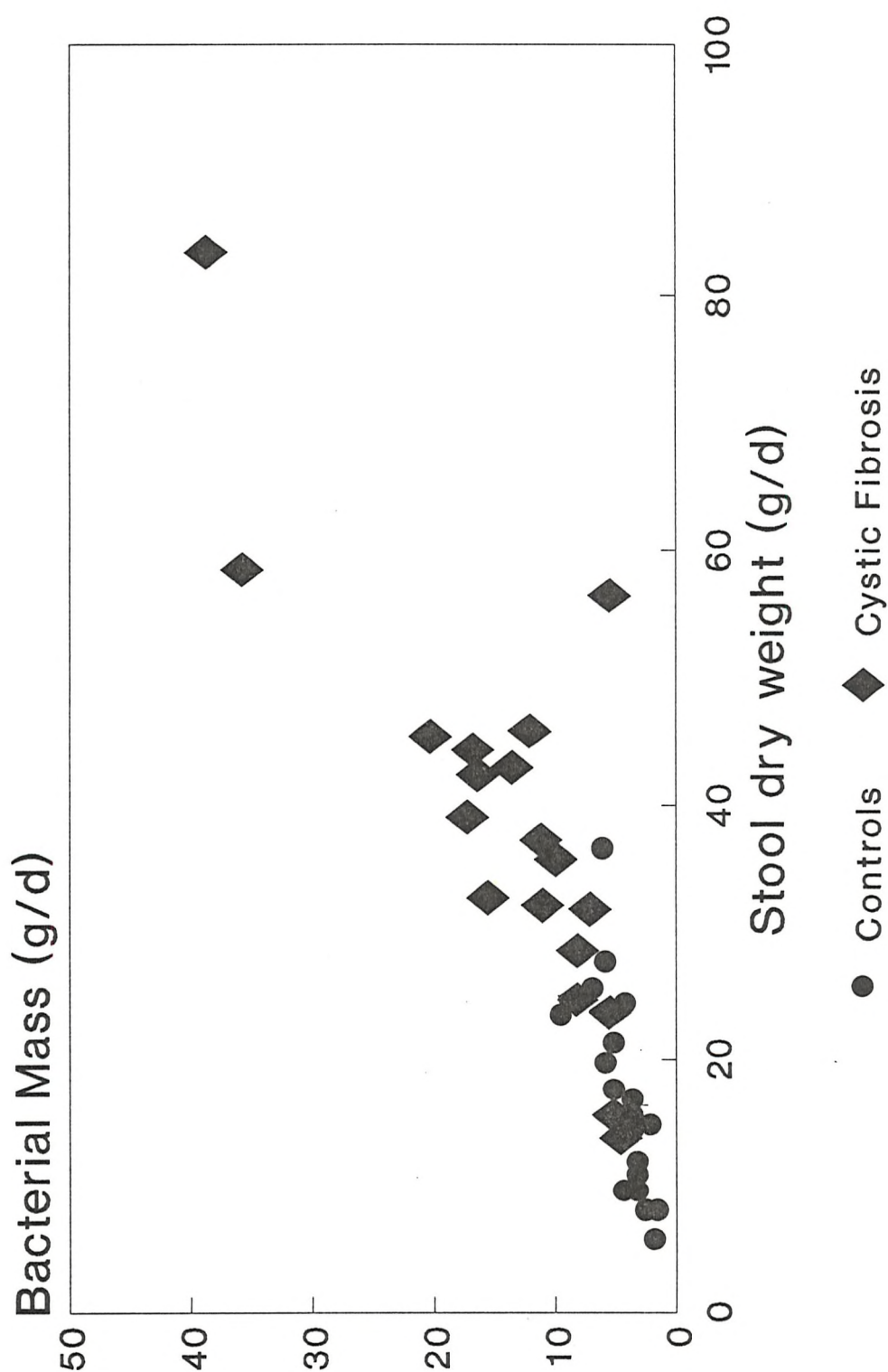


Figure 6.13 Daily energy content of faecal bacterial expressed as a percentage of stool energy for cystic fibrosis patients and healthy control subjects. The values are presented for each subject and the bars represent the median values.

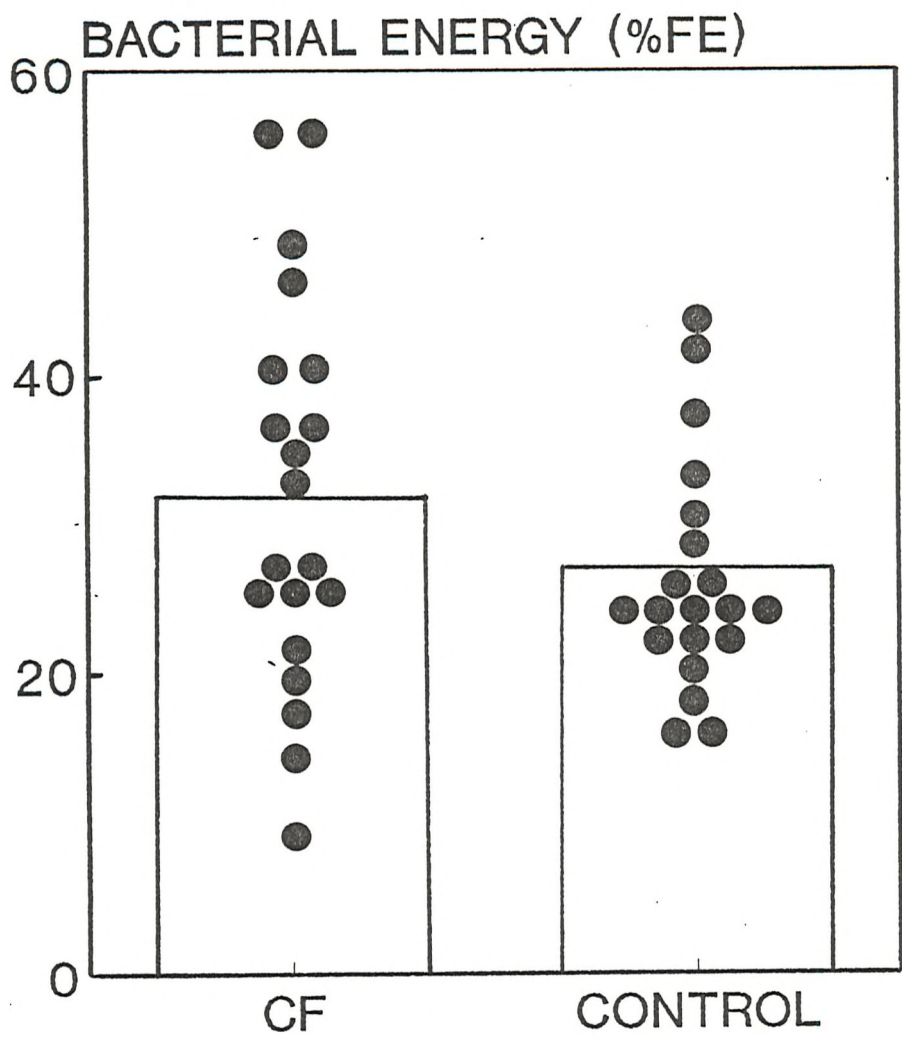


Figure 6.14 Daily faecal lipid versus bacterial content of faeces for cystic fibrosis patients and healthy control subjects. Correlation coefficients are 0.35, (NS) and 0.38, (NS) respectively.

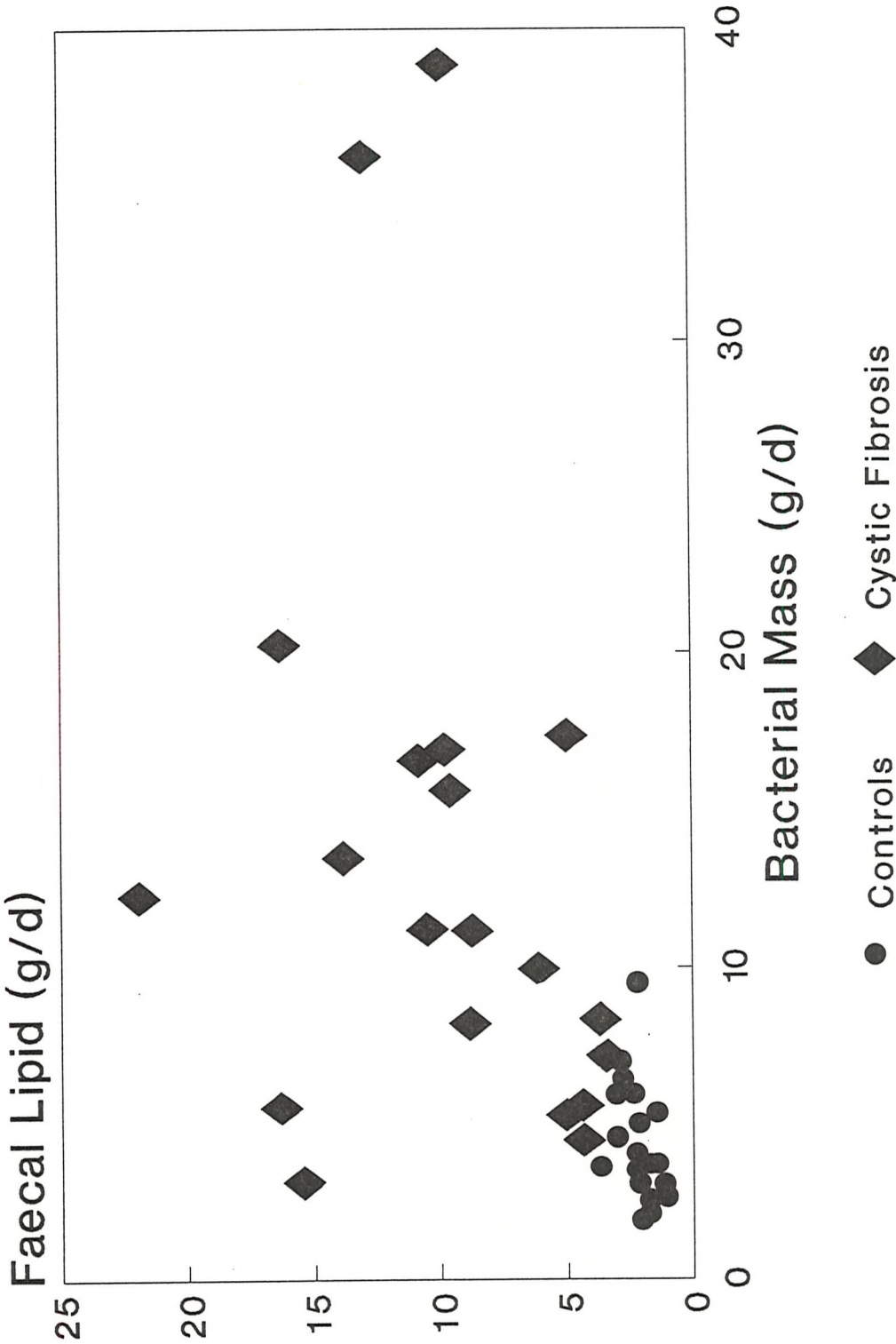


Figure 6.15 Lipid and nitrogen content of faecal bacteria expressed as a percentage of stool lipid and nitrogen respectively for cystic fibrosis patients and healthy control subjects. The values are presented for each subject and the bars represent the median values.

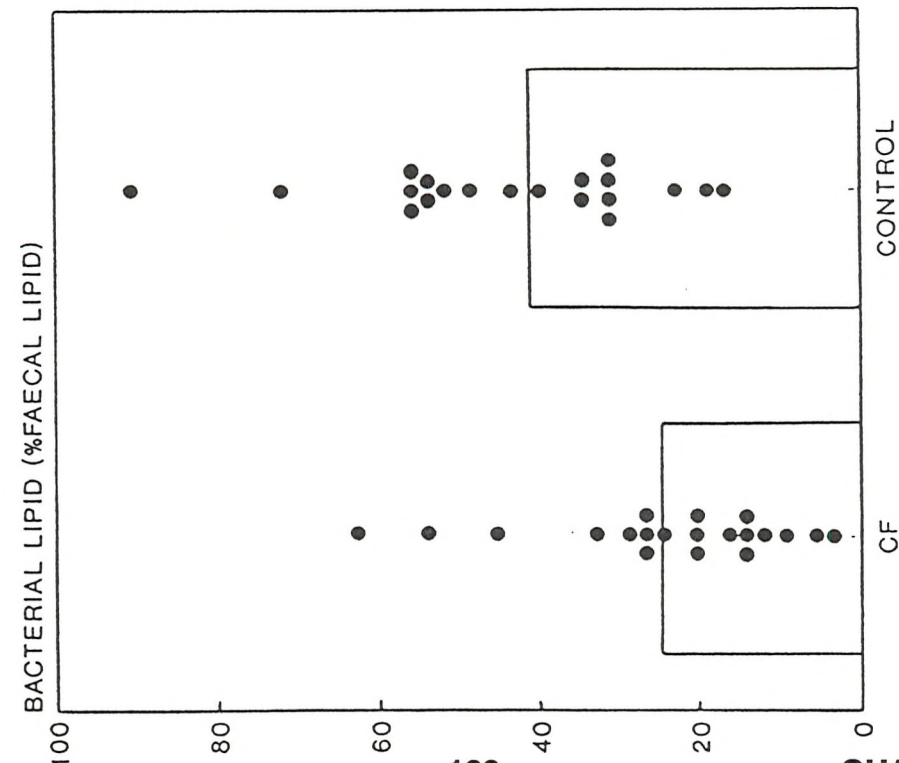
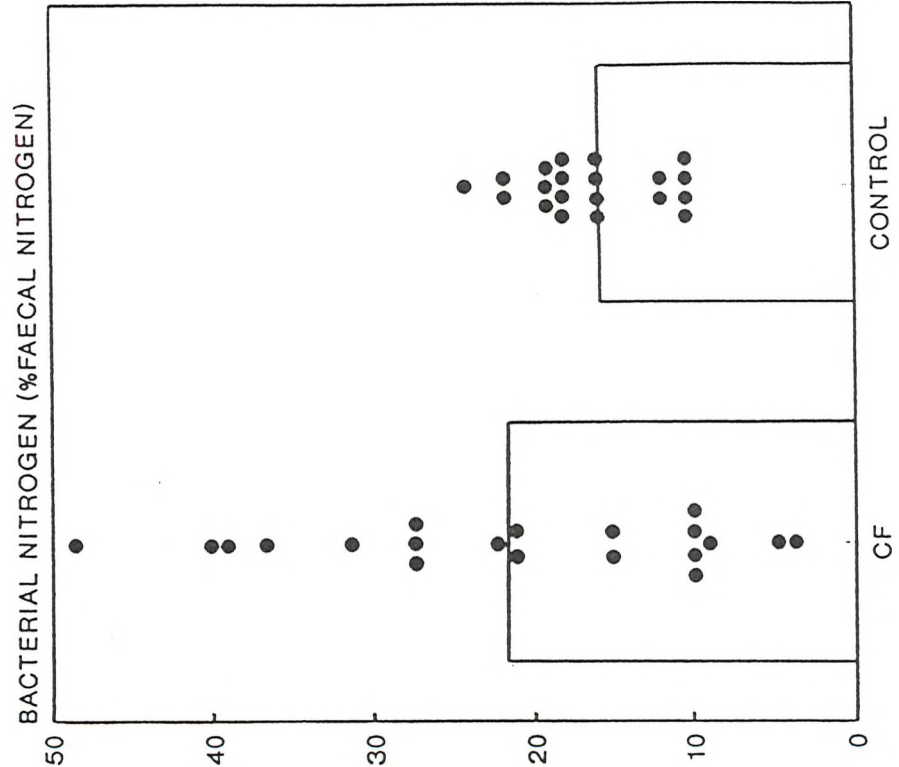
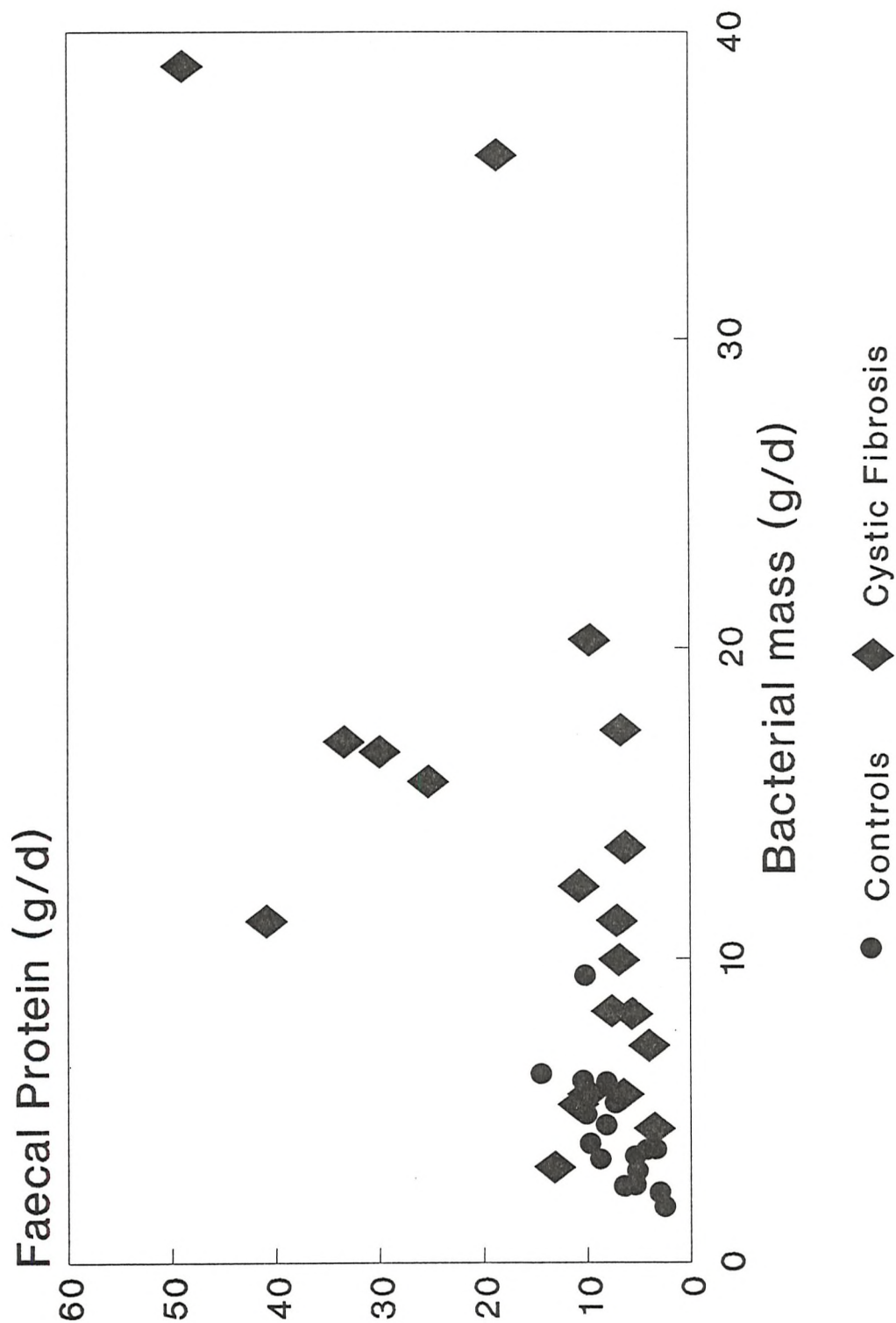


Figure 6.16 Protein content of faeces derived from the measurement of faecal nitrogen as $N \times 6.25$ versus faecal content bacterial for cystic fibrosis patients and healthy control subjects. Correlation coefficients are 0.59, ($p < 0.05$) and 0.74, ($p < 0.01$) respectively.



CHAPTER 7. EFFECT OF NON-STARCH POLYSACCHARIDES ON THE AVAILABILITY OF DIETARY ENERGY; ENERGY LOSS AND HUMAN COLONIC BACTERIA IN NORMAL HEALTHY ADULTS.

7.1 INTRODUCTION.

The potential role of dietary NSP in relation to human metabolism at the level of the colon remains unclear. In an attempt to explore the importance of this relationship, in this chapter, attention is directed towards how changes in dietary NSP may effect colonic function.

Without knowledge of the influence of diet on the metabolic activity of the colonic microflora, it is difficult to develop an understanding of the extent to which short chain fatty acids (SCFA) may contribute to energy requirements in man. Several studies have examined the effect of alterations in diet on the colonic bacteria by investigating bacterial profiles within the stool [Winitz *et al.* 1970; Drasar & Jenkins, 1976; Finegold & Sutter, 1978]. However, the overall findings of these studies have not been conclusive. It would appear that a different approach is required if we are to resolve this question.

With the interest in dietary NSP and its effect on bowel function, it has been known for some time that raised intakes of dietary NSP may be associated with increased faecal output and in the amounts of energy, lipid, nitrogen within the stool [Southgate & Durnin, 1970]. These relationships have been described in terms of a decrease in the apparent digestibilities of energy, lipid and nitrogen. Whilst this effect of dietary NSP has been explained by a number of mechanisms [Scheenan, 1978; Bender *et al.* 1979; Southgate, 1982; Saunders & Betschart, 1980](see section 2.7.2), the assumption that all of the energy within the stool is of dietary origin remains. It would appear that the potential contribution made by the colonic bacteria to energy, lipid and nitrogen losses within the stool has been clearly overlooked.

It may be that the decrease in the apparent digestibility of energy, lipid and nitrogen could be attributable to a greater faecal bacterial mass, by implication the growth of colonic bacteria [Stephen & Cummings, 1980b]. Whilst the majority of the nitrogen within faeces is thought to be largely bacteria on high NSP-diets

[Stephen & Cummings, 1980c], there is no information of the extent to which bacteria may contribute towards increased faecal energy, lipid and nitrogen losses by modifying the intake of NSP.

The aim of this study was to examine the effect of how alterations in dietary NSP may influence colonic bacterial activity. To answer this question a comparison of the energy, lipid and nitrogen contents of the faeces and the contribution made by the faecal bacteria to these components was determined in healthy adult men following the consumption of diets which provided different amounts and types of NSP.

7.2 METHODS.

Six normal adult men aged 22 to 43 years participated in the studies (Table 5.1). Three of the subjects were members of the academic staff at the University of Southampton and the other three subjects were medical students at Southampton General Hospital. Informed consent was obtained from all of the subjects after the nature of the investigation had been explained. They were all in good health at the time of the studies. None of the subjects had been taking any medication before or during the investigation. The study had the approval of the Southampton Hospitals and South West Hampshire Health Authority Ethical Subcommittee.

All of the subjects followed three study protocols. Two of the studies lasted for a period of 5 days where standard diets were provided. The third study was carried out over a seven day period during which the habitual food intake of each subject was determined by a weighed food diary record. Stools were collected between carmine markers during the final three days for each of the studies. All stool collections were complete.

7.2.1 DIETS.

For studies 1 and 2, the diet was based upon a commercial complete oral liquid formula (Ensure, Abbott Laboratories, UK). For the purpose of another investigation, the protein intake was reduced to 35 g/day. This would not influence the results of the present studies.

Study 1. (NSP-free diet)

During study 1, a diet containing no NSP was consumed by each of the subjects. Each day this diet provided 5.8 g nitrogen, 36g lipid, 447g carbohydrate, 9.2 MJ metabolisable energy, 10.1 MJ gross energy (expressed as medians). This was a liquid diet provided by Ensure with the energy intake maintained by the addition of a glucose polymer (Maxijul, Scientific Hospital Supplies, UK).

Study 2. (NSP-enriched diet)

In study 2, 50% of the daily energy intake was provided by Ensure and Maxijul in similar proportions to that taken in study 1. The diet was made isoenergetic by the addition of ripe bananas to provide fermentable carbohydrate. The intake of nutrients from bananas was determined by weighing the peeled banana immediately before ingestion and calculating the nutrient content from food composition tables (Paul & Southgate, 1979). This diet provided 5.7g nitrogen, 22.8g lipid, 524g carbohydrate, 45.4g starch, 52.5g NSP, 9.7 MJ metabolisable energy, 12.6 MJ gross energy (expressed as medians).

Study 3. (Habitual diet)

Weighed food intake was recorded for a period of seven days using digital electronic scales (Hanson, UK) and analyzed in the manner described in the methodology chapter. The habitual intake provided 17.5g nitrogen, 116g lipid, 326g carbohydrate, 30.3g NSP, 11.6 MJ metabolisable energy, 12.7 MJ gross energy (expressed as medians).

7.2.2 ANALYSES.

The procedures for the collection, weighing and preparation of stools and the analyses of energy, lipid, nitrogen and the amount of bacteria within the stool are previously described in the methodology chapter. A portion of dried faecal bacterial mass from all of the subjects for each diet was pooled to provide a representative sample of bacteria. The amounts of energy, lipid and nitrogen per gram bacteria were determined by the methods described in the methodology chapter within i) pooled samples of faecal bacterial material and ii) bacteria from

stool samples from each subject, for all diets. Using these values, the proportions of stool energy, lipid and nitrogen that may be attributable to faecal bacteria from both i) and ii) were estimated.

7.2.3 CALCULATIONS AND STATISTICS.

Results are given as medians and ranges. Comparisons between groups of data were assessed by the Wilcoxon matched pairs signed ranks test. The Pearson product moment correlation coefficient (R) was used to compare the relationship between variables. The apparent digestibilities of energy, lipid and nitrogen were calculated as the difference between dietary intake and faecal output expressed as a percentage of intake.

7.3 RESULTS.

Stool weights.

For each of the diets, the values for faecal wet and dry weights are illustrated in figure 7.1. Following the consumption of a diet with no NSP, there were reductions in the wet weights of stools by 72% and 66% respectively in comparison to NSP-enriched ($p < 0.05$) and habitual diets ($p < 0.05$) (Table 7.2). There were also reductions in the dry weights of stools from the NSP-free diet by 63% and 41% in comparison to the NSP-enriched ($p < 0.05$) and habitual diets ($p < 0.05$) respectively. Although there appeared to be a trend towards greater faecal weights from the NSP-enriched diet compared to the habitual diet, this difference was not significant. Whilst a five fold difference in dry stool weights was observed between subjects from the NSP-enriched diet, there were only two fold ranges for dry stool weights from both the NSP-free and habitual diets. Stool frequency from all the diets ranged between 0-2 stools passed each day. The percentage water content of stool was 6.9% greater for the habitual diet than NSP-free diet ($p < 0.05$). There were no associations between gross energy intake and faecal dry weight from both the NSP-free diet ($R = -0.5$; Not significant; NS) and NSP-enriched diet ($R = -0.34$; NS) and a weak association was observed from the habitual diet ($R = 0.59$; NS; figure 7.2). Whilst there was no association between dietary NSP and faecal dry weight from the NSP-enriched diet ($R = 0.35$; NS), a weak relationship could be demonstrated for the habitual

diet ($R=0.65$; NS; figure 7.3). Overall it appears that the associations between gross energy intake or dietary NSP intake with faecal dry weight were similar for the diets studied.

Stool energy.

The values for faecal energy are illustrated in figure 7.4. The consumption of the NSP-free diet resulted in decreases in stool energy losses by 64% and 46% respectively in comparison to the NSP-enriched ($p<0.05$) and habitual diets ($p<0.05$; table 7.3). There was a 5 fold difference in faecal energy from the NSP-enriched diet with only a 2 fold range in the values from the habitual diet. Whereas the amounts of energy within the stool for all the subjects from the NSP-free diet appeared to be relatively similar.

With the addition of NSP to the diet, faecal energy was equivalent to 7.2% of the gross energy intake. A five fold difference in these values was demonstrated. There was no difference between the values for the NSP-enriched diet and either the NSP-free or habitual diets (table 7.3).

The consumption of the NSP-free diet was associated with reductions in the amount of lipid within the stool by 40% and 63% when compared to the NSP-enriched ($p<0.05$) and habitual diets ($p<0.05$; figure 7.5). More of the lipid within the stool could be 'equivalent' to lipid intake with the addition of dietary NSP in comparison to both the NSP-free ($p<0.05$) and habitual diets ($p<0.05$). There were approximately 43% and 47% reductions in the amount of nitrogen within the stool from the NSP-free diet than the NSP-enriched ($p<0.05$) and habitual diets ($p<0.05$; figure 7.5). Stool nitrogen was 'equivalent' to more of the nitrogen intake with the addition of NSP to the diet when compared to the NSP-free diet ($p<0.05$) and habitual diets ($p<0.05$).

The addition of dietary NSP led to a reduction in the apparent digestibility of both lipid and nitrogen in comparison to both the NSP-free ($p<0.05$) and habitual diets ($p<0.05$)(table 7.4). Whilst there appeared to be a reduction in the apparent digestibility of energy from the NSP-enriched diet in comparison to the NSP-free and habitual diets, the difference did not attain statistical significance.

There were clear relationships between faecal energy and stool dry weight for all dietary groups (NSP-free diet $R=0.75$; $p<0.05$; NSP-enriched diet $R=0.99$; $p<0.01$; habitual diet $R=0.99$; $p<0.01$)(Figure 7.6). The values for faecal energy per gram dry weight of stool were similar from each of the diets (NSP-free diet 21.9 kJ/g; 20.3 - 24.2 kJ/g, NSP-enriched diet 21.3 kJ/g; 19.9 - 23.7 kJ/g, habitual diet 22.8 kJ/g; 20.4 - 23.4 kJ/g. Whilst more obvious differences were observed for the relationship between faecal wet weight and faecal energy (Figure 7.7), there were still close associations for each of the dietary groups (NSP-free diet $R=0.92$; $p<0.01$; NSP-enriched diet $R=0.80$; $p<0.05$; habitual diet $R=0.70$; NS). Faecal energy per gram wet weight of stool was greater from the NSP-free diet (5.7 kJ/g; 4.9 - 6.1 kJ/g) compared to the habitual diet (4.2 kJ/g; 2.8 - 5.6 kJ/g; $p<0.05$). There were no significant differences between the amounts of energy per gram wet weight of stool for the NSP-enriched (4.7 kJ/g; 3.2 - 8.2 kJ/g) and both the NSP-free and habitual diets. Overall it appears that the associations between faecal weight and the energy content of stools were similar for all the diets.

There was no association between faecal lipid and faecal energy for the NSP-enriched diet ($R= -0.43$; NS) and only a weak relationship could be demonstrated for the habitual diet ($R=0.41$; NS; figure 7.8). A moderate relationship was observed between faecal lipid and faecal energy for the NSP-free diet ($R=0.75$; NS). Overall it would seem that there were poor associations between faecal lipid and the energy content of stools for all the diets. Faecal lipid accounted for more of the energy within the stool for both the NSP-free diet (17.8%; 10.8 - 29.6%) and habitual diet (21.8%; 17.5 - 35.3%) than the NSP-enriched diet (8.7%; 3.1 - 33.3%). These differences were not significant.

There were weak relationships between faecal protein and faecal energy for both the NSP-free ($R=0.49$; NS) and NSP-enriched diets ($R=0.57$; NS)(Figure 7.9). There appeared to be a closer association between faecal nitrogen and faecal energy for the habitual diet ($R=0.81$; $p<0.05$). Overall no general association between faecal protein and the energy content of stools could be demonstrated for all the diets. Faecal protein ($N \times 6.25$) could account for more of the energy within the stool from the NSP-free diet (40.7%; 26.5 - 49.8%) than both the NSP-enriched diet (26.2%; 11.2 - 48.2%) and habitual diet (35.5%; 31.4 - 50.8%). None of these differences attained statistical significance.

Stool bacterial mass.

The amounts of bacteria within the stool for all dietary groups are illustrated in Figure 7.10. Table 7.5 shows that following the consumption of the NSP-free diet an 80% reduction in bacterial mass was observed when compared to the NSP-enriched diet ($p < 0.05$) and only a 39% reduction in bacterial mass in comparison to the habitual diet ($p < 0.05$). Bacterial mass was increased by 68% with the addition of dietary NSP from banana in comparison to the habitual diet ($p < 0.05$). Stool bacterial mass accounted for more of the faecal dry weight from the NSP-enriched diet when compared to the NSP-free ($p < 0.05$) and habitual diets ($p < 0.05$). Also stool bacterial mass was equivalent to a smaller proportion of the stool dry weight from the habitual diet in comparison to the NSP-free diet ($p < 0.05$). Whilst there was a 12 fold difference in the values for bacterial mass from the NSP-enriched diet, only a two fold range was observed from both the NSP-free and habitual diets. A close association for the NSP-enriched diet could be demonstrated between bacterial mass and faecal dry weight ($R = 0.96$; $p < 0.01$) (Figure 7.11). Whilst there was some relationship between bacterial mass and faecal dry weight from the habitual diet ($R = 0.69$; NS), a weaker association was obtained for the NSP-free diet ($R = 0.45$; NS). Overall no general association between faecal bacteria and dry weight of stools could be demonstrated for all the diets.

Approximately 54% and 23% of the differences in faecal dry weight could be attributed to differences in bacterial mass following the consumption of the NSP-enriched and habitual diets than the NSP-free diet respectively. There were more than twice the number of bacteria within faeces for the NSP-enriched compared to the NSP-free diet ($p < 0.05$; table 7.5). Whilst there appeared to be more bacteria within the stool from the habitual diet in comparison to the NSP-free diet, this difference was not statistically significant. There was a reduction in the amount of bacteria per gram of dry stool for the NSP-free diet in comparison to the NSP-enriched diet. This difference did not attain statistical significance. However the amount of bacteria per gram of dry stool was significantly reduced from the habitual diet when compared to both the NSP-free ($p < 0.05$) and NSP-enriched diets ($p < 0.05$).

Stool bacterial energy.

The values for energy, lipid and nitrogen within pooled samples of bacteria and bacteria from each of the subjects for all the dietary groups are shown in tables 7.6 and 7.7 respectively. The amounts of energy, lipid and nitrogen per gram of bacteria were similar for all the dietary groups. Figure 7.12 shows the relationship between estimated bacterial energy content using values from pooled and individual samples of bacteria for all the dietary groups.

Following the consumption of the NSP-free diet, there was a 80% reduction in the bacterial energy content of the stool when compared to the NSP-enriched diet ($p < 0.05$; table 7.8). In comparison to the habitual diet, the bacterial energy content from the NSP-free diet was reduced by 48% ($p < 0.05$). Nearly half and 24% of the differences in faecal energy could be attributed to reductions in bacteria from the NSP-free diet when compared to the NSP-enriched diet and habitual diets respectively. In addition, the bacterial energy content of the stool was almost twice that for the NSP-enriched diet when compared to the habitual diet ($p < 0.05$). There was a clear relationship between the amount of energy within the faeces and stool bacterial energy content for the NSP-enriched diet ($R = 0.95$; $p < 0.01$) with the bacteria accounting for 37% of the energy within the stool (Figure 7.13). Weaker relationships were observed between faecal energy and stool bacterial energy for both the NSP-free diet ($R = 0.61$; NS) and habitual diet ($R = 0.57$; NS), with bacteria accounting for 22% and 18% respectively of the energy within the stool ($p < 0.05$). Overall no general association between the energy content of bacteria within faeces and stool energy could be demonstrated for all the diets. Whilst there appeared to be a trend towards a greater proportion of the energy with the stool attributable to bacteria from the NSP-enriched diet in comparison to the NSP-free diet, this difference was not significant.

Figure 7.14 shows the relationship between estimated bacterial lipid content estimated from pooled samples of bacteria and bacterial samples from each of the subjects for all the diets. There were reductions in the bacterial lipid content of faeces by 80% and 69% respectively for the NSP-enriched diet when compared to both the NSP-free diet ($p < 0.05$) and habitual diet ($p < 0.05$; table 5.9). There was a 34% reduction in bacterial lipid content for the NSP-free diet in comparison to the habitual diet using values for bacterial lipid from pooled samples of bacteria. However, the estimated value for bacterial lipid from samples of bacteria from each of the subjects were similar for the NSP-free and

habitual diets. No relationships could be demonstrated between faecal lipid and the lipid content of bacteria for all dietary groups (NSP-free diet $R = -0.12$; NS, NSP-enriched diet $R = -0.80$; NS, habitual diet $R = -0.04$; NS; figure 7.15). A reduced proportion of the lipid within the stool could be attributed to bacteria from the habitual diet when compared to both the NSP-free ($p < 0.05$) and NSP-enriched diets ($p < 0.05$). Whilst bacteria accounted for more of the lipid with the stool for the NSP-enriched diet in comparison to the NSP-free diet, this difference did not attain statistical significance. It was noted in one of the subjects that there was an overestimation of the contribution made by bacteria to the lipid content of the stool.

Figure 7.16 illustrates the relationship between bacterial nitrogen content estimated from pooled samples of bacteria and individual samples of bacteria for all the diets. With no dietary NSP, there was a 79% decrease in the bacterial nitrogen content of faeces compared to the NSP-enriched diet ($p < 0.05$; table 7.10). Approximately 85% of the difference in faecal nitrogen could be attributed to a reduction in bacteria between the NSP-enriched diet and NSP-free diet. The bacterial nitrogen content was reduced by approximately 27% for the NSP-free in comparison to the habitual diet ($p < 0.05$). This was significantly different when the value from pooled samples of bacteria was used. Applying the values from both pooled samples of faecal bacteria and samples of bacteria from each of the subjects, approximately 19% and 8% respectively of the differences in faecal nitrogen could be accounted by reductions in bacteria between the habitual diet and NSP-free diet. A difference in the bacterial nitrogen content of stool could be demonstrated for the NSP-enriched diet in comparison to the habitual diet ($p < 0.05$). Whilst poor associations were demonstrated between bacterial nitrogen and faecal nitrogen for the NSP-free diet ($R = 0.30$; NS) and NSP-enriched diet ($R = 0.40$; NS), there appeared to be some relationship for the habitual diet ($R = 0.75$; NS; figure 7.17). Overall no general association between the nitrogen content of bacteria within faeces and stool nitrogen could be demonstrated for all the diets. More of the nitrogen within the stool could be accounted by bacteria from the NSP-enriched compared to the NSP-free and habitual diets ($p < 0.05$). It was noted that the difference between the habitual diet and the NSP-enriched diet was only significant for the values estimated from individual samples of bacteria. Table 5.11 shows the estimated additional energy that may be recovered from colonic fermentation using the same approach as McNeil (1984). From these calculations it would appear that colonic fermentation may provide

an additional 7.0% energy with the addition of dietary NSP from banana compared to 1.5% energy for the NSP-free diet ($p < 0.05$) and 2.1% energy for the habitual diet ($p < 0.05$).

7.4 DISCUSSION.

In this study the 'NSP-free' diet was composed of Ensure, a complete oral formula and Maxijul, a glucose polymer which can be used to increase the energy intake of the diet where required. The combination of both these liquid formulae provided a diet containing no complex carbohydrates starch or NSP such that it could be assumed that no dietary residue would reach the large intestine. Following the consumption of this diet, reductions in stool output and in the amounts of energy, lipid and nitrogen within the stool could be demonstrated. It would seem that even with a diet containing no NSP, stools were produced with a range in faecal weights of between 50-70 g/day. Other studies have confirmed the reduction in faecal volume and decreased frequency of stools in subjects consuming elemental, NSP-free diets [Winitz *et al.* 1970; Crowther *et al.* 1973; McCamman *et al.* 1977]. In the present study, it would seem that the quantity of stool loss rather than stool frequency may account for the differences in faecal weights between the diets. These results would suggest that in the absence of dietary residue within the stool, faecal output could be represented by the colonic bacteria and components of endogenous origin such as intestinal secretions and cellular debris. Whilst it has not been possible in the present study to determine the relative contribution made by endogenous losses to faecal output, the colonic bacteria represented a major component being equivalent to approximately 24% of the stool dry weight.

Apart from the study reported by Winitz and coworkers [1970], a reduction in the concentration and types of bacteria within the colon and faeces following the consumption of elemental diets could not be confirmed in other studies [Attebury *et al.* 1972; Crowther *et al.* 1973]. In the present study it is clear that both the amount and number of bacteria within the stool were reduced which would imply that the activity of the colonic bacteria had been compromised. Thus, bacterial metabolism may well be limited to the availability of fermentable carbohydrate from endogenous losses such as the carbohydrate from glycoproteins and mucopolysaccharides.

The effects of the addition of bananas to a NSP-free diet resulted in greater faecal weights and in the amounts of energy, lipid and nitrogen within the stool. Bananas were selected as a source of complex polysaccharides as they provide a varied source of potentially fermentable substrate for the colonic bacteria. Stephen & Cummings [1980b] have shown that the NSP from cabbage, the 'soluble' form of NSP can increase faecal output by the proliferation of bacterial growth in the large intestine rather than the 'insoluble' form of NSP such as in bran. In the present study it would appear that the bacteria could account for approximately 54% of the relative increase in stool dry weight. This would suggest that bananas may provide an additional fermentable substrate for the growth and maintenance of bacterial cells. The bacteria represented a major component of the faeces weight being equivalent to 43% of the faecal dry weight. Stephen & Cummings [1980b] have reported that bacteria were attributable to 55% of the faecal dry weight when a control diet was supplemented with cabbage within a group of adult subjects.

The present study has not attempted to determine the contribution made by any undigested dietary NSP within faeces which may account for part of the increase in faecal weight. However Stephen & Cummings [1980b] reported that only 3-4% of the increase in faecal weight could be accounted by NSP within stools with the addition of cabbage to the diet. Whilst it is unlikely that undigested substrate from banana present a major component of faecal output, further studies are required to examine this possibility.

The present study has also investigated the magnitude and origins of energy within the stool in this group of subjects when they consumed their habitual dietary food intake. Thus, the extent to which changes in diet may influence the colonic bacteria in comparison to the normal activity of the colonic bacteria could be explored. It was evident that both the amount and types of dietary NSP were variable. The intakes of NSP ranged from 14 to 40 g/day. The types of dietary NSP were provided from a wide variety of sources in particularly from wheat products, pulses and fruit and vegetables. Whilst an increase in faecal weight was observed in comparison to the NSP-free diet, only 21% of the increase in stool weight could be accounted by the bacteria. This would also indicate that the increased stool weight may well be explained by other physiological effects of dietary NSP within the colon. Some types of dietary NSP are believed to resist digestion by bacteria such as wheat NSP. The cells within wheat NSP are relatively small and have lignified cell walls which are able to exert a physical

effect on intestinal bulk [Stephen & Cummings 1980b]. As the intakes of dietary NSP are composed of different types of NSP, this mechanism may partly explain the increase in faecal weight. Wetter stools were also observed following the consumption of the habitual intake than the NSP-free diet which could be attributable to the water associated with undigested NSP.

Apart from the association between the amount of nitrogen and energy within the stool from the habitual diet, it is apparent that faecal energy losses could not be simply related to both lipid and nitrogen losses within the stool for all the diets. This would suggest that the increased faecal energy losses following the consumption of raised amounts of dietary NSP do not simply represent high losses of lipid or nitrogen within the stool. Despite alterations in the amounts and types of dietary NSP, there were strong associations between the amount of energy within the stool and both the wet and dry weights of stool. The apparent constancy of energy per gram of stool weight would imply that the composition of stool must remain relatively similar between individuals despite a wide range in faecal energy losses. At the turn of the century, Rubner noted a constancy of energy per gram dry faeces in man for mixed or meat based diets of approximately 25.9 kJ/g. In the present study, it appears for all the diets that approximately 22 kJ and 4 kJ of energy is present within each gram of dry and wet stool respectively.

Several investigators have reported a reduction in the apparent digestibilities of energy, lipid and nitrogen following the consumption of increased amounts of dietary NSP from a variety of different sources [Southgate & Durnin, 1970; Calloway & Kretsch, 1978; Goranzon *et al.* 1983; Wisker *et al.* 1988]. In the present study, reductions in the apparent digestibilities of energy, lipid and nitrogen were only observed when bananas were added to a NSP-free diet. It would seem from the present study that both the amount of dietary NSP and gross energy within the diet can not be simply related to the magnitude of stool output. With a clear association between faecal dry weight and bacterial mass, it is apparent that the colonic bacteria represented a major component of the faecal dry mass when increased amounts of dietary NSP were consumed. A relationship between bacterial mass and faecal dry weight was also confirmed in a previous study ($R=0.84$) [Stephen & Cummings, 1980b]. It could be demonstrated that individuals with raised faecal losses may have high amounts

of bacteria within the stool. In this study the reduction in apparent digestibility of energy, lipid and nitrogen with increased intakes of NSP could be attributable to a greater faecal bacterial mass.

Only one previous study has shown that nitrogenous faecal loss may be largely bacterial with increased intakes of dietary NSP (Stephen & Cummings, 1980b). Approximately 60% and 34% of the increases in faecal nitrogen could be accounted by nitrogen within bacteria with the addition of cabbage and wheat NSP to the normal diets of healthy individuals. To my knowledge there have been no other reports of the extent to which bacteria contribute to increased faecal energy and lipid losses with the stool following the consumption of raised intakes of NSP. In the present investigation bacteria could account for approximately 46% and 85% of the relative increases in faecal energy and nitrogen with the addition of bananas to a NSP-free diet. These results would suggest that the greater bacterial mass following the consumption of a NSP-free diet supplemented with bananas is a major component of the decrease in apparent digestibility of both energy and nitrogen. The proportion of the differences in faecal energy and nitrogen which could be attributable to bacteria from the bananas was greater than the differences from the habitual diet. This would suggest that the increases in energy and nitrogen within the stool from the habitual intakes may be accounted by other components such as undigested dietary residues.

The finding that the addition of banana to a NSP-free diet caused a greater bacterial mass may account for the increase in faecal lipid. However the extent to which bacteria contribute to the relative difference in faecal lipid could not be established. This could be partly explained by an overestimation of the proportion of faecal lipid which could be attributable to bacteria in one of the subjects. It was noted in this subject that the amount of bacteria within the stool was much greater than that for the other individuals. Whilst there was no doubt that the addition of banana caused a substantial increase in the metabolic activity of the colonic bacterial, the reasons why this effect was particularly amplified in this subject was not obvious. It is known that during the ripening of bananas there are various complex alterations in the contribution made by carbohydrates [Englyst & Cummings, 1986]. Whilst it was not possible to ascertain the precise composition of bananas consumed in this study, differences in the availability of fermentable substrate, in addition to endogenous sources may determine the extent of the metabolic activity of colonic bacteria.

It is known that there is considerable intra-individual variation in the composition and distribution of bacteria in the human large intestine [Hudson, Borriello, Hill, 1981]. Furthermore it is thought that the colonic bacteria adjusts to the breakdown and fermentation of a new substrate within 7 - 10 days [Harmouth-Hoene & Schelenz, 1980]. In the present study changes in stool weight occurred very quickly with both the NSP-free and NSP-enriched diets such that after three days of the five day study period stool weight had changed and remained relatively stable during the collection period (data not shown). It should be noted that the responsiveness to changes in diet rather than the extent to which adaptation may occur with alterations in dietary NSP was examined. Further studies would be required to investigate whether further changes in stool weight occurred over longer periods of time. Following the consumption of the NSP-enriched diet in the present study, differences in the amounts of faecal bacteria between the subjects would indicate that the period of time required for the growth and development of bacteria may vary between individuals. It may be that this difference could be determined by the composition and distribution of bacteria and in the availability of fermentable carbohydrate. Further studies are required to explore these possibilities.

The analyses of energy, lipid and nitrogen within bacteria showed that the bacteria represented approximately 20-26% energy, 8-13% lipid and 4-5% nitrogen. There have been no other studies which have determined the energy content of faecal bacteria. Previous reports have demonstrated comparable estimates of the nitrogen and lipid content of bacteria of approximately 6-7% nitrogen for faecal bacteria [Stephen & Cummings, 1980a], 7% nitrogen for mixed populations of bacteria [Smith & McAllen, 1974] and 10-15% lipid for pure cultures [Luria, 1960] and 12-20% lipid for mixed rumen populations [Czerkawski, 1976].

Sufficient quantities of bacteria are required for the analyses of energy, lipid and nitrogen content within bacteria. The isolation of bacteria within faecal material from each individual in a study group is relatively time-consuming. To overcome this difficulty the possibility that energy, lipid and nitrogen could be estimated from pooled samples of bacteria rather than samples of bacteria from each of the subjects was investigated. It would appear from the unity between estimates of energy and nitrogen within faecal bacteria from pooled samples of bacteria and faecal samples of bacteria from each of the subjects that both the bacterial energy and nitrogen content could be reliably estimated from pooled samples

of bacteria. This would provide a useful application in further studies within larger groups of subjects. However more difference was observed between the values for bacterial lipid from pooled and individual samples of faecal bacteria which would suggest that bacterial lipid could not be simply estimated from pooled samples of faecal bacteria. An overestimation in bacterial lipid was observed within one of the subjects in the present study following the consumption of the NSP-enriched diet despite repeating the analysis. This could be attributed to errors in bacterial mass measurements; errors in the measurement of total faecal lipid; errors in the measurement of the lipid content of bacteria. Further studies are required to explore the reasons for this observation.

In ruminants it is well established that bacterial fermentation is important for the conservation of energy from food components that are not absorbed within the small intestine [Grossklau, 1983]. In studies of human energy metabolism, the importance of short-chain fatty acid absorption from bacterial fermentation in relation to human energy requirements is unclear. Using the same approach as McNeil [1984], the present study has shown that with the addition of bananas to a NSP-free diet, the additional energy made available through SCFA absorption was equivalent to 7.0% (2.0 - 24.3%) in comparison to only 2.0% (0.7 - 3.5%) when the diet contained different amounts and types of NSP from the habitual diet. This would provide further evidence to suggest that bananas provide a readily available carbohydrate substrate for bacterial fermentation, whereas part of the NSP within the habitual diet remains largely undigested. Following the consumption of the diet containing no NSP, energy made available through short-chain fatty acid absorption could provide additional energy equivalent to 1.5% (1.1 - 2.6%). As the source of carbohydrate in order to account for the faecal weight of bacteria is clearly not from dietary NSP at the time of the study, colonic bacterial fermentation may continue to a reduced extent through the presence of carbohydrate substrate from endogenous secretions and cellular debris. However there may well be some dietary NSP remaining in the large intestine at the beginning of the study period. Alternatively, this reduction in the recovery of energy from short-chain fatty acid absorption may reflect a declining population of bacteria within the colon due to a reduction in the availability of undigested carbohydrate residues of the diet.

7.5 SUMMARY AND CONCLUSIONS.

- i. The present study has shown that the metabolic activity of the colonic bacteria can be modified by alterations in diet as shown by substantial changes in the magnitude and composition of stools, particularly with the addition of fermentable substrate from bananas.
- ii. When there is no NSP within the diet, the extent of metabolic activity of colonic bacteria is clearly reduced. It is likely that fermentable carbohydrate is provided from endogenous sources.
- iii. A greater bacterial mass could account for a major part of the reduction in the apparent digestibility of energy and nitrogen associated with the consumption of fermentable carbohydrate from bananas.
- iv. The observation that energy and nitrogen contents of bacteria could be reliably estimated from pooled samples of bacteria provides a simple method of estimating the bacterial energy and nitrogen contents of individuals within large groups.

On the basis of these results, it appears that dietary modifications of the activity of colonic bacteria influences the extent to which energy may be recovered through short-chain fatty acid absorption. This may be important in studies of energy balance particularly when attempts are made to assess energy requirements in individuals with high intakes of dietary NSP or in intestinal dysfunction where NSP-free, formula liquid diets may form part of therapeutic management.

Table 7.1 The characteristics of the healthy adult men who participated in the study.

	AGE Yrs	HEIGHT m	WEIGHT kg
A	43	1.63	74.1
B	31	1.74	74.0
C	22	1.84	74.2
D	22	1.70	65.0
E	34	1.75	73.7
F	22	1.82	75.0

Table 7.2 Faecal wet and dry weights and percentage water were measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results presented are medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched diets or habitual diets (* p < 0.05).

	NSP-FREE DIET	NSP- ENRICHED DIET	HABITUAL DIET
FAECAL WET WEIGHT (g/day)	57.0 (46.0 - 78.0)	205.0 * (120.0 - 333.0)	169.0 * (116.0 - 250.0)
FAECAL DRY WEIGHT (g/day)	15.1 (12.0 - 19.0)	41.2 * (19.0 - 108.0)	25.8 * (22.0 - 52.0)
PERCENTAGE WATER CONTENT (%)	75.2 (68.0 - 78.0)	79.0 (60.0 - 85.0)	80.4 * (76.0 - 88.0)

Table 7.3 Energy, lipid and nitrogen within faeces were measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results presented are medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched diets or habitual diets (* p < 0.05) and for NSP-enriched diet compared with habitual diet (# p < 0.05).

	NSP-FREE DIET	NSP-ENRICHED DIET	HABITUAL DIET
ENERGY			
FAECAL ENERGY (kJ/day)	326.0 (281.0 - 380.0)	898.0 * (405.0 - 2208.0)	599.0 * (504.0 - 1112.0)
FAECAL ENERGY AS % GROSS ENERGY INTAKE (%)	3.2 (2.8 - 4.3)	7.2 (3.2 - 17.7)	5.1 (3.2 - 7.2)
LIPID			
FAECAL LIPID (g/day)	1.5 (0.9 - 2.9)	2.5 * (1.5 - 4.7)	4.0 * (2.8 - 5.0)
FAECAL LIPID AS % LIPID INTAKE (%)	4.0 (2.5 - 9.1)	11.1 * (6.6 - 20.4)	3.9 # (1.9 - 4.5)
NITROGEN			
FAECAL NITROGEN (g/day)	0.9 (0.6 - 1.1)	1.6 * (1.3 - 2.1)	1.7 * (1.1 - 2.4)
FAECAL NITROGEN AS % NITROGEN INTAKE (%)	15.6 (10.1 - 20.5)	27.5 * (23.2 - 43.7)	8.3 # (5.1 - 18.7)

Table 7.4 Apparent digestibilities of energy, lipid and nitrogen estimated in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results presented are medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched or habitual diets (* p < 0.05) and for NSP-enriched diet compared with habitual diet (# p < 0.05).

	NSP-FREE DIET	NSP-ENRICHED DIET	HABITUAL DIET
APPARENT DIGESTIBILITY OF ENERGY (%)	96.8 (95.7 - 97.2)	92.8 (82.3 - 96.8)	95.0 (92.8 - 96.8)
APPARENT DIGESTIBILITY OF LIPID (%)	96.0 (91.0 - 97.5)	89.0 * (79.7 - 93.4)	96.2 # (95.5 - 98.1)
APPARENT DIGESTIBILITY OF NITROGEN (%)	84.4 (79.5 - 89.9)	72.5 * (56.3 - 76.8)	91.7 # (81.3 - 94.9)

Table 7.5 Bacterial mass, number and concentration within faeces were measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results presented are medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched or habitual diets (* p < 0.05) and for NSP-enriched diet compared with habitual diet (# p < 0.05).

	NSP-FREE DIET	NSP- ENRICHED DIET	HABITUAL DIET
BACTERIAL MASS (g/day)	3.5 (3.0 - 6.1)	17.6 * (5.0 - 63.8)	5.7 *# (3.8 - 8.2)
BACTERIAL MASS (%FAECAL DRY WEIGHT)	24.0 (17.7 - 33.1)	42.6 * (20.0 - 59.3)	19.1 *# (15.2 - 29.4)
BACTERIAL NUMBER (COUNTS/day X 10 ¹³)	1.8 (0.8 - 3.6)	4.2 * (1.6 - 5.8)	2.9 (1.5 - 5.9)
BACTERIAL MASS (g BACTERIA/g FAECAL DRY WEIGHT)	0.3 (0.2 - 0.3)	0.4 (0.2 - 0.6)	0.2 *# (0.2 - 0.3)

Table 7.6 Energy, lipid and nitrogen determined within pooled bacteria extracted from faeces were measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results are presented as medians.

	NSP-FREE DIET	NSP- ENRICHED DIET	HABITUAL DIET
BACTERIAL ENERGY CONTENT * (kJ/g)	20.05	18.38	26.24
BACTERIAL LIPID CONTENT * (g/g)	0.13	0.10	0.07
BACTERIAL NITROGEN CONTENT (g/g)	0.042	0.043	0.053

* Estimates from pooled samples.

Table 7.7 Energy, lipid and nitrogen determined within bacteria extracted from faecal material were measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results are presented as medians with ranges in parenthesis.

	NSP-FREE DIET	NSP- ENRICHED DIET	HABITUAL DIET	
BACTERIAL ENERGY CONTENT (kJ/g)	21.4 (17.0 - 22.9)	19.2 (17.9 - 20.9)	21.9 (20.9 - 24.8)	NS
BACTERIAL LIPID CONTENT (g/g)	0.10 (0.03 - 0.13)	0.08 (0.05 - 0.18)	0.09 (0.04 - 0.13)	NS
BACTERIAL NITROGEN CONTENT (g/g)	0.045 (0.038 - 0.050)	0.041 (0.035 - 0.049)	0.047 (0.034 - 0.054)	NS

Table 7.8 Faecal bacterial energy derived from i) pooled bacterial material and ii) bacteria from each of the subjects (individual samples) was estimated in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results are presented as medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched or habitual diets (* p < 0.05) and for NSP-enriched diet compared with habitual diet (# p < 0.05).

	BACTERIAL ENERGY (kJ/day)	BACTERIAL ENERGY (% FAECAL ENERGY)
NSP-FREE DIET		
POOLED SAMPLE	70.2 (59.2 - 122.3)	21.6 (17.8 - 32.2)
INDIVIDUAL SAMPLE	69.6 (57.8 - 139.5)	21.1 (17.9 - 36.7)
NSP-ENRICHED DIET		
POOLED SAMPLE	323.0 * (92.1 - 828.3)	36.6 (16.7 - 53.1)
INDIVIDUAL SAMPLE	333.0 * (92.1 - 1334.1)	37.2 (16.7 - 60.4)
HABITUAL DIET		
POOLED SAMPLE	133 *# (88.9 - 191.3)	18.9 * (15.2 - 30.6)
INDIVIDUAL SAMPLE	135 *# (83.4 - 177.3)	17.8 *# (15.4 - 32.5)

Table 7.9 Faecal bacterial lipid derived from i) pooled bacterial material and ii) samples of bacteria from each of the subjects (individual samples) was estimated in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results are presented as medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched or habitual diets (* $p < 0.05$) and for NSP-enriched diet compared with habitual diet (# $p < 0.05$).

	BACTERIAL LIPID (g/day)	BACTERIAL LIPID (% FAECAL LIPID)
NSP-FREE DIET		
POOLED SAMPLE	0.45 (0.38 - 0.79)	31.2 (25.8 - 45.3)
INDIVIDUAL SAMPLE	0.33 (0.10 - 0.49)	21.4 (8.2 - 32.9)
NSP-ENRICHED DIET		
POOLED SAMPLE	1.8 * (0.5 - 6.38)	78.2 (10.7 - 362.5)
INDIVIDUAL SAMPLE	1.6 * (0.35 - 3.19)	62.9 (7.5 - 181.3)
HABITUAL DIET		
POOLED SAMPLE	0.40 # (0.27 - 0.57)	9.9 *# (6.8 - 15.9)
INDIVIDUAL SAMPLE	0.46 # (0.19 - 0.97)	9.8 *# (6.6 - 27.3)

Table 7.10 Faecal bacterial nitrogen derived from i) pooled bacterial material and ii) samples of bacteria from each of the subjects (individual samples) was estimated in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results are presented as medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched or habitual diets (* p < 0.05) and for NSP-enriched diet compared with habitual diet (# p < 0.05).

	BACTERIAL NITROGEN (g/day)	BACTERIAL NITROGEN (% FAECAL NITROGEN)
NSP-FREE DIET		
POOLED SAMPLE	0.15 (0.12 - 0.26)	18.7 (11.0 - 24.5)
INDIVIDUAL SAMPLE	0.16 (0.13 - 0.23)	20.8 (12.1 - 24.4)
NSP-ENRICHED DIET		
POOLED SAMPLE	0.75 * (0.21 - 2.71)	45.0 * (17.8 - 161.4)
INDIVIDUAL SAMPLE	0.76* (0.24 - 2.23)	46.5 * (16.7 - 132.9)
HABITUAL DIET		
POOLED SAMPLE	0.30 *# (0.20 - 0.44)	17.2 (13.4 - 31.8)
INDIVIDUAL SAMPLE	0.22 # (0.19 - 0.44)	15.1 # (9.9 - 20.4)

Table 7.11 Additional energy that may be provided from colonic fermentation using the calculation of McNeil (1984) was calculated in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and their habitual diet. The results presented are medians with ranges in parenthesis.

Significant differences are for NSP-free diet compared with NSP-enriched or habitual diets (* p<0.05) and for NSP-enriched diet compared with habitual diet (# p<0.05).

	NSP-FREE DIET	NSP- ENRICHED DIET	HABITUAL DIET
HEXOSE REQUIRED TO SYNTHESIZE FAECAL BACTERIAL MASS (g/day) ¹	11.6 (9.7 - 20.1)	58.1 * (16.5 - 210.5)	18.9 *# (12.5 - 27.1)
FAECAL BACTERIAL ENERGY (MJ/day) ²	0.07 (0.06 - 0.14)	0.33 * (0.09 - 1.33)	0.14 *# (0.08 - 1.77)
TOTAL ENERGY YIELD (MJ/day) ³	0.20 (0.17 - 0.35)	1.02 * (0.29 - 3.68)	0.33 *# (0.22 - 0.47)
ENERGY AVAILABLE AS SCFA (MJ/day) ⁴	0.13 (0.10 - 0.21)	0.68 * (0.20 - 2.35)	0.20 *# (0.14 - 0.30)

¹ ASSUMES 0.1 MOL ATP TO SYNTHESIZE 1g OF BACTERIAL MASS AND THAT 33g HEXOSE FROM FERMENTATION WILL GENERATE 1 MOL ATP.

² CALCULATED USING ENERGY VALUES (kJ/g) MEASURED WITHIN FAECAL BACTERIAL SAMPLES FROM EACH OF THE SIX NORMAL MALE ADULTS.

³ ASSUMES 17.5 kJ/g HEXOSE.

⁴ TOTAL ENERGY YIELD - FAECAL BACTERIAL MASS.

Figure 7.1 Daily faecal wet weight and dry weight expressed as g/day measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The values are presented for each subject and the bars represent the median values.

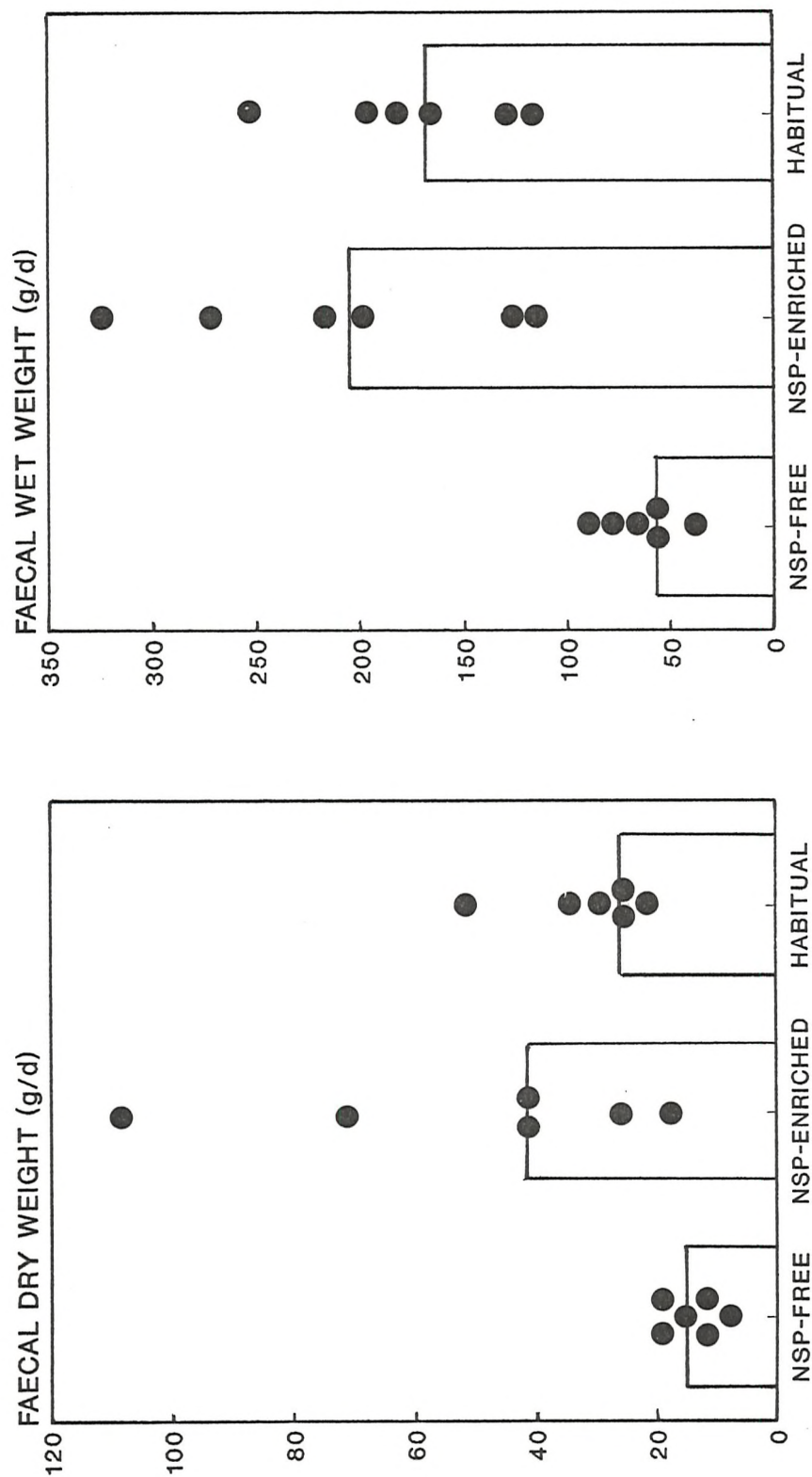


Figure 7.2 Daily gross energy intake estimated by applying heats of combustion values [Merrill & Watt, 1955] versus dry weight of faeces measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. Correlation coefficients are $R = -0.5$ (NS), $R = 0.35$ (NS) and $R = 0.59$ (NS), respectively.

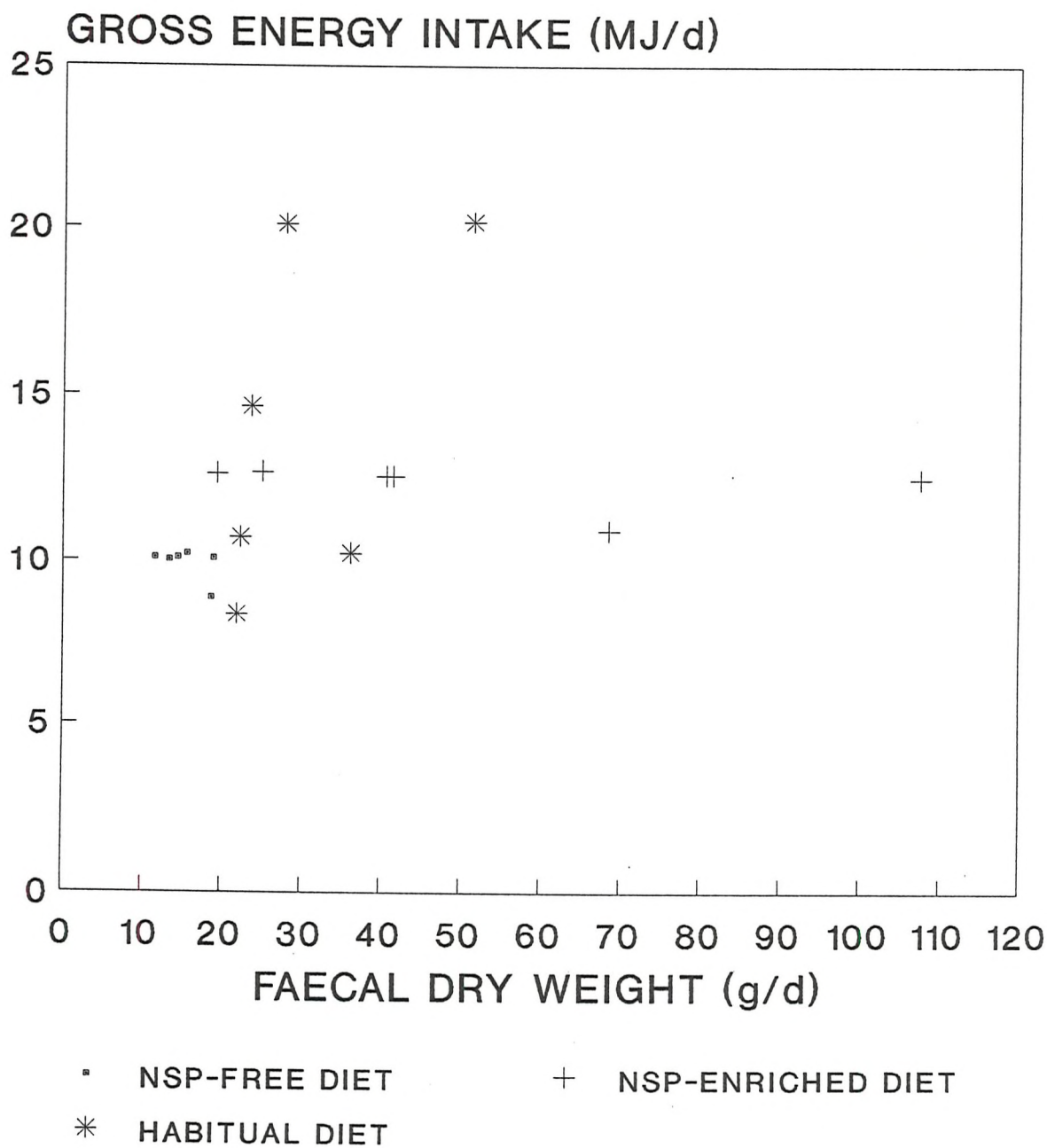


Figure 7.3 Dietary intake of non-starch polysaccharides (NSP) each day versus dry weight of faeces in six normal adult men following the consumption of both NSP-free and NSP-enriched diets.
Correlation coefficients are $R = 0.35$ (NS) and $R = 0.65$ (NS), respectively.

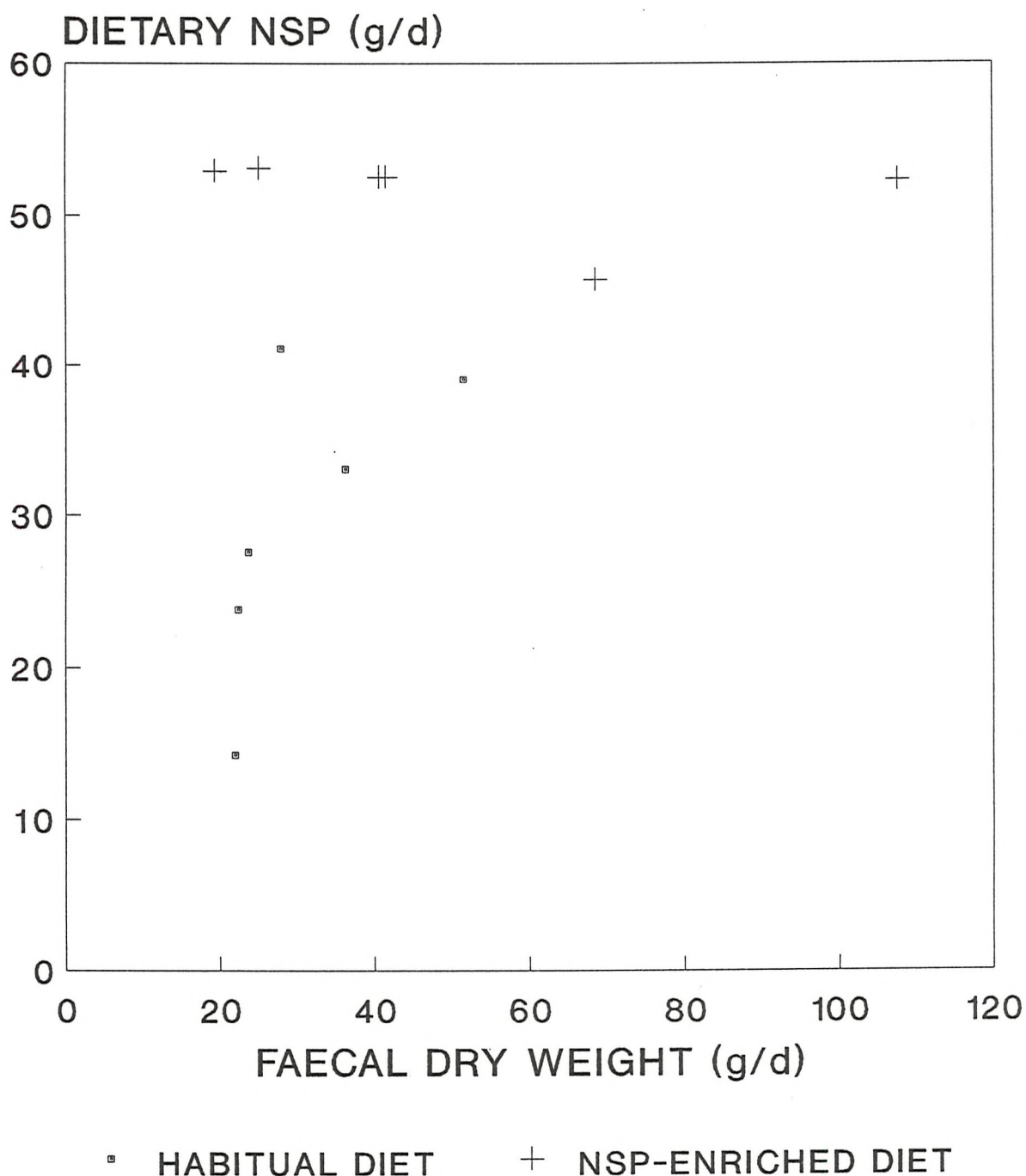


Figure 7.4 Daily faecal energy content as g/day measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The values are presented for each subject and the bars represent the median values.

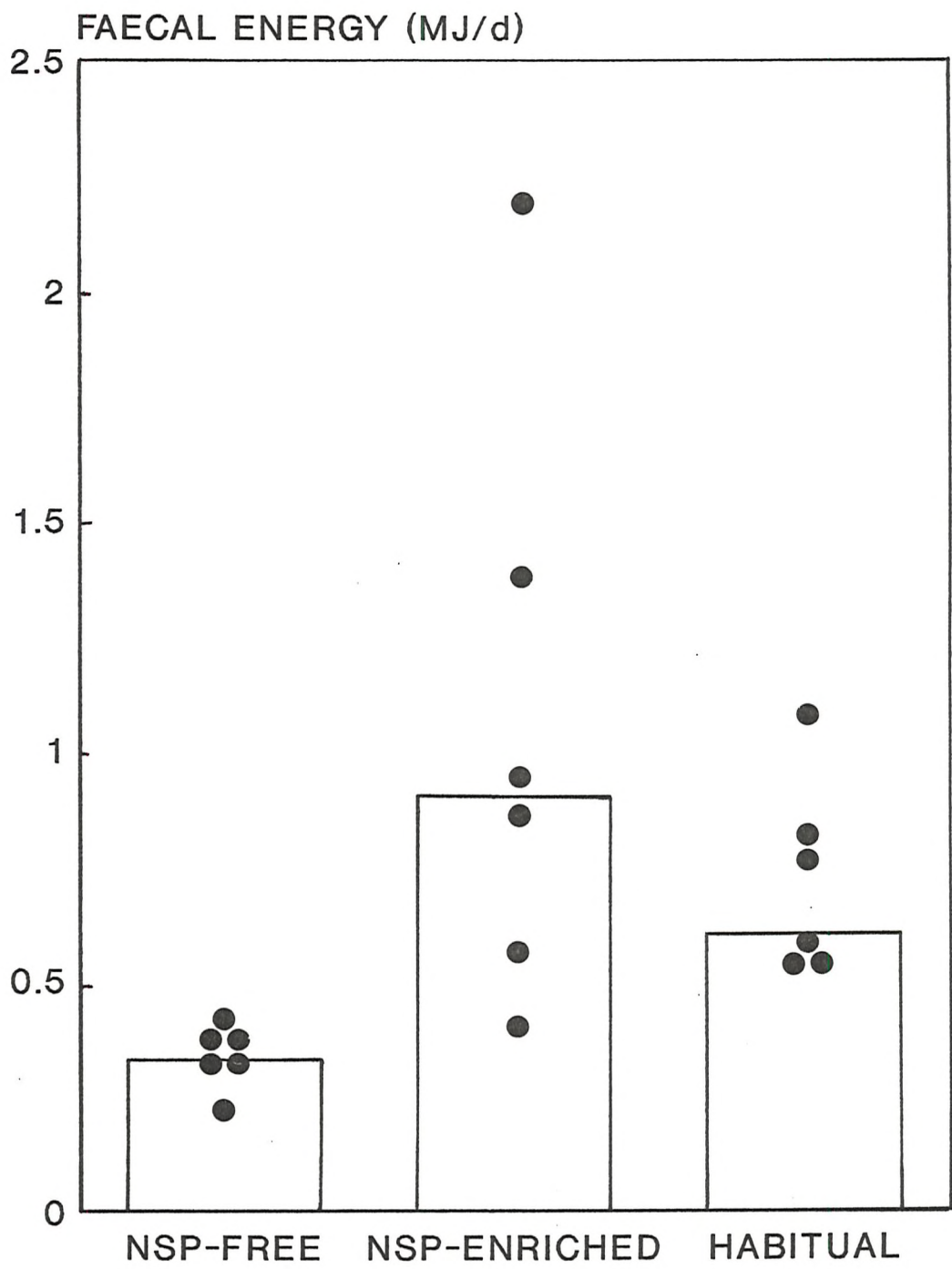


Figure 7.5 Daily faecal lipid and nitrogen content as g/day measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The values are presented for each subject and the bars represent the median values.

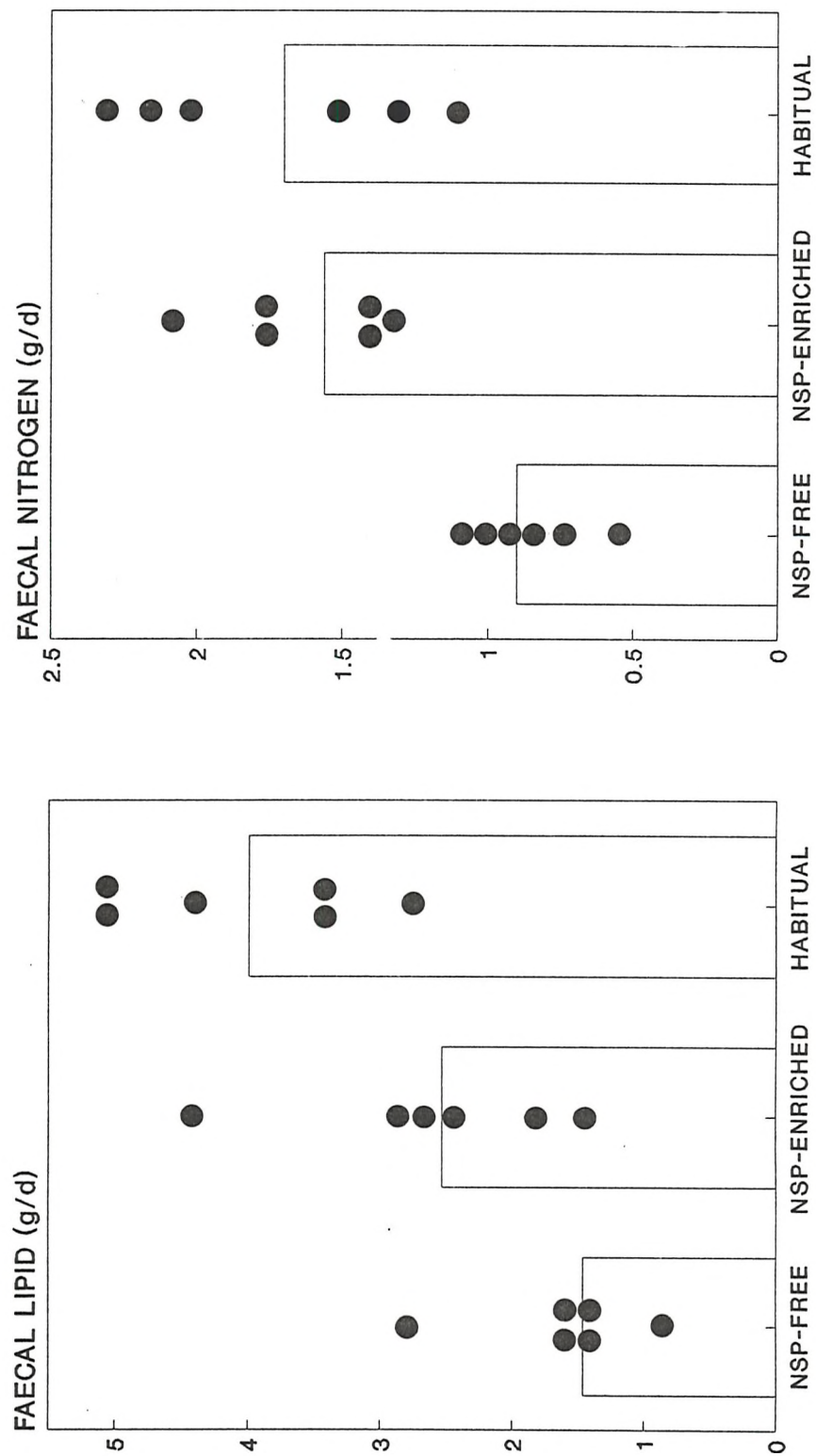


Figure 7.6 Daily faecal energy content versus dry weight of faeces in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. Correlation coefficients are $R=0.75$ (NS), $R=0.99$ ($p < 0.01$) and $R=0.99$ ($p < 0.01$) respectively.

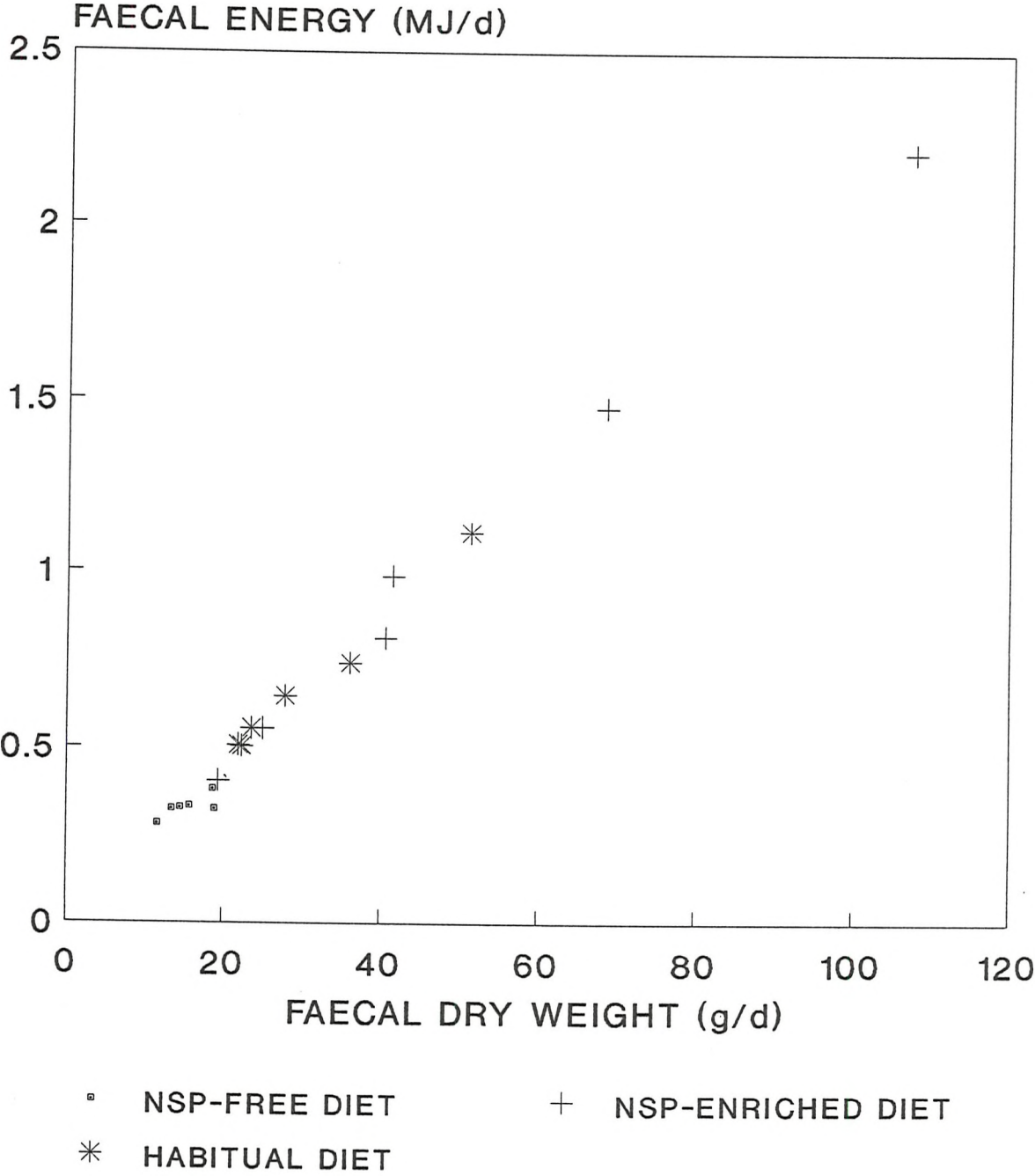


Figure 7.7 Daily faecal energy content versus wet weight of faeces measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet.

Correlation coefficients are $R = 0.92$ ($p < 0.05$), $R = 0.80$ ($p < 0.05$) and $R = 0.70$ (NS) respectively.

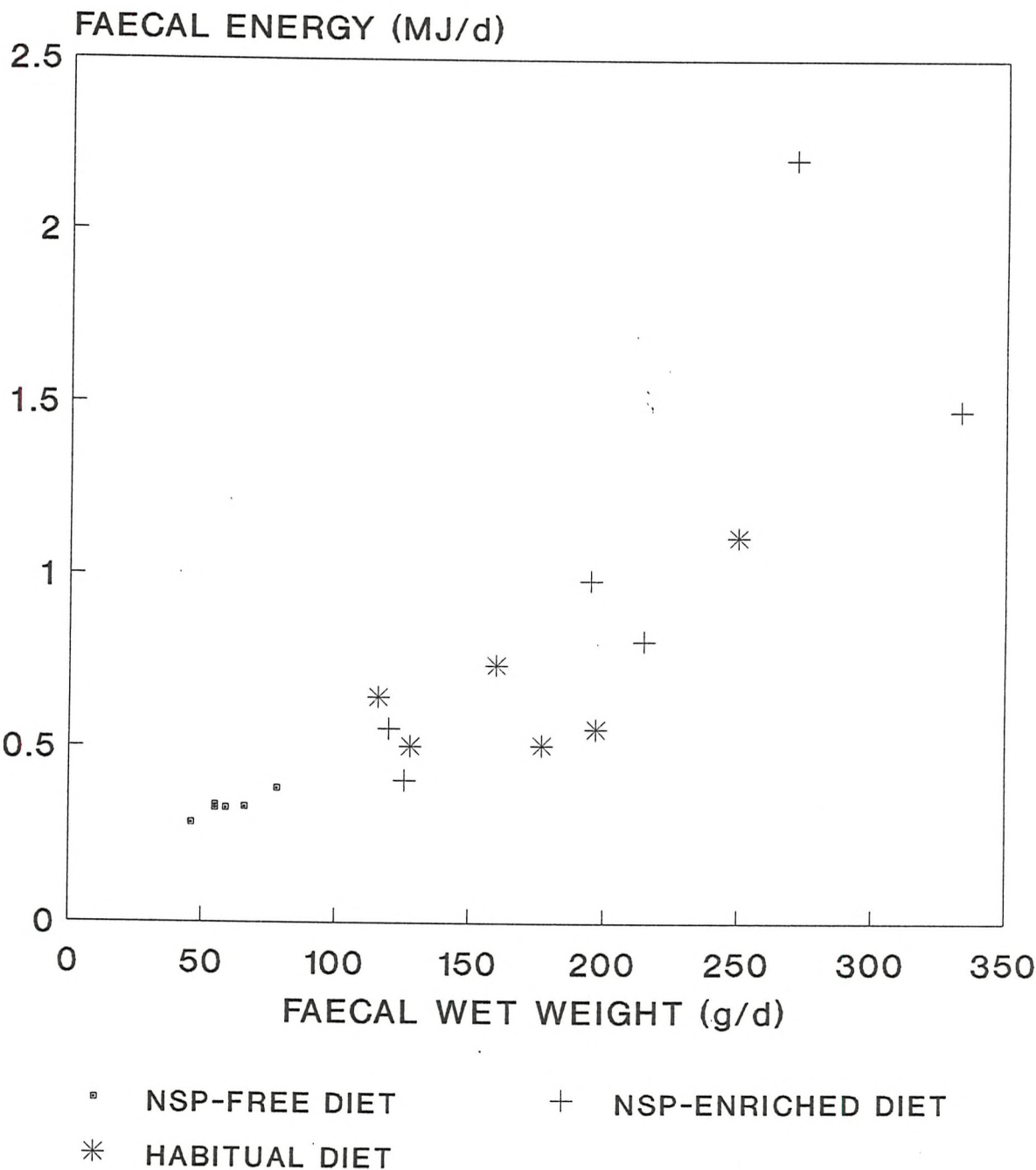


Figure 7.8 Daily faecal energy content versus lipid content of faeces measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet.
Correlation coefficients are $R = 0.75$ (NS), $R = -0.43$ (NS) and $R = 0.41$ (NS) respectively.

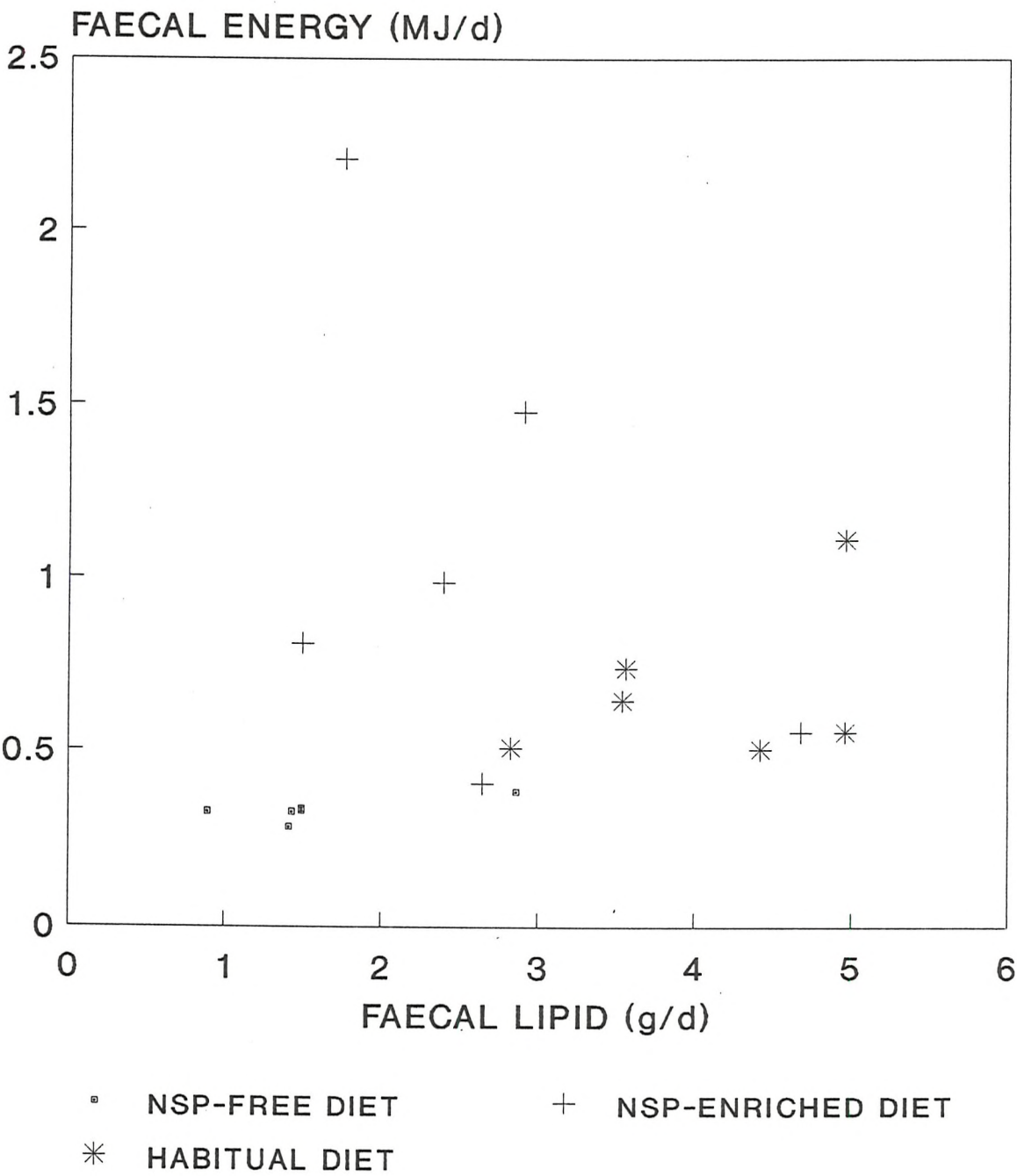


Figure 7.9 Daily faecal energy content versus protein content of faeces derived from the measurement of faecal nitrogen as $N \times 6.25$ measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. Correlation coefficients are $R=0.49$ (NS), $R=0.57$ (NS) and $R=0.81$ (NS) respectively.

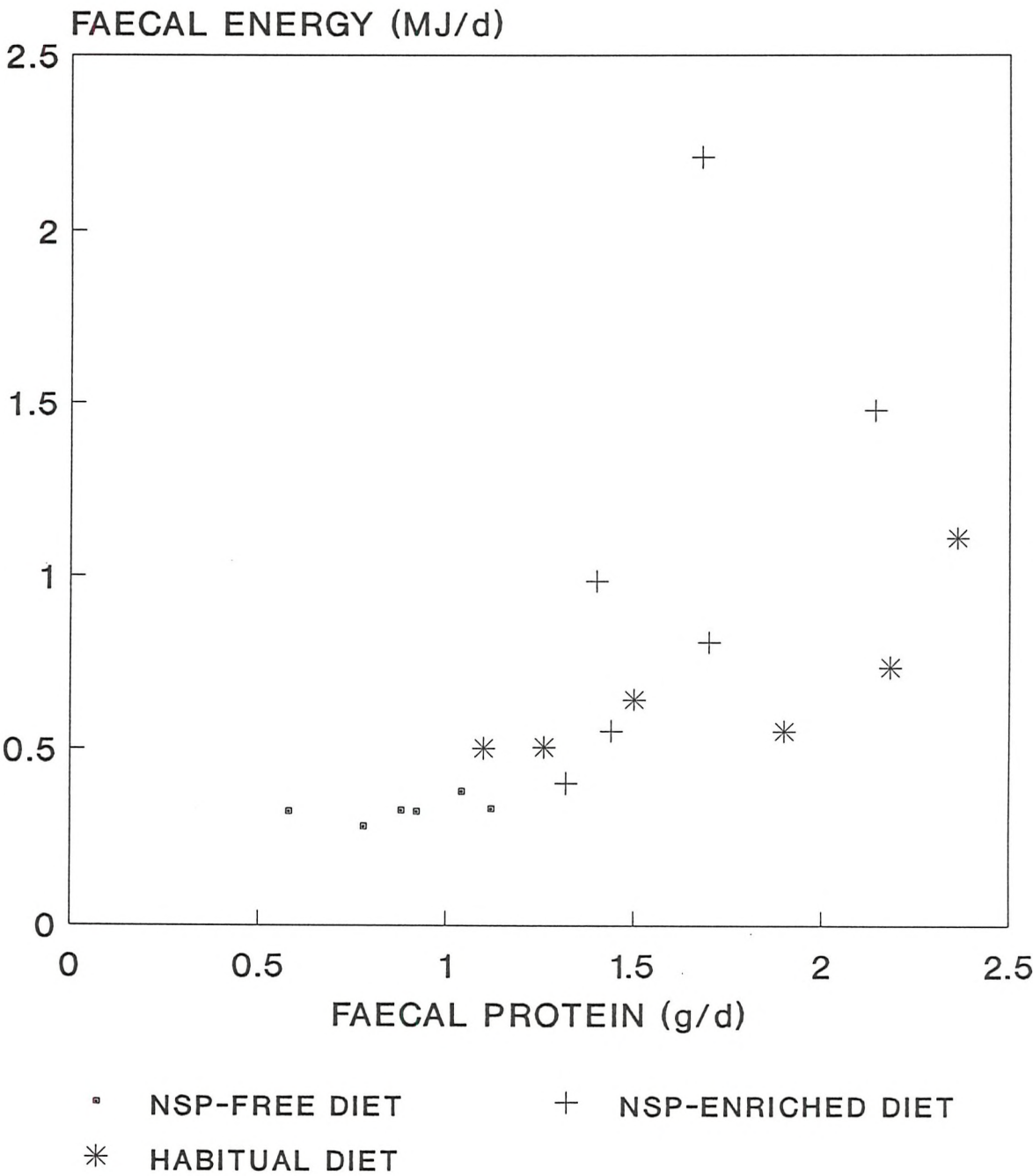


Figure 7.10 Daily bacterial content of faeces as g/day measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The values are presented for each subject and the bars represent the median values.

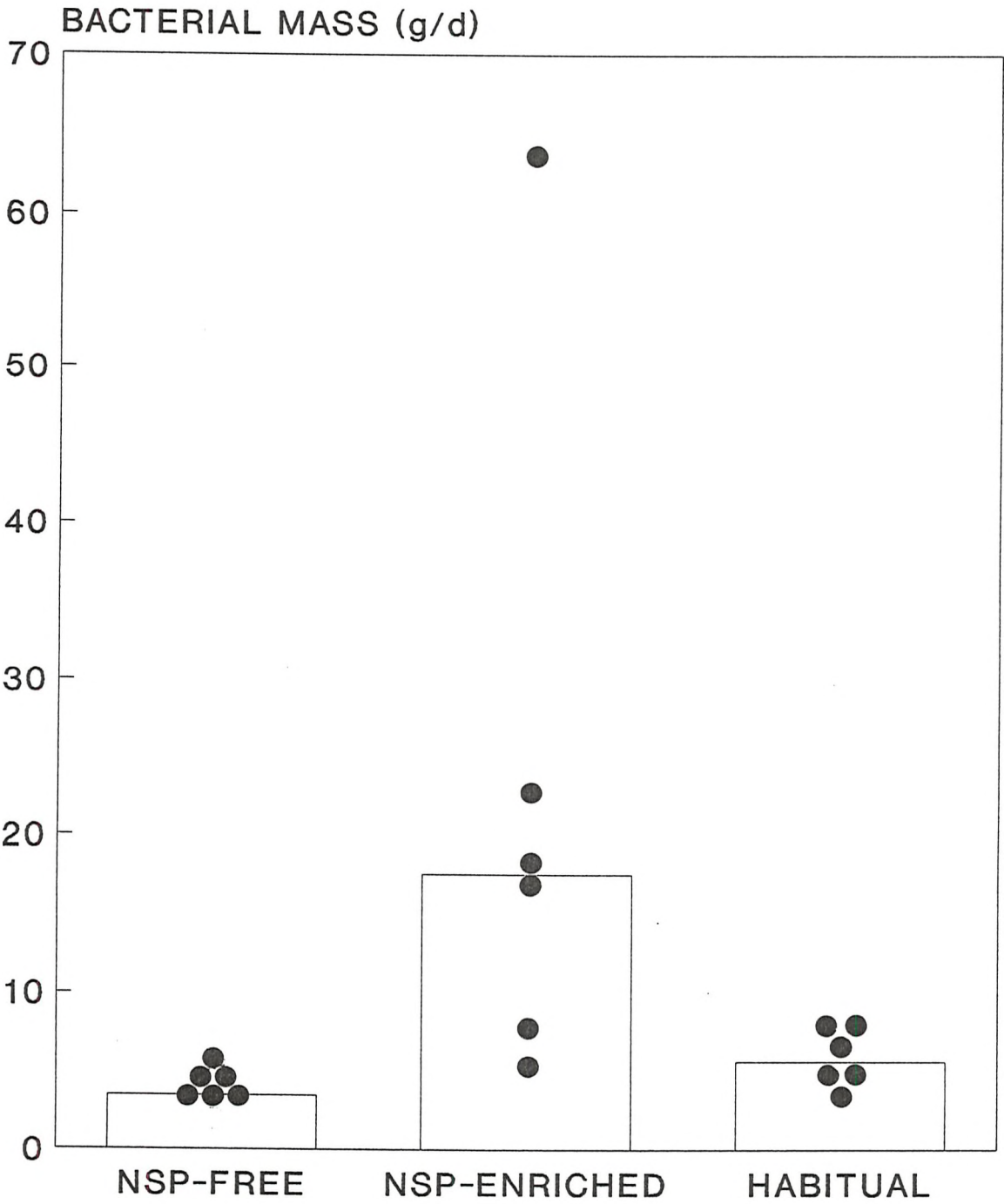


Figure 7.11 Daily faecal bacterial content of faeces versus dry weight of stool measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet.

Correlation coefficients are $R = 0.45$ (NS), $R = 0.96$ ($p < 0.01$) and $R = 0.69$ (NS) respectively.

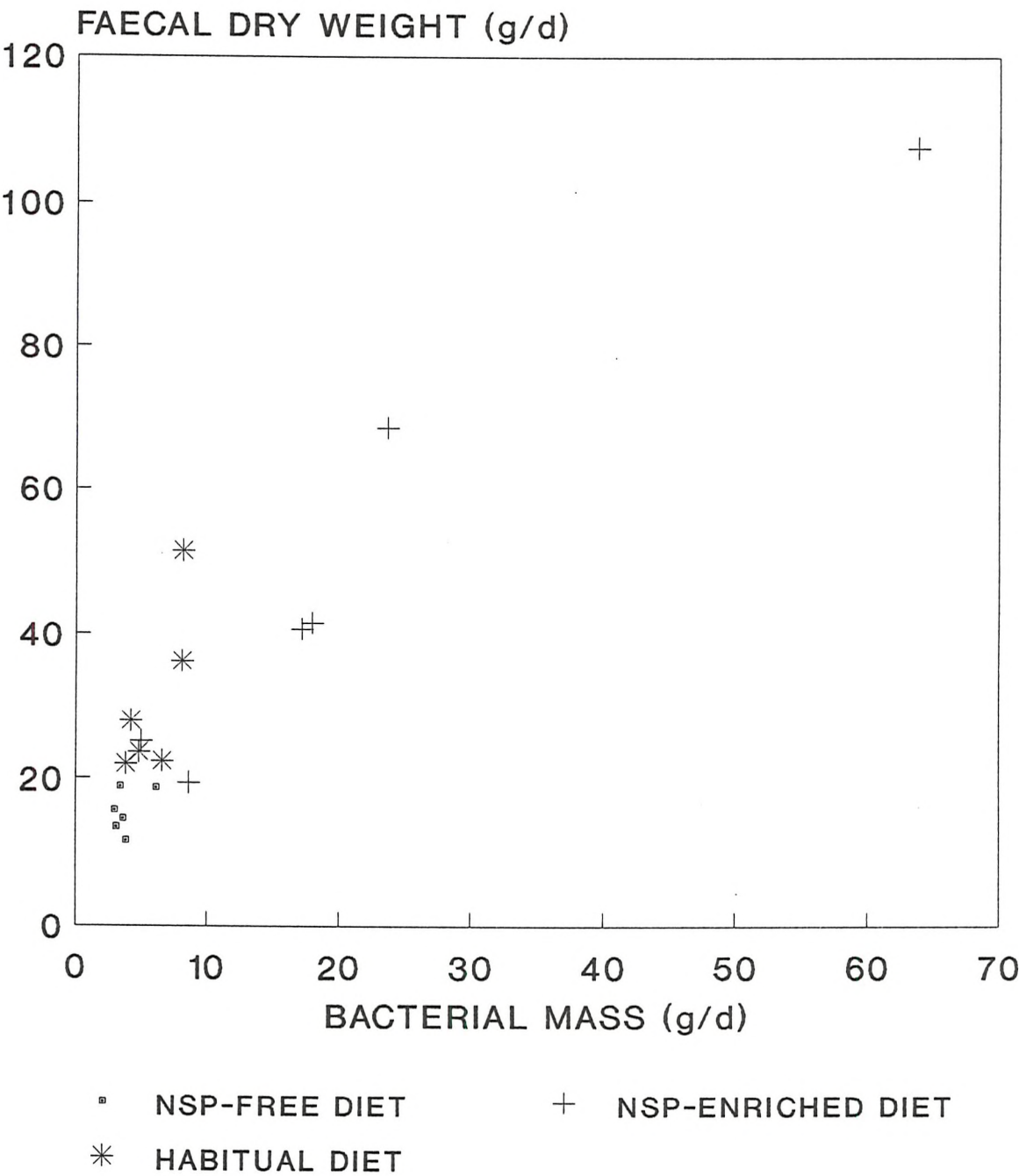


Figure 7.12 Daily energy content of bacteria within stools determined from pooled samples of faecal bacteria versus the energy content of bacteria within stool samples from six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The line of unity is given.

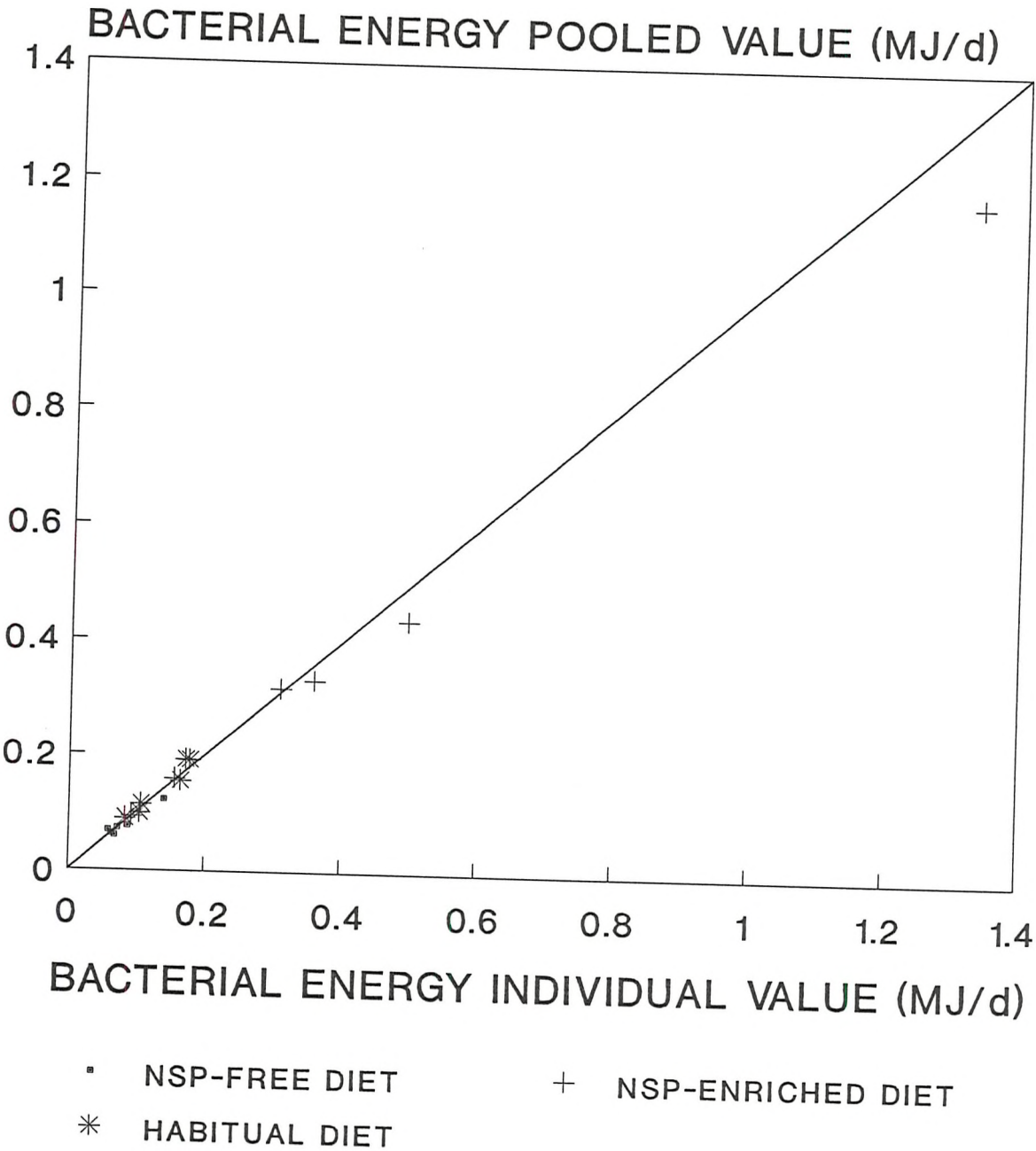


Figure 7.13 Daily faecal energy versus energy content of faecal bacteria measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet.

Correlation coefficients are $R=0.61$ (NS), $R=0.95$ ($p<0.01$) and $R=0.57$ (NS) respectively.

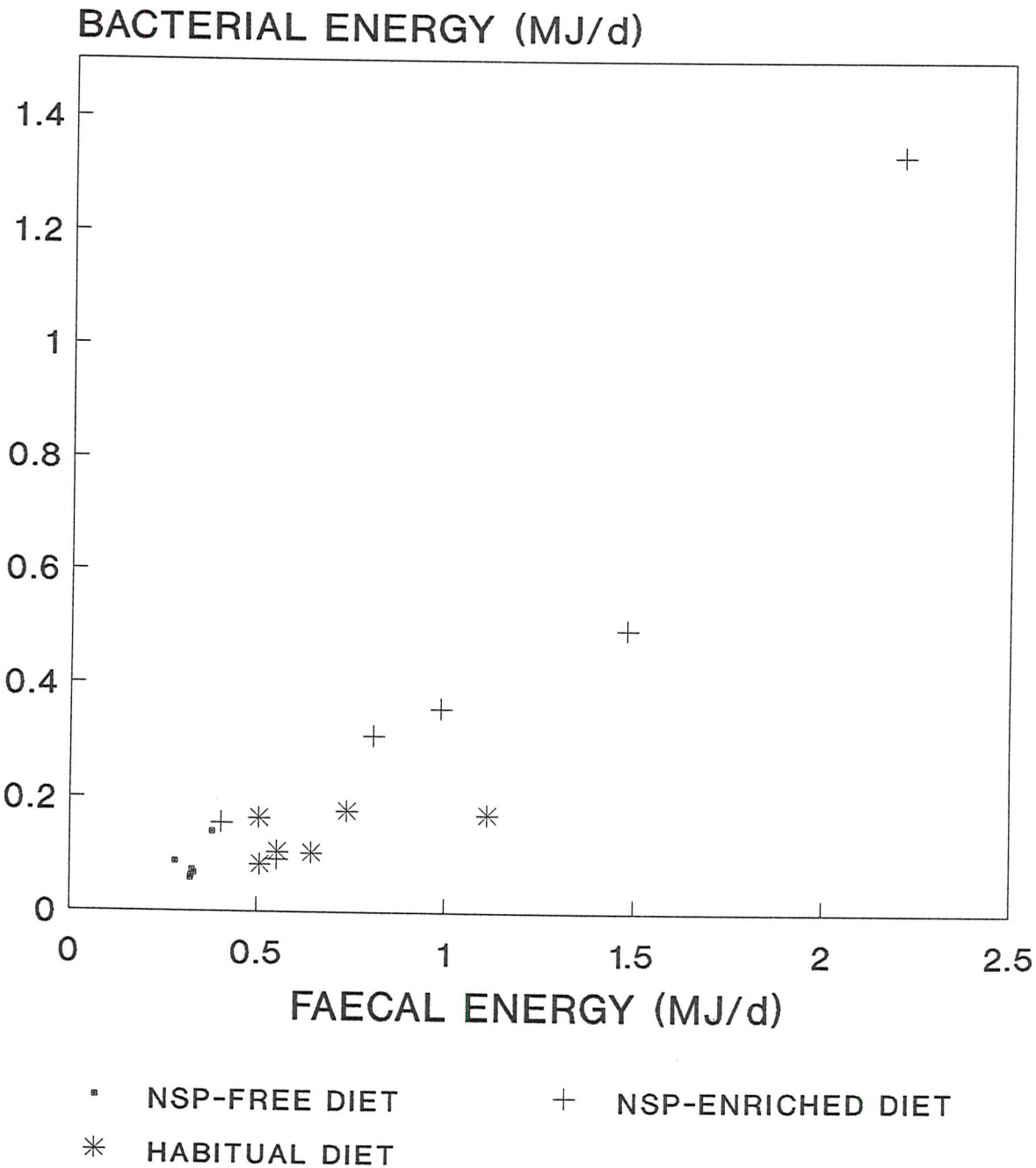


Figure 7.14 Daily lipid content of bacteria within faeces determined from pooled samples of faecal bacteria versus the lipid content of bacteria within stool samples from six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The line of unity is given.

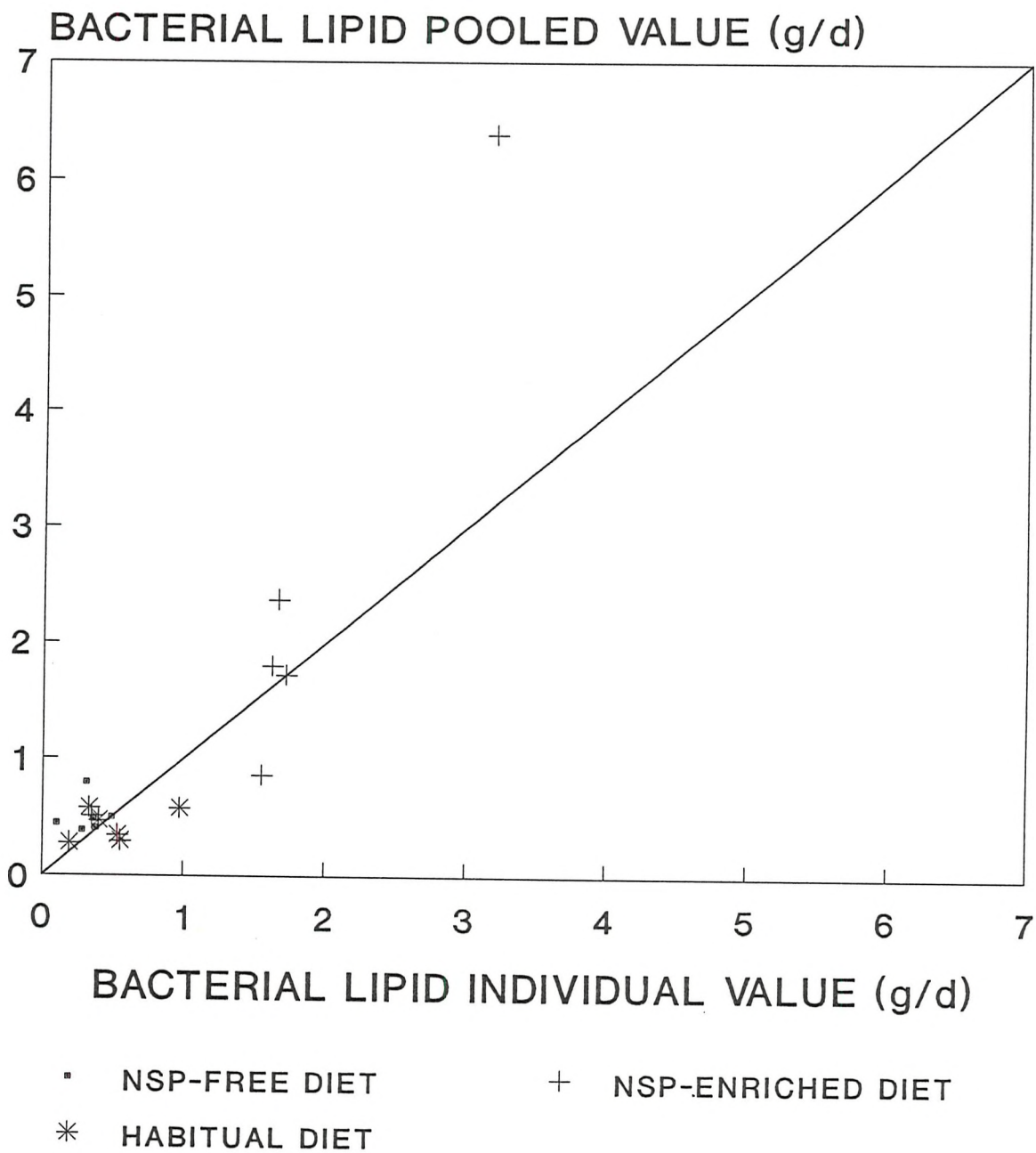


Figure 7.15 Daily faecal lipid versus the lipid content of faecal bacteria measured in six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. Correlation coefficients are $R = -0.12$ (NS), $R = -0.80$ (NS) and $R = 0.04$ (NS) respectively.

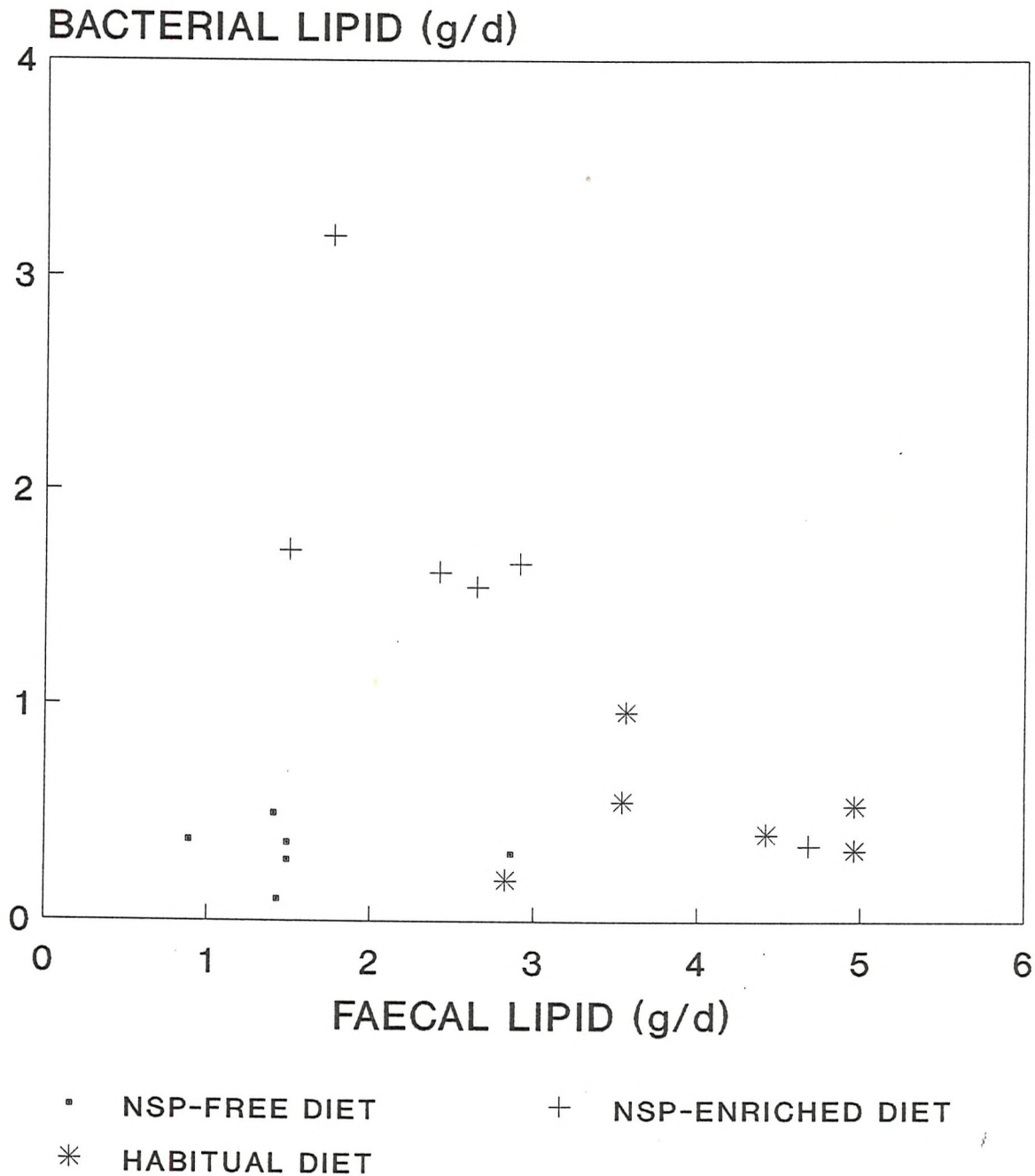
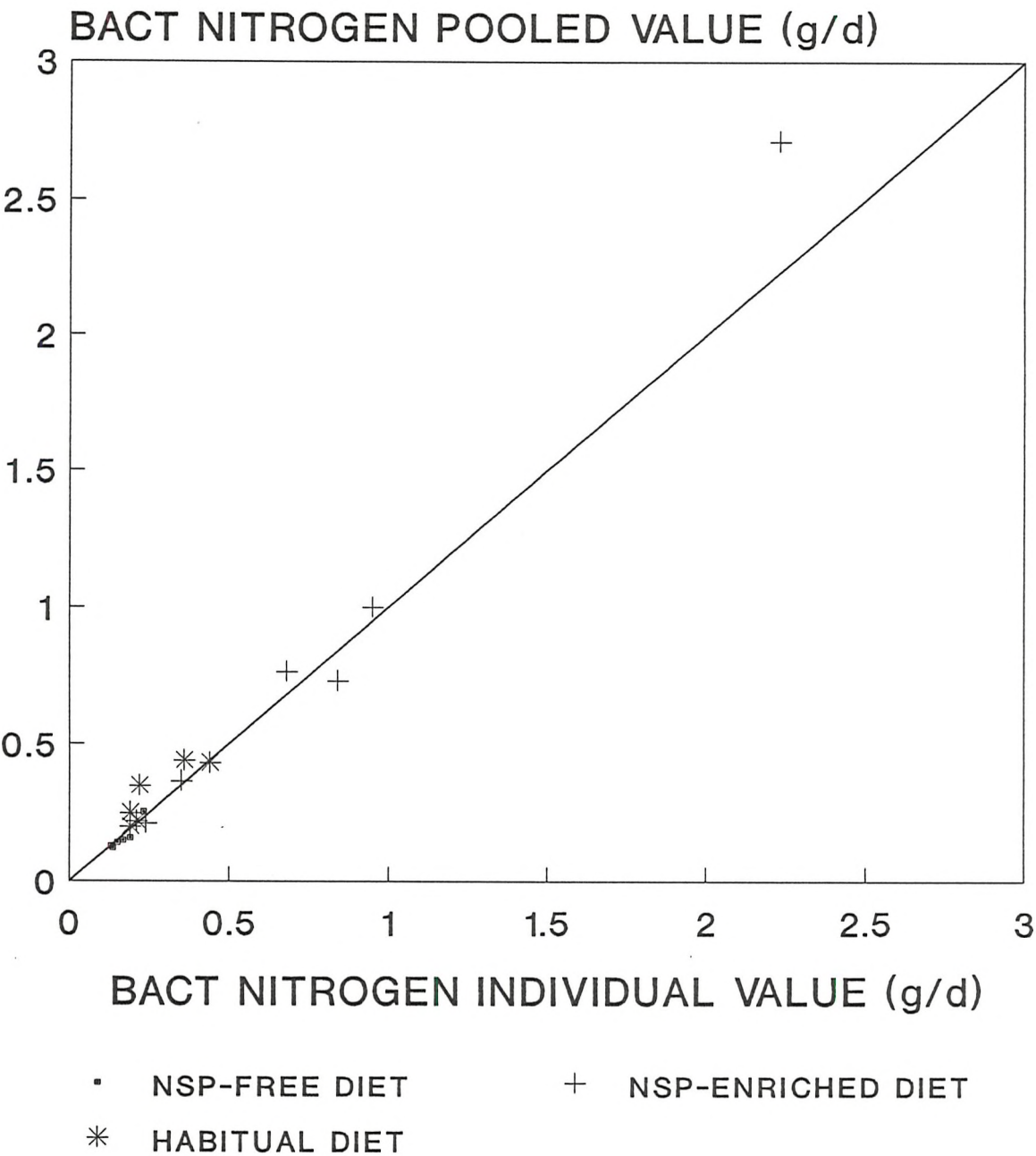
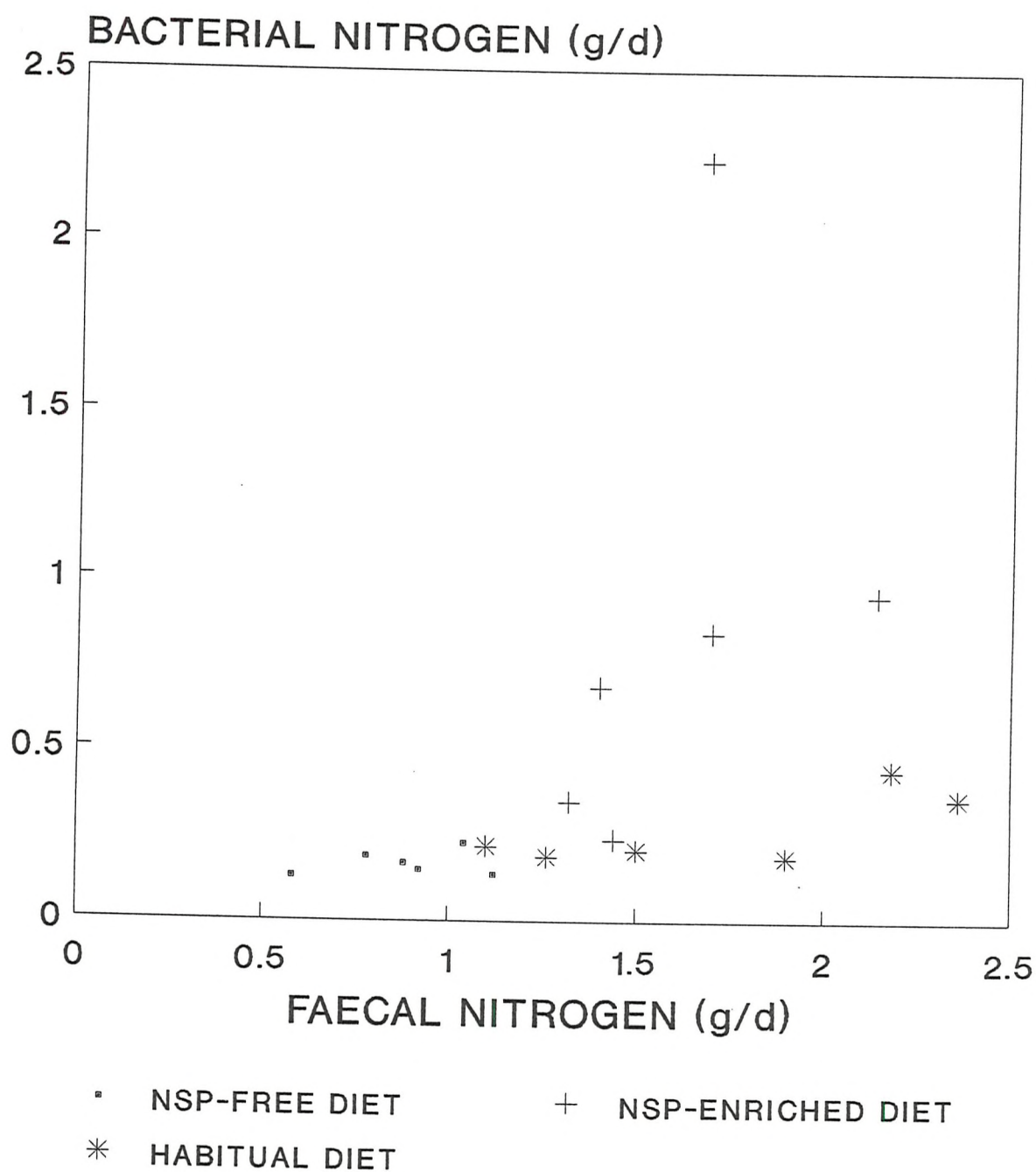


Figure 7.16 Daily nitrogen content of bacteria within faeces determined from pooled samples of faecal bacteria versus the nitrogen content of bacteria within stool samples from six normal adult men following the consumption of both NSP-free and NSP-enriched diets and habitual diet. The line of unity is given.



Correlation coefficients are $R=0.30$ (NS), $R=0.40$ (NS) and $R=0.75$ (NS) respectively.



CHAPTER 8. GENERAL DISCUSSION.

8.1 INTRODUCTION.

A clear understanding of how much energy may be made available from food is essential to assess the energy requirements of individuals under normal circumstances and during periods of ill health, during growth and development in childhood and in the therapeutic management of pathological states. It would appear from our present knowledge however, that there are a number of questions which remain outstanding. The extent to which faecal energy losses may limit the availability of energy from the diet remains unclear. The presumption is still made in studies of energy balance that all of the energy present within faeces is derived directly from the diet both in health and disease. Two other major components may also contribute to the material within faeces: endogenous losses and cellular debris into lumen of the gastro-intestinal tract and an extensive colonic bacteria microflora (figure 8.1). Whilst the relative proportion of each component may vary with i) alterations in dietary intake, ii) the effectiveness of digestion and absorption within the gastro-intestinal tract and iii) the extent to which dietary residue or endogenous losses may be fermented by colonic microflora, the relative contribution made by each of these components to faecal energy is not known. Similarly the relative proportions of energy within the stool derived from lipid, protein and carbohydrate have not been previously described. In addition, the metabolic activity of colonic bacteria microflora and the extent to which energy may be salvaged and be made available to the host in the form of short chain fatty acids (SCFA) is poorly understood.

This thesis has specifically addressed these issues in an attempt to develop a more clear understanding of the extent to which energy may be made available to the body both in health and disease. The successful utilisation of food available is determined by the functional integrity of the gastrointestinal tract. The metabolic activity of the resident microflora of the lower bowel may be of much greater significance in relation to the maintenance of energy balance than has been appreciated in the past.

This discussion examines the magnitude and composition of energy content of stools. Attention is then directed towards the availability of dietary energy in relation to the expression 'apparent digestibility'. The significance of colonic

bacterial activity and the extent to which energy may be made available to the host in the form of short chain fatty acids (SCFA) are also considered.

8.2 ENERGY CONTENT OF STOOLS; ITS VARIABILITY, COMPOSITION AND RELATIONSHIP WITH THE WEIGHT OF STOOL.

The amount of energy within the stools each day of healthy men and women from the present study ranged between 0.2-1.2 MJ/d (chapters 4 & 7) which is comparable to previous reports [Southgate and Durnin, 1970; Miles *et al.* 1984]. As stool energy losses are relatively small and collection and analysis of faecal material is both time consuming and unpleasant, measurements of faecal energy are not a common procedure.

Our knowledge of the magnitude and variability of stool energy content each day under normal circumstances would appear to be severely limited. We know from a previous study that faeces are not necessarily passed each day in healthy individuals [Rashgarian & Kashgarian 1967] which could be supported from the findings of the study reported in chapter 4. In the light of this information it might be expected for daily faecal energy within an individual to exhibit a high degree of variability which could be as much as nearly 60% from the present data. Due to this variation in stool frequency the best possible estimate of a representative value for daily faecal output must be determined over a period of days. Three or five day stool collections have been employed to measure representative daily faecal energy as well as the faecal lipid and nitrogen contents of stool.

It was demonstrated in the present investigation that the mean faecal energy content calculated from one day over each of the study periods was only 14.5% greater than average daily faecal energy calculated from both three and five day stool collections. There could be no benefit in measuring daily faecal energy from a period of time longer than three days as the variability and estimated representative daily faecal energy contents were similar from a three day and a five day stool collection. What was also demonstrated from this study was the relatively small variation in faecal energy between individuals although it has only been possible to determine differences in a group of six subjects.

Whilst there have been relatively few reports of the faecal energy contents of normal healthy adults, there is even less information of the extent of faecal energy loss in children between the ages of 5 years through to adulthood. Very early

studies within the literature indicated that faecal energy losses in childhood may be less than 500 kJ/d [Holt & Fales, 1921, Wang *et al.* 1936]. Information from the present group of healthy children aged between 6-11 years (chapter 5) demonstrated that the stool contained on average 298 kJ/d (range 123-647 kJ/d) which is comparable to these previous reports.

There would appear to be differences in the magnitude of faecal energy between normal adults and children. Whilst a greater amount of stool energy could be associated with increases in age, this is reflected by raised absolute energy intakes in adults when compared to children. To investigate the likelihood of an age relationship with normal faecal energy from the present data, it is possible to plot the faecal energy content of stools versus age (figure 8.2) From this information it would seem that there is a tendency towards a relationship between faecal energy and age ($R=0.63$, $p<0.01$) although it should be noted that two specific groups of individuals have been examined. Further studies are required to explore this relationship in more detail.

Having discussed the magnitude of faecal energy in health, it is important to consider the extent to which the energy content of stools may be influenced by changes in diet and disease. There have been relatively few studies which have examined the extent of faecal energy losses in gastro-intestinal disorders associated with maldigestion and malabsorption. From the information available, substantial alterations in the magnitude of faecal energy losses were evident in maldigestion and/or malabsorption [Brown *et al.* 1980; Heymsfield *et al.* 1981; Bijleveld *et al.* 1986]. In support of these observations, the present study illustrated the extent of faecal energy losses in patients with cystic fibrosis (CF) who believed themselves to be comparatively asymptomatic on their habitual PERT. Faecal energy losses were approximately 0.99 MJ/d (0.38-2.08 MJ/d) and therefore approximately three times greater than the energy content of stools within normal healthy children. This suggests that raised stool energy losses in CF patients may continue to contribute in part towards an energy deficit that may be sufficient to limit growth or cause weight loss.

It is well known that increasing the intake of foods providing different types of dietary NSP generally results in increased faecal output and the energy content of faeces [Kelsay *et al.* 1978; Goranzon *et al.* 1983; Stephen *et al.* 1986; Miles *et al.* 1988; Wisker *et al.* 1988; Wisker & Feldheim, 1990]. This effect could be confirmed in the present study following the consumption of a NSP-free diet

enriched with ripe bananas which contain complex carbohydrates. Previous studies have observed a reduction in stool weights following the consumption of so-called 'defined formula diets' which do not provide any dietary NSP [McCamman *et al.* 1977; Kien *et al.* 1981]. The present study could also demonstrate that when a diet does not contain any form of complex carbohydrate, starch or NSP, there is a reduction in stool energy content reflecting a reduction in stool weight.

Previous studies have examined the relationship between faecal lipid and faecal weight [Pinter & McClean, 1968; Skala *et al.* 1968; Pedersen & Halgreen, 1984; Bijleveld *et al.* 1986] and faecal lipid versus faecal energy [Heymsfield *et al.* 1981] in intestinal dysfunction. These studies attempted to determine whether stool weight or the lipid content of stools could be reliable methods to study maldigestion and/or malabsorption. Whilst strong associations between these variables could be described in these studies, the present investigation failed to demonstrate clear relationships between faecal lipid and the energy content of stools in gastro-intestinal dysfunction or in normal health despite alterations in diet. This would imply that the present approach to address the efficacy of PERT by measurements of faecal lipid should not be employed.

Previous studies have observed an association between faecal energy and wet stool weights in lactose-malabsorbing children [Brown *et al.* 1980] and between faecal energy and dry stool weights in the short bowel syndrome [Rodrigues *et al.* 1989]. These relationships could be confirmed within the disease CF in the present study (chapter 6). What is also clear is that these relationships appear to hold over a wide range of ages from childhood to adults, and is unaltered by the dietary content of complex carbohydrate (figures 8.3 and 8.4). It should be noted the constancy in energy density of stool on a wet weight basis and a dry weight basis despite a wide range in faecal energy losses. Very early studies have indicated that the energy content of dry human faeces remains constant on different diets at approximately 6.2 kcal (25.9 kJ) per gram [Rubner, 1902]. The present results from each of the experimental studies agree with this observation made by Rubner [1902] where the amount of energy per gram of dry faeces overall was approximately 22.6 kJ/g. The greatest energy content per gram of wet and dry stools was observed within cystic fibrosis patients which was approximately 8 kJ/g and 26 kJ/g respectively.

This information could have implications on the management of gastrointestinal problems associated with cystic fibrosis. Pancreatic enzyme replacement therapy (PERT) has been developed to limit the maldigestion in cystic fibrosis. In clinical practice the use of PERT has been directed towards symptomatic correction of steatorrhoea, abdominal pain relief and reduction in stool frequency and mass of stool passed each day. Steatorrhoea has frequently been used as the index of maldigestion [Mischler *et al.* 1982, Stead *et al.* 1987; George *et al.* 1990]. It is commonly assumed that when steatorrhoea and gastro-intestinal symptoms are improved, the amount of energy within stools is normalised. Despite this common belief, the present study has clearly demonstrated that this relationship does not hold in CF patients with PERT who believed themselves to be comparatively asymptomatic. It appears that CF patients with small faecal lipid losses may also have high stool energy losses. This would imply that a reconsideration of the present approach to address the efficacy of PERT by the measurement of faecal lipid may be required. Further attention should be directed towards enzymic activity and composition of pancreatic enzyme supplements with respect to the relative contribution made by lipase, amylase and protease. It could be that in CF where mucus production is raised, supplements which provide the enzymes for the digestion of mucus and other endogenous losses may allow the recovery of energy which may be otherwise lost within the stool.

The recommendation of Heymsfield and coworkers [1981] that the measurement of faecal energy may provide a simple and reliable assessment of malabsorption has been generally ignored as an index of the extent of maldigestion and/or malabsorption. The present information has demonstrated that faecal energy measurements may provide a more accurate routine assessment of the extent to which PERT normalises stool energy losses in patients with cystic fibrosis. However as the facilities required to measure stool energy directly are not generally available in clinical practice, it is not always possible to maintain this approach. Nevertheless whilst the energy density of stool remains constant on a wet weight basis and dry weight basis, an estimation of the extent of energy losses in stool could be derived by simply determining the weight of stool passed each day with the knowledge that 8 kJ/g is present in each gram of wet stool. This approach would provide a more simpler method to estimate faecal energy content without the need for complete stool collections over a fixed timed period. An increased number of stool measurements could be performed over an extended period of time which may be particularly useful in field investigations.

Whilst it may be difficult to weigh stools under these circumstances, a more simple method of weighing stools could be employed by a weighing device incorporated into a stool collection vessel.

Whilst we know that the energy within the diet is derived from carbohydrate, lipid and protein, the relative proportions of each of these components of the energy within the stool is not clear. Within the confines of the present studies in this thesis, attention has been focused towards the amount of energy represented by lipid and protein within stools. To gain this information from measurements of faecal lipid and nitrogen, a number of assumptions have been employed. Firstly, by assuming that most of faecal nitrogen is protein, the amount of protein within the stools is derived by applying the standard factor of 6.25. This factor is applied on the assumption that most proteins within foods contain 16% nitrogen. Secondly, the heats of combustion values from the lipid and protein energy content of foods per gram have been employed to estimate the lipid and protein energy content of faeces. Whilst it is recognised the errors that might be introduced by applying such factors, for the purpose of the present investigation they provide the best estimate of lipid and protein energy within the stool until further information is available. Further studies are required to determine the factors that may be applied to estimate the energy derived from lipid and protein (and carbohydrate) within different components of faeces and the proportion of nitrogen in protein compounds within faeces. These studies could only be achieved however, with further partitioning of each of the components of faecal material.

In the present studies the values for faecal lipid and nitrogen in children are comparable to those reported for healthy adult men. In addition these findings support previous reports in children [Schmerling *et al.* 1970] and in normal healthy adults [Wollaeger *et al.* 1947]. It is commonly presumed that energy is simply related to the lipid content of faeces because lipid is more energy dense per gram than protein or carbohydrate. However it is clear from the present studies that the relationships observed between the energy and lipid content of stools both in children and adults could not support this idea. The amount of protein within the stool could be related to the energy content of faeces. In the light of this observation, it is clear that more of the energy within the stool could be attributable to protein compared with lipid in normal childhood and adulthood. This observation is also unchanged with alterations in diet. However, it should

be noted the extent of the range of values between individuals within each subject group.

Whilst it has not been possible to determine the relative contribution made by carbohydrate to energy within the stool, the amount of carbohydrate within stools (both endogenous and exogenous losses) is not clear. It is thought that 3-5g of dietary NSP remains in the stool each day, representing approximately 3-4% of the stool dry weight [Cummings *et al.* 1979]. It may be that other carbohydrates possibly of endogenous origin such as endogenous secretions and cellular debris for example, intestinal glycoproteins and mucopolysaccharides or even some unabsorbed starch from the diet may represent part of the remaining energy within faeces. There may be however alterations in the carbohydrate content of stools with different types and amounts of dietary NSP depending upon their effect on human metabolism. In addition the carbohydrate components of mucus and epithelial cell losses within the faeces may form a substantial part of stool carbohydrate particularly where mucus production is raised in CF patients. The extent to which the carbohydrate from endogenous sources may be fermented by bacteria is not known. Bacterial cell lipid could be derived from maldigested and/or malabsorbed dietary residue and lipid from endogenous losses. In addition it could be that bacterial lipid could originate from the conversion of carbohydrate substrate once the requirements for energy for growth and maintenance of bacteria cells are met. Therefore it would seem that the origin of carbohydrate within faeces remains at a speculated level as it has not been possible to directly quantify the potential contribution made by the components of faecal carbohydrate.

8.3 APPARENT DIGESTIBILITY OF ENERGY AND THE METABOLIC ACTIVITY OF COLONIC BACTERIA.

The current approach to determine the 'apparent digestibility of energy' is gross energy minus faecal excretion divided by intake. Applying this approach the amount of energy within the stool each day in healthy adults is generally believed to be equivalent to approximately 5% of the gross energy intake [Southgate and Durnin, 1970]. From early studies [Holt & Fales, 1921, Wang *et al.* 1936] a similar value was observed within children. In this thesis stool energy losses were equivalent to 3.5% (1.3-5.8%) in childhood and 5.1% (3.5-6.7%) in healthy adults of gross energy intake, despite what would appear to be a greater absolute

faecal energy content in adults in comparison to children (see chapters 5 and 7).

Whilst there were substantial alterations in faecal energy with changes in dietary NSP or in maldigestion and/or malabsorption, differences in the apparent digestibility of energy from the diet were observed. Stool energy losses were equivalent to between 5-20% of the gross energy intake in maldigestion and/or malabsorption. Following the consumption of a diet containing no dietary NSP and a diet with the addition of fermentable carbohydrate from bananas to a NSP-free diet, stool energy losses were equivalent to 3.2% and 7.2% of GEI respectively.

Attention within the literature has particularly centred upon the use of the apparent digestibility relationship to explain the effect of increased intakes of dietary NSP on the digestibility of energy from lipid and protein. It is thought that increased intakes of dietary NSP may reduce the apparent digestibility of energy from lipid and nitrogen within the diet [Widdowson, 1960; Southgate & Durnin, 1970]. The results from the study, presented in chapter 7, with the addition of bananas to a NSP-free diet are therefore in agreement with this finding. In an attempt to explain this phenomenon, attention has been focused upon the upper gastro-intestinal tract and the idea that there is an effect of dietary NSP on the digestion and absorption of lipid and protein has been developed.

By applying this approach it assumes that all of the energy within the stool is of dietary origin. However this simple model of the digestibility of energy conceptually has important limitations. It makes no allowance for endogenous losses into the lumen of the gut in the form of digestive secretions, mucus and sloughed cells and the effects of the bacterial microflora within the colon which will both influence the magnitude and composition of faecal energy. Therefore it does not take into account for alterations in diet where there may be substantial changes in the activity of the bacterial microflora or in the disease cystic fibrosis where mucus production may be raised which may form a substantial part of stool energy losses.

Although it is not possible to quantify directly mucus and epithelial cell losses within faeces, it is comparatively simple to measure the amount of bacteria within human faeces using a technique developed by Stephen & Cummings [1980a]. Whilst bacterial microflora represent a major proportion of the stool dry weight

[Stephen & Cummings, 1980a], a major proportion of the energy within the stool could be attributed to bacteria both in health and disease. It should be noted however that whilst attention is focused upon colonic bacterial activity, it can only be assumed that measurements of the bacterial content of faeces reflect the activity of colonic bacterial microflora. Although studies from sudden death victims indicate that the faeces contain a representative population of those bacteria present in the colon [Moore & Holdeman, 1978], it is difficult to extrapolate such results from post mortems to living individuals.

Under normal circumstances a major component of stool weight may be attributable to colonic bacteria representing 25% of dry stool weight (range 17-42%) in childhood and 19% of dry weight of stool (range 15-29%) in healthy adult men. The proportions of bacteria within the stools of patients with cystic fibrosis would appear to be generally similar to healthy individuals with bacteria accounting for 33% (range 19-61%) of faecal dry weight. Therefore differences in the contribution made by faecal bacteria within stools may reflect between subject differences in colonic bacteria activity.

The metabolic activity of colonic bacteria is modified by dietary changes. Whilst this observation is in contrast to previous reports [Attebury *et al.* 1972, Crowther *et al.* 1973], it is most probably related to the approach used to measure faecal bacteria. In these studies the classical bacterial technique of counting and identifying organisms have been applied to determine the nature of faecal bacteria in contrast to the quantitative approach of Stephen & Cummings [1980a] employed in the present studies. Whilst a reduction in stool weight could confirm previous reports following the consumption of so called 'defined formula diets' which do not contain any NSP [Kien *et al.* 1981, McCamman *et al.* 1977], bacteria could still represent part of the faecal dry weight, approximately 24% (range 18-32%). This would suggest that components other than dietary NSP may provide carbohydrate to supply energy for colonic bacterial growth. This is likely to be from an endogenous source such as the carbohydrate from digestive secretions, mucus and sloughed cells as mentioned previously. However the relative importance of each of these components remains at the level of speculation as the amount of carbohydrate which may enter the large intestine from both endogenous and exogenous sources is not clear. From intubation studies within the terminal ileum it has been estimated that 3-4 g/d of endogenous carbohydrate as hexosamine, which constitutes about 40% of human mucus [Forstner 1978] may enter the colon from the upper gastro-intestinal tract

[Stephen *et al.* 1983]. However it should be noted that this figure does not allow for other sources of carbohydrate or mucus production within the colon. Whilst it is assumed that the components of elemental diets are totally absorbed in the small intestine, it could be that there is also some dietary residue entering the colon as evidence derived from studies with elemental diets in ileostomists show that some dry material is excreted each day [Hill *et al.* 1975].

It has been previously demonstrated that a diet containing additional NSP from cabbage to a normal diet provided a readily usable substrate for bacterial growth within the colon. In the present studies it could be demonstrated that with the addition of bananas to a NSP-free diet 43% (range 27-54%) of the faecal dry weight could be attributed to bacteria. This would suggest that the complex carbohydrate content of bananas as starch or NSP may provide an ideal substrate for the stimulation of bacterial growth within the colon. However, it could be that the increased carbohydrate load from bananas had exceeded the body's capacity to provide sufficient enzymic activity to digest carbohydrate substrate sufficiently such that more fermentable substrate was available for fermentation by colonic bacteria.

The studies reported in this thesis have demonstrated the proportion of energy within stools which could be attributed to bacteria with the knowledge of the bacterial content of faeces. Before these measurements could be made, information of the energy content of bacteria was required. Whilst nitrogen [Smith & McAllan, 1974; Stephen & Cummings, 1980a] and lipid contents [Luria, 1960] of bacteria have been previously reported, there have not been any previous reports of the energy that may be contained within bacteria. Whilst there may be differences in the types of bacteria within faeces between individuals [Gorbach *et al.* 1967], it would appear that there are comparable amounts of energy within faecal bacteria ranging between 20-24% from normal individuals, in disease and despite changes in dietary NSP intake.

Greater differences in the lipid content of bacteria was observed between the subject groups in the present study ranging between 9-20%. Luria [1960] has shown that the lipid content of bacteria ranges between 10-15%, although this value was derived from pure cultures. In contrast to the lipid content of bacteria, less of a difference could be demonstrated for the nitrogen content of bacteria which was approximately 3-5%. In comparison Stephen & Cummings [1980a] demonstrated a bacterial nitrogen content of 6-7%. However it should be noted

that the approach to measure the nitrogen content of bacteria in the present investigation was different to that in the previous studies (see chapter 3). Within each study the nitrogen, energy and lipid content of faeces was determined on a pooled sample of faecal bacteria from each subject thereby providing a representative sample of bacteria. Whilst there may be errors introduced by such an approach, a comparison between the energy, lipid and protein from pooled samples of bacteria and from faecal bacteria within each subject has been demonstrated (chapter 7). This study provides evidence to suggest that the energy and nitrogen contents of bacteria could be measured from pooled samples of bacteria within a group of individuals. However a similar finding could not be confirmed for lipid although it is not clear why this is apparent. It could be that this discrepancy may account for the observed difference in bacterial lipid content between the study groups.

With this knowledge of the energy, lipid and nitrogen contents of bacteria, it has then been possible to estimate the proportion of stool energy accounted by bacteria and how much of this energy from lipid and protein could be attributed to bacteria. A proportion of the energy within stools could be associated with faecal bacteria, around 25% (range 17-43%) of stool energy in healthy children and approximately 18% (range 14-28%) in healthy men. However in disease it would seem that the amount of energy attributed to bacteria exhibited more variability in comparison to normal individuals, ranging between 9-57%. This may suggest that the relative proportions of energy from bacteria, malabsorbed dietary residue and other endogenous components such as mucus within the stools of cystic fibrosis patients may also vary.

It has been indicated that the metabolic activity of the colonic bacteria could be modified by dietary change, as demonstrated by substantial alterations in the bacterial content of faeces. As might be expected the proportion of energy within the stool which may be attributed to bacteria could also be altered with the addition of fermentable substrate from bananas to a NSP-free diet, accounting for approximately 36% (range 22-48%) of the energy within the stool. This effect on colonic bacterial activity could also be supported from the data of the proportions of lipid and nitrogen within faeces attributable to bacteria. It has been previously reported that bacteria could provide approximately 60% of the nitrogen within faeces following the consumption of diets containing increased amounts of NSP [Stephen & Cummings, 1980d]. The present study has shown that bacteria could account for around 47% of the nitrogen within faeces

following the consumption of bananas with an NSP-free diet. In healthy individuals and in disease, bacteria could account for between 15-16% and 20% respectively of the nitrogen within faeces. However it should be noted that these values exhibited a high degree of variability between subjects reflecting the differences in the activity of colonic bacteria. It is difficult to derive firm conclusions from the proportion of stool lipid attributable to bacteria due to the limitations of the procedure as previously described in chapter 7. Information of the lipid content of stools from bacteria has not been previously reported in man, although an early report showed that 40% of faecal lipid could be attributed to bacteria in dogs [Sperry, 1929]. The present observations start to explain the composition of energy derived from lipid and nitrogen within faecal bacteria.

In conclusion it is clear that a major component of the stool is not simply maldigested and malabsorbed dietary residue but that part of the energy within the stool may be attributable to the bacterial microflora. This observation therefore has important implications on our understanding of the energy which is made available to the body in health and the way in which the body accommodates with alterations in diet and in disease. It appears that substantial alterations in the contribution made by bacteria towards energy content occurs as a result of dietary change. Therefore what appears to be a reduced availability with increased intakes of dietary NSP may simply reflect colonic bacterial growth resulting in an increase in the contribution made by faecal bacteria to faecal energy.

This information would also indicate that what we know about apparent digestibility from the studies of Atwater at the turn of the century and from other studies of energy balance requires re-evaluation as no attempt has been made to discriminate between dietary and endogenous sources of faecal energy. The actual digestibility of dietary substrates may thus be higher than indicated. The factors presently used to calculate metabolisable energy intake by applying McCance and Widdowson's modified Atwater factors [Paul & Southgate, 1978], are dependent upon a reliable conversion from gross energy to metabolisable energy which were derived from studies in adults. It is not clear however, whether such factors are necessarily applicable to growth and development in childhood or in diseases associated with maldigestion and/or malabsorption.

It is a well known that human large intestinal bacteria are capable of degrading carbohydrate to supply their energy requirements for growth by the process of fermentation. The origin of carbohydrate could be from maldigested and/or malabsorbed dietary residue such as dietary NSP [Williams & Olmstead, 1936; Hummel *et al.* 1943], starch [Englyst & MacFarlane, 1986; Stephen *et al.* 1983] or endogenous losses such as mucus [Miller & Hoskins, 1981] and some other glycoproteins [Hoskins & Boulding, 1976]. Whilst it is not known how much carbohydrate passes the terminal ileum under normal circumstances nor the quantity of carbohydrate from endogenous losses, there have been relatively few reports which have attempted to estimate the amount of energy that may be recovered by the host through SCFA absorption in normal men [Cummings, 1981; Wolin, 1981; McNeil, 1984].

From the data of the present studies, it has been possible to estimate colonic SCFA production using the same approach as McNeil [1984] which is based on the growth requirements of intestinal bacteria. From this information the impact of colonic function on energy metabolism in both health and disease and with alterations in diet could be determined. In both healthy children and adults the theoretical amounts of energy that could be made available through SCFA absorption were comparable, equivalent to approximately 1.9% (range 0.9-4.2) and 2.0% (range 0.7-3.5%) respectively. Whilst these values would appear to be less than previous reports [Cummings, 1981a; McNeil, 1984] in healthy individuals, it should be noted that these are estimated figures. Whilst this calculation does not allow for SCFA loss through faeces, it is unlikely that this may represent an important route of energy loss as faecal output of SCFA appears to be small [Rubinstein *et al.* 1969; Hoverstad & Bjornekleit, 1984]. Whilst the recovery of energy through SCFA absorption may make a relatively small contribution towards energy needs in health, it has been suggested that this mechanism of energy salvage may be more important in malabsorption or diets high in NSP where there may be increased amounts of carbohydrate entering the colon [Grosslaus, 1983; McNeil, 1984].

In pathological states where there is maldigestion and/or malabsorption it has been demonstrated that the energy made available through SCFA absorption may provide additional energy equivalent to approximately 6% which could be as much as 14% in some patients with CF (chapter 6). This confirms the claim made by Bond & Levitt [1980] that SCFA allows the recovery or 'salvage' of calories through the malabsorption of carbohydrate, starches and simple sugars

under pathological conditions which would be otherwise lost within faeces. Another additional factor to consider in the disease cystic fibrosis is the opportunity to recover at least part of the energy from endogenous secretions. This would suggest that apart from the availability of fermentable substrate from malabsorbed carbohydrate, the potential contribution made by endogenous carbohydrate for bacterial fermentation in the colon could be substantial. It would also seem that the potential contribution made by SCFA absorption varies between individuals with CF. Whilst this may reflect differences in the metabolic activity of the intestinal microflora it could be that for individuals on marginal intakes or in whom energy requirements are elevated, the relative contribution made by the absorption of SCFA may be a critical determinant to support energy needs.

Estimates of the amount of carbohydrate required for fermentation suggest that the intake of NSP alone in healthy children and CF patients could not produce the weight of bacteria within faeces. As outlined in chapter 5, carbohydrate substrate required for colonic bacterial fermentation must be derived from maldigested and/or malabsorbed starch and the carbohydrate within endogenous losses and cellular debris. Whilst the intakes of dietary NSP were comparable for CF patients and control subjects, the theoretical amount of hexose required was greater in CF patients than control subjects to produce the weight of faecal bacteria. This would suggest that the fermentable carbohydrate from maldigested and/or malabsorbed dietary residue such as starch and endogenous losses in cystic fibrosis where mucus production is raised may both form potentially important sources of fermentable substrate for bacterial fermentation within the large intestine.

Given that increased intakes of complex carbohydrates may alter the proportion of energy that can be recovered from SCFA absorption, additional energy made available as SCFA from colonic fermentation was equivalent to 7.0%. As demonstrated in maldigestion and/or malabsorption there is considerable variation between individuals and up to 18% of daily energy needs could be met by colonic metabolism. What is also evident from this study is that when the diet contains no NSP the activity of the colonic bacteria is maintained over the period of the study to a similar extent as individuals consuming normal diets with the result that between 1-2% of energy requirements could be recovered through SCFA absorption. This would suggest that sources other than dietary NSP provide substrate for the growth of intestinal bacteria which therefore must have been

endogenous origin and/or it could be that there was some remaining dietary residue within the large intestine from food consumed prior to the study period.

The recovery of energy from SCFA absorption following the consumption of a diet containing no NSP was comparable to that observed for a normal dietary intake. Does this mean that in the absence of dietary carbohydrate from diet, the colonic bacteria then utilise endogenous sources of carbohydrate which is not fermented under normal circumstances? Alternatively and probably more likely, does this reflect differences in bacterial species resident in the colon which have the capacity to utilise carbohydrate substrate preferentially from endogenous losses or that derived from dietary residue?

The quantitative effect of NSP on the availability of dietary energy is at present unclear. There is evidence that increasing the amount of NSP in the diet may lead to an increased excretion of lipid, nitrogen and energy; the result being a decrease in the apparent digestibility of lipid and protein and a reduction in available energy [Southgate & Durnin, 1970]. On this basis the report of the FAO/WHO/UNU [1985] suggest when diets contain only small amounts of NSP, no extra correction need be made to the energy available from food if the value is calculated from food composition tables based on the Atwater factors. It is stated that "when diets contain increased amounts of 'fibre', derived from fruit, vegetables, and wholemeal bread, values for available energy should be probably reduced by about 5%. This adjustment may not be enough for diets containing the high levels of 'fibre' typical of some populations in developing regions of the world". This concept however has been challenged by a number of investigators that bacterial fermentation of NSP and the subsequent absorption of SCFA may contribute towards human energy supply [Goranzon & Forsum, 1987; Miles *et al.* 1988; Wisker *et al.* 1988; Wisker & Feldheim, 1990]. The findings of the present study from estimations of the energy made available through SCFA absorption (see chapter 7) are therefore in support of these studies. How the increase in NSP contents of the diet affects the overall metabolisable energy of the diet and how the metabolisable energy content of high NSP diets should be calculated remains unresolved. In healthy individuals consuming the same diet, it is believed that the availability of energy could be different from person to person [FAO/WHO/UNU, 1985]. For example this would imply that a diet containing increased amounts of NSP may be utilised more efficiently by those who are habituated to such diets. In the light of the findings from the present studies, it appears that the recommendation made by

FAO/WHO/UNO [1985] should not be applied uncritically without an improved understanding of the potential contribution made by colonic fermentation to metabolisable energy and the nature and origin of the energy within the stool

8.4 CONCLUSIONS AND SUMMARY.

To summarise the principle findings of the studies presented in this thesis, the overall conclusions can be drawn.

Firstly the energy content of stools in a group of normal healthy children aged between 6-11 years of age contains 3.5% of the gross dietary energy. The magnitude of faecal energy may vary with alterations in the quantity and composition of the diet and where there may be maldigestion and/or malabsorption of lipid, protein and carbohydrate in gastro-intestinal disease.

Secondly, the composition of energy within the stool is variable with a major component of the energy represented by faecal bacteria. This would challenge the approach of the measurement of apparent digestibility which ignores the possibility that there may be an endogenous component of faecal energy. For example with alterations in dietary NSP, what appears to be reduced availability of energy could be reflected by colonic bacterial growth.

Thirdly, although the relative proportions of bacteria, malabsorbed dietary residue and other components may vary, the energy density of stool remains remarkably constant over a wide range of faecal energy losses. The close relationship appears to hold over a wide range of ages from childhood to adults, and is relatively unaltered by the dietary content of complex carbohydrate and in maldigestion and/or malabsorption associated with disease. As the energy content per gram remains relatively constant, this information would provide a more simple means of evaluating the extent of maldigestion and/or malabsorption and the efficacy of PERT than the determination of faecal lipid in diseases associated with gastro-intestinal dysfunction such as cystic fibrosis.

In conclusion, both the effectiveness of digestion and absorption and the metabolic activity of the resident microflora in the gastro-intestinal tract play a critical role in the maintenance of energy balance in health and disease. The findings of the studies presented in this thesis raise several important questions.

It is clear that not all of the energy within the stool is represented by maldigested and/or malabsorbed dietary residue as a major proportion of the energy within the stool is attributable to colonic bacteria. This observation has an important influence upon the expression of apparent digestibility which has been employed to describe the energy available from food, that is dietary intake of energy minus faecal energy losses ($DE = GE - FE$). We have no information of the proportion of energy attributable dietary residue and endogenous losses. Whilst the energy content of bacteria within faeces is derived from dietary and/or endogenous losses, the extent to which the energy content of faecal bacteria is derived from dietary residue or endogenous losses is not known [Figure 8.5]. This information would have an important influence on our understanding of the expression of apparent digestibility. For example if the energy content of bacteria is derived principally from endogenous losses rather than from dietary residue, digestible energy will not describe the energy available from food such that the energy available from food would be underestimated. Therefore the validity of such an approach requires further examination which may be related to both the extent of digestion and absorption of ingested food and fermentation of dietary residue.

In the light of these observations, it appears that there are a number of issues which remain and require further investigation. Whilst it is difficult to study interactions between diet and disease with body energy metabolism by conventional techniques, the development of more sophisticated techniques such as metabolic tracer technology may provide a further insight into these aspects of metabolism. Studies are required to further partition the stool into each of its components parts. How much of the energy, lipid and nitrogen content within the stool is derived from faecal bacteria, dietary residue and that originating from endogenous losses may be determined using non-radioactive stable isotope tracer techniques (^{13}C labelled carbohydrate, lipid and protein and ^{15}N labelled protein). Attention could be specifically focused upon following the label into the various stool compartments in healthy children and adults, with alterations in diet, in diseases associated with gastro-intestinal dysfunction. In the present studies, it has not been possible to examine the effect of antibiotic therapy on the activity of the colonic microflora. Attention could be focused upon the extent to which the proportion of stool energy attributable to bacteria may be altered. With this information, the validity of measurements to determine the availability of dietary energy during the therapeutic management of disease with antibiotics could be explored.

Figure 8.1 The passage of material into the human colon from the small intestine, bacterial fermentation and faecal excretion.

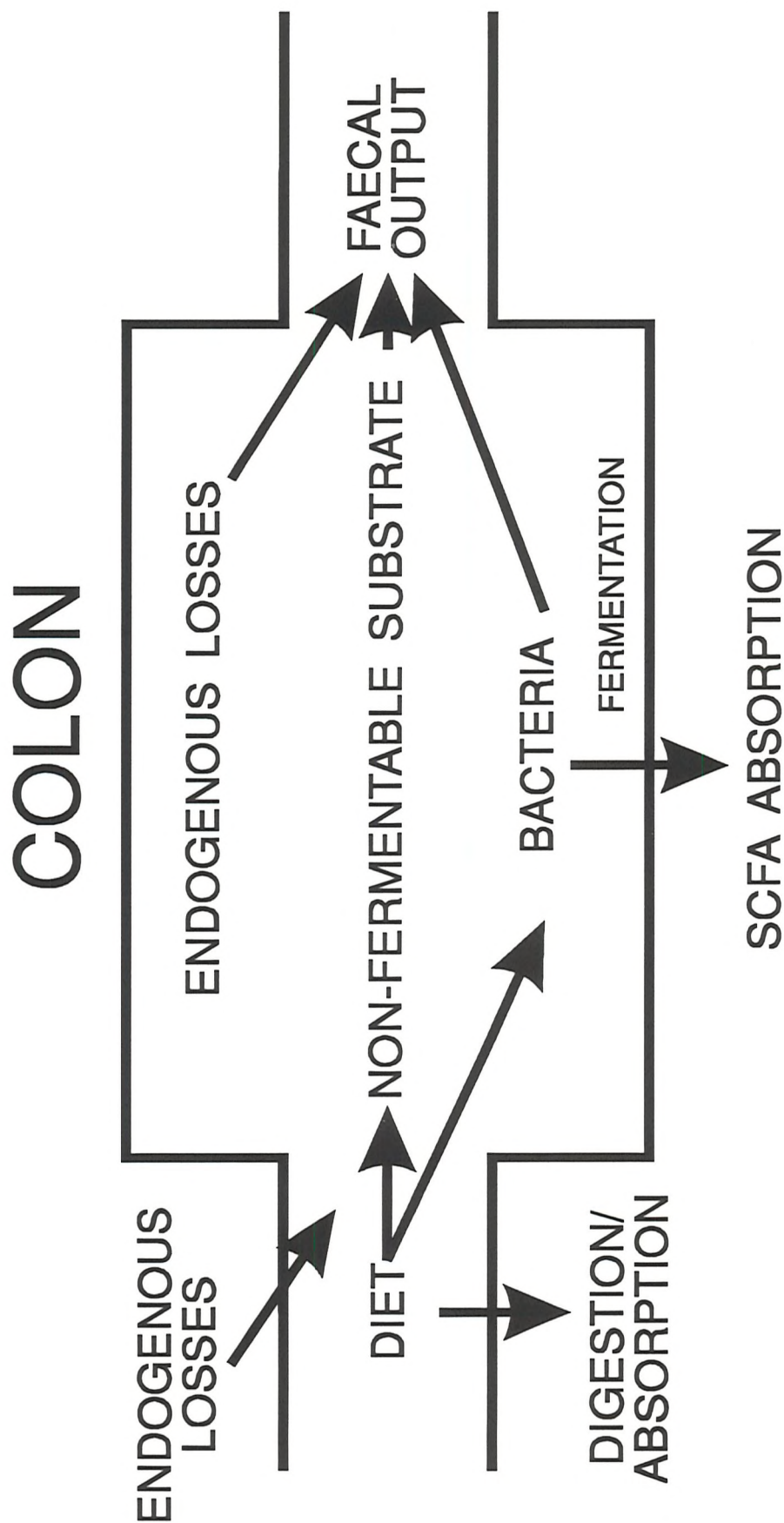


Figure 8.2 Daily energy content of faeces measured in twenty healthy children and six normal adult men and six normal adult women versus age.
The correlation coefficient is 0.63 ($p < 0.05$).

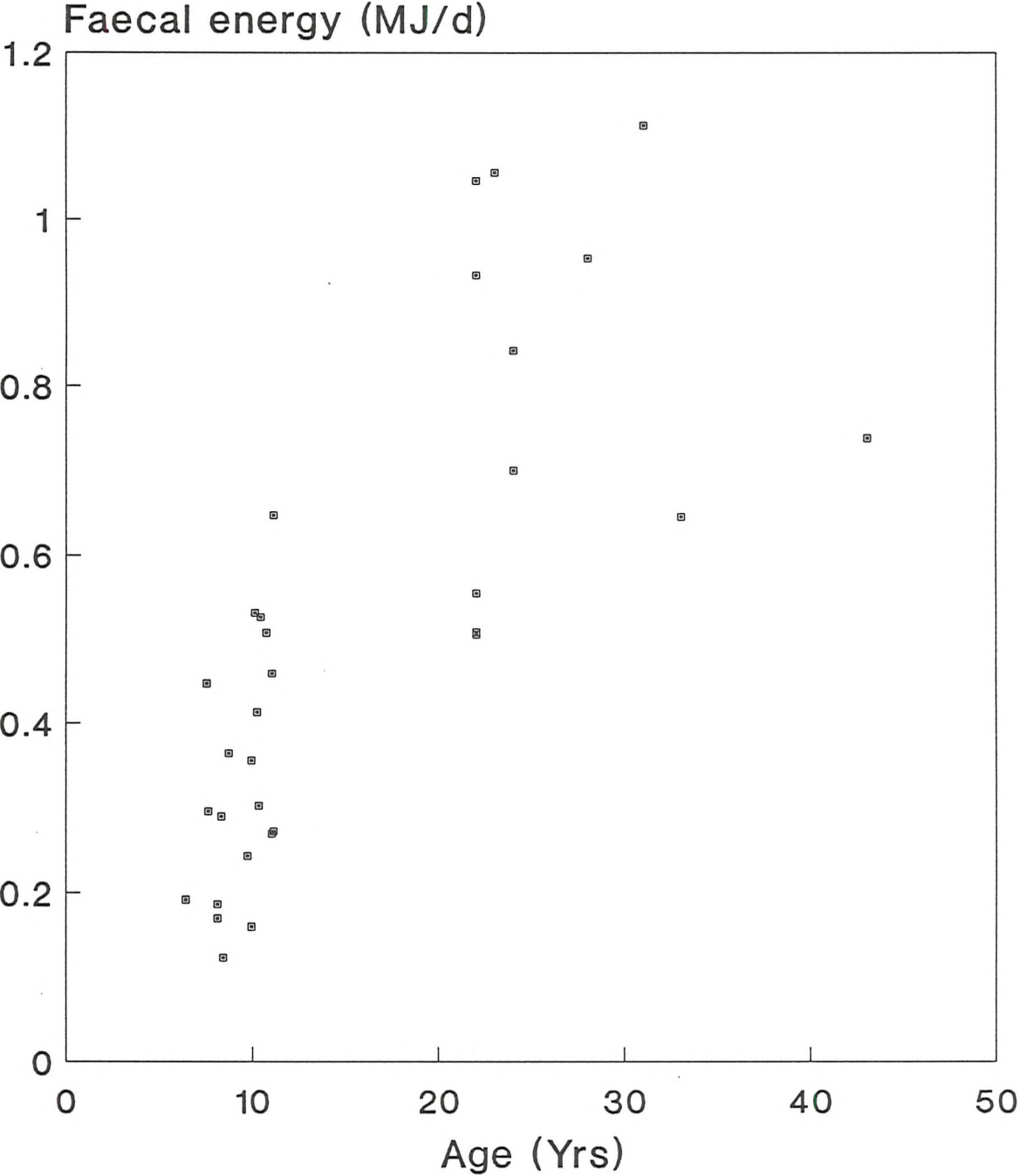


Figure 8.3 Daily faecal energy content versus faecal dry weight measured in twenty healthy children, twenty patients with cystic fibrosis and six normal adult men expressed as median values determined from each of the study periods and a adult healthy woman measured over the period of one menstrual cycle.
The correlation coefficient is 0.97 ($p < 0.01$).

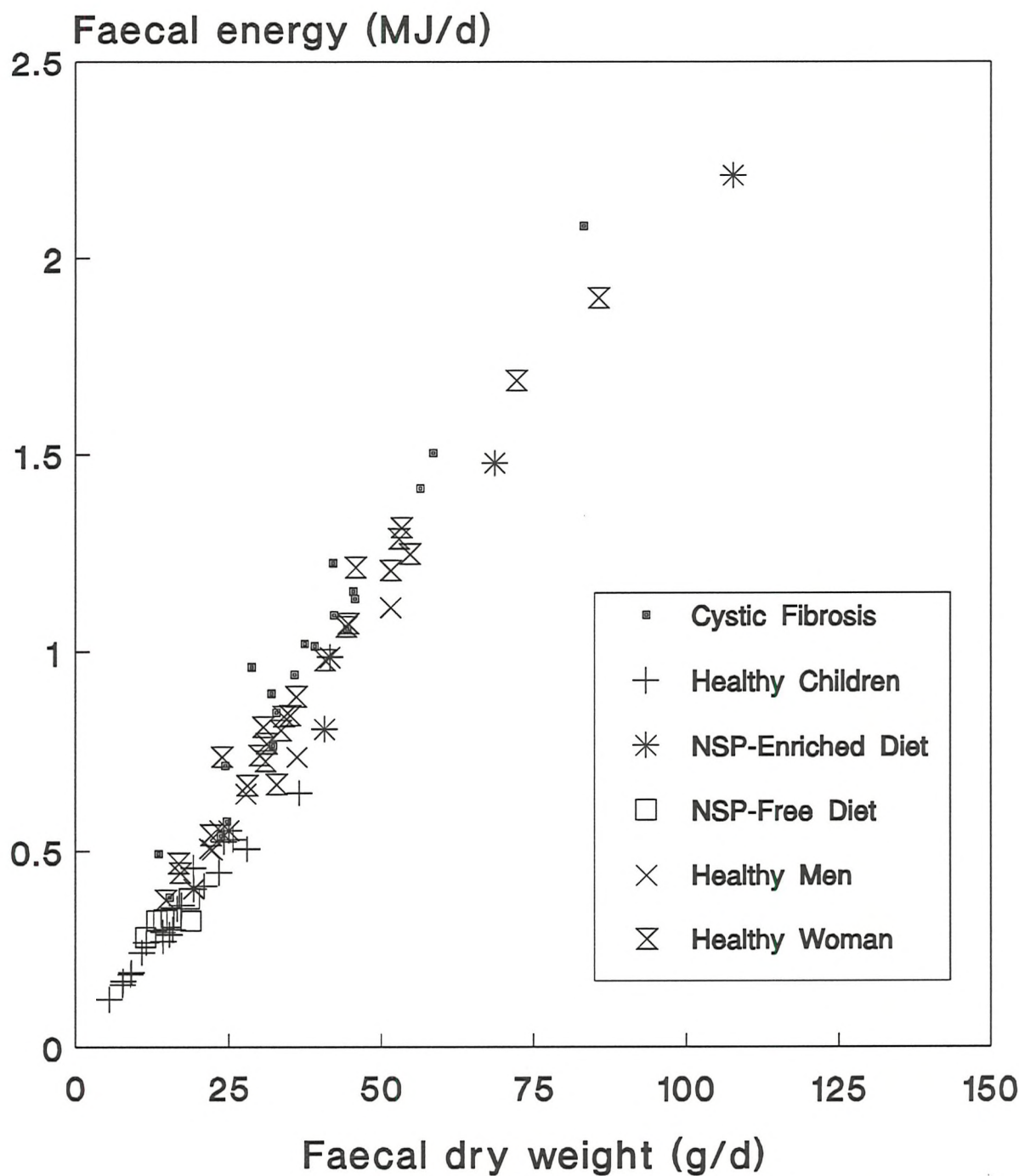


Figure 8.4 Daily faecal energy content versus faecal wet weight measured in twenty healthy children, twenty patients with cystic fibrosis and six normal adult men expressed as median values determined from each of the study periods and a healthy adult woman measured over the period of one menstrual cycle.

The correlation coefficient is 0.83 ($p < 0.01$).

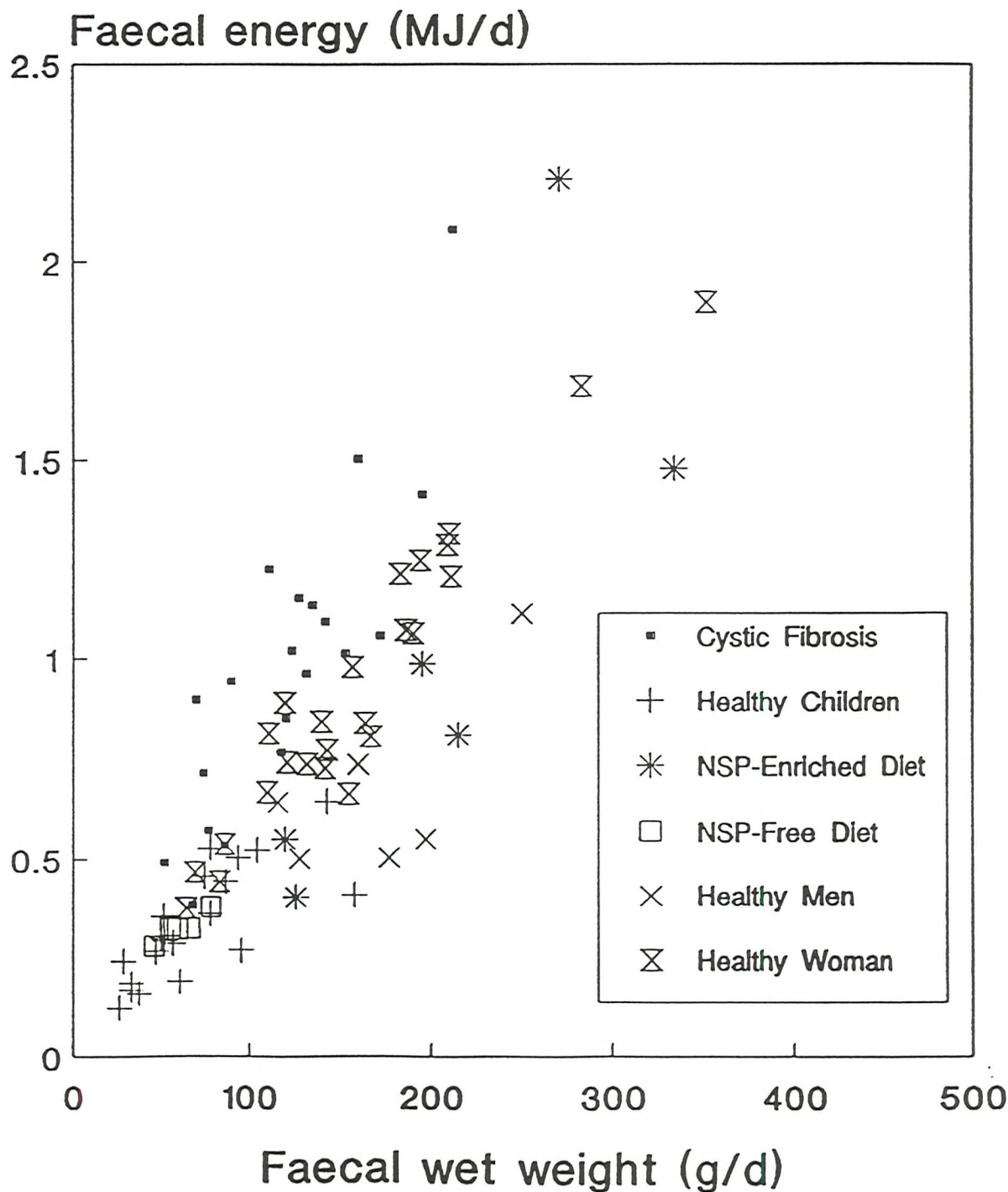
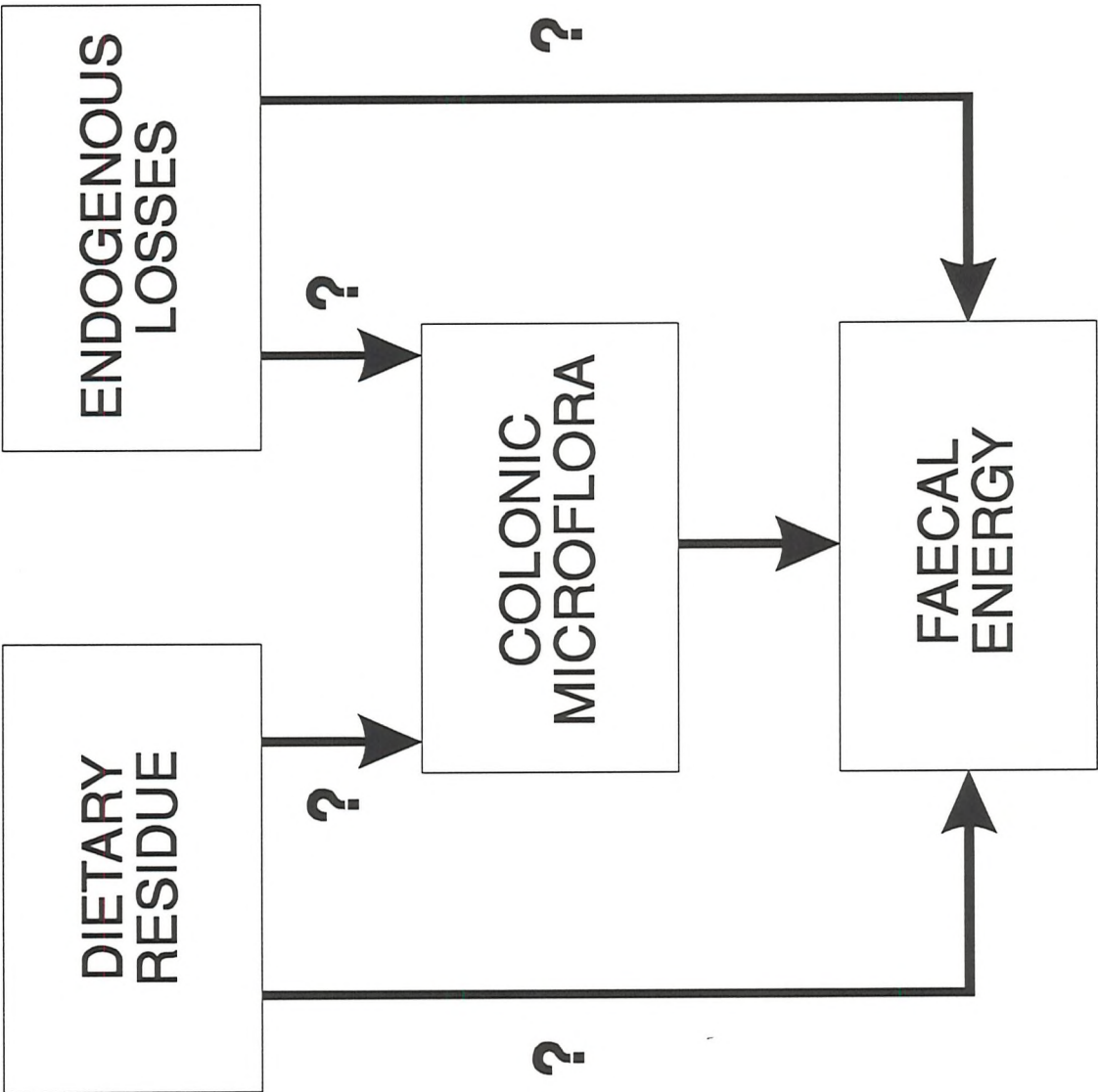


Figure 8.5 Faecal energy could be derived from dietary residue, endogenous losses and colonic bacteria. The proportion of the energy within the stool attributable to bacteria has been measured. The relative proportions of faecal energy attributable to dietary residue and endogenous losses and the extent to which the energy content of bacteria could be derived from dietary residue and/or endogenous losses are not known.



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