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Nitrogen Metabolism and Health of People with Ileostomy

by

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ABSTRACT

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NITROGEN METABOLISM AND HEALTH OF PEOPLE WITH ILEOSTOMY

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In clinical practice, the importance of colonic functions is often neglected. It is generally assumed that the loss of colon is of no consequence and people with ileostomy are free of health problems. This is compounded by the paucity of information on the metabolic and clinical effects of total colectomy, leading to a lack of awareness among clinicians of the potential ramifications on nutrition and health. Ileostomy patients who have had additional small bowel resection (SBR) may be particularly at risk from the effects of total colectomy due to reduced nutrient absorption and excessive stomal losses.

Apart from sodium and water absorption, the colon, through its commensal microflora, is capable of nitrogen salvage from urea hydrolysis and energy salvage from carbohydrate fermentation. Together with its regulatory role on the upper gastrointestinal tract, these colonic functions exert an influence on our physiology and metabolism, contributing to the preservation of our nutritional integrity and health. Urea-nitrogen salvage is an integral part of the body's adaptive response to changes in nitrogen availability and demands and aids in the maintenance of nitrogen equilibrium. For a fixed demand, urea-nitrogen salvage increases as intake reduces and for a fixed intake, urea-nitrogen salvage increases as demand increases. The aim of this thesis, therefore, was to examine the impact of the loss of the colon on nitrogen metabolism and health.

A comprehensive cross-sectional study was conducted on 60 ileostomy patients, 14 of whom had SBR. Compared to age- and sex-matched healthy controls, ileostomy patients had lower body mass index, lower lean mass and tended to be underweight. They were not only at risk of sodium depletion but this risk might also be associated with depleted body stores of calcium and magnesium. Ileostomy patients also had lower urinary nitrogen excretions due possibly to excess stomal nitrogen losses and/or the presence of urea-nitrogen salvage. When health status was assessed, ileostomy patients reported significant impairment in their physical, psychological and social well being. Despite these findings, however, haematological and biochemical blood indices were within normal limits of the general population suggesting that organ and biosynthetic functions are maintained at the expense of other metabolic processes.

Studies on nitrogen balances and urea kinetics were also conducted in ileostomy patients with and without SBR so that potential changes in nitrogen metabolism could be examined. In free-living conditions, ileostomy patients had higher stool nitrogen losses and hence, higher nitrogen intakes compared to healthy controls. They were capable of urea-nitrogen salvage in the absence of the colon and were also able to maintain nitrogen balance. However, under metabolic duress created by a significant reduction in nitrogen intake, ileostomy patients, particularly those who had SBR, suffered significant reduction in nitrogen balance as they were not able to up-regulate urea-nitrogen salvage.

Changes to nitrogen metabolism occur following the loss of colon. People who have had total colectomy and ileostomy appear to have compromised nitrogen status and impairment of overall health. They may be more vulnerable when under metabolic stress, seen commonly during illness or surgery, and under these circumstances, early intervention with nutritional support should be considered. The risk associated with total colectomy is increased in ileostomy patients who have had additional SBR.

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Abbreviations

ALT	alanine transaminase
BMI	body mass index
BIA	bioelectrical impedance analysis
BF	body fat
%BF	percentage body fat
°C	degree centigrade
^{14}C	labelled 14-carbon stable isotope
Ca	calcium
CNMU	Clinical Nutrition and Metabolic Unit
CoV	coefficient of variation
CPL	Chemical Pathology Laboratory
CRP	C-reactive protein
DEXA	Dual Energy X-ray Absorptiometry
dl	decilitre
EDTA	ethylenediamine tetraacetic acid
ESR	erythrocyte sedimentation rate
Eu	urea excretion
FBC	full blood count
Fe	iron
FFM	fat-free mass
FFQ	food frequency questionnaire
GLP-2	glucagon-like-peptide 2
g	gram
HbSS	homozygous sickle cell disease
HCL	hydrochloric acid
HD	habitual diet
H_2SO_4	sulphuric acid
K	potassium
kcal	kilocalorie
kg	kilogram
I	dietary nitrogen intake
INR	International Normalised Ratio

iu	international units
l	litre
LFT	liver function tests
LiOBr	lithium hypobromite
LP	low protein diet
m	metre
Mg	magnesium
mg	milligram
ml	millilitre
mm	millimetre
mmol	millimole
M	molar
N	nitrogen
^{15}N	labelled 15-nitrogen stable isotope
^{14}N	nitrogen
^{24}Na	labelled 24-sodium stable isotope
NaOCL	sodium hypochlorite
NaOH	sodium hydroxide
NH_3	ammonia
$(NH_4)_2SO4$	ammonium sulphate
NO	nitrous oxide
N_2O	nitric oxide
nm	nanometre
nmol	nanomole
NSBR	ileostomy subjects without small bowel resection
NSP	non-starch polysaccharide
OHLS	Oxford Healthy Life Survey
P	urea production
Pr	recycled urea going into urea production
PNP	phenol sodiumprusside solution
PYY	peptide YY
Qu	flux of urea
REE	resting energy expenditure
Ref	reference subjects

RMR	resting metabolic rate
S	recycled urea going into synthetic processes
SBR	ileostomy subjects with small bowel resection
SCFAs	short chain fatty acids
sd	standard deviation
SF-36	Short Form-36 health questionnaire
SUHT	Southampton University Hospital Trust
T	urea hydrolysis
U&E	urea and electrolytes
ug	microgram
ul	microlitre
umol	micromole
VCO_2	rate of carbon dioxide production
VO_2	rate of oxygen consumption
WTCRF	Wellcome Trust Clinical Research Facility

1

INTRODUCTION

Traditionally, the small intestine has occupied centre stage in relation to the handling of ingested food and the provision of nutrients required for metabolic interactions with the colon playing a role no more than the storage and disposal of human waste alongside a minor role in the absorption of sodium and water. However, in the last three decades, the colon has increasingly been under the spotlight and its roles in metabolism and maintenance of nutrient balance have been recognised.

The commensal microflora in the colon have been shown to be capable of fermenting undigested and unabsorbed carbohydrate that reaches the colonic lumen to produce short chain fatty acids (SCFAs) which are absorbed back into the bloodstream and utilised by the host as a source of energy. The colon is also known to have a regulatory role in the functions of the upper gastrointestinal tract which are mediated by hormones secreted from colonic cells. These hormones maintain the structural integrity and motility of the upper gastrointestinal tract, thereby influencing digestion and absorption. Furthermore, it has also been demonstrated that colonic microflora are capable of urea-nitrogen salvage. Urea, an end product of amino acid oxidation, passes from the bloodstream into the colon where it is hydrolysed by the microflora, resulting in the release of urea-nitrogen which is then returned to the host for metabolic engagement.

The focus of this thesis is on the contribution of the colon to overall nitrogen metabolism and health. Colonic urea-nitrogen salvage plays a significant role in maintaining nitrogen equilibrium and is an integral part of the host's adaptive response to changes in nitrogen availability and demands. When there is an imbalance between nitrogen supply and demand, urinary excretion of urea is reduced and the rate of colonic urea hydrolysis is up-regulated so that more nitrogen is salvaged and made available to the host for utilisation. In addition, there is also evidence to support that the salvaged nitrogen is returned to the host as essential and non-essential amino acids. Therefore, in essence, urea hydrolysis in the colon serves two functions which are crucial to the host's ability to

maintain nitrogen economy: firstly, it acts as the host's metabolic reserve by providing nitrogen in quantitative terms when nitrogen availability is insufficient to meet demands; and secondly, it improves the effectiveness of nitrogen supply in qualitative terms, thereby enhancing the 'goodness of fit' between nitrogen supply and demands.

Despite this evidence, clinicians tend to neglect the importance of the metabolic functions of the colon in the clinical management of their patients. People who have had total colectomy and formation of permanent ileostomy are generally assumed to be free of health problems following surgery and are usually discharged from specialist care unless they are known to have small bowel Crohn's disease. However, based on what we know about the colon's metabolic capabilities, its loss could have important ramifications, with detrimental effects on the ability of an individual to maintain nitrogen economy. This is likely to be particularly problematic in some ileostomy patients who also had additional small bowel resections where the ability to maintain adequate nitrogen supply is compromised further by malabsorption and excessive losses from the stoma. The development of potential problems in nutritional status and other aspects of health following total colectomy and additional small bowel resection are illustrated schematically in Figures 1.1 and 1.2 respectively.

In the current literature, information on the diet and health of ileostomy patients is not only limited but also inconsistent. Three studies had reported that these people have no clinically detectable nutritional deficiencies (Hill et al 1977, McNeil et al 1982, Baixas et al 1984) while one study reported the presence of subclinical malnutrition (Cooper et al 1986). Although there is some evidence to suggest that ileostomy patients may be capable of urea-nitrogen salvage in the absence of the colon, potential changes in urea kinetics and how these people maintain nitrogen balance in habitual free-living conditions have not been investigated. In addition, the ability of ileostomy patients to cope under metabolic stress, seen commonly during periods of illness, is also unknown.

The aim of this thesis was to investigate the impact of the loss of the colon on nitrogen metabolism and health. In a cross-sectional study, we conducted a dietary assessment and examined the health of ileostomy patients with respect to their body mass index (BMI), body composition, apparent nitrogen balance, organ synthetic functions and self-reported health status. We then conducted metabolic studies in ileostomy patients to assess: 1.

urea-nitrogen salvage capabilities and nitrogen balances under habitual free-living conditions and 2. their ability to up-regulate urea-nitrogen salvage capacity and maintain nitrogen balance under conditions of metabolic stress created by a significant reduction in nitrogen availability. The aim of these studies was to provide a clearer understanding of the changes that might have occurred in nitrogen metabolism following total colectomy and the impact of these changes on the health of ileostomy patients. The effects of additional small bowel loss were also examined.

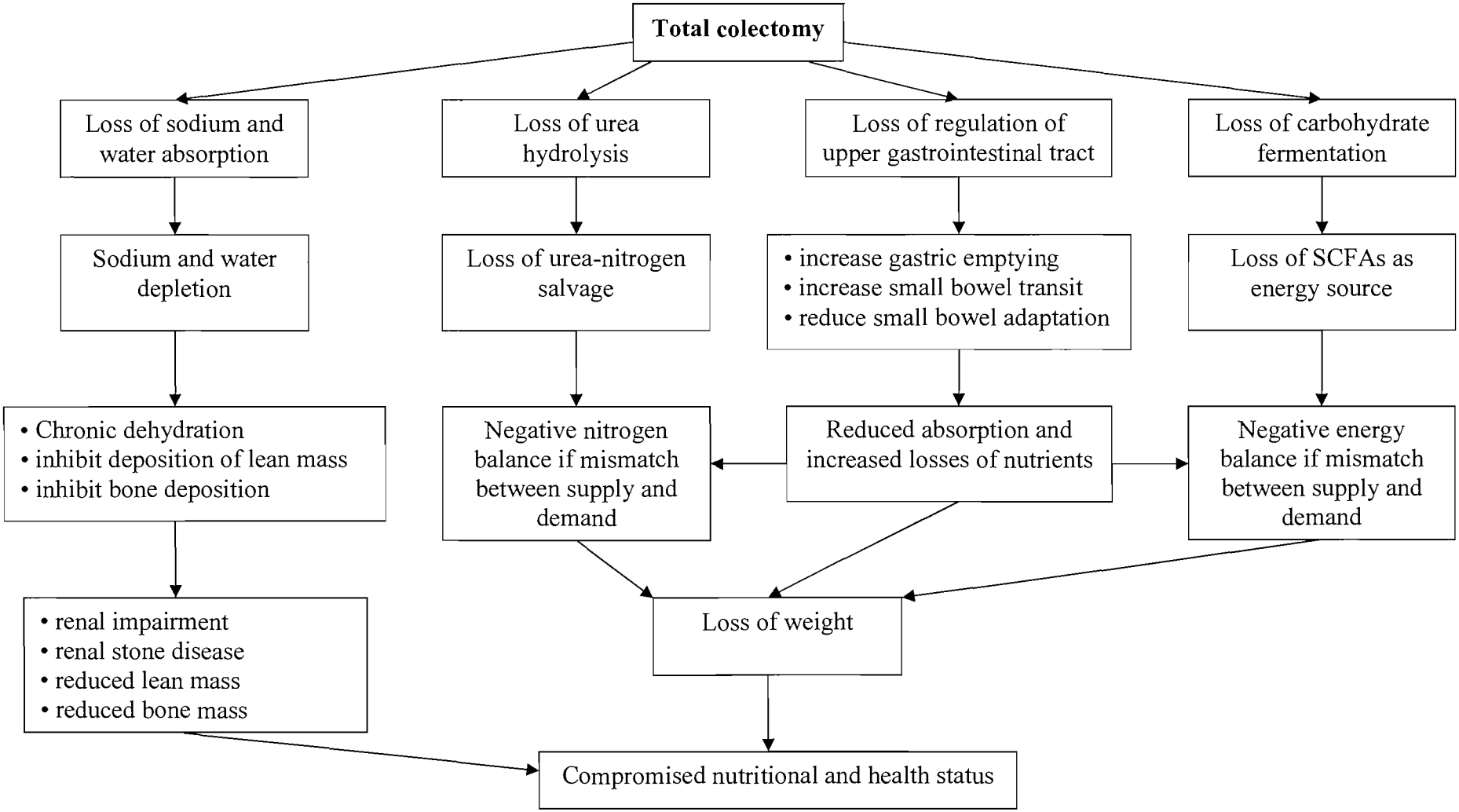


Figure 1.1 Potential metabolic and clinical consequences resulting from the loss of colonic functions

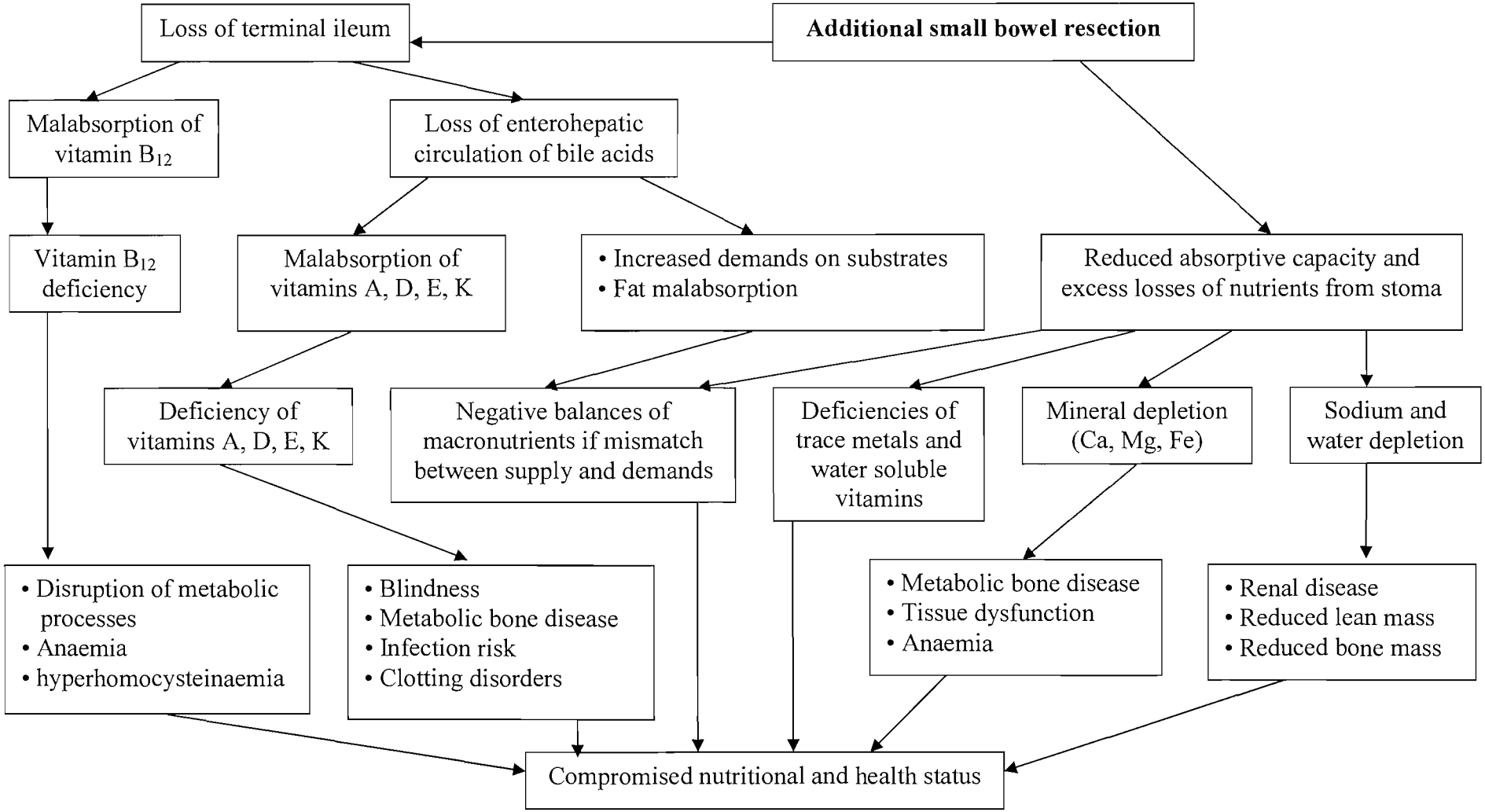


Figure 1.2 Potential metabolic and clinical consequences resulting from additional small bowel resection

2

LITERATURE REVIEW

2.1 The gastrointestinal tract

The gastrointestinal tract starts at the mouth, through which oral nutrients and fluids enter the body, and ends at the anus, from which unabsorbed materials are disposed. It is generally divided into the upper and lower gastrointestinal tract with each segment performing specific functions. The upper gastrointestinal tract consists of the mouth, oesophagus, stomach, jejunum and ileum and the lower gastrointestinal tract consists of the colon, rectum and anus. The ileum and colon is connected via the ileocaecal valve.

2.1.1 Functions of the upper gastrointestinal tract

The main functions of the upper gastrointestinal tract are ingestion, digestion and absorption of food and fluid. Apart from ingested food and fluid, secretions and cells shed from the gastrointestinal tract are also substrates for digestion and absorption.

Digestion is the process whereby ingested foodstuffs are broken down into small absorbable units by enzymes secreted in the upper gastrointestinal tract. Although digestion begins in the mouth by salivary enzymes, the major site of digestion occurs in the stomach and small intestine. Proteins are initially digested into polypeptides of different sizes by pepsin in the stomach and then into smaller di- and tripeptides by pancreatic enzymes such as trypsin, chymotrypsin, elastase, and carboxypeptidases in the small intestine (Ganong 2001a). Although some free amino acids are released, the final stages of digestion of these small peptides into amino acids are completed mainly at the brush border of the intestinal mucosa by peptidases (Ganong 2001a). Some di- and tripeptides are not completely digested but they are actively transported into the enterocytes where they are hydrolysed into free amino acids by intracellular peptidases (Ganong 2001a). Nucleic acids are digested by ribonuclease and deoxynuclease secreted by the pancreas (Ganong 2001a).

The digestion of carbohydrate is similar to that of proteins in that pancreatic amylase digests polysaccharides into di- and oligosaccharides which are in turn digested by disaccharidases and oligosaccharidases at the mucosal brush border into glucose, galactose and fructose (Ganong 2001a). Dietary fat is digested mainly in the upper small intestine by pancreatic lipase, phospholipase and cholesteryl ester hydrolase while lingual and gastric lipases play a smaller role (Ganong 2001a). The products of fat digestion are fatty acids, phospholipids and cholesterol (Ganong 2001a).

Absorption is the net passage of a substrate from the lumen of the intestine, across the epithelium, into the blood stream or lymph. Apart from amino acids, monosaccharides, fatty acids and cholesterol, electrolytes, water, minerals and vitamins are also absorbed. These substrates are absorbed across the intestinal mucosa through a variety of transport systems ranging from simple diffusion across concentration gradients to specific transporters which are energy dependent. The major site of absorption occurs in the small intestine with the colon playing a small but significant role (Ganong 2001a).

At the mucosal brush border, absorption of amino acids and monosaccharides are dependent on both the concentration of sodium in the intestinal lumen and the absorption of sodium across the mucosa (Bowler et al 1994, Ganong 2001a). As sodium concentration is higher in the lumen then it is in the enterocytes, sodium diffuses down the concentration gradient from the lumen into the cells. This creates a driving force for the passage of these macronutrients into the enterocytes and this process is called facilitated diffusion. Sodium is then actively pumped out of the cells into the blood stream thereby maintaining the concentration gradient across the luminal border of the cells. The movement of glucose from the cells into the blood stream is again facilitated by the Na-K ATPase pump (Bowler et al 1994, Ganong 2001a). Amino acids leave the enterocytes by a combination of simple diffusion and energy dependent carrier transport (Bowler et al 1994, Ganong 2001a).

Following the digestion of dietary fat, fatty acids and cholesterol are transported to the luminal surface of small intestinal mucosa by micelles (Bowler et al 1994, Ganong 2001a). The formation of micelles requires the presence of bile salts which are secreted from the gallbladder in response to cholecystokinin-pancreozymin, a hormone secreted from the duodenum stimulated by fatty acids (Bowler et al 1994, Ganong 2001a). At the

brush border, the lipids diffuse out of the micelles into the enterocytes, in which they re-esterified into triglycerides and cholesteryl esters. The triglycerides and cholesteryl esters are then coated with a layer of proteins and phospholipids to form chylomicrons. The chylomicrons leave the enterocytes by exocytosis and enter the lymphatic system via the lymphatic ducteals in the intestinal villi (Bowler et al 1994, Ganong 2001a). They then enter the systemic circulation via the thoracic duct. From the blood stream, the chylomicrons enter the liver which processes the fatty acids and cholesterol for metabolic utilisation (Bowler et al 1994, Ganong 2001a). As medium chain fatty acids (less than 12 carbons) are sufficiently water-soluble, they move directly from the enterocytes into the portal blood, where they are transported as free fatty acids to the liver (Bowler et al 1994, Ganong 2001a).

The absorption of water across the intestinal mucosa occurs in response to the osmotic gradients generated by the movements of sodium, amino acids and glucose (Bowler et al 1994, Ganong 2001a). All the water-soluble vitamins, apart from vitamin B_{12}, are absorbed in the upper small intestine (Bowler et al 1994, Ganong 2001a). Vitamin B_{12} binds to intrinsic factor, a protein secreted by the stomach, and is absorbed in the terminal ileum (Bowler et al 1994, Ganong 2001a). Fat-soluble vitamins are absorbed along with the dietary lipids. Most of the minerals are absorbed in the small intestine via specific transport carriers (Bowler et al 1994, Ganong 2001a).

Digestion and absorption are affected by several factors. When the rate of gastric emptying and small intestinal transit are increased, foodstuffs have less time to mix with digestive enzymes, thus affecting the efficiency of digestion. This will have a knock-on effect as partially digested food will not be absorbed. Similarly, an increase in gastric emptying or intestinal transit also compromises absorption due to a reduction in the time of contact between the nutrients and the mucosa. When the length of the small intestine is shortened due to small bowel disease or resection, the available surface area for digestion and absorption is reduced. Under these circumstances, the availability of nutrients for utilisation is effectively diminished and losses are increased. The rate of gastric emptying and structural integrity of the small intestine are regulated by hormones secreted by the colonocytes and these are described in the next section.

2.1.2 The colon and its microflora

Apart from its role in the storage and disposal of faeces, the colon performs other functions which are of substantial importance to the body. One of these functions is the absorption of sodium and water which contributes to the maintenance of sodium and fluid balance of the body. Approximately 1-2 litres of nearly isotonic fluid pass the ileocaecal valve and enter the colon each day. However, only 100-200 millilitres (ml) of water are excreted in the faeces, together with 5-10 millimoles (mmol) of sodium (Bowler et al 1994). The absorption of sodium from the colon, particularly in the distal colon and rectum, occurs through specific channels in the colonic mucosa and these channels are controlled by aldosterone (Bowler et al 1994). Water absorption is then coupled with the movement of sodium across the mucosa. The presence of other metabolically active solutes like SCFAs, which are produced from bacterial actions on luminal residues, also promote sodium and water absorption in the colon (Mortensen and Clausen 1996).

The colon has a regulatory role on gastric and small bowel functions which are mediated by hormones secreted from the colonocytes. Peptide YY (PYY) is a polypeptide consisting of 36 amino acids and its secretion is stimulated by nutrients entering the colon (Adrian et al 1985, Andrews and Irving 1992). PYY inhibits gastric emptying and controls the rate at which chyme enters the small intestine, thus allowing more time for food to be digested in the stomach (Allen et al 1984, Ganong 2001b). This phenomenon is known as the 'colonic brake' of the stomach (Nightingale et al 1996). Glucagon-like-peptide 2 (GLP-2) is a polypeptide consisting of 32 amino acids and it is secreted by the L-cells which are found mainly in the distal ileum and the colon (Ghiglione et al 1987, Roberge and Brubaker 1991, Xiao et al 1999). Its secretion is also stimulated by nutrients entering the distal ileum and colon. GLP-2 is a trophic hormone and is thought to play an important role in the renewal of enterocytes, thereby maintaining the structural integrity and the optimal function of the small intestine (McGregor et al 1987, Drucker et al 1996, Goodlad et al 1997, Tsai et al 1997).

The metabolic activities of the colon have been increasingly recognised. They are the functions of colonic microflora which exert a pronounce impact on our physiology and metabolism. The colon is more densely populated with microorganisms than any other

organ in the body. The microflora consists of an abundant but complex assembly of bacteria and they form a symbiotic relationship with their host. There are approximately 10^9 to 10^{11} organisms per gram (g) of colonic luminal content, accounting for 75% of faecal wet weight in man (Gorbach 1971, Wrong et al 1981). It has been estimated that there are over 400 different species of bacteria, 95% of which are obligatory anaerobes consisting mainly of bifidobacilli and bacteroides with smaller numbers of fusobacteria, clostridia, lactobacilli and Gram-positive cocci (Wrong et al 1981). Enterobacteria, diphtheroids, some lactobacilli and streptococci are the main aerobic bacteria in the colon (Wrong et al 1981).

The species and number of organisms present in the colon are regulated to prevent overgrowth or colonisation by other bacteria which are harmful to both the microflora and host. This is achieved by the interactions between the host and bacteria and amongst the microorganisms themselves. Bacterial multiplication within the colon is very much below the possible maximum rate, varying between 0.5 to 6 divisions a day in different mammals whereas in vitro, intestinal bacteria can divide every 12-50 minutes (Wrong et al 1981). Host factors like local immune mechanisms, antibody production and shedding of lymphocytes from the mucosa are thought to be involved in regulating bacterial multiplication (Wrong et al 1981). The production of organic acids and antibiotic substances (bacteriocins and colicines) by the bacteria themselves has been shown to inhibit the growth of micoorganisms which are not part of the commensal microflora (Wrong et al 1981). The microflora is also thought to be influenced by the oxidation-reduction potential within the colon. This is normally about –200 millivolts which is essential for the survival of anaerobic bacteria (Wrong et al 1981).

Collectively, the colonic microflora are intimately involved in driving the development and maintenance of the digestive functions of the gastrointestinal tract. The colonic microflora have an enormous amount of biochemical versatility, capable of performing reactions that their hosts are incapable of. No mammalian tissue has the capacity to break down cellulose or salvage nitrogen from urea except for the microflora in the colon (Walser and Bodenlos 1959, Cummings et al 1987). Their host in turn makes good use of these bacterial activities for the purpose of its own metabolism. Consequently, these metabolic activities of the microflora may be regarded as constituting an extra 'organ' of the mammalian body.

Besides urea-nitrogen salvage which is described in section 2.3, numerous studies have shown that the colonic bacteria are capable of fermenting food residues that reach the colon. This pool of fermentable substrates consists of non-starch polysaccharides (NSP) from dietary fibre and dietary starch and sugars that are incompletely absorbed in the small intestine (Levitt 1983, Scheppach et al 1988, Mortensen and Clausen 1996). As a result of bacterial fermentation, gases, water and SCFAs (mainly acetate, proprionate and butyrate) are formed. These SCFAs serve several functions. Apart from being the main source of nourishment for the colonocytes (Roediger 1980), SCFAs are also absorbed via the colonic mucosa into the blood stream (Ruppin et al 1980) where they provide the body with a useful source of energy. Butyrate and proprionate are utilised mainly by the liver and acetate by peripheral tissue (Mortensen and Clausen 1996). In healthy individuals with a fully functional gastrointestinal tract and taking a diet that is adequate in energy, the contribution from SCFAs to the total energy requirement was estimated to be about 5-10%, which is comparatively minor relative to dietary intake (Cummings 1981). However, in people with malabsorption from small bowel disease or extensive small bowel resection where intestinal digestive and absorptive capabilities are reduced, SCFAs can make a significant contribution to energy balance (Cummings 1981). In patients who have a short length of small bowel anastomosed to a normal colon, increasing carbohydrate intake from 20% to 60% can lead to an increase in energy salvage by approximately 465 kcal (Nordgaard et al 1994). Thus, in circumstances where there is a mismatch between energy supply and demand, the metabolic activities of colonic microflora become substantially important.

2.1.3 Clinical importance of colonic functions

Although the metabolic capabilities of colonic bacteria is well known, many people continue to hold the view that the colon is merely a reservoir for the storage and controlled disposal of human waste alongside a minor role in the absorption of sodium and water. The reason for this seemingly entrenched view is unclear but one possible explanation could be that as most people have unrestricted excess to food and thus, dietary supply of nutrients is often more than sufficient, the metabolic value of the colon is, therefore, hardly ever obvious. However, in a small group of people who have had extensive small bowel resection, the significance of colonic functions becomes evident.

Crohn's disease, thrombotic infarction and volvulus of the small bowel are the most common indications for extensive resection of the small intestine. A patient is considered to have short bowel syndrome when the residual length of the small intestine does not adequately absorb fluid or nutrients from an oral diet to maintain nutrient balance and health (Nightingale 1995). Besides malabsorption, excessive losses of fluid and nutrients from body stores can also occur. Nutrient absorption correlates with jejunal length (Lennard-Jones 1994). In clinical practice, it has generally been recognised that patients with a residual healthy small bowel length of less than 200 cm ending in a stoma can suffer from the effects of malabsorption (Lennard-Jones 1994, Nightingale 1995).

When nutrient supply is compromised, the presence of a functional colon becomes significant. In the largest review of 84 patients with short bowel syndrome who have residual small bowel length of less than 200 cm, Nightingale et al (1992) reported that preservation of the colon was beneficial and equated to about 50 cm of small intestinal function when the need for parenteral supplements was examined. Patients with a jejunostomy were more likely to require long-term parenteral supplements if the jejunal length was less than 100 cm while no patients with a jejunal length of 50 cm or more who had a colon required parenteral supplements (Nightingale et al 1992). For jejunostomy patients with small bowel lengths between 100 and 200 cm, only 1 did not require any supplements while 21 required oral supplements and 3 required parenteral supplements (Nightingale et al 1992). Out of 25 patients with similar small bowel length anastomosed to a colon, only 1 required oral supplements (Nightingale et al 1992). Gouttebel et al (1986) also reported similar findings in 39 patients where the minimum length required for the maintenance of nutritional integrity by oral supplementation was 110-150 cm without a colon, but 50-70 cm with a colon. Thus, preservation of the colon becomes increasingly vital with decreasing small intestinal length and the colon determines whether nutrient and fluid supplements, whether orally or parenterally, are required (Nightingale et al 1992, Lennard-Jones 1994, Nightingale 1995).

The colon aids in sodium and fluid balance by absorbing sodium and water which would otherwise have been lost via the stoma in the absence of the colon (Wright 1975, Ladefoged and Olgaard 1979). The production of SCFAs from colonic bacteria fermentation of undigested and unabsorbed carbohydrate would also have otherwise been lost via the stoma (Messing et al 1991, Nordgaard et al 1994). Furthermore, the presence

of SCFAs enhances sodium and water absorption. Therefore, patients with shortened small bowel length and intact colon are advised to consume diets high in carbohydrate (Nightingale 1995, Nordgaard et al 1994). The role of the colon in nitrogen metabolism in patients who had small bowel resection has not been studied. However, the evidence from animals with extensive small bowel resection suggested that the presence of the colon and its bacteria contributed to better weight gain, higher carcass protein content and nitrogen balance (Aghdassi et al 1994). Although the evidence is lacking in human beings, it is not unreasonable to expect that colonic bacteria, which have been shown to salvage energy in people who have small bowel resection with intact colon, can also salvage nitrogen from urea hydrolysis to aid in the maintenance of nitrogen balance in these people. The colon's role in enhancing mineral retention was also highlighted when calcium and magnesium absorption in patients who had small bowel resection with an intact colon were found to be significantly higher than ileostomy patients (Gouttebel et al 1986, Sandstrom et al 1986, Hylander et al 1990). The study of Sandstrom et al (1986) also showed better zinc retention in patients who had shortened small bowel length with intact colon compared to those who had stoma.

Gastric and small intestinal functions following small intestinal resection were also shown to be enhanced by the presence of the colon. In 18 patients who had undergone ileal resection of 40-200 cm, basal and post-prandial PYY were significantly higher than both the control groups and ileostomy patients (Adrian et al 1987). Nightingale et al (1996) also reported similar findings and believed that the presence of the colon had enabled PYY to be secreted following a meal causing inhibition of gastric emptying and contributed to the 'colonic brake', which in turn has a net effect of slowing the intestinal transit of food, allowing more time for digestion and absorption to take place. Similarly for GLP-2, two studies have shown significant elevation of fasting and post-prandial GLP-2 levels in 7 patients with short bowel and intact colon compared to healthy controls, while post-prandial GLP-2 responses were significantly lower in 7 short bowel jejunostomy patients with less than 150 cm small bowel compared to controls (Jeppesen et al 1999, Jeppesen et al 2000). Commercially manufactured analogue of GLP-2, ALX-0600, was used in a therapeutic trial for 35 days, given as a subcutaneous injection twice daily in 8 small bowel resected patients without a colon (Jeppesen et al 2001). The authors demonstrated a significant increase in intestinal absorption of energy and nitrogen and significant reduction in the wet weight of stoma effluents. There was also an

associated significant increment in body weight, lean body mass, urinary creatinine excretion and a reduction in fat mass. Intestinal biopsies of 5 of these 8 patients showed a modest increase in both the crypt depth and villus height by 10% (Jeppesen et al 2001).

The contribution of the colon in enhancing overall efficiency of the gastrointestinal tract in patients who have had extensive small bowel resection is undeniable. In these patients, the colon assumes not only as the role of an organ capable of energy and nitrogen salvage, it also serves to support the functions of the stomach and small bowel remnant and improves sodium, water and mineral balances.

2.2 Nitrogen metabolism

Nitrogen is an important constituent of the body. It is found in amino acids which are the structural components of protein and also in other compounds such as nucleotides which are equally important in sustaining bodily functions. Proteins and other nitrogenous compounds are present in every cell and tissue, serving a variety of functions that are at the very core of our existence. The metabolism of nitrogen describes the dynamic changes within the body and these changes involve the intimate but complex interactions between demands, supply and elimination of nitrogen so that a state of equilibrium can be attained.

2.2.1 Demands for nitrogen

When we discuss the body's requirement for nitrogen, it is important to state that the body's metabolism handles nitrogen containing compounds rather than elemental nitrogen per se. Most of these compounds are proteins which quantitatively dominate the nitrogen pool within the body, with nucleotides and other amino acids making up the rest. Hence, the body's demand for nitrogen is dominated by the demand for protein which in turn, is governed by numerous factors.

Our genetic composition determines our body habitus and physiology, and therefore influences the pattern and amount of protein required, while our lifestyle determines the amount of nitrogen that is necessary to sustain our activities (Jackson 1998b). In the 19th

century, Playfair and Voit had recognised the difference in protein requirements between an average working man and those involved in heavy physical work (Munro 1964). The stage of an individual's physiological development also plays an important factor in determining nitrogen requirement (Jackson 1998b). For an adult, the physiological aim is to maintain nitrogen equilibrium such that body weight and composition remain relatively constant whilst for infants and children, the purpose is to accrue nitrogen for growth and organ maturation (Jackson 1998b). Hence, the daily nitrogen requirement per kilogram (kg) body weight is higher for infants and children than for adults. The daily rate of protein synthesis is estimated to be approximately 12 g/kg in newborn infants, reducing to 6 g/kg by the age of 1 year (Jackson 1998b). For normal adults, the rate of protein synthesis is approximately 4 g/kg per day while in pregnant women, the requirement for nitrogen is increased to fulfil the demands for foetal growth and placenta function as well as to prepare for lactation (Jackson 1998b). In pathological states such as inflammation, surgery, trauma or disease, there is a major shift in the pattern of protein synthesis and degradation, designed to limit damage, remove foreign material and repair tissue (Jackson 1998b). For example, during an infection, emphasis is diverted towards the formation of cells and proteins necessary to mount an inflammatory response while the formation of other proteins such as albumin and nutrient carrier proteins (e.g. zinc and vitamin A binding proteins) is down-regulated (Jackson 1998b). Therefore, pathological processes change the utilisation of nitrogen and coupled with an increase in nitrogen losses, the overall demand for nitrogen is increased.

The body's protein pool is in a dynamic state, constantly turning over by processes of synthesis and degradation (Jackson 1998b). This dynamic state of protein was recognised as far back as 1829 by Magendi who described the continual renewal of body constituents, differing in rate in different tissues (Munro 1964). This concept of the instability of tissue components was further supported by the work of Schoenheimer in 1942 in which amino acids labelled with stable isotopes ^{15}N were fed to rats and the enrichment in proteins in different tissues was studied (Munro 1964). The effect of continual protein turnover results in the conceptual existence of a pool of amino acids from which amino acids for protein synthesis are derived and to which amino acids coming from protein degradation contribute. Other metabolic pathways also contribute towards the constant exchange of amino acids within the pool as illustrated in Figure 2.1 (Jackson 1998b).

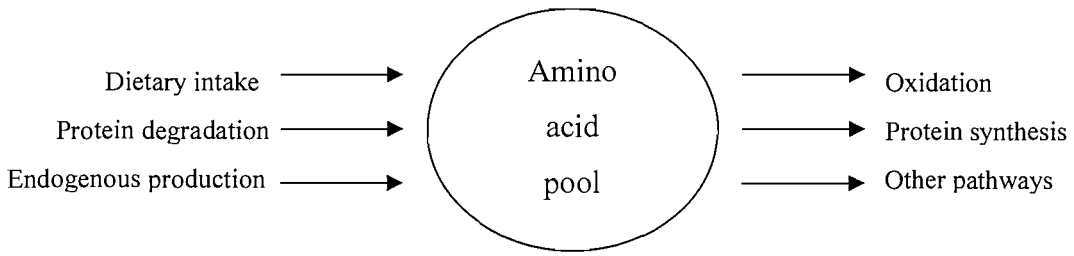


Figure 2.1 A model of the dynamic turnover of amino acids in the body

The pattern and amount of amino acids required to support protein synthesis is determined by the pattern and amount of protein required under a prescribed circumstance (Jackson 1998b). Thus, the nature of the demand for nitrogen can vary from day to day and from situation to situation. The key requirement is for the body to ensure that the supply of amino acids is not only adequate but that amino acids are present in the right mix to 'fit' what is needed to satisfy the demand. This brings us to another concept in nutrition, the '*goodness of fit*' which will be discussed in the next section.

2.2.2 Supply of nitrogen

An appreciation of the complexity of nitrogen demands emphasizes the need to ensure that nitrogen supply fits the demand both quantitatively and qualitatively. As the formation of a particular protein requires that all the appropriate amino acids are available at the point of synthesis, the supply must not only be adequate but must also meet a defined pattern of amino acids (Jackson 1998b). Therefore, the closer the pattern of available amino acids is to the pattern of protein demand, the less the total amount of amino acids needed for overall nitrogen balance (Jackson 1995). Conversely, if a high degree of nitrogen exchange is required, more total nitrogen will be needed to achieve balance (Jackson 1995). This is the concept of '*goodness of fit*' which allows us to consider the body as a demand-led system where the nature of nutrient supply is dictated by metabolic demands (Jackson 2000).

Plants are able to make their own amino acids by fixing nitrogen from inorganic compounds, but animals are unable to synthesize the amino group (Jackson 1998b). Human beings therefore obtain nitrogen by eating plants and animals, mainly as proteins but also as other nitrogen containing compounds such as nucleic acids and vitamins. In general, the pattern of plant proteins differs from that of human proteins, while the pattern of proteins in animal cells is similar. Hence, with adequate intakes of both plant and animal proteins, a mix of dietary supply of both essential and non-essential amino acids is likely to meet our demands for protein synthesis (Jackson 1998b). In the United Kingdom, the average daily protein intake of the general population is 67 g per day (National Food Survey 2000) and studies have also shown that for an average normal adult, the minimum physiological requirement for protein in order that nitrogen balance is maintained is between 30-35 g per day (Danielsen and Jackson 1992, Langran et al 1992). Below this level of protein intake, nitrogen balance cannot be sustained (Danielsen and Jackson 1992).

There are two components to protein requirement; that for total nitrogen and that for essential amino acids. Although a minimum intake of nitrogen is required to maintain nitrogen balance, dietary requirement for essential amino acids also appears to be a determining factor (Jackson 1998b). In 1946, Block and Mitchell described that the quality of a protein was determined by its essential amino acid content (Munro 1964). The relationship between the requirements of total nitrogen and that of essential amino acids is rather complex in that even on minimum required intakes of essential amino acids, growth can still be limited if total nitrogen intake is inadequate (Rose et al 1949). This is because the total nitrogen content of the diet is insufficient to support endogenous synthesis of non-essential amino acids (Rose et al 1949). In summarising the data from a series of experiments conducted by Rose (1957), Jackson (1995) proposed the following:

1. In order to achieve nitrogen equilibrium at the lowest level of total nitrogen intake of 3-4 g per day, essential amino acids must be supplied at 2-3 times the minimum level as determined by Rose, with a relatively low amount of non-essential amino acids.
2. Since a number of non-essential amino acids can substitute for essential amino acids, nitrogen balance can still be achieved with a minimum required intake of

essential amino acids but total nitrogen intake has to increase to about 6-8 g per day.

3. Non-essential nitrogen such as urea and ammonium can substitute for non-essential amino acids but, although nitrogen balance can be achieved readily with Rose's minimal requirements for essential amino acids, the total nitrogen intake might need to be increased to 12 g per day.

There is, however, another consideration regarding the requirements of non-essential amino acids. It has been recognised that the endogenous synthesis of some non-essential amino acids such as arginine, glycine, serine, cystine etc may not be sufficient to meet metabolic demands, either because the pathways for their formation are not sufficiently matured and/or there are unusually high physiological or pathological demands. These non-essential amino acids can therefore become 'essential' or 'indispensible' and have now been termed as conditionally essential amino acids (Jackson 1998b). For example, Rose (1957) argued whether arginine should be classified as essential or non-essential when he recognised that young rats were not able to synthesize arginine in sufficient amounts to maintain optimal rate of growth but fully matured rats can synthesize sufficient quantities for maintenance needs. Clearly, for young rats, arginine is an essential amino acid while in the adult rats it is non-essential. Glycine, which is required in collagen formation and the synthesis of glutathione, can become conditionally essential in a patient with trauma and oxidative stress (Jackson 1998b). The provision of nitrogen therefore requires consideration of prevailing circumstances which dictate the nature of demands.

The interaction between nitrogen and energy metabolism is also well established. In his extensive work investigating nitrogen equilibrium, Rose noted that despite provision of sufficient nitrogen in the diets of his study subjects, insufficient energy intakes invariably led to failure to attain nitrogen balance (Rose 1957). As the process of protein turnover requires energy, the energy balance of the body is an important factor in determining nitrogen balance and influences the utilization of dietary protein (FAO/WHO/UNU 1985). Studies have demonstrated that, at any given level of dietary protein, the addition of energy improves nitrogen balance until the response reaches a plateau, which represents the limitations imposed by dietary protein availability (Anderson et al 1969, Calloway 1975). This effect can then be extended further by raising protein intake

(Anderson et al 1969, Calloway 1975). The work of Motil et al (1981) also suggests that increasing energy intake enhances protein synthesis and reduces amino acid oxidation. Apart from dietary nitrogen, the body itself also contributes significantly to the overall nitrogen pool. Degradation of body protein releases amino acids which then enter the amino acid pool enhancing the mix of amino acids available for protein synthesis (Figure 2.1). Furthermore, in the last three decades, a wealth of evidence has accumulated describing the salvage of urea-nitrogen by colonic bacteria which has also been shown to contribute significantly towards overall nitrogen economy (Walser and Bodenlos 1959, Langran et al 1992, Jackson 1995, Meakins and Jackson 1996a). The importance of this colonic metabolic activity, which is a topic of interest in this thesis, will be discussed further in section 2.3.

2.2.3 Elimination of nitrogen

Although nitrogen is an essential constituent of the body, its balance has to be regulated. Ammonia, a product of nitrogen metabolism, is toxic and high levels can result in cerebral dysfunction and disturbances of acid-base balance (Gale and Anderson 1994). Tolerance to an excess of any single amino acid varies but sustained high levels invariably exert toxic effects. This phenomenon is best demonstrated by inborn errors of metabolism where an enzyme defect leads to a detrimental accumulation of an amino acid or its breakdown products (Gale and Anderson 1994, Mehler 1986). Hence, the body goes to some lengths to maintain amino acids at the required levels (Jackson 1998b).

Nitrogen is eliminated from the body via several routes. Urinary nitrogen accounts for 85-90% of the total nitrogen loss while 5-10% is lost in stool from unabsorbed food, cells from the gastrointestinal tract, gastrointestinal secretions and colonic bacteria nitrogen (Jackson 1998b). The loss of nitrogen through other routes such as skin, sweat and hair are less easy to measure but is estimated to be in the order of about 4% (Calloway et al 1971) or approximately 8 milligram (mg)N/kg/day for adults (FAO/WHO/UNU 1985). When an amino acid is oxidised, the amino group is removed from the carbon skeleton and ammonia is formed. Most of the ammonia is then removed from the blood stream by the formation of urea in the liver and a small amount is excreted in the urine as

ammonium. Ammonia is also formed from the degradation of pyrimidine nucleotides while degradation of purine nucleotides gives rise uric acid which is excreted in the urine. Creatinine, which is formed from the cyclization of creatine phosphate in muscle, is also excreted in the urine. Thus, the urine consists of 85% urea, 5% ammonium, 5% creatinine and 2-5% uric acid. Water-soluble vitamins and small amounts of proteins and amino acids are also lost in the urine.

The loss of nitrogen through the sustenance of body functions and metabolic processes continues in the absence of nitrogen intake. This is regarded as 'obligatory nitrogen loss', defined as the amount of nitrogen lost from the body when foods which have adequate energy but are essentially devoid of proteins are consumed (FAO/WHO/UNU 1985). A component of this loss is the 'obligatory oxidative loss' which is the oxidation of amino acids from protein degradation which are not re-utilised for protein synthesis (Millward and Rivers 1988). Obligatory nitrogen loss is related to basal metabolic rate and is equivalent to approximately 2.31mgN/kilocalorie (kcal) in adult men and 1.78mgN/kcal in adult women (FAO/WHO/UNU 1985).

2.2.4 Nitrogen balance

In an adult, despite the constant turnover of proteins and exchange of amino acids, the protein content of the body remains remarkably constant and nitrogen balance is maintained over long periods (Jackson 1998a). It is clear, therefore, that the concept of balance lies at the core of nitrogen metabolism and in adults, nitrogen equilibrium is essential for the attainment of stability and health.

Nitrogen balance can be discussed from two perspectives; at a metabolic level and as a tool for the study of nitrogen metabolism. At the metabolic level, when the supply of nitrogen matches the body's demand, nitrogen balance is achieved. When nitrogen balance is maintained over long periods, body weight, composition and functions remain constant and a state of well-being is attained. In pathological states such as trauma and infection however, the supply of nitrogen is often insufficient to meet demands and a state of negative balance occurs, often at a cost to the body. Under such circumstances, there is usually a combination of a reduction in dietary intake and an increase in the

utilisation of nitrogen for inflammatory responses and tissue repair. There may also be an increase in losses such as in burns and profuse diarrhoea, which would further increase the demands for nitrogen. As the body attempts to correct the mismatch between nitrogen supply and demand, changes in metabolism occur. Apart from a shift in the pattern of protein synthesis, reutilization of amino acids is maximised together with a reduction in amino acid oxidation. Urinary excretion of nitrogen is also reduced and body protein is utilised, initially from the liver and gut but later from muscle and skin (FAO/WHO/UNU 1985). This process of adaptation allows the body to reach a new steady state. If the pathological state is sustained however, and the limits of adaptation are exceeded, progressive depletion of body protein will ultimately result in the deterioration of tissue function and eventually death.

In the study of nitrogen metabolism, nitrogen balance is frequently used as a means for estimating nitrogen requirement. However, there are limitations to balance studies due to factors which cannot all be controlled or corrected. These factors include:

1. In most balance studies, only the nitrogen content of diet, urine and faeces are directly measured and miscellaneous nitrogen losses are not determined which results in more positive balances. This difficulty can, however, be overcome by making an allowance for miscellaneous nitrogen loss which are estimated to be 8 mgN/kg per day for adults, provided that the climatic conditions in which the study is conducted are taken into account (FAO/WHO/UNU 1985).
2. Nitrogen balance does not show a linear relationship to protein intake (Young et al 1973). When protein intake is changed, adjustments in urinary nitrogen excretion do not occur immediately and therefore an adequate time period is required for the body to achieve a new steady state. The difficulty is determining the time required to achieve this. Data from short-term studies (up to 3 weeks) on obligatory nitrogen losses when protein intake is reduced showed that there is an initial sharp drop in urinary nitrogen followed by a long period of relatively stable but slowly declining excretion (FAO/WHO/UNU 1985). The major adjustment appears to be complete by days 5-7 in most adults over a range of age and sex (Munro 1964, Rand et al 1976).
3. Total dietary energy intake and level of physical activity of study subjects must also be considered as these two factors affect protein utilization.

4. Although nitrogen balance is an overall phenomenon, it does exhibit a diurnal pattern, following diurnal rhythm in dietary intake and metabolic behaviour (Jackson 1998b). Food is normally ingested in the daytime and as urinary nitrogen excretion is more marked on higher protein intakes, urinary nitrogen excretion is higher in the day than at night (Steffee et al 1981). Furthermore, the rate of protein synthesis is also lower at night (Garlick et al 1980). Therefore, on average, over a 24-hour period, these diurnal variations even out and an overall nitrogen balance can be considered (Jackson 1998b).

2.3 Urea-nitrogen salvage

Urea is considered to be a waste product of amino acid oxidation which is generally thought to be metabolically inert and excreted entirely in the urine by the kidney. However, following initial evidence that the hydrolysis of urea occurred in the intestinal tract of animals, Walser and Bodenlos (1959) were the first authors to demonstrate, through the use of urea isotopically labelled with ^{15}N and ^{14}C, that urea hydrolysis and nitrogen salvage also occurred in the human gastrointestinal tract. This observation has since been confirmed by numerous studies which show that in healthy subjects on habitual diets consisting of 75 g protein per day, 70-80% of the urea produced was excreted in the urine and approximately 25% was hydrolysed in the colon (Jackson et al 1984, Hibbert et al 1992, Jackson et al 1993a). Hydrolysis of urea results in the release of nitrogen which is then made available to the body for utilisation. This process is termed 'urea-nitrogen salvage' and has been shown to be of substantial importance to overall nitrogen metabolism.

2.3.1 Mechanisms of urea-nitrogen salvage

Urea hydrolysis is attributed entirely to the metabolic activities of bacteria which normally reside in the colon (Giordano et al 1968, Tanaka et al 1980). This bacterial function is, however, not site specific and urea hydrolysis has been shown to occur in the distal small bowel of people who do not have a colon (Gibson et al 1976a, Wheeler et al 1993). Furthermore, other studies have shown that the use of antibiotics, which interfere

with the normal colonic microflora, reduces or abolishes urea hydrolysis (Walser and Bodenlos 1959, Richards 1972, Moran et al 1991).

In order for urea hydrolysis to occur in the colon, urea has to move from the systemic circulation, through the colonic mucosa into the lumen. Due to inconsistencies in the results of various studies, the permeability of colonic mucosa to urea has been a subject of debate. Billich and Levitan (1969) studied the pore size of colonic mucosa and suggested that urea molecules could not readily pass through. This claim was supported by the studies of Wolpert et al (1971) and Bown et al (1975) who failed to demonstrate significant exchanges of urea between colonic lumen and the systemic circulation following intravenous administration and colonic perfusion of urea. However, the study conducted by Moran and Jackson (1990a) indicated otherwise. In this study, isotopically labelled $^{15}N^{15}N$-urea was instilled separately into the caecum and splenic flexure of the colon of normal subjects using a colonoscope and labelled urea was recovered in the urine of these subjects as $^{15}N^{15}N$-urea and $^{15}N^{14}N$-urea. In another study, Moran and Jackson (1990b) instilled the same label into functioning and defunctioned colon via the colostomy of subjects and again recovered the label as $^{15}N^{15}N$-urea and $^{15}N^{14}N$-urea in the subjects' urine. From both studies, Moran and Jackson concluded that in order for $^{15}N^{15}N$-urea to be present in urine, the instilled labelled urea must be absorbed from the colon into the blood stream as intact molecules and therefore, the colonic mucosa must be permeable to urea.

The contradictory conclusions drawn from the studies cited above can be explained by the differences in the methods used. In the study carried out by Wolpert et al (1971), when urea-^{14}C was administered intravenously into healthy subjects, only 2.4% of the total labelled urea administered was recovered from the colon and this result was interpreted to be indicative of poor colonic permeability to urea. However, it is possible that more labelled urea had entered the colon but was rapidly hydrolysed by the microflora on entry, thereby resulting in the low recovery. If this was the case, the presence of ^{14}C in the breath of the subjects would support colonic urea hydrolysis and hence, that colonic mucosa was permeable to urea. Unfortunately, the authors did not explore this possibility. In the study of Bown et al (1975), the methodology can be criticised as the subjects' colon was perfused with 1200 ml of isotonic solution containing unlabelled urea over 2 hours. When the authors failed to demonstrate an

increase in blood urea concentration, they concluded that colonic permeability to urea was limited. There are two major flaws in this study; firstly, when unlabelled urea is used, the fate of the perfused urea cannot be traced and secondly, perfusing the colon with a large volume of fluid over a short time period is not only unphysiological, it effectively amounts to 'flushing' the colon which will undoubtedly affect the absorption of urea across the colonic mucosa. In contrast, Moran and Jackson used urea isotopes where both the nitrogen atoms of each urea molecule were labelled enabling the fate of the isotope to be traced with more precision. In addition, as opposed to colonic perfusion, the urea isotope was instilled into the colon in a much smaller amount (5 ml) of normal saline. Therefore, the conclusions drawn from the studies of Wolpert et al and Bown et al are questionable while that of Moran and Jackson are more robust. Furthermore, in more recent studies, specific urea transporters in the colon, similar to that found originally in the kidney by Olives et al (1996), have been described by Ritzhaupt et al (1998), adding further weight to the permeability of colonic mucosa to urea.

When urea is hydrolysed by the microflora, nitrogen is released as ammonia. If urea-nitrogen salvage is an integral part of the body's nitrogen metabolism, providing a useful source of nitrogen, the question one has to ask is in what form this nitrogen is returned to the body. If the salvaged nitrogen is absorbed as ammonia, one would expect to find that, following administration of $^{15}N^{15}N$-urea, a large proportion of the administered label will be recovered as urinary $^{15}N^{14}N$-urea since after absorption, ^{15}N-ammonia passing up the portal vein into the liver is preferentially incorporated into urea synthesis (Nissim et al 1981). However, the proportion of the salvaged nitrogen recovered as $^{15}N^{14}N$-urea in the urine was reported to be only about 26% compared to the 66% thought to be retained in the nitrogen pool as essential and non-essential amino acids (Jackson 1995).

The evidence that salvaged nitrogen may be absorbed as amino acids came initially from Giordano et al (1968). In this study, ^{15}N-urea was administered for 4-6 days to one normal subject and also to patients with renal failure and the authors were able to demonstrate enrichment of ^{15}N in both essential (eg. leucine, lysine) and non-essential amino acids (eg. glutamate, alanine) isolated from albumin. In the same study, enrichment of both groups of amino acids increased by threefold when the subjects were taking low protein diets compared to when they consumed normal levels of protein, suggesting an adaptive increase in nitrogen salvage. However, after the administration of

antibiotics which would have eradicated colonic bacteria, enrichment was reduced substantially (Giordano et al 1968). A study by Tanaka et al (1980) also demonstrated incorporation of ^{15}N into essential (lysine, methionine, tryptophan) and non-essential amino acids when isotopically labelled urea was administered orally to Papua New Guinea Highlanders who habitually eat a low-protein diet and more recently, Millward et al (2000) demonstrated enrichment of orally administered ^{15}N label in urinary lysine of male infants treated for severe malnutrition.

From these studies, it is reasonable to conclude that following urea hydrolysis by the microflora, although some of the ammonia formed is absorbed back into the body and returns directly to urea synthesis, a larger proportion is fixed by the microflora for the synthesis of amino acids and other nitrogenous compounds. These amino acids are then absorbed back into the body and become available for utilisation (Hespell and Smith 1983, Jackson 1983). However, there remains an important unanswered question: how do these amino acids pass from the colonic lumen into the host? The mechanism for this remains unidentified although there is a suggestion that a peptide transporter, rather than an amino acid transporter, is involved (Jackson 1998a).

Overall, following urea hydrolysis, it is estimated that approximately 10% of urea-nitrogen is lost in the faeces, with 26% returning to urea formation in the liver and the rest entering the nitrogen pool and becomes available for metabolic engagement (Jackson 1995). The significance of the provision by colonic urea hydrolysis of both essential and non-essential amino acids is far reaching in that the quality of overall nitrogen supply available to the body is enhanced, thereby improving the 'goodness of fit' between supply and demand (Jackson 2000, Millward et al 2000). Furthermore, the capacity for urea-nitrogen salvage can be up-regulated, supplementing nitrogen supply when dietary protein intake is compromised. The importance of this metabolic reserve will be discussed in section 2.3.2. Figure 2.2 illustrates the role of bacteria urea hydrolysis in the dynamic interchange in nitrogen metabolism (Jackson 1998b).

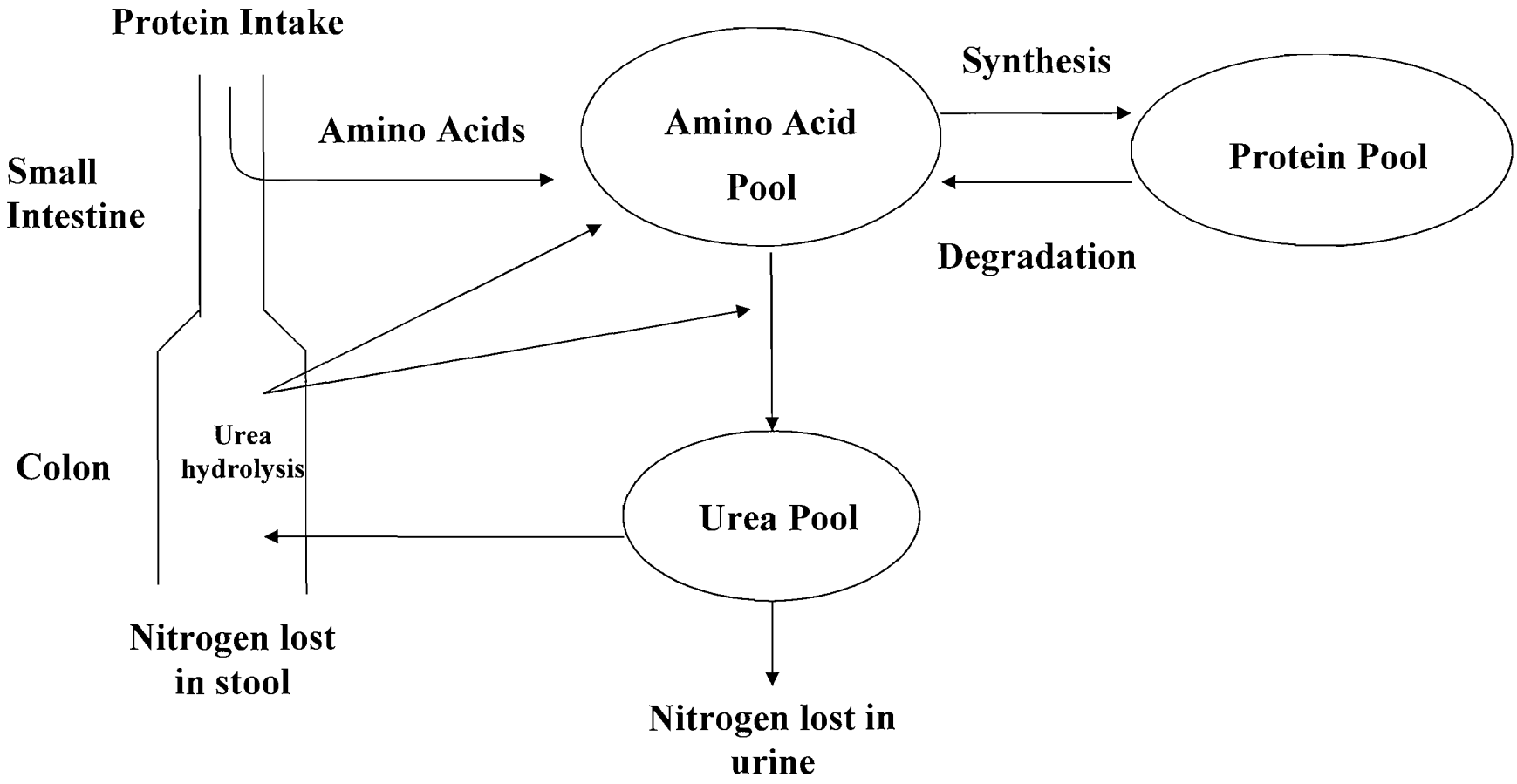


Figure 2.2 A model of the dynamic interchanges of protein, amino acids and nitrogen in the body encompassing urea hydrolysis and nitrogen salvage in the colon by commensal microflora

2.3.2 Functional significance of urea-nitrogen salvage

When there is a mismatch between nitrogen supply and demand, changes in nitrogen metabolism occur as adaptive processes attempt to address the discrepancy. One of the major ways in which the body adapts to limited nitrogen availability is through alteration in the rates of urea production and urinary excretion (Waterlow 1968). In association with changes in the body's handling of urea, numerous studies have shown that the capacity for urea-nitrogen salvage also alters in response to a reduction in nitrogen availability (Giordano et al 1968, Jackson et al 1988, Langran et al 1992, Meakins and Jackson 1996a).

When protein intake in an otherwise metabolically stable adult is adequate, the significance of the contribution of urea-nitrogen to overall nitrogen metabolism is unclear. However, when protein intake is reduced, studies have supported that urea-nitrogen becomes substantially important. In the study conducted by Langran et al (1992), they demonstrated that when protein intakes in healthy subjects were reduced from 70 g to 35 g per day, urea production relative to nitrogen intake increased from 132 % to 229%, urinary excretion of urea relative to nitrogen intake reduced from 101% to 61% and urea hydrolysis in the bowel relative to nitrogen intake increased from 63% to 149%, all of which were statistically significant. Overall, there was a significant increase in the retention of urea-nitrogen relative to nitrogen intake from 56% on a 70 g protein diet to 125% on a 35 g protein diet and consequently, the subjects were able to maintain nitrogen balance despite a reduction in protein intake. Similar conclusions were also drawn from other studies that examined the responses of urea kinetics to low protein diets (Picou and Phillips 1972, Meakins and Jackson 1996a). It would therefore appear that on a low protein diet, a series of adaptive responses occur, characterised by an increase in urea production, a reduction in urinary urea excretion and the movement of conserved urea to the colon for bacterial hydrolysis. These responses result in an increase in urea-nitrogen salvage which effectively compensates for the reduction in nitrogen supply and hence allow nitrogen balance to be achieved.

The adaptive capability of urea hydrolysis has obvious implication and importance to individuals who either habitually eat a low protein diet or in circumstances when nitrogen demands exceed availability (e.g. starvation, increased losses, infection,

pregnancy). Like the highlanders of Papua New Guinea, people who habitually take a diet that is low in protein and high in non-digestible carbohydrate appear to be in good health. In these people, it was felt that urea recycling contributes significantly to the maintenance of nitrogen equilibrium and thus body weight and function (Tanaka et al 1980, Miyoshi et al 1986). Furthermore, apart from the quantitative supply of nitrogen from urea recycling, the provision of salvaged nitrogen in the forms of both essential and non-essential amino acids, as discussed in section 2.3.1, also enhances the quality of dietary protein and is thus crucial in supporting nitrogen balance particularly when dietary supply is limited (Tanaka et al 1980, Millward et al 2000, Jackson 2000).

In the presence of metabolic stress resulting from unusual high demands which are not always met by adequate dietary protein intake, urea-nitrogen salvage has also been shown to be enhanced. In a study by Jackson et al (1988) the urea kinetics of 4 adults with homozygous sickle cell disease (HbSS) were compared with that of 6 adult controls. The authors found that the total rate of urea production was higher in HbSS compared to the controls (240 mgN/kg/day vs 139 mgN/kg/day) and 40% of the urea produced was excreted in the urine in HbSS compared to 71% in the controls. 60% of the urea produced was presumed to be hydrolysed in the bowel in HbSS compared to 29% in the controls. The authors concluded that the marked differences seen in the urea kinetics in HbSS might be a reflection of the metabolic demands for increased erythrocyte synthesis. Similarly, Moran et al (1991) demonstrated that following a single intravenous dose of $^{15}N^{15}N$-urea, 5 patients receiving total parenteral nutrition for intestinal failure retained a higher percentage of the isotope compared to 4 control subjects (38% vs 17% respectively). In another study, urea-nitrogen salvage was found to be higher in pregnant women, particularly in the first and second trimesters, compared to non-pregnant women, reflecting the higher demand for nitrogen required for fetoplacental growth (Forrester et al 1994).

From the above, it is clear that there is a wealth of evidence pointing to the vital role of the metabolic activities of colonic microflora in the nitrogen metabolism of their host and that urea hydrolysis contributes to the 'effective nitrogen supply' (Jackson 1998a). As the body can be considered as a demand-led system, the process by which that demand is satisfied are of fundamental importance (Jackson 2000). In essence, the colon and its microflora serve two vital functions; firstly, they improve the 'goodness of fit' by

enhancing the supply of nitrogen in qualitative terms and secondly, they act as a reserve capacity by increasing nitrogen availability quantitatively when supply is insufficient to meet demands. Thus, urea-nitrogen salvage is a fundamental mechanism for maintaining nitrogen balance, capable of adaptation, especially in situations of metabolic stress. For a fixed demand, salvage increases as intake reduces and for a fixed intake, salvage increases as demand increases (Jackson and Wootton 1990a).

There does appear, however, to be a limit to the metabolic capacity of the microflora in their response to changes in nitrogen supply and demand. Danielsen and Jackson (1992) showed that there was a critical level of protein intake beyond which nitrogen balance cannot be sustained. In this study, the authors measured the urea kinetics of 6 healthy men taking diets adequate in energy but containing 74 g and 30 g of protein per day in two separate series of experiments. They demonstrated that while on a 30 g protein diet per day, the subjects exhibited a reduction in both urea hydrolysis and nitrogen salvage and an increase in urinary urea excretion, and consequently, nitrogen balance could not be maintained. This finding was supported by Meakins and Jackson (1996a) who also reported a 30% reduction in urea production, a 50% reduction in urea hydrolysis and significantly more negative nitrogen balance in 6 healthy subjects when they were put on a diet consisting of 30 g protein daily compared to 70 g protein daily.

Considering the studies of Langran et al (1992), Danielsen and Jackson (1992) and Meakins and Jackson (1996a), Jackson proposed that the minimum physiological requirement for protein needed to maintain nitrogen balance in normal adult men must lie between 30-35 g per day (Jackson 1998b). When the daily protein intake is 35 g, urea-nitrogen salvage is enhanced and nitrogen balance is attained, but when protein intake drops beyond 30-35 g per day, the mechanisms of urea-nitrogen salvage fall apart and nitrogen balance cannot be supported (Jackson 1998b).

2.3.3 Control of urea-nitrogen salvage

It has long been recognised that renal adaptation takes place on a low protein diet, through active resorption of urea in the collecting ducts via urea transporters, mediated by vasopressin (Gillin and Sands 1993). As discussed in section 2.3.1, similar urea

transporters were identified in the mucosa of the human colon (Ritzhaupt et al 1998). Therefore, the presence in both the kidney and colon of similar urea transporters could provide the body with a co-ordinated response to a low protein diet, by directly linking an increase in urea retention by the kidney with an increase in the movement of urea into the colon for hydrolysis (Jackson 1998a). Indeed, as urea production remains constant, urea hydrolysis in the colon was shown to increase reciprocally as urinary excretion falls during the night, and conversely during the daytime (Meakins 1996b). However, although the urea transporters in the collecting ducts are regulated by vasopressin, the mechanism through which control is exerted over the movement of urea into the colon is unclear although it is evident that neither the excretion of urea in the urine, nor the movement of urea to the colon, are simple clearance phenomena (Meakins and Jackson 1996a).

Contrary to wide belief, it has been observed that urea production does not show a simple linear relationship with protein intake (Danielsen and Jackson 1992, Jackson 1998a). It is also erroneous to presume that the rate of urea excretion is a measure of the rate of urea production, since urea production has been shown to remain constant despite obvious diurnal changes in urea excretion (Meakins 1996b). However, data have supported the suggestion of a fundamental biological link between body weight, body composition (lean body mass), resting energy expenditure (REE) and the rate of urea production (Jackson 1998a). Urea production is estimated to be approximately 7.7 gN/basal kcal REE (Jackson 1998a).

Urea production is also intricately linked to urea hydrolysis in the colon (Jackson 1993b) and the studies conducted by Meakins and Jackson (1996a) had shed some light on this relationship. In these studies, healthy subjects who went into negative nitrogen balance on low protein diets of 30 g per day came into balance when their diets were supplemented with 13.7 g of urea (equivalent to 11.2 g of nitrogen). The urea supplementation led to an increase in urea hydrolysis and urea-nitrogen salvage, but the enhanced salvage was not related to either the concentration of urea in blood or the urea pool size. Instead it varied directly with the rate at which urea appeared in the urea pool and the authors therefore suggested that urea hydrolysis was being driven by urea appearance. Similar observations were also recorded by the study of Kies (1972). Jackson (1998a) concluded that in order to maintain the movement of urea to the colon

while consuming a low protein diet, the rate of urea appearance (production) has to be a minimum of about 150-170 mgN/kg/day. While consuming diets providing 25-30 g protein daily, urea production was approximately 121 mgN/kg/day and this was insufficient to drive urea hydrolysis (Jackson 1998a). Consequently, the system for nitrogen conservation failed and nitrogen balance could not be sustained. However, with a daily dietary supplement of 13.7 g of urea, urea production increased to 204 mgN/kg/day and urea-nitrogen salvage was enhanced with improvement in nitrogen balance (Jackson 1998a).

From this discussion of the association between urea production and hydrolysis in relation to dietary protein intake, it is clear that as dietary intake falls to a level at which the metabolic demands of the host are barely satisfied, the availability of urea nitrogen from urea production needed to satisfy the nitrogen requirement of the microflora is also compromised (Danielsen and Jackson 1992). The dynamics of urea kinetics and the responses of and interactions between the kidney and the colonic microflora are evidently complex and not fully understood. Nevertheless, they bear hallmarks of a controlled process, which is a fundamental characteristic of metabolism.

2.4 Total colectomy

Removal of the colon is sometimes required as a life-saving procedure. Amongst the numerous medical conditions that require this, inflammatory bowel disease (ulcerative colitis and Crohn's disease) is by far the most common indication. Other less common conditions include familial polyposis coli, colonic neoplasia, volvulus and colonic infarction. After the colon has been removed, the distal ileum can either be fashioned into a stoma to allow drainage of small intestinal contents into a bag attached to the abdominal wall or an internal reservoir (pouch) can be created to serve the purpose similar to that of a rectum.

2.4.1 Changes in the small bowel after total colectomy

Following a total colectomy, structural and functional changes in the remaining small intestine have been described in animal models but the evidence is less convincing in

human beings. Histological examinations of the small intestine of rats following extensive small or colonic resections show an increase in the rate of crypt cell proliferation, height of intestinal villi and weight of intestinal remnant (Woo and Nygaard 1978, Miazza et al 1985, Burgess and Sizeland 1990, Bilchik et al 1995). These observed changes are thought to have occurred to replace the lost intestinal functions. In human beings however, mucosal changes following intestinal resections are less clear. In one study, hypertrophy of distal ileal mucosa was described in patients with ileostomy (Wright 1975) while in another study, biopsies taken from distal ileum of ileostomy patients showed normal mucosa, partial villous atrophy or inflammatory changes only (Miettinen and Peltokallio 1971, Bechi et al 1981). It is not uncommon to experience discrepancies when animal data are applied to human beings. Firstly, very few human beings have more than 75% of their small bowel resected, as was the case in the animal models. Secondly, the ability to conduct a comprehensive examination of human small intestinal tissue is limited while in animal models, the animals are often sacrificed, allowing examination of the entire small bowel remnant. In human beings, small intestinal tissues are difficult to obtain technically and tissues obtained from biopsies are often very small and hence, may not be representative of the changes that that have taken place. On the other hand, the evidence for functional changes is more consistent. In normal individuals, approximately 1-2 litres of ileal fluid enter the colon each day, but the average daily output of an ileostomy following total colectomy only is about 600 ml (Bambach et al 1981, Kennedy et al 1983, Delin et al 1984). In his study, Weinstein et al (1969) described an increased in the capacity for water absorption in the remaining jejunum in people who had small bowel resection while the studies of Dowling and Booth (1966) and Gouttebel et al (1989) also reported an increase in the absorption of glucose and calcium respectively in the small bowel remnant following small bowel resection.

Changes in small intestinal microflora have also been described following total colectomy. The small intestine normally harbours a sparse microflora, increasing in numbers towards the terminal ileum (Drasar et al 1969). In the normal terminal ileum, the number of microorganisms is estimated to be approximately 10^3 to 10^5 per gram of ileal content compared to 10^{12} per gram of faeces (Gorbach 1971, Christl and Scheppach 1997). In the ileostomy effluent, there are approximately $10^5 - 10^7$ bacteria per gram (Gorbach et al 1967, Percy-Robb et al 1969, Finegold et al 1970). The microflora of the

distal ileum of ileostomy also appear to be different from that of the colon in that the ratio of anaerobic to aerobic organisms is lower, i.e. there are proportionately less anaerobic bacteria and more aerobic bacteria, which is opposite to the composition of colonic microflora (Gorbach et al 1967, Finegold et al 1970, Percy-Robb et al 1971a, Natori et al 1992, Sandborn et al 1995). Anaerobic lactobacilli species are notably absent from ileostomy effluent cultures (Percy-Robb et al 1969, Percy-Robb et al 1971a). Thus, the distal ileum of ileostomy patients appears to be colonised by a unique microflora different to that of the colon. This can perhaps be explained by the availability of oxygen in the distal ileal lumen via the stoma which results in alterations in the species of bacteria. In the colon, aerobic organisms are found in greater numbers immediately adjacent to the intestinal mucosa compared to the lumen, thought to be due to the availability of traces of oxygen reaching the luminal surface of the mucosa by diffusion from the blood stream (Wrong et al 1981).

Like colonic bacteria, the microflora of the distal small intestine of ileostomy patients has also been shown to be capable of responding to changes in the diet. Berghouse et al (1984) studied the effects of high-fibre/low-sucrose and high-sucrose/low fibre diets on the microflora of ileostomy effluent and noted a general increase in the number of all genera when ileostomy patients were on the high-fibre/low-sucrose diet. This observation is in agreement with the findings of Stephen and Cummings (1980a) who had previously reported that in normal subjects, high fibre diets increased the bacterial content in stool. When the microflora of ileostomy effluents of 8 people were examined, Fernandez et al (1985) found that there the number of bacteria increased significantly when these people were on high protein diets compared to low protein diets. Similar observations were also noted on high fat diets compared to low fat diets (Fernandez et al 1985). However, although the small intestinal microflora do respond to dietary changes, it does not necessarily imply that they are capable of urea hydrolysis and carbohydrate fermentation since the microflora in the distal end of the ileostomy is different to that of the colon. Urea recycling and carbohydrate fermentation in the absence of the colon will be discussed in sections 2.4.2 and 2.4.3 respectively.

Colonisation of bacteria in the small intestinal remnant may influence functional adaptation following total colectomy. Through the application of modern technologies in molecular science and genomics, a study conducted by Hooper et al (2001) provides us

with some insight into how these bacteria exert their influence on their host. In this study, germ-free mice were inoculated with Bacteroides thetaiotaomicron, a prominent component of normal mouse and human intestinal commensal. After colonisation, this bacterium was shown to modulate the expression of genes involved in nutrient absorption, gut motility, mucosal barrier fortification and angiogenesis in the mice. This suggests one mechanism by which functional adaptation can occur in the small intestine without obvious structural changes following an intestinal resection.

2.4.2 Effects of total colectomy on nitrogen metabolism

There is very little information in the literature concerning the impact of the loss of the colon on nitrogen metabolism. With a full length of functional small intestine, it is often assumed that there are no detrimental effects on overall nitrogen metabolism since most of the dietary nitrogen is absorbed in the small intestine (Ganong 2001a). Under normal circumstances when protein intake is adequate and there is no unusual nitrogen demand, it is perhaps not unreasonable to assume that ileostomy patients are maintaining nitrogen balance at no cost to their weight and health. However, we still need to ask whether it is safe to make such an assumption, what is the evidence to support this assumption, can ileostomy patients operate urea-nitrogen salvage and what happens to these people when they are under metabolic stress?

In the current literature, there is no information concerning the nitrogen balance of ileostomy patients but two studies have suggested that urea hydrolysis can occur in the absence of the colon. Using ^{14}C-urea, Gibson et al (1976a) demonstrated urea hydrolysis in two ileostomy patients and in the study of Wheeler et al (1993) using $^{15}N^{15}N$-urea, a neonate with congenital absence of the colon (cloacal exstrophy) was shown to operate urea-nitrogen salvage which increased from 5% of urea production on day 15 to 79% by day 23 of age. The salvage activities in these studies were thought to be the result of active microflora in the terminal end of the small bowel.

Despite the above evidence and bearing in mind that colonic urea-nitrogen salvage can be up-regulated in response to reduced nitrogen availability or increase nitrogen demands, the crucial question is whether ileostomy patients have the same salvage capacity as

normal individuals. While this issue has never been formally investigated, some information concerning this can be extracted from the study conducted by Gibson et al (1976a). When urea kinetics of the two ileostomy patients were compared to six healthy controls with all subjects taking diets consisting of 40 g protein and 2600 kcal, urea production was 28% lower and urea hydrolysis was 66% lower in ileostomy patients. Although the data from this study must be interpreted with care as it was not designed specifically to examine urea kinetics in response to changes in nitrogen intakes, nevertheless, these results do raise the possibility that ileostomy patients may not be able to up-regulate urea-nitrogen salvage in the same manner as normal individuals.

From the above, we can only conclude that our understanding of the impact of the loss of the colon on nitrogen metabolism is limited and thus, it is unsafe to assume that total colectomy is of no consequence even when nitrogen intake is adequate or when there is no unusual nitrogen demand. Ileostomy patients are likely to be more vulnerable to the effects of metabolic stress than normal individuals.

2.4.3 Effects of total colectomy on energy salvage

In normal individuals with an intact colon and eating typical Western diets, Cummings (1981) estimated that the energy salvaged in the form of SCFAs from colonic fermentation is about 7% of total energy intake. Although the contribution to total energy intake is small, in individuals where the diets consist primarily of non-absorbable carbohydrate from plant fibre, the energy provided by colonic bacteria fermentation is substantially more significant (Christl and Scheppach 1997). The importance of bacteria energy salvage to patients with extensive small intestinal resection and an intact colon has been described in section 2.1.3.

Since urea recycling has been shown to occur in people who have had total colectomy and ileostomy, it would be reasonable to expect that carbohydrate fermentation also takes place to some degree in the distal small bowel of these people. However, despite evidence indicating that the microflora in ileostomy patients respond to dietary changes, the evidence for significant bacterial fermentation is lacking. In ileostomy patients who had total colectomy only, the amount of absorbable carbohydrate in ileostomy effluent

was found to be insignificant, suggesting that carbohydrate absorption in the small bowel is almost complete (Christl and Scheppach 1997). On the other hand, the excretion of solids in ileostomy effluents, probably consisting of dietary fibre, was found to be 2-3 times greater than normal faecal content (McNeil et al 1982) and the study of Schweizer et al (1990) also found complete recovery of dietary fibre in the effluents of ileostomy subjects. The pH of the normal terminal ileum is approximately 7.4-7.6 while in the caecum, there is a sharp drop to 5.7 due to the presence of SCFAs. However, the pH of the distal ileum of ileostomy patients was found to be 7.2-7.3 (Fallingborg 1999), supporting the absence of SCFAs. Therefore, collectively, these studies indicate the lack of bacterial degradation and fermentation of dietary fibre in the absence of the colon.

2.4.4 Effects of total colectomy on sodium and water status

The average volume of ileal effluents in ileostomy patients has been reported to be about 600 ml, containing approximately 70 mmol of sodium (Bambach et al 1981, Kennedy et al 1983, Delin et al 1984). This is compared to 100-200 ml of water and 5-10 mmol of sodium excreted in the faeces of normal individuals (Bowler et al 1994). When 24-hour urinary sodium excretions and urine volumes were examined, there were significant reductions in both urinary sodium excretions and urine volumes in ileostomy patients compared to healthy controls (Bambach et al 1981, Kennedy et al 1983). In addition, significantly raised levels of serum aldosterone and renin were also recorded in ileostomy patients occurring in response to sodium deficiency (Kennedy et al 1983, Huber et al 1999). Collectively, these results indicated that the loss of colon had led to a state of chronic sodium and water deficit. Clarke et al (1967) studied total body water using deuterium oxide and total exchangeable sodium using isotope ^{24}Na in 21 ileostomy patients and reported an 11% reduction in total body water and 7% reduction in total exchangeable sodium. However, it was not clear from the study as to whether these patients also had additional small bowel resection.

It has also been reported that ileostomy patients have an increased risk of developing renal stones which is a known complication of chronic dehydration (Baker and Tomson 1994). A postal questionnaire conducted by Bambach et al (1981) involving 426 patients who have had small intestinal resection and/or total colectomy found an overall

prevalence of urinary stone disease of 9.4% compared to 1-5% reported in the general population of industrialised countries (Walker 1999). The prevalence of urinary stone disease in patients with ileostomy only was 8.9% and in ileostomy patients who had additional small bowel resection was 14.8% while in patients who had small bowel resection but intact colon, the prevalence was lower at 6.7% (Bambach et al 1981). Evaluation of the composition of urine showed that the urine volume, urinary sodium and urinary pH were all lower while urinary uric acid concentration was higher in ileostomy patients compared to healthy subjects (Clarke and McKenzie 1969, Bambach et al 1981, Kennedy et al 1983). Patients who had small bowel resection but intact colon had higher urine volume, higher urinary sodium, higher urinary pH and lower urinary uric acid levels compared to those who had ileostomy only and ileostomy with small bowel resection (Bambach et al 1981). The authors postulated that hydrogen ions were excreted in exchange for sodium and the combination of low urinary volume, low urinary pH and high urinary uric acid concentration led to the increased risk of urinary stone formation.

All the above studies indicate unequivocally the importance of the colon in the maintenance of sodium and fluid balance. Ileostomy patients are therefore vulnerable to the effects of chronic dehydration and are at risk of developing renal impairment and renal stone disease.

2.5 Health and diet of people following total colectomy and ileostomy

The consequences of total colectomy can be summarized as follows:

1. Following the loss of the colon, bacteria are established in the distal small bowel remnant but these bacteria are different from colonic microflora in both genera and numbers.
2. Despite the differences, these bacteria appear to be capable of urea hydrolysis although the extent to which this metabolic activity can operate is unknown.
3. Little is known about how ileostomy patients maintain nitrogen economy in habitual living conditions and the changes that may have occurred in urea kinetics following colectomy.

4. The ability of ileostomy patients to up-regulate urea-nitrogen salvage in response to reduced nitrogen availability is also uncertain although there is some information to suggest that this is limited. If this is the case, ileostomy patients may be susceptible to the effects of metabolic stress.
5. In terms of carbohydrate fermentation and energy salvage, the evidence to support the presence of this metabolic activity in the absence of the colon is lacking.
6. With regard to sodium and water status, it is clear that ileostomy patients are chronically depleted in sodium and water and are therefore at risk of developing renal impairment and renal stones.

From the above, it is apparent that the loss of colon has resulted in significant metabolic and physiological changes. The important questions we need to ask are what are the clinical manifestations of these changes and what is the state of health of people following total colectomy and ileostomy.

Several studies have examined the health issues of ileostomy patients. In the study of McNeil et al (1982), the levels of haemoglobin, albumin, total protein, electrolytes, vitamin B_{12} and erythrocyte folate were measured, together with body weight and percentage body fat using skinfold thickness, in 36 ileostomy patients, 26 of whom had had ulcerative colitis and 10 who had had Crohn's colitis with mean ileal resections of 6.4 cm (range 0-25 cm) and 40.2 cm (range 1-150 cm) respectively. The results from this study showed that apart from one female ileostomy patient who had mild iron deficiency anaemia, the blood indices of all the other 35 ileostomy patients were within the normal range of the general population. Percentage body fat was also not significantly different from that of age-match controls but ileostomy subjects were significantly lighter by 4.1 kg, thought to be due to a combination of chronic dehydration, absence of the colon and lack of SCFAs as energy source. Baixas et al (1984) examined the nutritional status, defined by body weight, serum albumin, prealbumin, transferrin, haemoglobin and urinary creatinine, of 21 ulcerative colitis subjects who had total colectomy with ileorectal anastomosis. When the results were compared to age- and sex-matched controls, the authors did not find any significant differences in the measurements. Hill et al (1977) examined the nutritional status of 16 ileostomy patients, defined by body weight, percentage weight loss, serum albumin, prealbumin, transferrin and haemoglobin

and reported no evidence of protein-energy malnutrition. Thus, all these studies did not detect any clinical manifestations of nutritional deficiencies and all the authors concluded that patients who have had total colectomy were in good health.

In contrast, the study by Cooper et al (1986) suggested that ileostomy patients might have subclinical malnutrition that was not clinically obvious. In this study, haemoglobin, plasma electrolytes, serum albumin and protein were measured together with body weight and body composition of 12 ileostomy patients who had had ulcerative colitis with minimal small bowel resection (range 2-10 cm). Percentage body fat was measured using skinfold thickness and total body nitrogen and potassium were measured using neutron activation analysis. The measurements of body weight and composition were compared with predicted values determined from regression equations based on previous measurements obtained from healthy subjects. The results of this study showed that despite normal levels of blood indices and similar body weight and percentage body fat as compared to predicted values, the 12 ileostomy patients had significantly lower total body nitrogen and total body potassium compared to predicted values suggesting that there was a reduction in fat-free mass (FFM). The authors concluded that ileostomy patients have evidence of subclinical malnutrition and went further to recommend prudent fluid and nutritional support during intercurrent illnesses and when requiring surgery.

Dietary assessments in people who have had extensive small bowel resection have reported a tendency to consume hypercaloric diets to compensate for a reduction in nutrient absorption caused by shortened intestinal length (Messing et al 1991, Lennard-Jones 1994, Nightingale 1995). For ileostomy patients who have had total colectomy with minimal small bowel resection, the average energy intake was reported to be 2323 kcal with 83.1 g of protein, 251 g of carbohydrate and 108 g of fat (Cooper et al 1986). For patients with total colectomy and ileorectal anastomosis, the average intakes were 2187 kcal in energy and 96.3 g of protein and intake of dietary fibre was also reduced compared to healthy subjects (Baixas et al 1984). Although care must be taken when comparing data from different time periods, these reported levels of intakes are higher than those of the general population which are 1880 kcal in energy, 67.0 g of protein, 239 g of carbohydrate and 76 g of fat (National Food Survey 2000).

Apart from total nutrient and energy intakes, changes in the pattern of intake have also been reported in ileostomy patients, mainly due to the adverse effects of certain foods on stomal function (Thomson et al 1970). Bingham et al (1982) studied this phenomenon in ileostomy patients where the pattern of dietary intake was compared with control subjects. In the first part of her study, mean daily intakes of energy was 2381 kcal, of protein was 75 g, of carbohydrate was 293 g and of fat was 106 g in 37 ileostomy subjects who recorded 7-day weighed food diaries and these levels of intakes were similar to that of 37 control subjects. However, like Baixas et al (1984), the intakes of dietary fibre, iron, vitamins A and C were significantly lower in ileostomy subjects compared to controls. In the second part of her study, 79 ileostomy subjects were interviewed, along with 70 control subjects, for their opinion of the effect of 200 food items on stomal function. She reported that there was a significant difference in food choice between ileostomy and control subjects due to adverse effects of certain foods on stomal functions like excess flatus and increase effluent. 50-95% of ileostomy patients in her report avoided or modified the intakes of nuts, food with pips, skins or seeds and vegetables like peas, sweet corn and onion. With regard to nutrient absorption, Langkilde et al (1990) observed that in 7 ileostomy patients with minimal small bowel resection, the mean absorption of dietary energy was 89% of 2280 kcal total energy intake (equivalent to 2092 kcal), of nitrogen was 88% of 108 g total protein intake (equivalent to 95g protein or 15.2 g nitrogen) and of fat was 92% of 42 g total fat intake (38.6 g of fat).

Collectively, all these studies, apart from that of Cooper et al (1986), concluded that ileostomy patients were in good health as determined by percentage body fat and synthetic functions of biological processes. They were also taking diets which were adequate in energy and protein and in the presence of fully functional small intestine, absorption did not appear to be compromised. However, several issues are raised by the study of Cooper et al (1986) which indicated the presence of subclinical malnutrition in ileostomy patients despite normal synthetic functions. Firstly, it is possible that the parameters used as indicators of health in these studies are inappropriate. Secondly, the measurements of blood indices and assessment of body composition using anthropometry may not be sufficiently sensitive in detecting reduced lean body mass. Thirdly, synthetic functions may be maintained at the expense of other biological processes such as muscle mass and function which are less easily measured. The possibility that subclinical

malnutrition may be present in ileostomy patients with other health consequences remains unclear.

2.6 Effects of small bowel resection on people with ileostomy

Some patients who have had total colectomy may also have compromised small bowel function due to additional small bowel resection or residual Crohn's disease. Unlike patients with frank short bowel syndrome, most of these patients may only have had modest resection of their small bowel, which does not render them dependent on nutritional support but may nevertheless impact upon the efficiency with which they can digest and absorb food and fluid and hence cause increased losses of fluid, electrolytes and nutrients. The average ileostomy effluent have been shown to be significantly higher in patients who have had mean small intestinal resections of 40-55 cm compared to ileostomy patients with total colectomy alone (wet weight 1084 g vs 635 g respectively) (Cooper et al 1986, McNeil et al 1982). Furthermore, the higher the ileostomy output, the greater the loss of sodium from the stoma (124 mmol vs 74 mmol respectively) (Cooper et al 1986, McNeil et al 1982) with significantly lower 24-hour urine volume and urinary sodium excretions (Bambach et al 1981). As a result, these patients have a higher prevalence of renal stones compared to those with total colectomy alone (14.8% vs 8.9% respectively) (Bambach et al 1981).

Energy absorption is shown to correlate significantly with residual small bowel length (Nightingale et al 1990). Nutrient absorption is further impaired by rapid intestinal transit rate and a significant correlation between energy absorption and transit rate (time to 50% marker recovery) has also been demonstrated (Rodrigues et al 1989). As the absorption of amino acids is dependent on the movement of sodium across intestinal mucosa, absorption of amino acids can, therefore, be affected by excessive losses of sodium. When dietary intakes were examined using 7-day food diary, the mean daily intakes of fluid, energy and nitrogen were consistently greater by 15-20% in ileostomy patients who have had mean small bowel resection of 54 cm (range 50-120) compared to those with very minimal resection of 4 cm (range 2-10 cm) (Cooper et al 1986). However, excessive stoma losses in ileostomy patients with small bowel resections do not appear to have any obvious impact on synthetic processes since the levels of haemoglobin, plasma

electrolytes, serum protein and albumin were within the normal range of the general population (Cooper et al 1986, McNeil et al 1982). Despite normal blood indices, ileostomy patients with a median small bowel resection of 54 cm (50-120 cm) had significant reductions in weight, total body fat and total body nitrogen compared to predicted values matched for age and sex but no significant differences were detected when compared to ileostomy patients with total colectomy alone (Cooper et al 1986).

In patients who have had total colectomy with additional small bowel resection, the terminal ileum is often excised. The terminal ileum is known to perform specific functions, namely the absorption of vitamin B_{12} and bile salts. The transition of the 'usual' ileum to terminal ileum is not structurally demarcated. However, studies investigating absorption of vitamin B_{12} and bile metabolism have suggested that the last 50-60 cm of the distal ileum constitute the terminal ileum and resection of 50 cm or more of the distal small bowel will invariably lead to malabsorption of vitamin B_{12} and bile salts (Heaton 1968, Lenz 1975, Andersson et al 1979). Vitamin B_{12} is an essential micronutrient which is required for the formation of haemoglobin, function of the nervous system and other metabolic processes. The importance of vitamin B_{12} in nitrogen metabolism is characterised by its role as a coenzyme in the methionine cycle where methionine is the precursor for the formation of cysteine from serine and other reactions in which the methyl group is made available for other metabolic processes (Figure 2.3) (Jackson 1998b). If vitamin B_{12} is deficient, methionine cannot be reformed from homocysteine and this will result in the accumulation of homomcysteine. Hyperhomomcysteinaemia is associated with an increased risk of dementia, Alzheimer's disease, venous and arterial thromboses (Compher et al 2001, Schnyder et al 2001, Seshadri et al 2002).

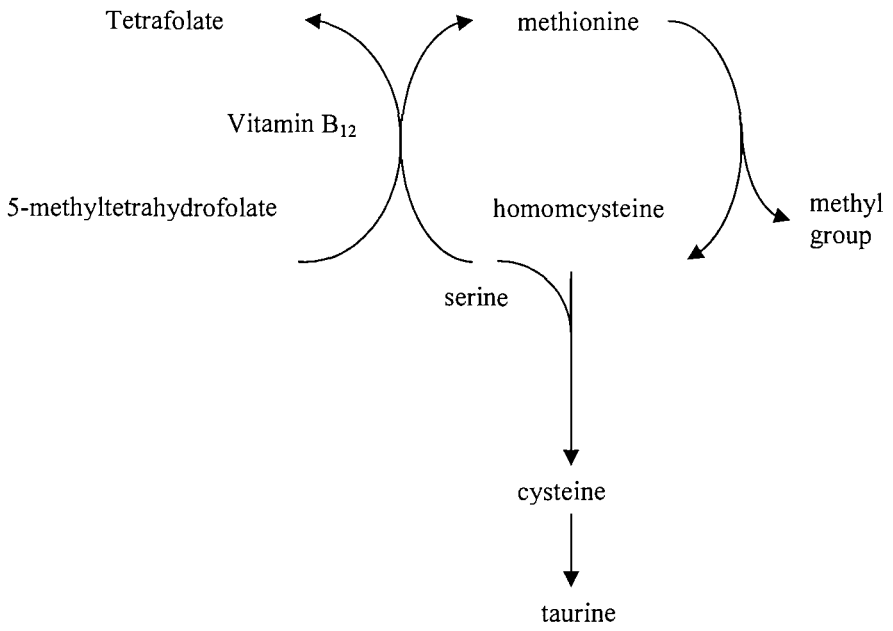


Figure 2.3 The methionine cycle

Bile salts are formed from the conjugation of bile acids with either glycine or taurine and they are necessary for the absorption of fatty acids, cholesterol and fat-soluble vitamins. Bile acids are synthesized in the liver from cholesterol at a rate of 0.2-0.4g per day. 90-95% of the bile salts are reabsorbed in the small intestine, mainly in the terminal ileum by an efficient sodium dependent secondary active transport. The remaining 5-10% enters the colon and is converted to salts of deoxycholic acid and lithocholic acids (known as secondary bile acids). Lithocholate is relatively insoluble and is mostly excreted in the stools, while deoxycholate is reabsorbed by non-ionic diffusion in the colon. The reabsorbed bile salts are returned to the liver via the portal vein and are re-excreted into bile. This enterohepatic recycling of bile salts plays an important role in economising both the total bile salt pool which is approximately 3.5g and the substrates required for bile salt synthesis. It has been calculated that the entire bile salt pool recycles twice per meal and approximately six to eight times in one day (Bowler et al 1994).

Ileostomy patients have higher bile acid losses in the ileal effluents compared to normal individuals (Fiasse et al 1983) and bile acid losses are also higher in ileostomy patients who had ileal resection compared to those who did not (Percy-Robb et al 1971b,

Akerlund et al 1994). As a result of the higher losses, bile acid composition of the ileal effluents consisted mainly of primary bile acids and the duodenal aspirates of bile secretion following a fatty meal showed a reduction in total bile acid concentrations and the absence of secondary bile salts (Percy-Robb et al 1971b, Fiasse et al 1983). In order that bile acid levels are maintained, the synthesis of bile acids in these patients is also increased as indicated by a several fold rise in both the serum concentrations of lathosterol and 7 alpha-hydroxycholesterol which are markers of cholesterol biosynthesis and bile acid biosynthesis respectively (Akerlund et al 1994). This increase would inadvertently place a demand on substrates like glycine and taurine which are required for conjugation of bile acids. Glycine, a non-essential amino acid in normal adults may therefore become conditionally essential in these patients, placing an additional burden on nitrogen economy. Similarly, taurine is synthesized from cysteine which again may become conditionally essential. If the demand cannot be met, a constraint in substrate availability will lead to bile salt insufficiency, manifesting as malabsorption of fat and fat-soluble vitamins.

2.7 Summary of literature review

From the literature, it is clear that the colon and its commensal microflora are of substantial importance in influencing our physiology and metabolism. Apart from the colon's capacity for sodium and water absorption, colonic microflora are capable of energy salvage from carbohydrate fermentation and nitrogen salvage from urea hydrolysis. Secretions of peptides from colonocytes also exert an influence on gastrointestinal motility and maintain small intestinal mucosa. Each of these functions contributes towards the preservation of our nutritional integrity on a day-to-day basis and their clinical importance is evident in patients who have short bowel with an intact colon.

Nitrogen metabolism is an integral part of our daily function and survival. Our body is a demand-led system where the supply of nitrogen must be effective in meeting nitrogen demands in order that nitrogen equilibrium is attained. Colonic microflora play a vital role in contributing towards this supply of nitrogen by salvaging nitrogen from urea, an end product of amino acid oxidation. The salvaged nitrogen is returned to the body in the forms of essential and non-essential amino acids for utilisation. Although the true value

of nitrogen recycling for an adult taking a diet adequate in energy and nitrogen and with no unusual metabolic demands is unclear, studies have shown that when nitrogen availability is insufficient to meet nitrogen demands, colonic urea-nitrogen salvage is increased and becomes essential in maintaining nitrogen balance. Colonic urea-nitrogen salvage, therefore, serves two functions:

1. Improve the effectiveness of nitrogen supply qualitatively, thereby enhancing the 'goodness of fit' between nitrogen supply and demand.
2. Act as a reserve capacity by the provision of nitrogen in quantitative terms when nitrogen availability is insufficient to meet demands.

In people who have had total colectomy and ileostomy, studies have suggested that the microflora present in the distal ileum may be capable of urea hydrolysis. However, as the small intestinal microflora are different from colonic microflora, their metabolic capabilities are unclear although one study had raised the possibility that their urea-nitrogen salvage capabilities are limited. While from several studies that have examined the diet and health of ileostomy patients have concluded that ileostomy patients take diets which are adequate in energy and protein, are in good health and have no clinically detectable nutritional deficiencies, the mechanisms through which health, and presumably nitrogen balance, are maintained are unclear. Detailed examination of their nitrogen balances and urea kinetics have not been conducted. In addition, there is also no information in the literature pertaining to the ability of the small intestinal microflora to act as a metabolic reserve. If nitrogen salvage capacity of small intestinal microflora is indeed limited, then ileostomy patients might be vulnerable to the effects of metabolic stress. Furthermore, contrary to the studies that reported that ileostomy patients were in good health, the study of Cooper et al (1986) reported a significant reduction in lean body mass in ileostomy patients raising the possibility that these people may in fact have subclinical malnutrition. If this is true, then ileostomy patients carry with them a burden of ill health which would become more apparent under metabolic stress. For ileostomy patients who also had small bowel resection, they have an added burden on nitrogen balance through reduced nutrient absorption and increased nutrient loss.

In conclusion, although the importance of colonic urea-nitrogen salvage in nitrogen metabolism is unequivocal, there is a gap in our understanding surrounding the impact of

the loss of this metabolic function on nitrogen metabolism and health of ileostomy patients. In order to clarify the uncertainties highlighted in the literature review, potential changes that may exist in nitrogen balance and urea kinetics in ileostomy patients, under both normal habitual condition and metabolic stress, must be thoroughly investigated. The findings will not only provide an insight into the mechanisms through which nitrogen metabolism is maintained in ileostomy patients, it will also highlight the presence of metabolic vulnerabilities of these people who have lost a vital organ.

3

HYPOTHESES AND PROPOSED STUDIES

3.1 Hypotheses

The colon and its metabolic activities have been shown to contribute substantially to the preservation of our nutritional integrity and health. Colonic urea-nitrogen salvage in particular, plays a significant role in maintaining overall nitrogen balance, especially when nitrogen availability is reduced and/or metabolic demands are high. Furthermore, patients with stomas might have excessive losses of nitrogen and other nutrients such as sodium that can influence lean tissue deposition. We therefore hypothesized that:

1. following total colectomy, ileostomy patients may have compromised nitrogen status and relatively poor overall health; and
2. following total colectomy, ileostomy patients may be incapable of operating urea-nitrogen salvage and will therefore, be particularly prone to negative nitrogen balance if nitrogen intake is reduced.

The effects of the loss of colonic functions on nitrogen metabolism and health were studied by comparing patients who have had total colectomy and permanent ileostomy with people who have intact colon.

The first hypothesis was explored by examining the following questions:

1. Do ileostomy patients have altered nitrogen status?
2. Do ileostomy patients have higher dietary nitrogen intakes?
3. Do ileostomy patients have sodium depletion?
4. Is the overall health of ileostomy patients impaired?

The second hypothesis was explored by examining these questions:

5. Without the colon, can ileostomy patients operate urea-nitrogen salvage?
6. In the presence of metabolic stress created by a reduction in nitrogen availability, can ileostomy patients up-regulate urea-nitrogen salvage and maintain nitrogen balance?

3.2 Outline of studies

To address questions 1 to 4, a cross-sectional study was conducted on 60 ileostomy patients and 60 reference subjects with no history of gastrointestinal disease or surgery. Nitrogen and health status were examined with reference to:

a. weight, BMI and body composition
b. dietary nitrogen intakes and urinary nitrogen excretions
c. apparent nitrogen balance
d. urine volume and urinary sodium excretions
e. assessment of clinical health
f. assessment of general health
g. organ and biosynthetic functions

The additional effects of small bowel resection were also examined by comparing ileostomy subjects who had small bowel resection with those who had total colectomy alone.

To address question 5, metabolic studies on nitrogen balance and urea kinetics were conducted on 18 subjects; 6 ileostomy subjects with total colectomy alone (NSBR), 6 ileostomy subjects with total colectomy and additional small bowel resection (SBR) and 6 subjects with no history of gastrointestinal disease or surgery. Habitual nitrogen intakes were assessed using weighed food diaries over a 5-day period along with the measurement of urinary nitrogen excretions and stool nitrogen losses. Hence, nitrogen balance could be calculated. The use of doubly labelled stable isotope $^{15}N^{15}N$-urea enabled the study of urea kinetics and urea-nitrogen salvage capabilities in these subjects.

By comparing these three groups of subjects, the effects of total colectomy and the additional burden of small bowel resection on nitrogen metabolism could be examined. Question 6 was examined by repeating the metabolic studies outlined above on the same subjects but on this occasion, metabolic stress was created by a 40% reduction in daily nitrogen intake. By comparing urea kinetics and nitrogen balance from the two diets, the responses of each group of subjects to reduced nitrogen availability could be studied.

4

CROSS-SECTIONAL SURVEY: THE HEALTH OF PEOPLE WITH ILEOSTOMY

4.1 Introduction

Despite the wealth of information highlighting the importance of colonic functions, the effects of total colectomy on nitrogen metabolism and health of ileostomy patients have not been thoroughly examined. Although several studies have reported on the diet and health of ileostomy patients, the evidence is not only scarce but also contradictory. Furthermore, there is no information in the current literature relating to the nitrogen balance in ileostomy patients and a formal health assessment, using an accepted health measure, has also never been described in these patients. The objective of this study, therefore, was to conduct a comprehensive review of the nitrogen status and overall health of ileostomy patients so as to gain a better understanding of the effects of losing the colon. This study will address Questions 1-4 and hence, our first hypothesis as set out in Chapter 3.

4.2 Identification and recruitment of subjects

Ileostomy subjects

A total of 161 patients who have had total colectomy and ileostomy and surgery related to ileostomy or small bowel resection between 1994 and 2000 were identified from the surgical database and gastroenterology outpatient clinic of the Southampton University Hospital Trust (SUHT). Of these, only 121 sets of medical case notes were available for review, following which 76 patients were identified as suitable subjects for this study. Patients who have moved out of Hampshire or who have had their ileostomy reversed with pouch formation, ileorectal or ileoanal anastomosis were excluded. As the study protocol required the subjects to fast overnight and there was no overnight admission facility, patients who had insulin-dependent diabetes were also excluded from the study.

Suitable study subjects were contacted initially by a letter, followed by a telephone call a week later, inviting them to participate in the study. The first 60 subjects, out of the 76 identified, who agreed to participate were recruited. A mutually convenient appointment was made for each subject to attend the Clinical Nutrition and Metabolic Unit (CNMU) at SUHT for the study. Each subject was provided with a patient information sheet and written consent was obtained after the purpose of the study and all investigative procedures were explained. The general practitioners and hospital consultants of all the subjects were informed of their participation in this study. The review of case notes and recruitment of patients were conducted by the Clinical Research Fellow who is the author of this thesis.

Reference subjects

60 subjects with no history of gastrointestinal disease or surgery were recruited to establish a reference population for comparison with the ileostomy subjects. Staff of the hospital and university and their associates were approached in person and invited to participate in this study. The recruitment criteria were:

1. Male and female subjects between 35-85 years and 26-77 years respectively.
2. Age of subjects within $\pm$ 5 years of the ileostomy subjects.
3. BMI of subjects within $\pm$ 1 standard deviation (sd) of population mean for their age groups. [Data for population means and standard deviations of age groups 16-24, 25-34, 35-44, 45-54, 55-64, 65-74 and above 75 for the year 2000 were obtained from Health Survey for England (2000)].
4. Height of subjects within $\pm$ 2 sd of population mean for their age groups. [Data for population means and standard deviations of age groups 16-24, 25-34, 35-44, 45-54, 55-64, 65-74 and above 75 for the year 2000 were obtained from Health Survey for England (2000)].
5. Subjects must have no history of liver, renal or significant heart disease.
6. Subjects must not be on drugs known to interfere with renal excretion of electrolytes or minerals (e.g. diuretics, non-steroidal anti-inflammatory analgesia, angiotensin converting enzyme inhibitors) and they must not be taking any nutritional supplements.

The reason for selecting subjects according to defined BMI and height criteria was to ensure that the reference population represented the normal healthy population, excluding unusually underweight or overweight individuals who might have abnormal eating habits or metabolism.

Subjects who met the above criteria and agreed to participate in the study were provided with an information sheet and written consent was obtained after the purpose of the study and all investigative procedures were explained.

4.3 Study protocol

Ileostomy subjects

60 subjects who have had total colectomy and permanent ileostomy were recruited into the study, which was approved by the South and West Local Ethics Committee (reference 086/00). Each subject attended the CNMU as out-patients in the morning following an overnight fast from 2200 hours. The following assessments were made:

a. BMI and body composition
b. clinical health determined by clinical questionnaire designed for ileostomy subjects and physical examination
c. general health using self administered Short Form-36 health questionnaire (SF-36)
d. dietary intake using self administered food frequency questionnaire (FFQ)
e. 24-hour urinary nitrogen, sodium and potassium excretions
f. biological functions determined by haemoglobin level, presence of inflammatory response, markers of renal function, markers of liver function, markers of bone health and micronutrient status

Reference subjects

60 subjects who fulfilled the recruitment criteria were recruited to form a reference population. This study received the approval of the South and West Local Ethics Committee (reference 332/02/w). Each subject had the following assessments made

either at the Wellcome Trust Clinical Research Facility (WTCRF) at the SUHT as out-patients or in their home:

a. BMI and body composition
b. clinical health determined by clinical questionnaire modified for reference subjects
c. general health using self administered SF-36
d. dietary intake using self administered FFQ
e. 24-hour urinary nitrogen, sodium and potassium excretions

4.4 Methods

4.4.1 Assessment of body mass index and body composition

All subjects were weighed using a digital scale (Seca Alpha, mpdel 770, CMS Weighing Equipment Ltd) and height was measured in metre (m) using a stadiometer (CMS Weighing Equipment Ltd). BMI was calculated using the Quetelet's index (kg/m^2). Body composition was determined as FFM, body fat (BF) and percentage body fat (%BF) using bioelectrical impedance analysis (BIA) (Bodystat 1500, Bodystat Limited, Douglas, Isle of Man). The validity of the body composition obtained from BIA using Bodystat 1500 was examined in a separate study where measurements were compared with those obtained from skinfold thickness readings, predictive equations based on BMI and age and dual energy x-ray absorptiometry (DEXA) (Ng et al 2002a). The correlation coefficients of BF% using BIA compared with each of the alternative methods were 0.805, 0.913 and 0.877 respectively ($p=0.01$ for all values). The weight, height and body composition measurements were taken by the Clinical Research Fellow, research assistant and research nurses from the WTCRF.

4.4.2 Assessment of clinical health

A specifically designed clinical questionnaire was used to assess the clinical health of ileostomy subjects. This questionnaire was not validated but it conformed to standard medical practice. In order that consistency was maintained, this questionnaire was administered by the Clinical Research Fellow only. The information obtained included:

a. subject's perception of his/her general health after having had total colectomy and ileostomy
b. presence of lethargy and level of activity
c. appetite and whether subject had loss or gain weight
d. presence of gastrointestinal symptoms (nausea, vomiting, abdominal discomfort, bloating, blood loss from stoma)
e. presence of symptoms suggestive of nutritional deficiencies (condition of hair, skin and nails, loss of taste, mouth ulcers and tendency to suffer from infections like flu and colds)
f. stoma function i.e. consistency of stool and an estimate of daily volume (Every ileostomy subject was asked the type and size of bag used, the number of times the bag was changed or emptied each day and how full the bag usually was before changing or emptying. The actual volume of the bag was obtained from the manufacturer and an estimate of daily stomal volume was then calculated.)

The clinical questionnaire used to assess the clinical health of the reference population was the same as that used for ileostomy subjects but excluding stoma function. This questionnaire was administered by a research assistant and two research nurses from the WTCRF.

The Clinical Research Fellow also examined the ileostomy subjects for the following:

a. general health appearance
b. clinical evidence of malnutrition (underweight, muscle wasting, ascites, dependent oedema)
c. signs of specific nutritional deficiencies (state of hair, skin, nails and tongue)
d. supine and standing blood pressure

4.4.3 Assessment of general health

The general health of both ileostomy and reference subjects was assessed using the SF-36 health survey questionnaire which contain 36 items examining 8 health scales and encompasses 3 broad aspects of health (Table 4.1) (Brazier et al 1992). This

questionnaire was self administered in the subject's home and collected at a later date. The responses to items in each scale (certain items are weighted) are summed to provide scores between 0 (worst health) to 100 (best health) based on methods set out by its developers (Jenkinson et al 1996).

Table 4.1 Health scales of SF-36

Aspect of health	Health scales	No. of items
Functional status	Physical function	10
	Social function	2
	Role limitations by physical problems	4
	Role limitations by emotional problems	3
Well being	Mental health	5
	Vitality	4
	Pain	2
Overall health evaluation	Perception of general health	5
	Health change *	1
Total		36

* This item is not included in the 8 health scales and is not scored

The SF-36 health survey questionnaire was developed from the Rand Corporation's health insurance experiment in the United States of America and the original questionnaire was lengthy containing 108 items. Since its development, the SF-36 has been assessed in several studies involving a total number of 12419 randomly selected subjects from general practitioners' surgeries across England and Scotland. All the studies consistently reported that it is a reliable and valid tool for the measurement of health status in the general population and that it is acceptable to subjects across a wide age group, including those above 75 years of age (Brazier et al 1992, Jenkinson et al 1993, Garratt et al 1993, Hayes et al 1995).

4.4.4 Assessment of dietary intakes

Daily intakes of energy, protein, carbohydrate, fat, minerals and a wide range of micronutrients were assessed using a FFQ which included over 200 items of food and drink. This FFQ was previously used in another study where validity against a 7-day weighed record and repeatability were examined (Shaheen et al 2001). The FFQ was self administered in the subject's home and collected at a later date. The ileostomy subjects were also asked if they avoid specific foods as a direct result of having a stoma.
Before deciding on an appropriate dietary assessment tool, factors such as study design, number of patients involved, dietary information required and manpower constraints were taken into consideration. On the whole, while accepting its limitations, it was felt that a FFQ was the most appropriate tool as it is easy to use, can be self administered, less labour intensive and suitable for obtaining the required dietary data. The FFQ and software programme required for data analysis were provided by Dr Rachel Thompson, Public Health Nutrition, Institute of Human Nutrition, University of Southampton.

4.4.5 Determination of 24-hour urinary nitrogen excretion

Protocol

Each subject completed a 24-hour urine collection. Verbal and written instructions were given and urine was collected into clean, disposable 2-litre (l) containers provided by the Chemical Pathology Laboratory (CPL) of the SUHT. Each container contained 20 ml of 6 molar (M) hydrochloric acid (HCL) as preservative. The weights of the container before and after collection were measured and the difference in these weights was taken as the volume of urine collected in a 24-hour period. 20 ml aliquots of the urine were placed in sterile containers and the rest discarded. One aliquot was sent immediately to the CPL for analysis of sodium and potassium concentrations while one aliquot was frozen at -20 degree centigrade (°C) for analysis of nitrogen concentration later.

Principle of the Kjeldahl method

The total amount of nitrogen in urine was measured using the Kjeldahl method devised by the Danish chemist Johann Kjeldahl (1849-1900) for estimating the nitrogen content of cereals (Fleck and Munro 1965). The organic matter in the urine is digested by oxidation with sulphuric acid (H_2SO_4) which reduces nitrogen to ammonium sulphate

($(NH_4)_2SO_4$). This is then distilled with sodium hydroxide (NaOH) to liberate ammonia which is trapped in an acidic buffer with indicator. The liberated ammonia is subsequently titrated with standardised HCL. The process was carried out using a Tecator Kjeltec semi-automated system (FOSS UK Ltd, Warrington, United Kingdom).

The Kjeldahl Method

1. To 0.5 ml of urine or 1 ml of standard (3.3% $(NH_4)_2SO_4$ = 700 mg nitrogen) in a large distillation tube, 2 copper sulphate Kjeltabs (act as catalyst) and 12 ml of 98% H_2SO_4 were added.
2. The distillation tubes were placed in a preheated block at 420 °C for 10 minutes until the solution had turned green, which indicated that digestion of the organic matter had completed.
3. After leaving the solution to cool, 70 ml of distilled water was added to each tube.
4. Sufficient quantities of 40% NaOH was added to the digested solution until it turned black and the solution was then steam distilled in a semi-automated system for 3 minutes. The ammonia was collected into a conical flask containing 30 ml of 4% boric acid with pH 4.5 indicator.
5. The dissolved ammonia solution was then titrated against 0.1 M HCL until the solution changed from blue to orange. The amount of acid used for titration was used for the calculation of nitrogen content in the urine sample.
6. Each urine sample was measured in duplicate and the average used in calculations.

Calculation

Urinary nitrogen concentration (g/l) = (volume of acid used x 0.1 x 14.008) / volume of sample

Total urinary nitrogen per 24-hour (g/day) = urinary nitrogen concentration (g/l) x volume of urine passed in 24 hours (l/day)

Repeatability

The determination of total nitrogen in all the urine samples collected in this thesis was conducted by the Clinical Research Fellow and Mrs Angela Hounslow, research assistant of the Institute of Human Nutrition, University of Southampton. The analytical precision of the measurement of urinary nitrogen was determined by repeating the assay on one

urine sample 10 times and the coefficient of variation (CoV) obtained for the Clinical Research Fellow and Mrs Hounslow were 1.44% and 2.10% respectively. Between assays repeatability was determined by the results from the standard assays and qualitative urine control, Lyphochek 2. The CoVs for the Clinical Research Fellow for 5 separate assays were 1.68% and 4.64% for the standard and Lyphochek 2 respectively and the CoVs for Mrs Hounslow were 6.50% and 3.66% respectively.

4.4.6 Determination of 24-hour urinary sodium and potassium excretions

The protocol for urine collection was described in section 4.4.5. A 20 ml urine aliquot was sent to the CPL for analysis of sodium and potassium concentrations in mmol/l. Total urinary sodium excreted over a 24-hour period was obtained by multiplying urinary sodium concentration with volume of urine void in that 24 hour period. Total urinary potassium excreted was calculated using the same formula.

4.4.7 Determination of biological synthetic functions

Fasting blood samples were obtained from ileostomy subjects for the following analyses, all of which were conducted by the CPL:

a. 5 ml of blood was drawn into ethylenediamine-tetraacetic acid (EDTA) vacutainers for determination of haemoglobin levels and leucocyte count.
b. 5 ml of blood was drawn into separate EDTA vacutainer for determination of erythrocyte sedimentation rate (ESR) and heparinised vacutainer for determination of C-reactive protein (CRP).
c. 10 ml of blood was drawn into heparinised vacutainers for determination of renal function (plasma levels of sodium, potassium, urea and creatitine), liver function (plasma levels of alanine transaminase (ALT), aspartate transaminase, alkaline phosphatase, bilirubin, total protein and albumin) and markers of bone health (plasma levels of calcium, magnesium, inorganic phosphate and alkaline phosphatase).

d. 10 ml of blood was drawn into plain vacutainers for determination of plasma micronutrient levels (vitamin A, vitamin B_{12}, 25-hydroxyvitamin D (inactive), 1,25-dihydroxyvitamin D (active), vitamin E, folate, iron, ferritin and transferrin). 2 ml of blood was drawn into sodium citrate vacutainers for determination of International Normalised Ratio (INR) which is widely used as a surrogate marker for vitamin K and a further 2 ml of blood was drawn into special heparinised tubes for determination of zinc, copper and selenium levels.

4.4.8 Statistical analysis

Statistical analyses were carried out using the SPSS for Windows software. As most of the data were not normally distributed, results are expressed as median and range, 25^{th} and 75^{th} percentiles, frequencies and percentages. Non parametric tests (Mann-Whitney Test and Chi-Square Test) were performed to detect statistical significant differences at 95% confidence intervals.

4.5 Results

4.5.1 Characteristics of subjects

The age and sex of all the subjects are shown in Table 4.2. The mean (sd) age difference between the two cohorts is 0.30 (1.98) years. These results showed that the two cohorts were matched for age and sex and thus fulfilled the recruitment criteria.

Table 4.2 Age and sex of ileostomy and reference subjects.

	Ileostomy subjects	**Reference subjects**
Sex	25 females, 35 males	25 females, 35 males
Age (years) : median (range) mean (sd)	56.00 (26–85) 55.83 (14.28)	55.00 (25-87) 55.62 (14.44)

For the 60 ileostomy subjects, the clinical diagnosis, time from surgery to recruitment, age and whether they have had additional small bowel resection are detailed in Table 4.3. The extent of small bowel resection could not be assessed and none of the ileostomy subjects with Crohn's disease had active disease or were receiving steroid treatment for Crohn's disease at the time of recruitment.

Table 4.3 Characteristics of ileostomy subjects.

	NSBR	SBR	Total
N (%)	46 (76.7%)	14 (23.3%)	60 (100%)
Diagnosis:			
Ulcerative colitis	32	6	38 (63.3%)
Crohn's disease	11	8	19 (31.7%)
Others	3	0	3 (5%)
Months post surgery (months): median (range)	49.00 (12-405)	84.50 (51-252) *	
Age (years): median (range)	57.00 (27-82)	59.00 (26-85)	
mean (sd)	57.39 (13.81)	50.71 (15.13)	

Mann-Whitney Test: * p=0.002

4.5.2 Body mass index and body composition

Body weight, height, BMI and body composition of ileostomy and reference subjects are shown in Table 4.4 and comparisons of these measurements by sex are shown in Table 4.5. On the whole, ileostomy subjects had significantly lower body weight and BMI compared to reference subjects. While BF% was similar in both groups, both male and female ileostomy subjects had significantly lower lean body mass compared to reference subjects.

Table 4.4 BMI and body composition of ileostomy and reference subjects

		Ileostomy (n=60)	Reference (n=60)
Height (m)	Median	1.68	1.70
	25th	1.61	1.60
	75th	1.73	1.80
Weight (kg)	Median	67.8*	77.7
	25th	61.7	67.8
	75th	86.2	87.9
BMI (kg/m^2)	Median	25.0*	27.3
	25th	22.0	25.2
	75th	28.4	29.4
Body fat (kg)	Median	20.2	22.9
	25th	16.9	19.6
	75th	25.2	28.4
BF %	Median	29.2	28.4
	25th	23.8	24.5
	75th	35.4	37.2
FFM (kg)	Median	47.8*	52.9
	25th	41.1	45.9
	75th	60.0	64.5

Mann-Whitney Test): *$p<0.05$ ileostomy vs reference

Table 4.5 BMI and body composition of ileostomy and reference subjects by sex

		Ileostomy		Reference	
		Male (n=35)	Female (n=25)	Male (n=35)	Female (n=25)
Height (m)	Median	1.72	1.60	1.70	1.60
	25th	1.69	1.57	1.70	1.60
	75th	1.75	1.65	1.80	1.67
Weight (kg)	Median	79.0*	63.3	83.9	65.0
	25th	65.8	54.4	76.2	61.6
	75th	87.2	66.8	92.1	77.3
BMI (kg/m^2)	Median	26.1	23.6	27.7	26.9
	25th	22.9	20.3	26.0	23.5
	75th	29.1	27.1	29.7	28.8
Body fat (kg)	Median	20.0	21.3	21.9	25.9
	25th	15.9	17.0	19.9	18.9
	75th	24.4	26.3	24.4	31.7
BF %	Median	26.0	35.6	26.7	38.6
	25th	22.8	31.1	24.0	29.3
	75th	29.3	43.3	28.8	41.4
FFM (kg)	Median	58.9*	39.8†	63.8	45.4
	25th	49.5	37.0	55.0	39.8
	75th	64.2	44.5	68.4	46.8

Mann-Whitney Test: * $p<0.05$ male ileostomy vs male reference; †$p<0.05$ female ileostomy vs female reference

Table 4.6 shows the percentage of ileostomy and reference subjects falling into BMI categories of under-, normal and over-weight compared to the general adult population as reported by the National Diet and Nutrition Survey (NDNS) (1998, 2004). The proportion of underweight ileostomy subjects was twice that seen in the general adult population and fewer were overweight. Similar results were evident when compared with the reference cohort although our selection criteria had ensured that our reference cohort contained no individuals who were very underweight or very overweight.

Table 4.6 BMI category of ileostomy, reference and NDNS cohorts

BMI	Category	Ileostomy (%)	Reference (%)	NDNS (%)
<20	Underweight	10	0	4.9
20-25	Normal	40	23.3	33.8
>25	Overweight	50	76.7	61.3

When the effects of additional small bowel resection on BMI and body composition were examined, no significant differences were seen between SBR and NSBR subjects (Appendix 1A). The BMI and body composition of ileostomy subjects with Crohn's disease and those who had ulcerative colitis were also similar (Appendix 1B).

In order to confirm that the reference subjects were of representative body weights, BMI and body composition of the general population, comparisons were made with published national data. The weight and BMI were compared with United Kingdom NDNS data according to age and sex (Appendix 1C) while the body composition indices were compared with the United States Third National Health and Nutrition Examination Survey (NHANES III) (Chumlea et al 2002) of non-Hispanic white adults according to age and sex (Appendix 1D). The results confirmed that our reference group were representative of the general population.

4.5.3 Clinical health

Clinical history

Clinical history was recorded in all 120 subjects and the results are shown in Table 4.7

Table 4.7 Clinical history of ileostomy and reference subjects

	Ileostomy n=60 (%)	Reference n=60 (%)
Perception of general health:		
Good	44 (73.3%)	43 (71.7%)
Fair	10 (26.7%)	8 (13.3%)
Poor	6 (10.0%)	9 (15.0%)
Lethargy:		
No	24 (40.0%)	51 (85.0%)
Yes	36 (60.0%)*	9 (15.0%)
Activity level:		
Active	48 (80.0%)	57 (95.0%)
Not active	12 (20.0%)*	2 (3.4%)
Appetite:		
Good / Normal	56 (93.3%)	58 (96.7%)
Poor	4 (6.7%)	2 (3.3%)
Weight in last 6 months:		
Gain	10 (16.7%)	14 (23.3%)
Stable	42 (70.0%)	41 (68.3%)
Lost	8 (13.3%)	5 (8.4%)
Renal stone – No	57 (95.0%)	57 (95.0%)
- Yes	3 (5.0%)	3 (5.0%)
Gall stone – No	55 (91.7%)	57 (95.0%)
- Yes	5 (8.3%)	3 (5.0%)
Gastrointestinal symptoms:		
Nausea – No	57 (95.0%)	58 (96.7%)
- Yes	3 (5.0%)	2 (3.3%)
Vomiting – No	59 (98.3%)	59 (98.3%)
- Yes	1 (1.7%)	1 (1.7%)
Abdominal pain / discomfort – No	50 (83.3%)	54 (90.0%)
- Yes	10 (13.3%)	6 (10.0%)
Bloating – No	46 (77.7%)	54 (90.0%)
- Yes	14 (23.3%)†	6 (10.0%)
Blood loss / Malaena – No	59 (98.3%)	60 (100%)
- Yes	1 (1.7%)	0
Nutritional symptoms:		
Loss of taste – No	58 (96.7%)	59 (98.3%)
- Yes	2 (3.3%)	1 (1.7%)
Skin – Normal	37 (61.7%)	50 (83.3%)
- Dry	23 (38.3%)*	10 (16.7%)
Hair – Normal	57 (95.0%)	57 (95.0%)
- Hair loss	3 (5.0%)	3 (5.0%)
Nail – Normal	54 (90.0%)	53 (88.3%)
- Brittle	6 (10.0%)	7 (11.7%)
Tendency for infection – No	52 (86.7%)	57 (96.6%)
- Yes	8 (13.3%)	2 (3.4%)

Chi-square: * $p<0.01$ and †$p=0.05$

The results on health perception, appetite and weight were similar in both groups of subjects with only 10% of ileostomy subjects reporting poor health. The incidence of renal and gall stone diseases also were similar in both groups of subjects. However, significantly more ileostomy subjects complained of lethargy and inability to lead an active life compared to the reference subjects and 56.7% (n=34) of ileostomy subjects have 1 or more gastrointestinal symptoms compared to 16.7% (n=10) in reference subjects ($p<0.001$). 56.7% (n=34) of ileostomy subjects also reported 1 or more nutritional symptoms compared to 30.0% (n=18) in reference subjects ($p=0.003$), the commonest complaint being of dry skin.

The BMI of ileostomy subjects who reported lethargy [24.3 kg/m^2 (14.2-42.8)] was similar to those who did not [25.9 kg/m^2 (19.1-32.2)]. The BMI of ileostomy subjects who reported reduced activity levels [25.7 kg/m^2 (17.2-39.9)] was also similar to those who did not [25.0 kg/m^2 (14.2-42.8)], suggesting that lower body weight could not explain these symptoms.

Examining the effects of additional small bowel resection on clinical health, significantly more SBR subjects reported poor health, lethargy, not leading an active life, abdominal pain or discomfort and a tendency to develop infection compared to NSBR subjects (Appendix 2A), and more subjects with Crohn's disease reported lethargy and bloating than those who had ulcerative colitis (Appendix 2B).

Clinical examination

Clinical examination of ileostomy subjects found that only 3 subjects had signs which might indicate malnourishment although there were 11 subjects (18.3%) who had dry skin, 2 of whom had SBR. 1 SBR subject was found to have brittle nails but no other signs of nutrient deficiencies and 2 subjects, one with SBR and 1 without SBR, had mouth ulcers with no other signs of nutrient deficiencies.

57 ileostomy subjects had their blood pressure recorded. The median systolic blood pressure was 132 mmHg [mean (sd): 132 mmHg (28)] and median diastolic blood pressure was 80 mmHg [mean (sd): 84 mmHg (17)]. 17 subjects had blood pressure above 140/90 mmHg. None of the subjects had orthostatic hypotension.

Details of the 3 ileostomy subjects who had clinical evidence suggestive of malnutrition are shown in Table 4.8. The common features in these 3 subjects were:

a. Over 70 years of age
b. Had ulcerative colitis
c. More than 3 years post surgery at the time of recruitment
d. Reported lethargy
e. Reported dry skin
f. Evidence of muscle wasting and dry skin
g. Low urinary excretion of sodium
h. Urinary nitrogen excretions in the 25^{th} percentile and highly positive apparent nitrogen balances suggest high stomal nitrogen losses

Table 4.8 Details of three subjects with clinical evidence of malnutrition

	Subject 023	Subject 021	Subject 001
Sex	Male	Male	Female
Age	71	85	71
Clinical diagnosis	Ulcerative colitis	Ulcerative colitis	Ulcerative colitis
SBR	No	No	No
Months post surgery	42	104	94
General health	Poor	Fair	Good
Lethargy	Yes	Yes	Yes
Activity	Not active	Not active	Active
Appetite	Poor	Good	Good
Weight loss	No	No	No
GI symptoms	No	Nausea only	No
Nutritional symptoms	Dry skin only	Dry skin only	Dry skin only
Stomal function	200ml/semi-form	200ml/semi-form	200ml/liquid
Muscle wasting	Yes	Yes	Yes
Oedema	Yes	No	No
Ascites	No	No	No
Nutritional signs	Dry skin only	Dry skin only	Dry skin only
BMI	21.4	17.2	14.3
Energy intake	2812 kcal/day	3599 kcal/day	2224 kcal/day
Nitrogen intake	26.6 g/day	19.7 g/day	16.6 g/day
Urinary nitrogen excretion	6.4 gN/day	6.8 gN/day	3.3 gN/day
Urinary sodium excretion	Low	Low	Low
Apparent nitrogen balance	20.4 gN/day	19.7 gN/day	16.6 gN/day
Vitamins status	Low A,D,E,K	Low B_{12}	Normal
Trace elements status	Low selenium	Normal	Normal
Inflammatory markers	Normal	Raised	Normal
Albumin	45	31	39
Status after recruitment	Deceased	Deceased	

Although subject 023 had a BMI of 21.7, he also had dependent oedema. If the weight related to this fluid retention was taken into account, this subject, like the other two, might also be underweight. 2 out of these 3 subjects had micronutrient deficiencies, both of whom have died since participating in this study.

Stomal function

Stomal function was also assessed in ileostomy subjects. The average estimated stool volume reported by these subjects was 400 ml (range 200–2300 ml). The estimated stool volume for SBR subjects [500 ml (range 200-2300ml)] was similar to NSBR subjects [400 ml (range 200-1200ml)]. 2 out of 14 SBR subjects (14.3%) reported estimated stool volumes of 1800 ml and 2300 ml and 2 out 46 subjects (4.3%) in the NSBR group reported estimated stool volumes of 1100 ml and 1200 ml. Comparing subjects who had ulcerative colitis to those with Crohn's disease, the estimated stool volumes were also similar [400 ml (range 200-900 ml) and 400 ml (range 200-2250 ml) respectively]. Of the 60 ileostomy subjects, 12 (20%) reported passing liquid or loose stools while the remainder had semi-formed stools. 6 of these 12 subjects had SBR. Thus, proportionately more SBR subjects had liquid or loose stools compared to NSBR subjects (42.6% vs 13.0%, p=0.024).

All 4 subjects who reported estimated daily stool volume in excess of 1000 ml complained of lethargy and 1 of these 4 subjects also had reduction in activity level. Proportionately more subjects who had liquid or loose stools reported lethargy (91.7% vs 52.1% respectively, p=0.019) and reduced activity levels (50% vs 12.5 % respectively, p=0.004) compared to subjects who had semi-formed stools. However, although there was a tendency for lower BMIs [24.3 kg/m^2 (14.2-38.0) vs 25.8 kg/m^2 (17.2-42.8)] and more underweight subjects (16.7% vs 8.3%) amongst those with liquid stools compared to those who had semi-formed stools, these differences are not significant. The incidence of gastrointestinal and nutritional symptoms were similar in subjects who had liquid or loose stools and those who had semi-formed stools.

In summary, although only 5% of ileostomy subjects had clinical evidence of nutrient deficiencies and 10% were reported to be in poor health, a significant proportion of ileostomy subjects reported a reduction in their physical function. Furthermore, more than half of these ileostomy subjects also reported gastrointestinal and nutritional

symptoms. The health of ileostomy subjects would therefore appear to be compromised. The incidence of poor health and reduced physical function were also higher in SBR and Crohn's subjects compared to NSBR and ulcerative colitis subjects respectively and proportionately more SBR subjects reported passing liquid stools compared to NSBR subjects, presumably due to reduced small bowel availability causing malabsorption.

4.5.4 General health

The SF-36 questionnaire was completed by all 120 reference and ileostomy subjects. The scores are presented in median, range, 25th and 75th percentiles except in Table 4.9 where they are presented in mean and standard deviation so that comparisons could be made with normative data from the Oxford Healthy Life Survey (OHLS) (Jenkinson et al 1993).

Table 4.9 Mean health scores of OHLS, reference and ileostomy subjects.

	OHLS (n=9332) Mean (sd)	Reference (n=60) Mean (sd)	Ileostomy (n=60) Mean (sd)
Physical function	88.40 (17.98)	91.42 (9.70)	70.42 (28.33)*
Role - Physical	85.82 (29.93)	93.33 (22.01)	68.75 (41.84)*
Role – Mental	82.93 (31.76)	91.11 (25.21)	74.44 (40.88)*
Social function	88.01 (19.58)	96.48 (7.79)	80.93 (24.64)*
Mental health	73.77 (17.24)	81.40 (11.43)	71.80 (19.96)*
Energy	61.13 (19.67)	68.92 (14.29)	50.75 (23.84)*
Pain	81.49 (21.67)	87.41 (15.37)	73.15 (30.84)*
Health perception	73.52 (19.90)	74.77 (15.73)	58.83 (25.73)*

Independent sample T-Test: *p<0.01 ileostomy vs reference

The data show that the mean score for each of the eight health dimensions was slightly higher in our reference population than in the normative data, whereas the mean scores for all eight dimensions were lower in the ileostomy subjects compared to the normative data and to the reference population.

Tables 4.10 and 4.11 show the median health scores for reference and ileostomy subjects respectively. The median scores for all eight health dimensions were significantly lower in ileostomy subjects than in reference subjects.

Table 4.10 Health scores of reference subjects.

	Median (range)	25th percentile	75th percentile
Physical function	95.00 (60-100)	86.25	100
Role - Physical	100 (0-100)	100	100
Role – Mental	100 (0-100)	100	100
Social function	100 (0-100)	100	100
Mental health	84.00 (44-100)	76.00	92.00
Energy	70.00 (35-95)	60.00	80.00
Pain	88.89 (33-100)	77.78	100
Health perception	77.00 (35-100)	67.00	87.00

Table 4.11 Health scores of ileostomy subjects.

	Median (range)	25th percentile	75th percentile
Physical function	80.00 (0-100)*	50.00	95.00
Role - Physical	100.00 (0-100)*	25.00	100
Role – Mental	100.00 (0-100)†	33.33	100
Social function	100.00 (11-100)*	66.67	100
Mental health	76.00 (8-100)†	61.00	100
Energy	52.50 (0-90)*	35.00	68.75
Pain	88.89 (0-100)†	47.22	100
Health perception	62.00 (0-100)*	42.75	82.00

Mann-Whitney Test: *$p<0.001$, †$p<0.05$ ilesostomy vs reference

The data were examined by sex since inherent differences were observed between men and women in the OHLS (Jenkinson et al 1993). In the reference cohort, all the health scores apart from physical function were similar between male and female subjects (Appendix 3A). In the ileostomy cohort, all the health scores were also similar between

male and female subjects (Appendix 3B). Comparisons of health scores between reference and ileostomy cohorts by sex are shown in Tables 4.12 and 4.13.

Table 4.12 Health scores of female reference and ileostomy subjects

	Female reference (n=25) Median (25^{th}-75^{th} percentile)	Female ileostomy (n=25) Median (25^{th}-75^{th} percentile)
Physical function	100.00 (90-100)	75.00 (47.50-97.50)*
Role - Physical	100.00 (100-100)	100.00 (50.00-100)†
Role – Mental	100.00 (100-100)	100.00 (66.67-100)†
Social function	100.00 (100-100)	100.00 (61.11-100)
Mental health	84.00 (76-90)	68.00 (52.00-88.00)†
Energy	75.00 (65-80)	50.00 (32.50-65.00)*
Pain	88.89 (83.33-100)	88.89 (50.00-100.00)
Health perception	82.00 (72-91)	67.00 (37.50-84.50)†

Mann-Whitney Test: *p<0.001, †p<0.05 ileostomy vs reference

Table 4.13 Health scores of male reference and ileostomy subjects

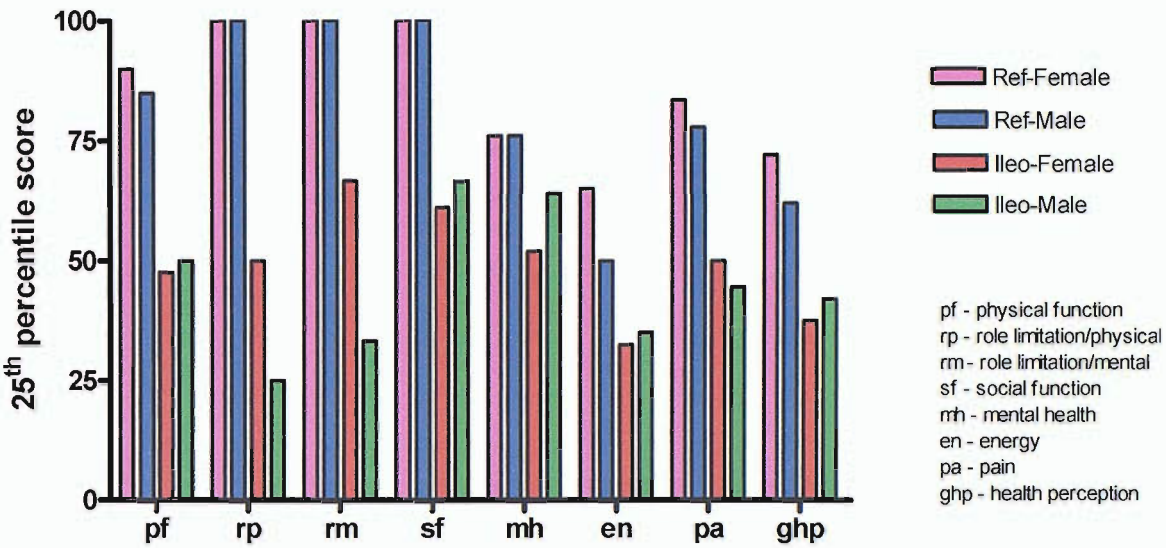
	Male reference (n=35) Median (25^{th}-75^{th} percentile)	Male ileostomy (n=35) Median (25^{th}-75^{th} percentile)
Physical function	95.00 (85.00-100)	80.00 (50.00-90.00)*
Role - Physical	100.00 (100-100)	100.00 (25.00-100)*
Role – Mental	100.00 (100-100)	100.00 (33.33-100)
Social function	100.00 (100-100)	77.78 (66.67-100)*
Mental health	84.00 (76.00-92.00)	76.00 (64.00-92.00)
Energy	70.00 (50.00-80.00)	55.00 (35.00-75.00)†
Pain	88.89 (77.78-100)	88.89(44.44-88.89)†
Health perception	77.00 (62.00-85.00)	62.00(42.00-77.00)†

Mann-Whitney Test: *p<0.001, †p<0.05 ileostomy vs reference

Both female and male ileostomy subjects had significantly lower scores in six out of the eight health dimensions compared to their reference counterparts and mark differences in

the 25th percentile scores were also evident between the two cohorts indicating that the health of the most unwell was substantially worst in ileostomy subjects than in reference subjects (Figure 4.1).

Figure 4.1 25th percentile health scores of reference and ileostomy subjects



Inherent differences by age were also reported in the OHLS (Jenskinson et al 1993) and this was examined in our data. In the reference cohort, all the health dimensions apart from physical function were similar among age groups 25-44, 45-64 and 65-87 (Appendix 3C). In the ileostomy cohort, all the health dimensions were also similar among these three age groups (Appendix 3D). Comparisons between the two cohorts by age groups are shown in Tables 4.14, 4.15 and 4.16.

Table 4.14 Health scores of reference and ileostomy subjects between 25-44 years

	Reference (n=10) Median (25^{th}-75^{th} percentile)	Ileostomy (n=10) Median (25^{th}-75^{th} percentile)
Physical function	100.00 (95.00-100)	82.50 (41.25-96.25)*
Role - Physical	100.00 (100-100)	62.50 (0-100)*
Role – Mental	100.00 (100-100)	66.67 (0-100)*
Social function	100.00 (94.44-100)	72.22 (44.44-100)*
Mental health	82.00 (67.00-88.00)	62.00 (45.00-74.00)*
Energy	67.50 (50.00-76.25)	37.5 (11.25-52.50) †
Pain	88.89 (77.78-100)	77.78 (33.33-91.67)
Health perception	82.00 (63.25-87.50)	59.50 (35.25-67.75)*

Mann-Whitney Test: †p=0.003, *p<0.05 ileostomy vs reference

Table 4.15 Health scores of reference and ileostomy subjects between 45-64 years

	Reference (n=35) Median (25^{th}-75^{th} percentile)	Ileostomy (n=32) Median (25^{th}-75^{th} percentile)
Physical function	95.00 (90.00-100)	85.00 (61.25-95.00)*
Role - Physical	100.00 (100-100)	100.00 (75-100)*
Role – Mental	100.00 (100-100)	100.00 (100-100)
Social function	100.00 (100-100)	100.00 (66.67-100) †
Mental health	84.00 (76.00-88.00)	78.00 (64.00-92.00)
Energy	60.00 (60.00-75.00)	62.50 (45.00-75.00)*
Pain	88.89 (77.78-100.00)	88.89 (33.33-100)
Health perception	77.00 (67.00-87.00)	64.50 (42.75-82.00)*

Mann-Whitney Test: †p=0.005, *p<0.05 ileostomy vs reference

Table 4.16 Health scores of reference and ileostomy subjects between 65-87 years

	Reference (n=15) Median (25^{th}-75^{th} percentile)	Ileostomy (n=18) Median(25^{th}-75^{th} percentile)
Physical function	90.00 (90.00-95.00)	65.00 (50.00-81.25) †
Role - Physical	100.00 (100-100)	50.00 (0-100)*
Role – Mental	100.00 (100-100)	100.00 (33.33-100)
Social function	100.00 (100-100)	100.00 (66.67-100)
Mental health	88.00 (76.00-92.00)	76.00 (63.00-89.00)
Energy	80.00 (60.00-80.00)	42.50 (23.75-67.50)†
Pain	88.89 (77.78-100.00)	66.67 (88.89-100)
Health perception	77.00 (62.00-82.00)	62.00 (43.75-83.25)

Mann-Whitney Test: †p=0.005, *p<0.05 ileostomy vs reference

For subjects between 25-44 years, the scores of seven out of eight health dimensions were significantly lower in the ileostomy cohort than in the reference cohort, whereas in the 45-64 and 65-87 age groups, the scores were significantly lower in five and three health dimensions respectively comparing the ileostomy cohorts to the reference cohorts. These results suggest that total colectomy has a greater impact on the general health of the younger age group than in the older age group.

The effects of additional small bowel resection on the health dimensions are shown in Table 4.17. SBR subjects scored significantly lower than NSBR subjects in five out of eight health dimensions, although no differences were seen in all eight health dimensions between ileostomy subjects with Crohn's disease and those who had ulcerative colitis (Appendix 3E).

Table 4.17 Health scores of NSBR and SBR ileostomy subjects

	NSBR (n=46) Median (25^{th}-75^{th} percentile)	SBR (n=14) Median (25^{th}-75^{th} percentile)
Physical function	80.00 (58.75-95.00)	52.50 (20.00-82.50)*
Role - Physical	100.00 (50.00-100)	50.00 (0-100)*
Role – Mental	100.00 (91.67-100)	66.67 (0-100)*
Social function	100.00 (66.67-100)	72.22 (55.56-100)
Mental health	78.00 (64.00-89.00)	58.00 (39.00-89.00)*
Energy	57.50 (35.00-75.00)	40.00 (15.00-61.50)*
Pain	88.89 (63.89-100.00)	22.22 (61.11-100)
Health perception	62.00 (46.50-82.00)	40.00 (13.75-74.50)

Mann-Whitney Test: *p<0.05 ileostomy vs reference

4.5.5 Dietary intake

The FFQ was completed by all 120 reference and ileostomy subjects. However, as 1 ileostomy subject (subject 52) did not complete a 24-hour urinary collection and for the ease of data examination for nitrogen balance, this subject's dietary intake was not included in the analysis.

Protein intakes reported by both ileostomy and reference subjects were distributed over a wide range but were similar to each other [median (range) g/day: 99.5 (42.7-212.9) vs 91.3 (38.9-231.8) respectively] (Figure 4.2). The equivalent median (range) nitrogen intakes for ileostomy and reference subjects were 15.9 g/day (6.8-34.1) and 14.6 g/day (6.2-37.1) respectively. Energy intakes between ileostomy and reference subjects were also similar [median (range) kcal/day: 2443 (1151-5203) vs 2282 (1043-6255) respectively] (Figure 4.3) but the intakes of NSP, starch, magnesium, iron, carotene and vitamin C were all significantly lower in ileostomy subjects compared to reference subjects (Appendix 4A).

Figure 4.2 Protein intakes of ileostomy and reference subjects

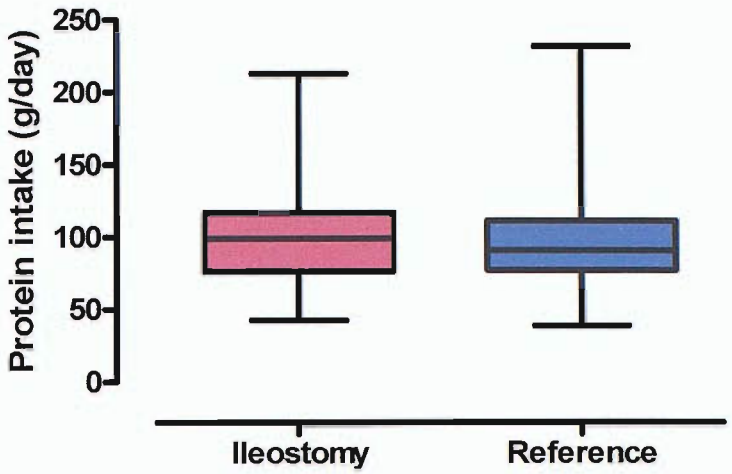
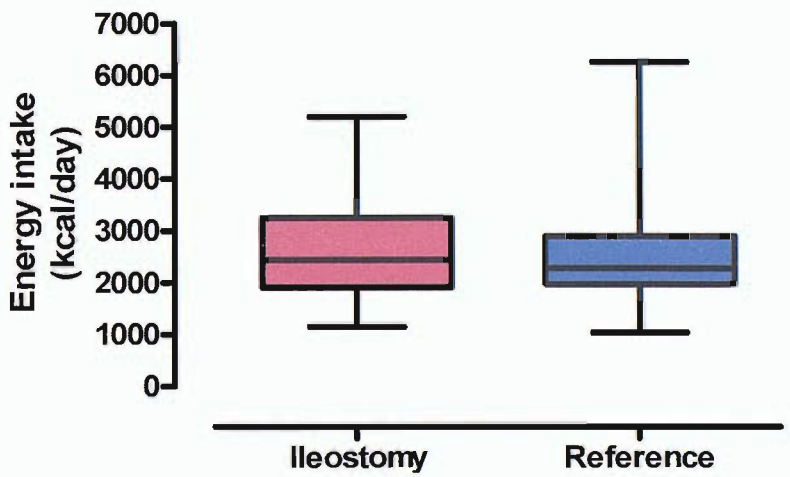


Figure 4.3 Energy intakes of ileostomy and reference subjects



The intakes of protein, energy and all other nutrients measured were similar between SBR and NSBR subjects (Appendix 4B) and between subjects with Crohn's disease and ulcerative colitis except for carbohydrate and calcium which were significantly lower in subjects with Crohn's disease compared to those with ulcerative colitis (Appendix 4C).

Food avoidance

37 out of 59 ileostomy subjects (62.7%) reported avoidance of certain foods as a direct result of having a stoma. The characteristics of these subjects are illustrated in Table

4.18. Foods such as nuts, apples, pears, salads, raw vegetables, peas and sweet corn were avoided due to the fear of stomal blockage. Baked beans were also avoided due to excessive flatus and several subjects reported avoiding fatty foods which caused diarrhoea. A few subjects reported that they were specifically advised by dieticians and stoma nurses to avoid these foods and one subject was asked to follow a low residue diet by a stoma care nurse.

Table 4.18 Characteristics of ileostomy subjects who reported food avoidance

	NSBR (total n=46)	SBR (total n=14)
Crohn's disease	7 (64% of 11)	4 (50% of 8)
Ulcerative colitis	21 (66% of 32)	5 (83% of 6)
Others	0 (0% of 3)	

As previously mentioned, the intakes of NSP, starch, magnesium (Mg), iron, carotene and vitamin C were significantly lower in ileostomy subjects than reference subjects, but these observations did not appear to be due to deliberate food avoidance by some subjects and Table 4.19 suggest that the intakes of these nutrients were low in ileostomy subjects regardless of specific dietary measures. In addition, no significant differences were found in all nutrient intakes apart from selenium when comparing ileostomy patients who avoided certain foods with those who did not (Appendix 4D).

Table 4.19 Nutrient intakes of reference and ileostomy subjects who avoided and did not avoid certain foods [median (range)]

Nutrient intake (per day)	Reference n=60	Food avoidance n=37	No food avoidance n=22
Energy (kcal)	2282 (1043-6255)	2380 (1151–4167)	2619 (1243–203)
Protein (g)	91.3 (38.9-231.8)	99.5 (47.5–212.9)	100.6 (42.7-188.2)
NSP (g)	23.0 (6.8-50.7)	16.7 (5.6–40.4)*	18.5 (9.9-30.6)†
Starch (g)	131.3 (38.0-559.2)	150.5 (13.1–284.8)	161.3 (94.2–289.8)†
Mg (mg)	388.8 (150.4-829.8)	343.2 (169.7–667.6)*	331.7 (142.3–620.4)
Iron (mg)	15.5 (4.5-37.1)	13.7 (4.0–23.9)*	14.3 (6.4–24.2)
Carotene (ug)	3277 (1333-41024)	2632 (572–6059)*	2829 (747–6632)†
Vitamin C (mg)	178.5 (55.0-398.2)	128.4 (42.7–476.2)*	113.3 (47.5–261.7)†

Mann-Whitney Test: * $p<0.05$ reference vs food avoidance, †$p<0.05$ reference vs no food avoidance

4.5.6 Urinary nitrogen excretion

All 60 reference subjects and 59 ileostomy subjects completed the 24-hour urinary collection. Although urinary nitrogen excretions were widely distributed, particularly in the ileostomy subjects, the median value was significantly lower in the ileostomy group [median (range) g/day: 9.8 (2.7-21.9) vs 12.0 (3.8-18.1) respectively, p=0.002] (Figure 4.4). Urinary nitrogen excretions of SBR subjects were also significantly lower compared to NSBR subjects [median (range) g/day: 6.8 (3.0-13.4) vs 10.3 (2.7-21.9) respectively, p=0.018] (Figure 4.5). Ileostomy subjects with Crohn's disease and ulcerative colitis had similar urinary nitrogen excretion [median (range) g/day: 8.3 (3.0-19.8) vs 9.9 (5.3-21.9) respectively].

Figure 4.4 Urinary nitrogen excretions of ileostomy and reference subjects

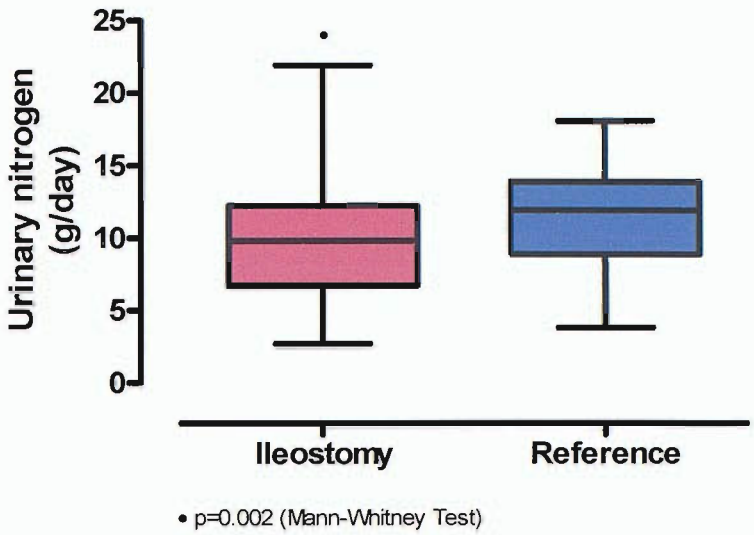
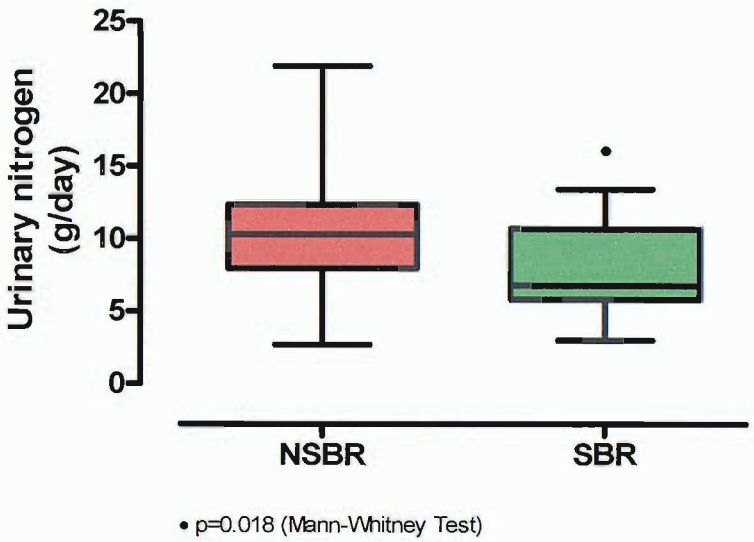


Figure 4.5 Urinary nitrogen excretions of NSBR and SBR ileostomy subjects



4.5.7 Apparent nitrogen balance

Nitrogen balance is normally calculated by subtracting urinary, faecal and other miscellaneous nitrogen losses from dietary nitrogen intake. In this study however, we could merely calculate apparent nitrogen balance since only dietary nitrogen intake and urinary nitrogen excretion were measured. Both cohorts were in positive balance with ileostomy subjects significantly more positive than reference subjects (Table 4.20).

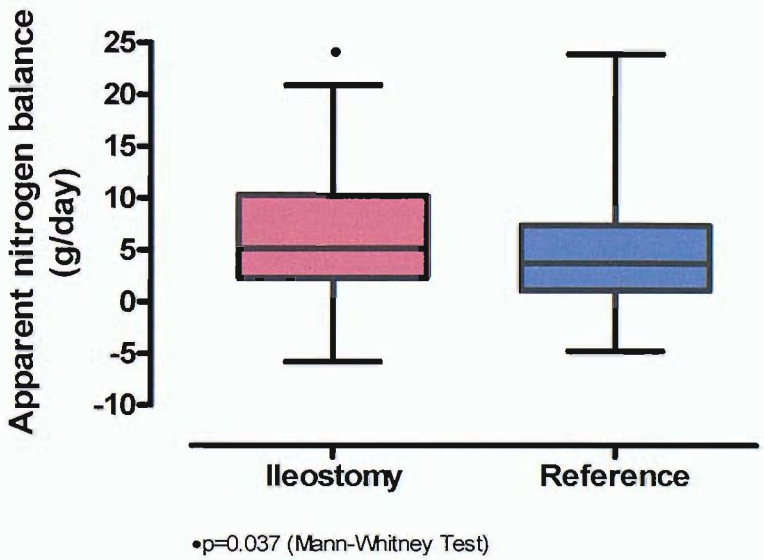
However, apparent nitrogen balances were distributed over a wide range for both groups (Figure 4.6). There were no significant differences in the apparent nitrogen balances between SBR and NSBR subjects or between subjects with Crohn's disease and those who had ulcerative colitis (Table 4.20).

Table 4.20 Apparent nitrogen balance of ileostomy and reference subjects [median (range)]

	Nitrogen intake g/day	**Urinary nitrogen g/day**	**Nitrogen balance g/day**
Ileostomy subjects	15.9 (6.8-34.1)	9.8 (2.7-21.9)†	5.1 (-5.8-20.9)*
Reference subjects	14.6 (6.2-37.1)	12.0 (3.8-18.1)	3.6 (-4.8-23.8)
Ileostomy subjects :			
NSBR	16.2 (7.6-34.1)	10.3 (2.7-21.9)	4.9 (-4.8-20.4)
SBR	14.1 (6.8-28.4)	6.8 (3.0-13.4)‡	7.9 (-5.8-20.9)
Ileostomy subjects:			
Crohn's disease	14.2 (6.8-34.1)	8.3 (3.0-19.8)	4.9 (-5.820.9)
Ulcerative colitis	16.4 (7.6-29.0)	9.9 (5.3-21.9)	4.9 (-4.8-20.4)

Mann-Whitney Test: *p=0.037 & †p=0.002, ileostomy vs reference, ‡p=0.018, SBR vs NSBR

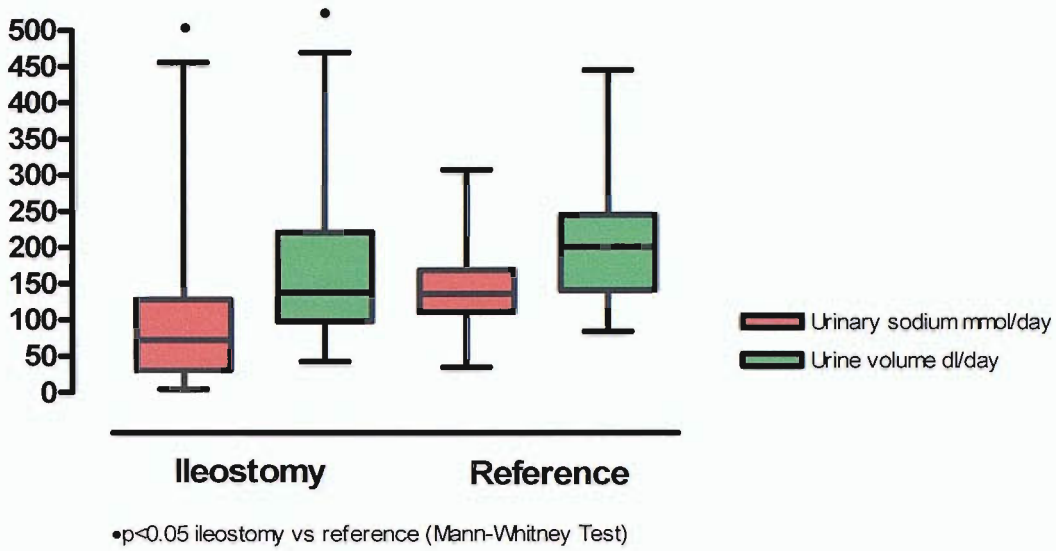
Figure 4.6 Apparent nitrogen balance of ileostomy and reference subjects



4.5.8 Urinary excretions of sodium and potassium and urine volume

All 60 reference subjects and 59 ileostomy subjects completed a 24-hour urinary collection. Figure 4.7 illustrates the distribution of urinary sodium excretions and urine volume of ileostomy and reference subjects.

Figure 4.7 Urinary sodium and urine volume of ileostomy and reference subjects



Urinary sodium excretions were significantly lower in ileostomy subjects compared to reference subjects [median (range): 73 mmol/day (5-456) v 137 mmol/day (35-307), $p<0.05$]. Urine volumes were also significantly lower in ileostomy subjects compared to reference subjects [median (range): 138 decilitre (dl)/day (43-469) v 200 dl/day (84-444), $p<0.05$]. Comparing SBR subjects with NSBR subjects, both urinary sodium excretions subjects [median (range): 27 mmol/day (5-456) v 88 mmol/day (6-328), $p<0.05$] and urine volumes [median (range): 96 dl/day (43-459) v 148 dl/day (46-469), $p<0.05$] were significantly lower in SBR subjects. For subjects who had Crohn's disease versus those who had ulcerative colitis, only urine volume was significantly lower in the Crohn's group [median (range): 104 dl/day (43-389) v 152 dl/day (57-469), $p<0.05$] while urinary sodium was similar [median (range): 58 mmol/day (5-456) v 90 mmol/day (5-263)].

The results of urinary potassium excretion are shown in Table 4.21. No significant differences were seen in urinary potassium excretion between ileostomy and reference subjects or between NSBR and SBR subjects but the urinary sodium:potassium ratio was

significantly lower in ileostomy subjects, particularly those who had SBR. Urinary potassium and urinary sodium:potassium ratio between subjects with Crohn's disease and those who had ulcerative colitis were similar.

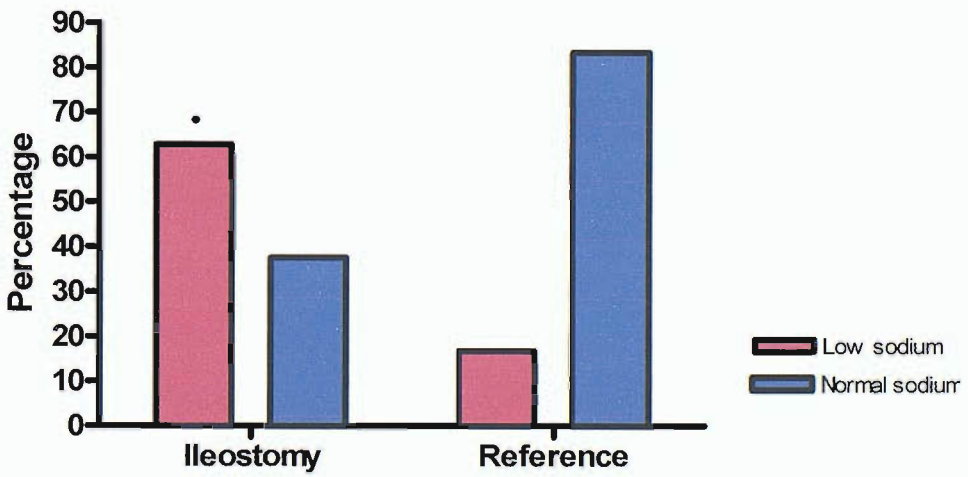
Table 4.21 Urinary potassium excretion and urinary sodium:potassium ratio of ileostomy and reference subjects [median (range)]

	Urinary potassium (mmol/day)	**Urinary sodium:potassium**
Ileostomy subjects	78 (24-155)	0.99 (0.06-5.85) *
Reference subjects	81 (24-149)	1.72 (0.79-3.79)
NSBR subjects	80 (32-137)	1.10 (0.10-4.05)
SBR subjects	62 (24-155)	0.39 (0.06-5.85) †
Crohn's disease subjects	68 (24-109)	0.64 (0.06-5.85)
Ulcerative colitis subjects	80 (46-155)	1.09 (0.11-3.16)

Mann-Whitney Test: *p<0.001 vs reference; †p<0.001 vs NSBR

Figure 4.8 shows the frequency of subjects who had normal or low urinary sodium excretions, defined as less than 100 mmol/day excretions based on CPL reference values.

Figure 4.8 Frequency of subjects with low and normal urinary sodium excretions



•p<0.001 ileostomy v reference (Chi-square)

There were significantly more ileostomy subjects with low urinary sodium (62.7%) than reference subjects (16.7%). However, the proportion of SBR subjects who had low urinary sodium excretions (78.6%) was not significantly different to NSBR subjects (57.8%). The proportion of subjects who had Crohn's disease with low urinary sodium excretions (68.4%) was also similar to subjects who had ulcerative colitis (56.8%).

Table 4.22 shows the relationship between urinary sodium excretion and BMI, FFM, nitrogen intake, urinary nitrogen and apparent nitrogen balance of ileostomy and reference subjects.

Table 4.22 BMI, FFM, nitrogen intake, urinary nitrogen and nitrogen balance of subjects with low and normal urinary sodium excretions [median (range)]

	Ileostomy (n=59) Urinary sodium		**Reference (n=60) Urinary sodium**	
	Low (n=37)	**Normal (n=22)**	**Low (n=10)**	**Normal (n=50)**
BMI (kg/m^2)	23.9* (14.3–33.0)	28.4 (16.6-43.0)	24.9† (21.6-32.2)	27.7 (20.2-31.7)
FFM (kg)	44.1* (19.3-73.0)	59.5 (36.6-67.9)	46.0 (38.8-73.8)	55.7 (34.0-73.5)
Nitrogen intake (g/24 hours)	14.9 (7.6-30.1)	16.8 (6.8-34.1)	15.1 (6.2-17.7)	14.6 (8.3-37.1)
Urinary nitrogen (g/24 hours)	7.9* (2.7-13.7)	11.8 (5.3-21.9)	10.4 (3.8-18.1)	12.3 (5.9-17.6)
Nitrogen balance (g/24 hours)	6.9 (-1.0-20.9)	4.4 (-5.8-17.9)	3.0 (-2.3-12.3)	3.6 (-4.8-23.8)

Mann-Whitney Test: *$p<0.001$ ileostomy, †$p=0.035$ reference; low urinary sodium vs normal urinary sodium

In both cohorts, BMI was significantly lower in subjects who had low urinary sodium compared to those who had normal urinary sodium. However, significantly lower FFM was only seen in ileostomy subjects with low urinary sodium compared to those who had normal urinary sodium. Despite similar nitrogen intakes, urinary nitrogen was significantly lower in ileostomy subjects who had low urinary sodium compared to those

who had normal urinary sodium although apparent nitrogen balance between these two groups of ileostomy subjects was not significantly different. For reference subjects, nitrogen intake, urinary nitrogen and apparent nitrogen balance were similar between subjects who had low and normal urinary sodium.

The results of urinary sodium and nitrogen excretions, urine volume and apparent nitrogen balance were also analysed for differences among subjects who were underweight, normal weight or overweight (Table 4.23).

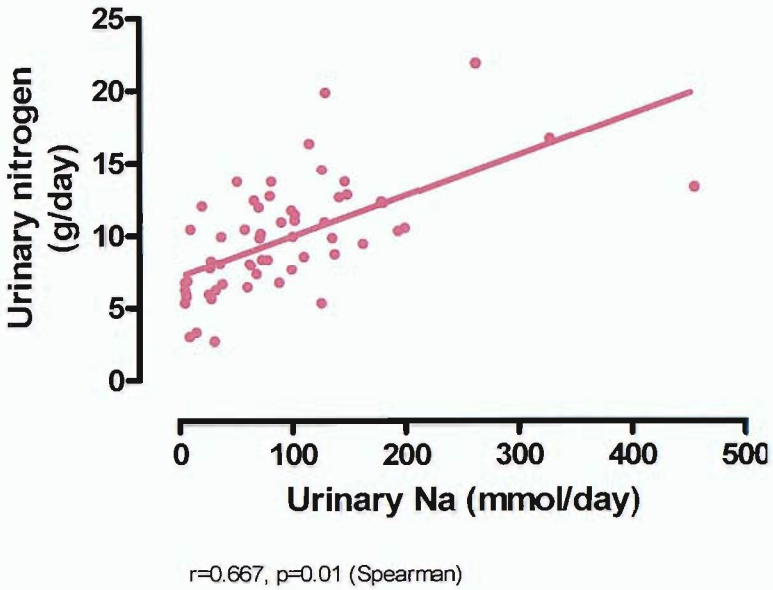
Table 4.23 Urine volume, urinary sodium, urinary nitrogen and nitrogen balance of subjects based on BMI classification [median (range)]

	Urine volume l/day	**Urinary sodium mmol/day**	**Urinary nitrogen g/day**	**Nitrogen balance g/day**
Ileostomy: n=59				
BMI<20, n=6	0.8 (0.5-1.5)*	31 (7-110)*	7.4 (3.3-10.4)*	9.9(-1.0-17.9)*
20<BMI<25,n=23	1.2 (0.5-4.2)	51 (32-328)	8.2 (2.7-16.7)	6.0(0.04-20.9)
BMI>25: n=30	1.7 (0.4-4.7)	101 (5-456)	11.0 (3.0-21.9)	4.2 (-5.8-16.3)
Reference: n=60				
20<BMI<25,n=14	2.1 (0.9-3.2)	118 (65 (242)	10.1 (4.8-18.1)	3.5 (-3.6-12.8)
BMI>25: n=46	2.0 (0.8-4.4)	146 (35-307)	12.3 (3.8-17.6)	3.6 (-4.78-23.8)

Kruskal-Wallis Test: *p<0.05

Ileostomy subjects who were underweight had significantly lower urinary sodium excretion, urinary nitrogen excretion, urine volume and more positive apparent nitrogen balance compared to those who were classified as average weight or overweight. There were no underweight reference subjects and no significant differences were seen between those who were of average weight compared to those who were overweight. Figure 4.9 shows a significant correlation between urinary sodium and nitrogen excretions indicating that subjects who were sodium depleted were also at risk of nitrogen depletion.

Figure 4.9 Correlation of urinary nitrogen and urinary sodium excretions in ileostomy subjects



4.5.9 Blood indices

Due to financial constraints, blood indices were measured only in the 60 ileostomy subjects and the results were compared to SUHT CPL reference ranges. It should also be noted that although 30 ileostomy subjects took no nutritional supplements, 11 took cod liver oil and/or evening primrose oil alone and 19 took combinations of multivitamins, calcium and vitamin D supplements and cod liver oil. 3 ileostomy subjects also received regular vitamin B_{12} injections. Since the nutritional supplements consisted of various formulations, it was not possible to ascertain the exact content and the doses of supplements taken and hence the effects of these supplements on the following blood indices could not be examined.

Haemoglobin level

Only 2 ileostomy subjects (3.3%) (1 NSBR, 1 SBR), had haemoglobin levels below the normal range. One subject had a haemoglobin level of 73 g/l associated with low iron status and the other subject had a haemoglobin level of 115 g/l with normal iron levels. The subject who had low iron status took multivitamin supplements regularly while the other subject took only zinc and vitamin C supplements.

4 ileostomy subjects (6.7%) (3 NSBR, 1 SBR) had polycythaemia with haemoglobin levels between 170-174 g/l and raised pack cell volume. Dehydration could not account for these results as their urea and electrolytes were within normal range. 2 of these subjects were smokers which could explain the raised levels of haemoglobin but it was not clear why the haemoglobin levels should be raised in the other 2 subjects.

Inflammatory status

11 (6 NSBR, 5SBR) (18.3%) out of 60 ileostomy subjects had raised ESRs ranging from 21 mm to 62 mm. 7 (4 NSBR, 3 SBR) (11.7%) out of 60 ileostomy subjects had mildly raised CRP ranging from 9.0-25.5 mg/l. Of these subjects, 5 (2 NSBR, 3 SBR) had raised ESR and CRP. No subject had a raised leucocyte count.

It is not clear why these subjects should have an elevated inflammatory response although 8 of these subjects had suffered from Crohn's disease and some of these may have had a low grade, ongoing inflammatory gastrointestinal problem.

Renal function

All the subjects had normal plasma sodium concentration and only 1 NSBR subject had hypokalaemia (potassium 3.2 mmol/l). 9 subjects (15%) (7 NSBR, 2 SBR) had mildly raised urea concentrations between 6.9-9.7 mmol/l while 2 NSBR subjects had slightly low urea levels of 2.7 and 2.9 mmol/l. All subjects apart from one had normal creatinine levels. The creatinine level in this NSBR subject was marginally raised at 128 mmol/l and his urea level was 9.7 mmol/l. Overall, there was no evidence of renal impairment in this cohort of ileostomy subjects. The slight elevation in urea and creatinine levels could reflect mild dehydration as all the blood samples were drawn following an overnight fast.

Liver function

4 subjects (2 NSBR, 2 SBR) (6.7%) had mildly elevated bilirubin levels (20-25 umol/l) with normal levels of alkaline phosphatase and only one of these 4 subjects had slight elevation of ALT at 53 international units (iu)/l. 10 other subjects (5 NSBR, 5 SBR) (18.3%) had elevated ALT levels (46-122 iu/l) and one of these (ALT 46 iu/l) also had raised alkaline phosphatase level of 696 iu/l, a slightly low albumin of 31 g/l but a normal bilirubin level. This subject's calcium and inorganic phosphate levels were

normal. All other subjects' alkaline phosphatase, albumin and total protein levels were normal.

In this study, it was not possible to ascertain the cause or extent of liver abnormality in these 14 subjects. Nevertheless, it is worth noting that most abnormalities were mild and there was no evidence of decompensated chronic liver disease in any of these subjects. Liver synthetic function was normal and none had stigmata of chronic liver disease. However, of these 14 subjects, 6 had raised ESR and or CRP which might indicate the presence of an inflammatory process within the liver. 6 other subjects had raised fasting lipid levels which might suggest steatohepatitis. 1 subject was known to have viral hepatitis B infection and 1 subject had non-insulin dependent diabetes which could lead to fatty infiltration of the liver.

Markers of bone health

All the ileostomy subjects had normal plasma calcium levels while 3 out of 60 (5%) (2 SBR, 1 NSBR) had low plasma magnesium levels (0.51-0.69 mmol/l). Of the 3 subjects with hypomagnesaemia, 2 were taking multivitamin supplements (both SBR) and one was not. 1 of the SBR subjects whose plasma magnesium level was 0.51 mmol/l also had low inorganic phosphate level (0.61 mmol/l) although his alkaline phosphatase level was normal. All the other ileostomy subjects had normal inorganic phosphate levels. Only 1 subject, who had additional small bowel resection, had a raised alkaline phosphatase level of 690 iu/l although this was not associated with low levels of plasma calcium, magnesium or inorganic phosphate.

From the above, there is no evidence that this cohort of ileostomy subjects, apart from 1 subject, had compromised bone health. However, these markers of bone health do not reflect bone mineral density and therefore do not exclude osteoporosis. Further assessments of both urinary calcium and magnesium excretions (Ng et al 2004) and bone mineral density by DEXA (Ng et al 2002b) were conducted in separate studies to examine the bone health of ileostomy subjects.

Micronutrient status

Iron stores

Out of the 60 ileostomy subjects, 1 subject (NSBR) had reduced iron stores as indicated by low iron (3 mmol/l) and ferritin (3 microgram (ug)/l) levels with a raised transferrin (4.5 ug/l) level. This subject had anaemia (haemoglobin 73 g/l) and was taking multivitamin supplements. 1 subject (NSBR) also had low iron (10 mmol/l) and ferritin (8 ug/l) but normal transferrin (3.4 ug/l) levels. This subject did not take any nutritional supplements and had polycythaemia (haemoglobin 170 g/l). Another subject (SBR), who was taking multivitamin supplements, had raised iron (42 mmol/l) and ferritin (1446 ug/l) but normal transferrin (3.21 ug/l) levels. The cause for these abnormal levels was not apparent and genetic test conducted for haemochromatosis was negative.

Trace elements status

Trace element measurements on 60 ileostomy subjects showed: 2 (both SBR) had low copper levels (8.9 and 11.4 micromole (umol)/l) with one was taking a multivitamin supplement, 2 (1 NSBR, 1 SBR) had low zinc levels (10.6 and 10.7 umol/l) with one taking a multivitamin supplement and 8 (4 NSBR, 4 SBR) had low selenium levels (0.61-0.77 umol/l) with 3 taking a multivitamin supplement. For the 2 subjects with low zinc levels, 1 had raised ESR and CRP which makes interpretation of low zinc levels difficult since inflammation leads to reduction in zinc binding protein. Of the 12 abnormal results, 3 subjects (1 NSBR, 2 SBR) had 2 low trace element levels but none had low levels of all 3 trace elements.

Vitamin B_{12} and folate status

Only 2 (both SBR) ileostomy patients had low levels of vitamin B_{12} (105.8 and 112 nanomole (nmol)/l) and all folate levels were normal. These 2 subjects were not receiving vitamin B_{12} supplements.

Fat-soluble vitamins status

1 out of the 60 ileostomy subjects had a raised INR (1.4). This subject did not have small bowel resection and was not on warfarin. 11 subjects (6 NSBR, 5 SBR) (18.3%) had low vitamin A levels (0.9-1.4 umol/l), 4 of whom were taking a multivitamin supplement. 2 subjects (both NSBR) had low vitamin E levels (5.3 and 7.7 umol/l) and both were not taking any nutritional supplements. Although none of the 60 subjects had low 25-

hydroxy-vitamin D levels, 2 (both NSBR) out of 41 subjects who had measurements of 1,25-dihydroxy-vitamin D had low levels (13.3 and 31 umol/l). Both were not taking multivitamin supplements.

Overall, 1 subject had abnormal levels in all 4 fat-soluble vitamins while the rest of subjects had low levels of only 1 vitamin. This subject was not taking any nutritional supplements and did not have small bowel resection but had clinically detectable muscle wasting and dependent oedema. He also had low selenium level but his other blood indices were normal.

In summary, only 1 ileostomy subject had low levels of five micronutrients (selenium and all the fat-soluble vitamins) and 1 had low levels of 3 micronutrients (iron, zinc and selenium). 8 subjects had low levels of two micronutrients and 8 had deficiency in one micronutrient. These deficiencies were probably caused by reduced dietary micronutrient intakes and or excess stomal losses. Some of the ileostomy subjects had low micronutrient levels despite taking nutritional supplements suggesting that these subjects had malabsorption. As 70% of ileostomy subjects had no micronutrient deficiencies, there was no evidence to suggest that ileostomy subjects as a whole were prone to micronutrient deficiencies although the adequacy of nutritional supplements in maintaining micronutrient levels is uncertain.

4.6 Discussion

This is the first comprehensive study to describe the nitrogen and health status of a substantial number of ileostomy subjects with respect to their apparent nitrogen balance, body mass index, body composition and physical, mental and biological capacities. Although the nutritional health of ileostomy patients were described previously (Hill et al 1977, McNeil et al 1982, Baixas et al 1984), it is doubtful that the results from these reports are robust since assessments were based mainly on anthropometric measurements and blood indices as surrogate markers of nutritional competence. Furthermore, information on formal measurements of health status was lacking and considering that the loss of the nitrogen-salvage capabilities of the colon could quite clearly be detrimental to overall nitrogen status, assessments of nitrogen balance of ileostomy

patients have never been described. Therefore, with these issues in mind, this study was conducted to provide a better overall picture of the metabolic and clinical effects of total colectomy. The results suggest that following the loss of the colon, changes to the handling of nitrogen do occur and ileostomy patients have significantly lower BMI, lower FFM and are more likely to be underweight. The results also demonstrate that despite normal blood indices, ileostomy patients are at risk of chronic sodium and water depletion and their overall health status and well being are compromised.

Patient characteristics

As in previous studies, this cohort of ileostomy subjects was heterogeneous with respect to age, gender, diagnosis and whether there was additional small bowel resection. The duration post surgery when this study was conducted was also widely distributed but all subjects had had a stoma for at least 12 months. For subjects who had ulcerative colitis, it is widely accepted that they are cured of the disease following total colectomy while for those who had Crohn's disease, although all the subjects were thought to be in remission, it remained possible that there was on-going small bowel inflammation which might have additional adverse effects on their health. All these factors presented a major challenge since they could impact on the outcomes of our measures. Therefore, besides examining the consequences of total colectomy, the additional effects of small bowel resection and Crohn's disease were also investigated when examining the results of the different components of this cross-sectional study.

BMI and body composition

In this study, the BMIs of ileostomy subjects were significantly lower than those of reference subjects. While none of the reference subjects were underweight due to the recruitment criteria, 10% of the ileostomy cohort was underweight. This figure is twice the incidence of underweight individuals found in the NDNS population. Proportionately, there were also more overweight subjects in the reference and NDNS population than in the ileostomy cohort. Our results were similar to the findings of previous studies where ileostomy subjects were reported to be lighter than the control groups (Clarke et al 1967, McNeil et al 1982, Cooper et al 1986) but in our study, it was evident that ileostomy subjects also had significantly less FFM than reference subjects. Total colectomy therefore appears to have an adverse impact on the BMI and body composition of ileostomy subjects but no effect was seen with additional small bowel resection or

Crohn's disease. Our findings could be due to reduced total body water or actual reduction in lean tissue mass.

Clinical assessment

Traditionally, the most fundamental tools used by clinicians in assessing the health of patients involve taking a history and performing a physical examination. In this study, these tools were used as part of health assessment in ileostomy subjects only. Although the clinical questionnaire was not previously validated, the format of the questionnaire conformed to standard medical practice. To maintain consistency, both the clinical history and physical examination were conducted by the Clinical Research Fellow only.

Although most ileostomy subjects had reported to be in good and fair state of health with good appetite and no weight loss, a significant proportion of these subjects complained of lethargy and had reduced level of activity. These functional symptoms suggest some degree of compromise in their health. As the colon is regarded as an organ capable of responding to reduced nitrogen availability by actively engaging in urea-nitrogen salvage (Giordano et al 1968, Jackson et al 1988, Langran et al 1992, Meakins and Jackson 1996a), it is possible that following total colectomy, ileostomy subjects have reduced metabolic reserve and are therefore unable to respond to increase physical demands. Consequently, their level of activity is reduced and they have a tendency to tire easily compared to normal individuals with intact colon. Proportionately, significantly more ileostomy subjects reported gastrointestinal symptoms compared to reference subjects. This may be caused by intra-abdominal adhesions, known to occur following such surgery, or alternatively, since hormones secreted by colonocytes exert control on the upper gastrointestinal tract (Allen et al 1984, Nightingale et al 1996, Goodlad et al 1997, Tsai et al 1997), these gastrointestinal symptoms may relate directly to their total colectomy. Compared to reference subjects, significantly more ileostomy subjects reported nutritional symptoms, particularly dry skin, suggesting the possible presence of underlying nutrient deficiencies caused by excess losses through the stoma and or reduced micronutrient intakes. Relating these symptoms to their weight, ileostomy subjects who complained of lethargy and reduced activity were more likely to be underweight compared to those who did not (13.9% vs 4.2% and 16.7% vs 8.3% respectively). Thus, these symptoms may indicate that the health of ileostomy subjects is adversely affected by the loss of the colon with small bowel resection as an added risk

factor. In the physical examination, only 3 ileostomy subjects were found to have clinical evidence of undernutrition.

As part of our clinical assessment, stomal function with reference to stool volume and consistency were recorded. Valuable information can be derived from stomal function since high output and liquid stool suggest poor intestinal absorption and high nutrient losses. In this cohort of ileostomy subjects, the average estimated daily stool volume was 400 ml (range 200-2300 ml) with 4 subjects reporting stool volume in excess of one litre. This figure is lower than the average reported volume of 600 ml (Bambach et al 1981, Kennedy et al 1983, Delin et al 1984). In these previous reports, actual volumes of stoma effluents were collected and measured but in this study, an estimate of output was obtained from the subjects. Thus, our subjects could have under estimated the volume of stomal effluent. In terms of stool consistency, only 20% of the subjects passed liquid stool whiles the stomal effluent of the rest of the subjects were semi-formed. No differences were seen in the stool volumes between SBR and NSBR subjects and between those with Crohn's disease and ulcerative colitis. However, proportionately, more SBR subjects passed liquid stool compared to NSBR subjects. This is clearly due to the reduced small bowel length which has affected nutrient absorption.

Relating stomal function to the clinical history, all 4 subjects who had daily stool volumes in excess of one litre complained of lethargy and proportionately more subjects who had liquid stools also complained of lethargy and reduced activity compared to those who had semi-formed stools. Therefore, high output stoma and liquid stools have a detrimental impact on the physical capacity of these subjects.

General health assessment

Health is defined as a state of complete physical, psychological and social well being (World Health Organisation 1958). In evaluating the impact of a specific disease or the outcome of a treatment on health, one of the most important modalities of assessment is to ask the patients themselves how they feel. The health of ileostomy patients have been described previously but the markers of health were based on assessments of dietary intake, body mass index, body composition and blood indices (Thomson et al 1970, Hill et al 1977, Bingham et al 1982, McNeil et al 1982, Baixas et al 1984, Cooper et al 1986). Although quality of life has been examined by numerous studies, these concentrated

mainly on the changes to life style, the impact on sex life and issues related to the practical aspects and complications of the stoma (McLeod et al 1985, Kennedy 1988, Awad et al 1993, Nugent et al 1999). The patients' perception of their health in general following total colectomy and ileostomy, using an accepted health status measure, has never been described.

We conducted a health survey using SF-36 to ascertain the subjects' assessment of the impact of total colectomy and ileostomy on their health as compared to normal individuals. SF-36 is a generic health status measure which has been widely used in the general population including old people (Brazier et al 1992, Fletcher et al 1992, Jenkinson et al 1993, Hayes et al 1995) and patient groups like those with Parkinson's disease (Jenkinson et al 1995), renal replacement therapy (Khan et al 1995), stroke, ischaemic heart disease, arthritis, diabetes and asthma (Lyons et al 1994). It has been designed to provide a short, comprehensive and easy to administer tool for use in the clinical setting, covering three aspects of health (functional status including physical, psychological, and social functions, well being and overall health evaluation) that may be affected by illness. Compared to another widely used generic health status measure, the Nottingham Health Profile, the SF-36 was found to be more sensitive to low levels of ill health (Brazier et al 1992). Its validity and repeatability has also been demonstrated among community and patient populations in the United Kingdom (Brazier et al 1992, Jenkinson et al 1993, Garratt et al 1993). Another added advantage is that population normative data are available for comparison (Brazier et al 1992, Jenkinson et al 1993).

In our study, the mean scores of all eight health dimensions of our reference population were slightly higher than the OHLS normative data. This could be due to our smaller number and/or selective bias resulting from the type of people who volunteered for the study. The mean and median scores of all eight health dimensions of our ileostomy subjects were significantly lower than the scores of reference subjects suggesting that ileostomy subjects have poorer health. The scores of all eight health dimensions at the 25^{th} percentile were also lower in the ileostomy subjects compared to the reference subjects indicating that the health of the most unwell was substantially worst in the ileostomy cohort than in the reference population. When the results were analysed separately by sex and age as there were inherent differences in the scores (Jenkinson et al 1993), similar results were obtained. In addition to total colectomy, SBR also appear to

increase the vulnerability to develop poor health but the health scores of Crohn's and ulcerative colitis subjects were similar.

It is generally recommended that assessment of the health of a patient group should consist of both disease-specific measure designed to be sensitive to outcomes of that particular disease and generic broad base health status measure (Hayes et al 1995, Jenkinson et al 1996). It is important to highlight that although SF-36 has been used in other patient groups, it has never been used in ileostomy patients. Without the supplement of such a disease-specific health measure, we cannot be fully confident of our results. However, when the SF-36 health scores were validated against the clinical history of lethargy and reduced activity, we found that ileostomy subjects who complained of these symptoms had significantly lower scores on all eight health dimensions compared to subjects who did not (Appendices 3F and 3G). Therefore, compared to an age and sex matched reference population, the results of SF-36 indicate that the overall health of ileostomy subjects is compromised following the loss the colon, and ileostomy subjects who had SBR is particularly vulnerable.

Dietary intake

Assessment of dietary intake is an integral part of health and nutritional survey and it is particularly important in ileostomy subjects as alterations to intakes following total colectomy have been described (Thomson et al 1970, Bingham et al 1982, Baixas et al 1984). Numerous tools for dietary assessment are available but for this study which involved 120 subjects, FFQ was thought to be the most appropriate tool on the basis that it is easy to complete, can be self administered and is less labour intensive. More subjects can therefore be evaluated and it may give a better approximation to the usual diet of the population (Margetts et al 1989). Furthermore, comparison studies with 24-hour recall and weighed food diary have demonstrated reasonable agreement in nutrient intakes with subjects ranked appropriately and very few misclassified (Margetts et al 1989, Little et al 1999). The FFQ used in this study had previously been used to assess the dietary intakes of adults with asthma (Shaheen et al 2001). The validity of this FFQ was compared with a 7-day weighed food record in 61 individuals and significant correlations were seen in the intakes of key foods and nutrients (Shaheen et al 2001). This FFQ also demonstrated fair repeatability when two separate estimates on flavonoid-rich foods were compared in 99 individuals (Shaheen et al 2001).

In this study, the intakes of protein and energy of ileostomy and reference cohorts were similar. The results therefore suggest that the loss of the colon and the additional loss of small bowel in some ileostomy subjects do not lead to a compensatory increase in protein and energy intakes. Since this FFQ has never been validated in ileostomy subjects, the quality of the data was examined by comparing with other similar studies and the results from 24-hour dietary recall assessments which were also conducted in the ileostomy subjects (Table 4.24).

Table 4.24 Comparison of mean energy and protein intakes of this study with published data

		Energy (kcal/day)	**Protein (g/day)**
This study – ileostomy subjects	FFQ	2619	102.0
This study – reference subjects	FFQ	2464	99.0
This study – ileostomy subjects	24-hour recall	1865	64.9
Bingham et al – 1982	Weighed record	2380	75
Baixas et al - 1984	Unknown	2187	96
National Food Survey – 2000	Questionnaire	1880	67

Compared to this FFQ, both energy and protein intakes were substantially lower in ileostomy subjects recorded by 24-hour dietary recall. The protein intakes of 37 ileostomy subjects reported by Bingham et al (1982) were also lower than the values reported by our ileostomy cohort using FFQ whereas the protein intakes reported by 21 subjects who had total colectomy and ileo-rectal anastomosis in the study of Baixas et al (1984) were similar to the FFQ findings. Compared to the National Food Survey, the energy and protein intakes of both our ileostomy and reference subjects using FFQ were substantially higher. It would therefore appear that the FFQ used in this study over-estimates energy and protein intakes in our subjects and similar findings were also reported by Bingham et al (1994) when their FFQ results were compared to a 16-day weighed food diary, and by Robinson et al (1996) when their FFQ was compared to a 4-day food diary.

As seen in previous studies (Thomson et al 1970, Binghma et al 1982, Baixas et al 1984), a significant proportion of ileostomy subjects avoid specific foods, mainly fruits and vegetables, as a direct result of having a stoma. However, it appears that regardless of whether ileostomy subjects avoid these foods intentionally or not, the intake of NSP as a cohort was lower than the reference subjects. This could have two effects: firstly, it could account for the lower intakes of magnesium, vitamin C and carotene in ileostomy subjects and secondly, since dietary fibre is associated with increased bacteria mass in the colon (Stephen and Cummings 1980a), lower NSP intake could reduce bacteria mass in the terminal end of the small bowel of ileostomy subjects which may in turn diminish the ability of these subjects to engage in urea-nitrogen salvage.

Urinary nitrogen excretion

When assessing nitrogen balance, the measurement of urinary nitrogen excretion is crucial since it usually accounts for 85-90% of total nitrogen loss from the body. 85% of urinary nitrogen is excreted in the form of urea and it has been known that one of the major ways in which the body adapts to limited nitrogen availability is through changes in the rates at which urea is produced and excreted in the urine (Waterlow 1968).

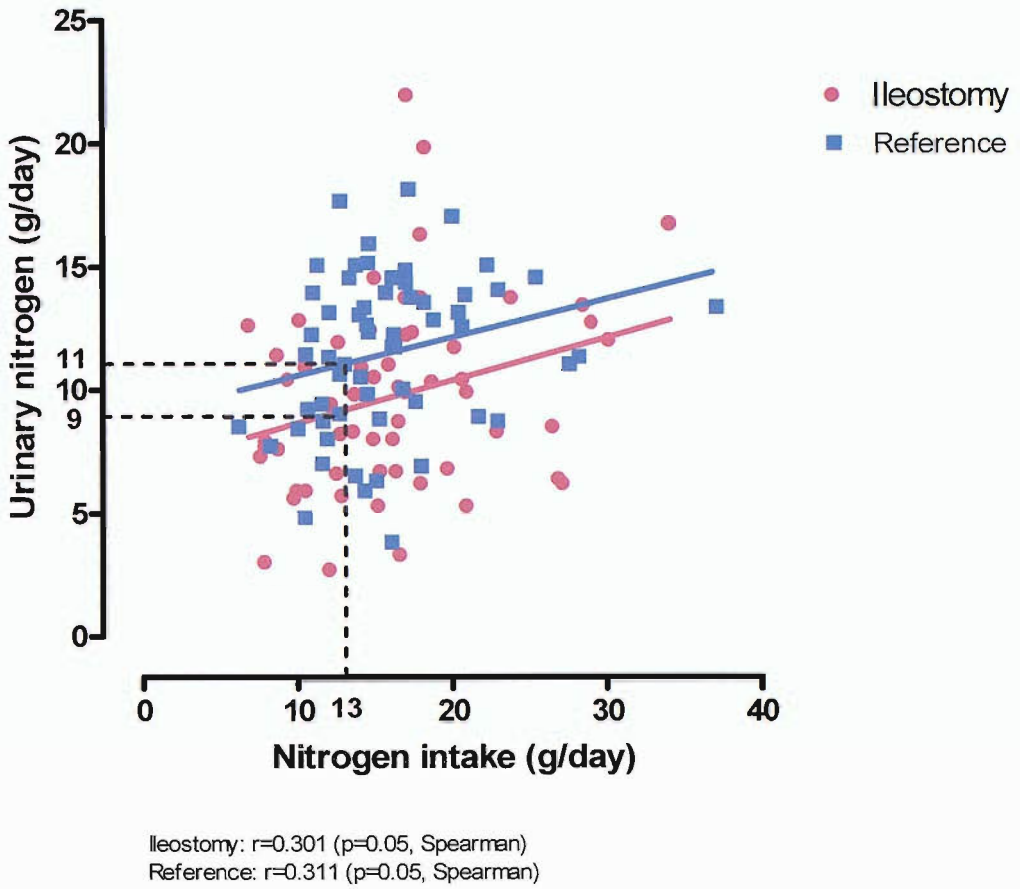
In this study, urinary nitrogen excretions in ileostomy subjects were found to be significantly lower than the reference subjects and within the ileostomy cohort, SBR subjects also had significantly lower urinary nitrogen excretion compared to NSBR subjects. These results may simply reflect lower nitrogen availability, due to reduced nitrogen intakes or high stomal nitrogen losses in ileostomy subjects, or they may also be due to ileostomy subjects operating active urea-nitrogen salvage despite the loss of the colon. However, when protein intakes were assessed using FFQ, the results were similar between ileostomy and reference subjects and between SBR and NSBR subjects indicating that the cause of lower urinary nitrogen excretion is not due to reduced nitrogen intake but the result of high stomal nitrogen losses and/or active urea-nitrogen salvage. No differences in urinary nitrogen excretions and protein intakes were observed between subjects with Crohn's disease and those who had ulcerative colitis implying that there was probably no significant underlying Crohn's disease in the small bowel.

Apparent nitrogen balance

In adults, despite the constant turning over of protein and exchanges of amino acids, the protein content of the body remains relatively constant over long periods (Jackson 1998a). There is a tendency towards the attainment of nitrogen equilibrium which when maintained, leads to functional stability and a state of well being. Hence, assessments of nitrogen balance provide a valid statement concerning the health of an individual. In this study, an estimate of nitrogen balance, i.e. apparent nitrogen balance, was examined as only urinary nitrogen excretion and protein intake were measured. The positive apparent nitrogen balance in both groups of subjects is likely to be attributable, in part, to faecal nitrogen loss which was not accounted for. The significantly more positive apparent nitrogen balance in ileostomy subjects suggests a greater amount of stomal nitrogen loss compared to normal faecal loss, and is augmented further by better renal nitrogen retention possibly for urea-nitrogen salvage. Given that the apparent nitrogen balance was somewhat greater in SBR subjects than in NSBR subjects although the difference did not reach statistical significance, stomal nitrogen loss and urea-nitrogen salvage may be more pronounced with additional small bowel loss.

Figure 4.10 shows the relationship between urinary nitrogen excretions and nitrogen intakes for ileostomy and reference subjects.

Figure 4.10 Correlation of nitrogen intake and urinary nitrogen excretion



Although there is a similar correlation between estimated nitrogen intake and measured urinary loss for both ileostomy and reference subjects, there is a huge variation in the relationship for individuals. This probably reflects the shortcoming of our study protocol in which FFQ provides an estimate of average nitrogen intake over time but by chance may over- or under-estimate actual intakes of nitrogen during the 24-hour period related to the urine collection.

Figure 4.10 also illustrates that on similar intakes, urinary nitrogen excretions were lower in ileostomy subjects compared to reference subjects. This supports earlier suggestions of higher stomal nitrogen losses and/or more active urea-nitrogen salvage ileostomy subjects. Our results also suggest that the FFQ systematically over-estimates nitrogen intakes as even allowing for faecal nitrogen loss of 1-2 g in normal individuals (Gibson

et al 1976b, Stephen and Cummings 1980b, Jackson 1995), the reference subjects would still be in positive nitrogen balance by 1.6-2.6 g/day. A further feature of Figure 4.11 is that for both groups of subjects, it appears that at lower levels of nitrogen intakes, urinary nitrogen losses are similar to the amount of nitrogen consumed whereas at higher levels of nitrogen intakes, urinary nitrogen losses were very much less than nitrogen consumed. The cut-off appears to be at 13 g nitrogen intake where urinary nitrogen excretions for reference subjects would be approximately 11 g and assuming stool nitrogen loss of 2 g, the reference subjects would be in balance as expected (Figure 4.10). Above this level of nitrogen intake, FFQ appears to progressively over-estimate nitrogen intakes in individuals who consume more protein. By applying the same calculation for ileostomy subjects whom we also expect to be in balance, an intake of 13 g nitrogen would give rise to approximately 9 g of urinary nitrogen loss (Figure 4.10), and hence the predicted stomal nitrogen loss for these subjects would be approximately 4 g.

Urinary excretions of sodium and potassium and urine volume

Our assessments of urinary sodium and urine volume provided information relating to the sodium and hydration status of each individual. This is particularly important in ileostomy subjects as the colon is known to have a large capacity for sodium and water absorption. Previous studies have found that ileostomy subjects had lower urinary sodium and volume following total colectomy (Bambach et al 1981, Kennedy et al 1983). Such sodium and water depletion can cause weight loss and may also affect muscle function leading to lethargy. Excessive losses of sodium in the small intestine may also compromise amino acid absorption.

In our study, we confirmed that ileostomy subjects, particularly those who had SBR, were at risk of sodium and fluid depletion with urinary sodium excretions and urine volumes significantly lower than reference subjects. Urinary sodium excretions and urine volumes from our study are compared with other published reports in Tables 4.25 and 4.26.

Table 4.25 Comparison of urinary sodium of this study with published data

	Reference (mmol/day)	NSBR (mmol/day)	SBR (mmol/day)
This study – median (range)	137 (35-307)	88 (6-328)	27 (5-456)
Bambach et al (1981) – mean (sd)	172 (8)	79 (8)	23 (9)
McNeil et al (1982) – mean (sd)	-	67 (11)	-
Kennedy et al (1983) – mean (sd)	142 (59)	113 (65)	-
Delin et al (1984) – mean (sd)	-	92 (14)	7 (2)

Table 4.26 Comparison of urine volume of this study with published data

	Reference (dl/day)	NSBR (dl/day)	SBR (dl/day)
This study – median (range)	200 (84-444)	148 (46-469)	96 (43-459)
Bambach et al (1981) – mean (sd)	170 (7)	99 (7)	72 (11)
McNeil et al (1982) – mean (sd)	-	129 (59)	-
Kennedy et al (1983) – mean (sd)	144 (56)	134 (40)	-
Delin et al (1984) – mean (sd)	-	112 (13)	73 (9)

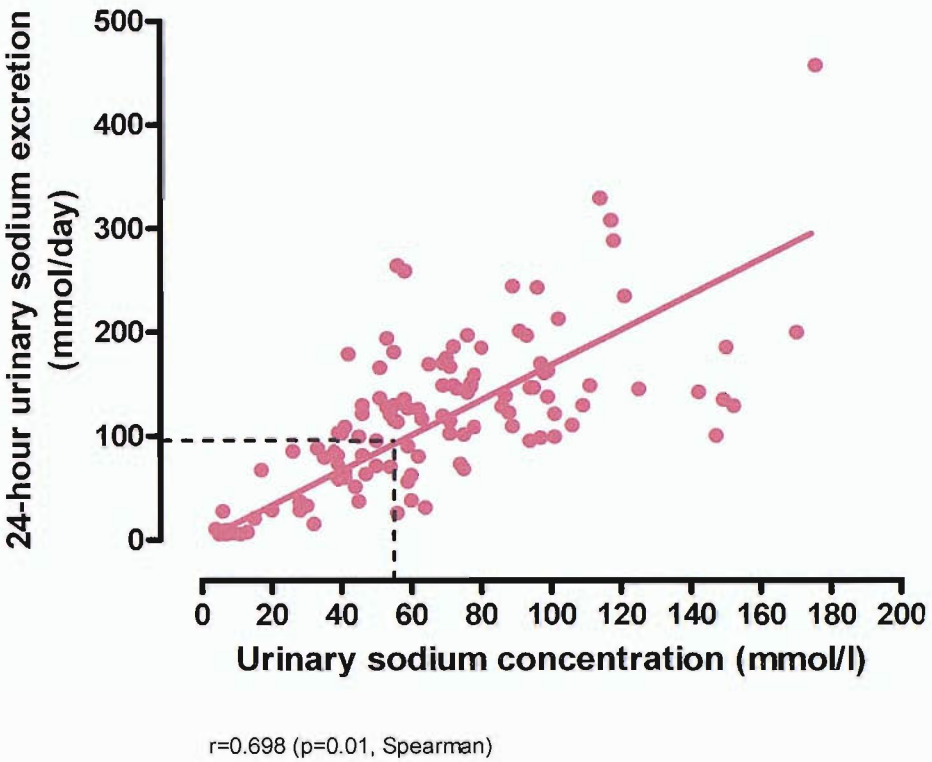
Although these direct comparisons have limitations, we can say that the median urinary sodium excretions in our subjects were similar to the mean urinary sodium excretions of other studies, whereas our median urine volumes were marginally higher. The mean stomal output in ileostomy subjects who had total colectomy only were reported to be 606-635 ml/day in volume containing 71-74 mmol/day of sodium (McNeil et al 1982, Kennedy et al 1983, Delin et al 1984) while the stool volume of normal individuals with an intact colon is 100-200 ml/day containing 5-10 mmol/day of sodium (Bowler et al 1994). The capability of the colon to contribute towards sodium and fluid balance was also demonstrated when higher urinary sodium excretion and urine volume were found in people who have had extensive small bowel resection but with intact colon compared to people who had total colectomy and ileostomy but without SBR (Bambach et al 1981).

In the presence of sodium depletion, one might expect that the renin-angiotensin pathway to be activated which, in turn, would increase the secretion of aldosterone from the adrenal gland. Under the influence of aldosterone, sodium resorption is increased in the renal tubules with a net loss of potassium, producing potassium diuresis. In our study, despite evidence of lower sodium excretion in ileostomy subjects, urinary potassium excretion was not higher than reference subjects. However, similar to previous studies (Clarke and McKenzie 1969, Kennedy et al 1983), the sodium:potassium ratio was significantly lower in ileostomy subjects, particularly in those who had SBR, indicating higher urinary excretion of potassium relative to sodium excretion in ileostomy subjects compared to reference subjects. Therefore, despite normal serum potassium levels, total body potassium may be diminished in ileostomy subjects as previously described by Cooper et al (1986). The levels of renin and aldosterone were not measured in our subjects but raised levels had been described in ileostomy subjects compared to control groups (Kennedy et al 1983, Huber et al 1999) and in ileostomy subjects who had SBR compared to those who did not (Delin et al 1984). The ileostomy subjects described in these studies also had low urinary sodium excretions, high urinary potassium excretions and low urinary sodium:potassium ratios.

The results from our study also suggest that there is a group of ileostomy subjects who have excess stomal sodium losses and these subjects also have a low BMI and FFM. Although fluid depletion may be a contributory factor since body weight has been shown to correlate significantly with total body water and total exchangeable sodium (Clarke et al 1967), this observation may also reflect genuine changes in body composition. In a study of elemental balances during intravenous hyperalimentation of underweight adult subjects, the withdrawal of sodium and potassium separately from intravenous nutrition has been shown to halt lean tissue deposition and any weight gain then largely consists of adipose tissue (Rudman et al 1975). In the study of Cooper et al (1986), ileostomy subjects showed significant reductions in body weight, total body potassium and total body nitrogen but had similar total body water compared to predicted normal values. The same group with excess stomal sodium losses also have low urinary nitrogen excretions which, as commented previously, are probably due to high stomal nitrogen losses although active urea-nitrogen salvage may also contribute.

In a separate study where urinary calcium and magnesium excretions were examined in relation to urinary sodium excretions, we found that urinary calcium and magnesium excretions were not only significantly lower in ileostomy subjects but those who were sodium depleted also tended to show greater depletion of calcium and magnesium (Ng et al 2004). These results are again consistent with the elemental studies reported by Rudman et al (1975) where calcium retention and bone mineral repletion were shown to be halted in the presence of sodium deficiency. When bone mineral density was examined in these 60 ileostomy subjects in another study using DEXA, 49% of the subjects had osteopenia and 12% had osteoporosis by WHO definition (Ng et al 2002b). Clearly, the risk of continual bone demineralization is substantial in ileostomy patients who are already vulnerable to osteoporosis due to their inflammatory bowel disease and previous steroid use. Figure 4.11 shows a significant correlation between urinary sodium concentration and 24-hour urinary sodium excretion. In clinical practice, urinary sodium concentration is simple to measure and therefore, could be used to identify ileostomy patients who may be vulnerable to compromised fluid, sodium, mineral and nitrogen status. Since urinary sodium excretion less than 100 mmol/day is thought to be low, urinary sodium concentration less than 50 mmol/l may be use as a cut-off to identify vulnerable patients.

Figure 4.11 Correlation of urinary sodium concentration and 24-hour urinary sodium excretion of ileostomy and reference subjects



Blood indices

Excess nutrient and fluid losses following total colectomy and ileostomy may be detrimental to organ function which in turn may be reflected in symptom reporting. In the haemopoietic system where nitrogen, in the form of amino acids and nucleic acids, is vital for the formation of blood cells, reduction in nitrogen availability may give rise to anaemia. However, all apart from 2 ileostomy subjects were not anaemic indicating that on the whole, there was no abnormality in the haemopoietic system. The renal function, which may affect nitrogen handling, was also found to be normal in all the ileostomy subjects. Apart from the handling of bile and nutrients, the liver also synthesizes urea from ammonia and other proteins. Therefore, changes in nitrogen metabolism following total colectomy may affect liver function. In this study, 23% of the ileostomy subjects had some abnormality of liver function. These abnormalities were in general mild and without further detailed investigations, it was not possible to ascertain the exact cause of these abnormalities. Based on one random blood test, it was also not possible to say if

these abnormalities were transient or persistent. However, based on albumin and INR levels, the synthetic functions of the liver in 98.3% of the ileostomy subjects were normal suggesting no significant impairment. Bone health was assessed by the levels of calcium, magnesium, phosphate and alkaline phosphatase. From these measurements, no abnormality was seen in the bone profile but as previously mentioned, a significant number of ileostomy subjects had reduced BMD by DEXA (Ng et al 2002b). Various micronutrients were also measured and 70% of the ileostomy subjects had no micronutrient deficiency while 30% had low levels of at least one micronutrient. Although dietary intakes of zinc, selenium, copper, folate and B vitamins, apart from iron, carotene and vitamin C, were similar to reference subjects, it is possible that there were higher stomal losses of these micronutrients in a selected number of ileostomy subjects.

4.7 Summary

In summary, the findings of our cross-sectional study are:

1. Ileostomy subjects had lower BMI and lower FFM and were also more likely to be underweight compared to national data.
2. Ileostomy subjects had lower urinary sodium excretion and urine volume and were therefore at risk of sodium depletion and the effects of chronic dehydration.
3. Some ileostomy subjects with low urinary sodium excretion might also have depleted mineral stores and were therefore at risk of osteoporosis and fragility fractures.
4. Ileostomy subjects had similar dietary nitrogen intakes, lower urinary nitrogen excretions and more positive nitrogen balance compared to reference subjects. This was most certainly due to excessive stomal nitrogen losses which were not accounted for but they might also be operating active urea-nitrogen salvage.
5. Ileostomy subjects had lower intakes of NSP due to alterations in the diet and food choices. This could potentially hamper urea-nitrogen salvage which is bacteria dependent.
6. Although the lower body weight could be due to a reduction in total body water, ileostomy subjects might have genuine reduction in lean tissue mass caused by

changes in nitrogen metabolism and possibly, also by sodium and potassium depletion which is known to limit lean tissue deposition.

7. On both informal and formal health assessments, ileostomy subjects reported significant impairment in their health compared to reference subjects.
8. Despite the above findings, measurements of haematological and biochemical blood indices in ileostomy subjects indicated normal organ and biosynthetic functions.

From the above, we can therefore conclude that the results of this comprehensive study support our hypothesis that following the loss of the colon, ileostomy patients have compromised nitrogen status and poor overall health. Furthermore, our study also suggests that the risks associated with total colectomy are increased in ileostomy patients who had additional small bowel resection. From our study, although ileostomy patients with Crohn's disease did not appear to be more susceptible than those who had ulcerative colitis, this may simply reflect the absence of significant Crohn's disease in the remaining small bowel of our subjects. It is most probable that ileostomy patients who have small bowel Crohn's disease are as susceptible to the increased risks of total colectomy as those who have had small bowel resection. In addition, this study also suggests that the use of anthropometry and blood tests alone as surrogate markers of nutritional and health status is inadequate if underlying metabolic perturbations and subclinical malnutrition are to be detected. It is very likely that organ and biosynthetic functions are maintained at the expense of other metabolic processes such as lean tissue deposition and muscle functions, which are less easily measured but nonetheless, are important in the maintenance of physical, psychological and social well-being and overall health.

Despite the above conclusions, our understanding of the changes relating to the handling of nitrogen and the mechanisms through which nitrogen equilibrium is maintained in the absence of the colon remain unclear. There is, therefore, a need to further investigate the following issues:

1. Considering the limitations of the FFQ highlighted in the cross-sectional study, the habitual nitrogen intakes of ileostomy patients should be reassessed using weighed food diary, a gold standard for measuring dietary intakes.

2. Stomal nitrogen losses in ileostomy patients are potentially high and should be measured so that a more accurate assessment of nitrogen balance can be made.
3. The ability of ileostomy patients to operate urea-nitrogen salvage and the extent to which this metabolic activity contribute to nitrogen balance in free-living conditions should be investigated.
4. The ability of ileostomy patients to up-regulate urea-nitrogen salvage and maintain nitrogen balance under metabolic stress created by a reduction in nitrogen availability should also be investigated.

All the above issues were examined and reported in the next chapter.

5

NITROGEN BALANCE AND UREA KINETIC STUDIES

5.1 Introduction

The importance of colonic functions in the maintenance of our physiological and metabolic requirements is well described in the literature. Apart from its capacity for sodium and water absorption and its role in the preservation of upper gastrointestinal functions, colonic urea-nitrogen salvage has been shown to contribute significantly towards overall nitrogen balance, especially when there is an imbalance between nitrogen availability and demands (Langran et al 1992, Meakins and Jackson 1996a, Millward et al 2000).

In our cross-sectional study, we demonstrated that the loss of colon is detrimental to health and nitrogen status. These findings were significant as they refute evidence from previous studies (Hill et al 1977, McNeil et al 1982, Baixas et al 1984) that the loss of colon is of no consequence to nutrition and health. However, our understanding of the changes relating to the handling of nitrogen and the mechanisms through which nitrogen equilibrium is maintained in the absence of the colon remain unclear. There is therefore a need to further investigate the following issues:

1. Although the loss of colon did not lead to a compensatory increase in protein and energy intakes as determined by the FFQ, this finding may be erroneous since the FFQ was shown to consistently over-estimate intakes. A better dietary assessment method is therefore needed to assess the true effects of total colectomy on protein and energy intakes.
2. The more positive apparent nitrogen balances in ileostomy patients suggest that stomal nitrogen losses are high, possibly at least 3-4 g per day since this amount flows through the ileocaecal valve in normal individuals (Jackson 1995). By measuring dietary nitrogen intakes and stomal nitrogen losses, a more accurate

assessment of nitrogen absorption and nitrogen balance in ileostomy patients could be made.

3. The ability of ileostomy patients to operate urea-nitrogen salvage in the absence of the colon is unclear although the lower urinary nitrogen excretions may indicate that they are capable of this metabolic activity. If urea-nitrogen salvage is indeed present, it would mean that ileostomy patients are actually actively salvaging nitrogen under habitual free-living conditions and hence contributing to nitrogen balance whereas in normal individuals under similar conditions, urea-nitrogen salvage is usually minimal, hence the implication that the handling of nitrogen is altered by the loss of colon.
4. If ileostomy patients are capable of urea-nitrogen salvage, it would be important to know if they have the capacity to increase this metabolic activity in response to reduced nitrogen availability. Considering that NSP intakes were shown to be lower in ileostomy subjects in the cross-sectional study, it is possible that these people may have a limited capacity for increasing urea-nitrogen salvage due to reduced bacterial mass in the terminal end of their small bowel. This aspect of nitrogen metabolism has crucial clinical implications because if ileostomy patients have limited capacity for urea-nitrogen salvage, they will be vulnerable during periods of metabolic stress.

In order to understand the dynamics of nitrogen metabolism that occur following total colectomy, further studies were therefore undertaken. These included assessments of nitrogen and energy intakes under habitual free-living conditions using weighed food diary, together with measurements of stomal nitrogen losses, urinary nitrogen excretions, nitrogen absorption and nitrogen balance. Urea kinetics and urea-nitrogen salvage were also studied using doubly-labelled $^{15}N^{15}N$-urea isotope and the effects of metabolic stress, simulated by imposing a 40% reduction in dietary nitrogen intake, on nitrogen balance and urea kinetics were examined by repeating all the above measurements. These studies will address Questions 5 and 6 and hence, our second hypothesis as set out in Chapter 3.

5.2 Urea kinetics

Studies reporting a direct linear relationship between protein intake, urea excretion and urea production had led to the assumption that urinary urea excretion rates are a direct reflection of urea production rates and hence, the rate of amino acid oxidation. This assumption is, however, unsafe since other studies have shown that urea production does not show a simple linear relationship with protein intake or urea excretion (Jackson 1998a). Although changes in urea excretion do occur with varying levels of protein intake, urea production tends to remain constant over a range of intakes (Langran et al 1992, Child et al 1997) and furthermore, while there are obvious diurnal changes in urinary urea excretion, similar diurnal variation in urea production is not seen (Meakins 1996b). Evidence therefore suggests an alternative fate for urea other than excretion via the kidney and studies have shown that urea can be moved into the colon for bacterial hydrolysis with the derived nitrogen made available for further metabolic interaction (Walser and Bodenlos 1959, Jackson 1993b) (see Section 2.3). In view of the above, it is more appropriate to measure urea production rather than urinary urea excretion, when examining the dynamics of nitrogen metabolism.

By the application of tracer methodology, urea kinetics can be measured by following the fate of isotopically labelled urea through the body. Furthermore, by using urea in which both atoms of nitrogen are labelled, it is possible to follow the extent to which nitrogen coming from hydrolysed labelled urea is reincorporated into endogenously synthesized urea (Picou and Phillips 1972, Wolf 1981, Jackson et al 1984). ^{15}N is a stable isotope with an extra neutron in the nucleus and it is usually expressed as atoms percent excess which is a ratio of the isotopic content of a sample over natural abundance. In this thesis, the capability of small bowel bacteria to hydrolyse urea in the absence of the colon was studied using doubly labelled stable urea isotope, $^{15}N^{15}N$-urea.

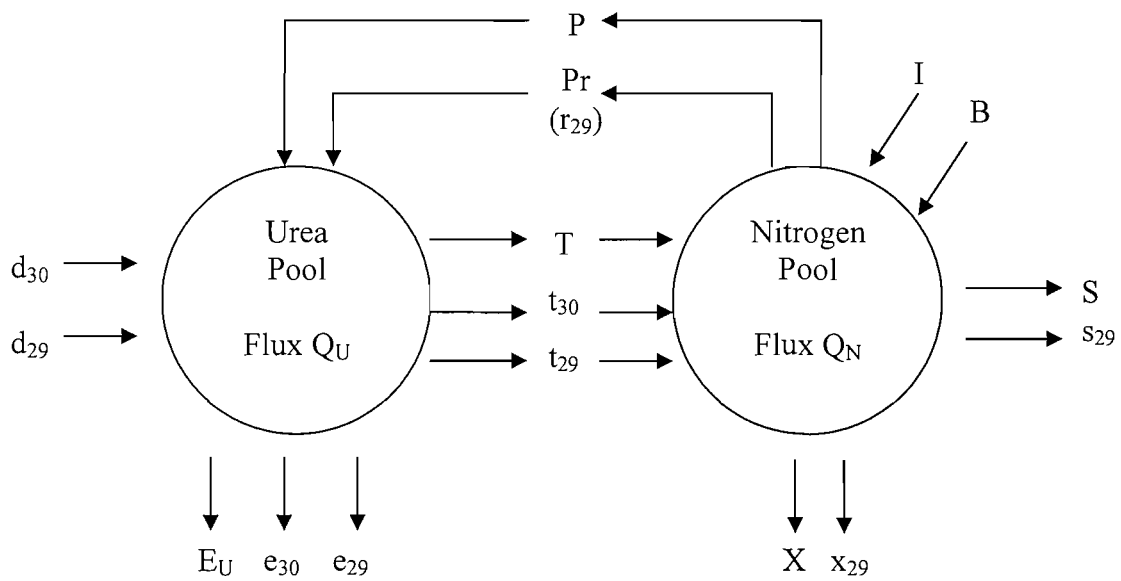
5.2.1 Principles of urea kinetics

Urea kinetics were measured based on the model described in the study of Jackson et al (1984) (Figure 5.1). In this model, the following assumptions were made:

1. The presence of a metabolic steady state i.e. constant pool size with constant input into and output from the pool.

2. The existence of two pools, a urea pool, and a nitrogen pool within which nitrogen derived from degraded urea moves freely.
3. The administered isotopic urea label is handled in the same manner by the body as that of endogenously produced urea.

Figure 5.1 A two-pool model of urea kinetics



Following the administration of urea isotope $^{15}N^{15}N$-urea, three species of urea molecules are present: $^{15}N^{15}N$-urea (urea-30), $^{15}N^{14}N$-urea (urea-29) and $^{14}N^{14}N$-urea (urea-28). The movement of the isotope label within and between the urea and nitrogen pools describes the movement of urea-30 and urea-29 which are present in excess of natural abundance. Calculations are made by considering the urea and nitrogen pools individually and are based on the single pool model of Shipley and Clarke (1972) which states that the turnover rate is equivalent to the input-output ratio of material through a pool in a steady state. When an isotope is infused continually into an unlabelled substrate pool, the relative concentration of the isotope to unlabelled substrate is the same as the proportion of infused tracer to the endogenous input of unlabelled substrate in the pool and as long as input rates remain constant, enrichment will reach an isotopic steady state reflected by a plateau.

The urea pool is conceived has having an inflow from urea produced in the body (P) and two outflows as urea excreted in the urine (E_u) and as urea hydrolysed in the bowel (T). The flux of urea (Q_u) through the pool is given by $Q_u = P = E_u + T$. T is the proportion of urea production that cannot be accounted for in terms of urinary excretion. Label flows into the urea pool from an exogenous dose as urea-30 (d_{30}) and urea-29 (d_{29}). In addition, some labelled urea is formed endogenously from nitrogen recycled in the bowel. Virtually all this urea is of molecular species urea-29 (r_{29}), as the chance of two ^{15}N atoms combining to form one molecule of urea-30 is negligible. Label is lost into the urine as e_{30} and e_{29} and to hydrolysis in the bowel as t_{30} and t_{29}.

In the nitrogen pool, inputs come from dietary nitrogen (I), nitrogen from endogenous breakdown of protein, amino acids and other nitrogenous compounds (B), and nitrogen from urea hydrolysis in the bowel (T). Outputs from the nitrogen pool go to urea synthesis (P), other nitrogenous end product of metabolism (X) and synthetic and metabolic processes (S), which will be predominantly amino acid and protein syntheses. All entry of isotope comes from the hydrolysis of urea, t_{30} and t_{29}. Any recycling from other labelled nitrogenous compounds is assumed to be negligible. Label leaves the pool as r_{29} to urea synthesis (Pr), as x_{29} to other end products and as s_{29} to synthetic processes. Therefore, the flux through the nitrogen pool, Q_N is $Q_N = I + B + T = P + X + S$.

5.2.2 Prime-constant infusion model

In this thesis, the prime-constant infusion model was employed. This model was adapted from the studies of Picou and Phillips (1972) and Wolfe (1981) and measures the rate of urea production, i.e. entry of urea into the pool. In the study of Picou and Phillips, the urea label was administered as a constant infusion until an isotopic steady state had been achieved, as measured by the enrichment in urinary urea. As it had been recognised that it could take at least 30 hours of constant infusion to achieve an isotopic steady state (Jackson et al 1984), a prime dose of urea label was given to shorten the time to reach isotopic steady state, followed by the constant infusion to maintain isotopic steady state (Wolfe 1981). However, the choice of the correct priming dose is important. If the prime dose is incorrect, the tracer enrichment plateau may artificially reflect the priming dose. In this thesis, a prime dose that is equivalent to 15 hours of constant infusion was given

which is similar to those used previously by other authors (Hibbert and Jackson 1991, Hibbert et al 1992, Langran et al 1992, Danielsen and Jackson 1992, Meakins and Jackson 1996a).

Urea kinetics can also be measured using the single dose model described in the study of Jackson et al (1993a). In this model, the fate of urea passing from the urea pool is traced and by comparison of the cumulative excretion of the isotope in urea-30 and urea-29, an estimate of the rate of nitrogen recycling is obtained (Jackson et al 1984). The obvious advantage of this model over prime-constant infusion model is the simplicity of administration of urea label. However, a major disadvantage is that the accuracy of the results of this model relies on the quantitative assessment of the total amount of urea excreted over the period of study (Jackson et al 1993a). As can be seen from the data of Long et al (1978), the choice of the timing over which urine is collected and the actual points included in the analysis can produce widely different results. Thus, the single dose model is susceptible to any errors in the collection of urine. In the prime-constant infusion model, as we are dealing with an isotopic steady state, the rate of nitrogen recycling can be calculated with greater confidence (Jackson et al 1984).

The urea label can be administered orally or intravenously. With oral dosing, the measurement of urea kinetics requires that the urea label is absorbed intact. Infection of the upper gastrointestinal tract by Helicobacter Pylori is known to be associated with hydrolysis of urea label before absorption (Graham et al 1987, Hibbert et al 1992). In this case, the study subjects will have an abnormally high rate of excretion of urea-29 and a low rate of excretion of urea-30 in the urine (Hibbert et al 1992). Therefore, in order to circumvent the question of hydrolysis of the urea label, this thesis opted for the intravenous protocol at the expense of a relatively more invasive approach.

5.2.3 Calculations

The basic principle for the calculations of urea kinetics is that urea leaving the urea pool by any route must contain the same proportion of urea-28:urea-29:urea-30, and nitrogen leaving the nitrogen pool by all routes must contain the same abundance of ^{15}N (Jackson et al 1984). The calculations are somewhat complex because one molecule of urea-30

transfers its label to two molecules of urea-29 and that the administered doubly labelled urea-30 contains 1% of urea-29.

The quantities that will be determined are: urea production, P; the amount of urea transferred to the colon for hydrolysis, T; the component of P that represents recycled urea, Pr; the amounts of nitrogen derived from bacterial hydrolysis which enter synthetic and metabolic pathways, S; and the flux through the nitrogen pool, Q_N. The information available from the urea kinetics study for determining these quantities is nitrogen intake, I, excretion of urea, E_u, and of total nitrogen, E_T, and the relative amounts in urine of urea-28, urea-29 and urea-30. These relative amounts are given by the relative intensities (I_{28}, I_{29}, I_{30}, $I_T = \Sigma I$) measured in the mass spectrometer at m/e 28, 29 and 30. δI_{29} and δI_{30} are the increases in intensity which result from the increase in enrichment over natural abundance in urea-29 and urea-30. Therefore the ratios $\delta I_{29}/I_T$ and $\delta I_{30}/I_T$ represent the proportions of total urea derived from exogenous labelled urea. The calculations for determining the unknown listed above from these experimental data are set out in Appendix 9 (Jackson et al 1984). In simplified terms, $P = E_u + T$ and $T = Pr + S$.

5.3 Identification and recruitment of subjects

Ileostomy subjects who had participated in the cross-sectional study described in Chapter 4 were invited to take part in this study. 6 adults with no gastrointestinal disease or surgery, 6 NSBR ileostomy subjects and 6 SBR ileostomy subjects were recruited to form three separate groups. Five of the adults in the reference group were staff of the hospital and university and one adult was the spouse of an ileostomy subject.

As this study required the time and commitment of all participants, some difficulties were encountered in the recruitment. Consequently, the subjects in all 3 groups could not be matched for age or sex. In the NSBR group, all the subjects had previous ulcerative colitis, omitting those with Crohn's disease. This was to ensure that the subjects in NSBR group had no previous undiagnosed or residual small bowel disease which could be present in some patients with Crohn's disease. In the SBR group, the subjects had varying degrees of small bowel resection and it was not possible to assess adequately the extent of small bowel loss for each of the subjects.

5.4 Study protocol

The studies were carried out in all 18 subjects. Every subject was provided with a patient information sheet and written consent was obtained after the nature of the study and all investigative procedures were explained. The study was approved by the South and West Local Ethics Committee (reference 319/01).

Every subject underwent two separate studies which were carried out at least 2 weeks apart. Both studies had similar investigative protocols but differ only in dietary protein intake. The first study was conducted when the subject consumed his/her habitual free-living diet while the second study was conducted when the subject consumed prescribed diets which had only 60% of the protein content of the habitual diet. The energy intake in the reduced protein diet was kept similar to that of the habitual diet. Each study lasted 5 days during which assessments of BMI, body composition, dietary intake, urinary nitrogen excretion, faecal nitrogen losses and overall apparent nitrogen balance were made. All the subjects were engaged in their normal activities during the study period except on Day 5 when urea kinetics was measured in the hospital.

5.4.1 Dietary intake

Habitual free-living diet

Previous studies of similar kind had fixed the level of protein intake at 70 g/day and energy intake at 1.4 times of basal metabolic rate for all the subjects (Langran et al 1992, Danielsen and Jackson 1992, Meakins and Jackson 1996a). These levels of intakes were thought to be sufficient to meet the subjects' metabolic demands but also had the added advantage of maintaining conformity and hence, allowing easier comparisons across the study groups. However, as one of the aims in this study was to investigate the effects of the loss of colon on metabolic requirements, the studies were conducted with each subject taking his/her habitual free-living diet so that, despite losing dietary conformity, assessments could be made of the free-living energy and protein intakes in each group of subjects in relation to their clinical status.

Prior to the study, personal preferences concerning the type of food normally consumed were identified to improve compliance. For this, each subject filled in a FFQ which was

identical to that used in Chapter 4 and a telephone interview was also conducted. Based on the information obtained, each subject was provided with a variety of foods, sufficient for the entire duration of the study, consisting of main meals (e.g. meat pies, fish fillets, chicken fillets, ham, pasta, chips), snacks (e.g. biscuits, chocolate bars, crisps) and drinks (e.g. soft drinks, squash) bought from supermarket stores. However, other food items such as bread, margarine, butter, jam, milk, beverages, cereals, fruits and vegetables had to be provided by the subjects themselves and he/she was also free to eat any other food. A daily weighed food diary was then recorded from Days 1 to 4 and each subject was instructed to keep all food package labels so that food consumed could be identified and nutrition values calculated. Food for Day 5 was provided during the urea kinetics study (see section 5.4.3)

Low protein diet

In previous studies of urea kinetics where responses to adequate and low protein diets were measured, the levels of protein intake were set at 35 g/day or 30 g/day, a reduction of 50% and 57% respectively from 70 g/day (Langran et al 1992, Danielsen and Jackson 1992, Meakins and Jackson 1996a). These levels of intakes were considered to be at the physiological minimum for normal adults (Jackson 1998b). However, since the potential changes to metabolism and the minimum physiological protein intakes following total colectomy with and without small bowel resection were unknown, it was felt that the well-being of the ileostomy subjects could be compromised if protein intakes were reduced to the same extent as in previous studies. In view of this, for the purpose of the second part of this study where the responses of urea kinetics to low protein diets were examined, protein intake was reduced by 40% of their habitual intakes in all the subjects.

Once the study on habitual intake was completed, energy and protein intakes on each of the five days of the study period were analysed and an overall average habitual intake for energy and protein was calculated. Based on these figures, diets for each subject were designed where daily protein intake was reduced by 40% but the amount of energy was kept at the same level as that of habitual diet. As personal preferences were also taken into account, the diets for each subject were different from one another. To add variety and improve compliance, the diets on Days 1 to 4 for every subject were different but energy and protein contents were kept at the same level (Appendix 7). Food for Day 5 was provided during the urea kinetics study (see section 5.4.3). As in the study on

habitual diet, main meals, snacks and drinks were provided but each subject had to provide other food items such as bread, beverages, milk etc (as listed above). Each subject had to consume every item listed on the diet sheet for each day and was not allowed to eat anything else. Each subject was allowed to drink any quantities of water or non-calorific drinks as required. A weighing scale was provided to weigh out the stipulated food portion on the diet sheet.

5.4.2 Collection of urine and stool

Prior to the studies, each subject was given verbal and written instructions on urine collection. Clean, disposable plastic bottles were ordered from the CPL of the hospital and collecting receptacles were provided to facilitate collection. Each 2-litre bottle contained 20 ml of 6 M HCL as preservative. On the first day of each study period, each subject started the urine collection on an empty bladder, i.e. the first urine sample passed on awakening on the first day was discarded. Thereafter, all urine voided was collected for the entire study period. After each 24-hour period, a new bottle was used and dated. The urine bottles were collected from each subject every two days. The weights of the container before and after collection were measured and the difference in these weights was taken as the volume of urine collected in a 24-hour period. 20 ml aliquots from each 24-hour period were placed in labelled sterile containers marked and frozen at -20°C for later analysis of urinary nitrogen content.

Each subject was also given verbal and written instructions on stool collection which was carried out for each of the 5-day study period. Clean freezer bags, collecting receptacles and insulated boxes containing dry ice were provided. Once stool was passed, each sample was labelled and placed in dry ice immediately. The insulated boxes containing the stool samples were collected from each subject every two days and there was sufficient dry ice to ensure that stool samples remained frozen prior to collection. After collection, the stool samples were stored in the freezer at -20 °C for later analysis of stool nitrogen content.

5.4.3 Urea kinetic study

Administration of $^{15}N^{15}N$-urea isotope

The urea kinetics study was conducted on Day 5 of each study period and the prime-constant intravenous infusion protocol was used (see section 5.2.2). Each subject was required to attend the SUHT at the CRF at 2100 hours on Day 4 in preparation for the study. The $^{15}N^{15}N$-urea isotope (99 AP; Lot No. SHO-08096-B; Research Chemical, Mass Trace, Woburn, Massachusetts, USA) was formulated into a solution for intravenous administration by the Aseptic Services Division of the Pharmaceutical Department of SUHT on Day 4 of the study and stored at 4°C until use. Each syringe contained 300 mg of $^{15}N^{15}N$-urea isotope in 48 mls of normal saline. At 0000 hours on Day 5, a prime dose of 300 mg of urea isotope, equivalent to 15 hours of continuous infusion at a rate of 20 mg/hr, was administered intravenously as a bolus via a cannula placed in a forearm vein. 6 hours later, a continuous infusion was commenced using the same cannula at a rate of 20 mg/hr (3.2 ml/hr) for 15 hours. The infusion was stopped at 2100 hours on Day 5. This protocol is illustrated schematically in Table 5.1

Table 5.1 Schematic illustration of the urea kinetics study protocol

Day 5 - Time	**0000**	**0600**	**0900**	**1200**	**1500**	**1800**	**2100**
Urea isotope	Prime dose	← Continuous infusion →					
Urine collection	X	X	X	X	X	X	X
Food intake		X	X	X	X	X	

Urine collection

On arrival at the CRF at 2100 hour on Day 4, each subject was required to void so that a 3-hour baseline urine could be collected prior to the administration of the prime dose of urea isotope at 0000 hours on Day 5. Thereafter, urine was collected every 3 hours from 0600 hours prior to starting the infusion to 2100 hours after which the infusion was stopped (see Table 5.1). At each collection, urine is voided before food intake. The subject was then allowed to leave after 2100 hours and complete the Day 5 urine and stool collection at home.

Urine was collected into clean containers. The volume of urine voided was given by the difference in the weight of the container prior to and after urine collection. 1 ml of 6M HCL was added to every 100 ml of urine as preservative. Aliquots were than marked clearly and stored at -20°C for later analysis. The rest of the urine was pooled together to complete the 24-hour urine collection for Days 4 and 5.

Food

Food for Day 5 was provided during the urea kinetics study. For the study on habitual diet, the weighed food diary for Days 1 to 3 were collected from each subject on Day 4 and analysed. The average energy and protein intakes over the 3 days were calculated and based on these result, a diet for Day 5 was designed for each subject. For the study on low protein diet, the energy and protein content of Day 5 was similar to that on Days 1 to 4 as these levels of intakes had already been calculated from habitual intakes.

When measuring urea kinetics, a steady metabolic state is required for the duration of the study but not all contributory factors can be controlled (Jackson et al 1984). However, the pattern of food intake, which is one of the major influences on metabolic state, can be regulated and hence, each subject was fed at three hourly intervals to maintain relatively constant ingestion rates during the study from 0600 to 1800 hours (see Table 5.1). The intakes of energy and protein for Day 5 were divided into five portions so that the subjects were taking the same amounts of energy and protein at each three hourly interval. Sample Day-5 diets on habitual and low protein intakes are shown in Appendix 8. Despite a lessened sense of accuracy in protein intakes, whole foods were used instead of a liquid diet.

BMI, body composition and resting metabolic rate

The weight, height and body composition of every subject were measured shortly after arrival at the CRF on Day 4. These measurements were taken by state registered research nurses assigned by the CRF to support this study. Between the time period of 0000 and 0500 hours following the administration of the prime dose of urea isotope, the subjects slept. Resting metabolic rate (RMR) was measured at 0500 hours by one of the research nurses. The subjects were woken from their sleep and were instructed not to get out of their beds. Their beds were then wheeled to the calorimeter by the nurses where RMR was measured over 20 minutes.

5.5 Methods

5.5.1 Assessment of body mass index and body composition

For all the subjects, weight was measured using a digital scale (Seca Alpha, Model 770, CMS Weighing Equipment Ltd) and height was measured using a stadiometer (CMS Weighing Equipment Ltd). BMI was determined as the Quetelet's index (kg/m^2). Body composition was determined as FFM, BF, and %BF using BIA (Bodystat 1500). All the measurements were taken by the research nurses assigned to support this study and the study described in Chapter 4. All three pieces of equipment were also used in the study described in Chapter 4.

5.5.2 Assessment of resting metabolic rate

RMR was measured for 20 minutes after 5 hours of rest (see section 5.4.3) using indirect calorimetry (GEM Gas Analyser, Europa Scientific, Crewe, UK). This device measures the rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The respiratory quotient is derived from these values and energy expenditure is then calculated from the Weir equation (Weir 1949). The subjects were shown the calorimeter and the procedure was explained to them when they arrived at the CRF on Day 4 to reduce anxiety. The measurements were taken by the research nurses who were trained to use the calorimeter.

The calorimeter was calibrated at the beginning of each measurement using a mixture of 5% CO_2 and 95% O_2. It was also validated for flow rates and respiratory exchange ratio every month by burning a known quantity (5 ml) of pure ethanol. Between July 2003 and January 2004 when urea kinetics studies were conducted, the mean VCO_2 and respiratory quotient were 3513 – 3740 ml/min and 0.610 – 0.651 respectively. The mean recovery of ethanol was 95% - 98%.

5.5.3 Dietary analysis

All the dietary analyses and design of menus were carried out by the Clinical Research Fellow. Weighed food diaries were analysed using a computerised food composition database (Foodbase 2000, The Institute of Brain Chemistry and Human Nutrition,

University of North London). Additional information such as weight of food and manufacturer's nutritional values available on food packaging was also used in the calculation of energy and protein intakes. Food portion sizes were estimated using a published guide on typical weights and portion sizes of food eaten in Britain (Food Portion Sizes 1993). The above tools were also used to design the diets for all the subjects.

5.5.4 Determination of total nitrogen in urine

The total nitrogen content of urine was measured by the Kjeldahl method as described in section 4.4.5. These analyses were carried out Mrs Angela Hounslow.

5.5.5 Determination of total nitrogen in stool

The total nitrogen content in stool was measured using the Kjeldahl method described in section 4.4.5. The stool samples were homogenised prior to analysis.

Method

1. All stool samples passed in a single day, which was defined as starting from 0700 to 0659 hours the next day, were removed from the plastic bags and placed in a clean household blender. The weight of the stool was obtained by subtracting the weight of the blender from that of the blender and the stool.
2. Water, measuring one and a half times that of the weight of the stool was added to the blender, and the stool was homogenised. Therefore, the total weight was taken as the amount of stool sample plus water added. As the stool samples from ileostomy subjects were either loose or liquid, the stool was homogenised without the need to add water.
3. Once the stool is homogenised, a known amount of stool weighing approximately 0.5 g, was placed on a piece of nitrogen-free grease proof paper. The stool and paper were then placed in a large distillation tube and the determination of nitrogen content in the stool was carried out exactly as described in section 4.4.5.

Calculation

Nitrogen content in stool per day (gN/day) =

(volume of acid used x 0.1 x 14.008 x weight of stool passed in 24 hours)/ 0.05 g faeces

Repeatability

The analyses of stool nitrogen content were conducted by Mrs Angela Hounslow. Analytical precision of the measurement of stool nitrogen was determined by duplicate analysis of samples. At a mean concentration of 1.90 g/day, the CoV for 12 samples was 1.45%.

5.5.6 Determination of ammonia nitrogen in urine

Ammonia in the urine was determined using the Berthelot method (Kaplan 1965). The concentration of ammonia is estimated by reacting phenol with alkaline hypochlorite to form p-quinone chloroimine. The p-quinone imine reacts with another molecule of phenol to form indophenol which then dissolves to yield a blue indophenol dye. The reaction is catalysed by sodium nitroprusside.

Method

1. 10 microlitres (ul) of each standard was pipetted into the bottom of labelled test tubes.

   Stock: 500 mg NH_3-N / 100 ml. Dry $(NH_4)_2SO_4$ in glass beaker at 100 °C for 12 hours. 2.36 g of dried $(NH_4)_2SO_4$ is dissolved in 100 ml of deionised water.

   Working standard:

S/0 Deionised water (blank)	0.0 µg NH_3-N / 10 ul
S/1 1 ml stock + 49 ml deionised water	1.0 µg NH_3-N / 10 ul
S/2 2ml stock + 48 ml deionised water	2.0 µg NH_3-N / 10 ul
S/3 3 ml stock + 47 ml deionised water	3.0 µg NH_3-N / 10 ul
S/4 4 ml stock + 46 ml deionised water	4.0 µg NH_3-N / 10 ul

2. 10 ul of urine was pipetted into the bottom of labelled test tube.
3. 4.0 ml of working phenol nitroprusside solution (PNP) was added into the test tube and mixed.

Stock: 50.0 g of phenol (Analar) was added to 500 ml deionised water and 250.0 mg of sodium nitroprusside to 100 ml deionised water. Both solutions were mixed together and then made up to 1 litre.

Working PNP: 100 ml of stock was added to 300 ml of deionised water

4. 5.0 ml of working alkaline hypochlorite solution was added into the test tube and mixed.

   Stock: 25.0 g sodium hydroxide pellets was dissolved in 800 ml of deionised water. 40 ml of 'Chlorax' bleach (5% sodium hypochlorite, NaOCl) or 'Vortex Industrial' bleach (5% NaOCl) or 66.6 ml of 'Chloro-do' bleach (3% NaOCl) was also added and the solution made up to 1 litre.

   Working alkaline hypochlorite: 100 ml of stock was added to 400 ml of deionised water.
5. The test tube was incubated in a water bath at 37 °C for 20 minutes.
6. The intensity of the blue indophenol dye in the test tube was then read in a spectrophometer at a wavelength of 560 nanometer (nm).
7. Each urine sample was measured in duplicate and the average used for calculation.

Calculation

A graph was plotted from results of the standards. The concentration of urinary ammonia nitrogen was calculated from the standard graph.

Repeatability

The analyses of urinary ammonia nitrogen were conducted by Dr Chandrasekar Persaud, Senior Research Fellow at the Institute of Human Nutrition, University of Southampton. The analytical precision of this assay was determined by within assay repeatability and a CoV of 2.08% was achieved. The CoV for between assays repeatability was 2.40%.

5.5.7 Determination of urea nitrogen in urine

Urea in the urine is hydrolysed by the specific enzyme urease, converting urea to ammonia and carbon dioxide, with carbamic acid as a probable intermediate. The reaction is buffered with EDTA, which also serves to chelate any heavy metal ions that

might otherwise inactivate the urease. The ammonia is then estimated by the Berthelot method as described in section 5.5.6.

Method

1. 25 ul of each urea standard was pipetted into the bottom of labelled test tubes.
   Stock standard: 1.0717 g of urea, together with 100 mg of sodium azide as a preservative, were dissolved in 100 ml of deionised water to make up a stock solution of 500 mg urea-N / 100ml.
   Working standards:

S/0 Deionised water (blank)	0.0 µg Urea-N / 25 ul
S/1 1 ml stock + 49 ml deionised water	2.5 µg Urea-N / 25 ul
S/2 2 ml stock + 48 ml deionised water	5.0 µg Urea-N / 25 ul
S/3 3 ml stock + 47 ml deionised water	7.5 µg Urea-N / 25 ul
S/4 4 ml stock + 46 ml deionised water	10.0 µg Urea-N / 25 ul

2. Each urine sample was diluted to 1:20 (100 ul of urine + 1.9 ml of deionised water). 25 ul of the diluted urine was then pipetted into the bottom of labelled test tube.
3. To each tube, 1 ml of working urease was added and mixed well. The tube was incubated in a water bath at 37 °C for 20 minutes.
   Stock urease: 30.69 units / ml. 19.8mg of Jack Bean Urease Type III (31000 units / g) was dissolved in 10 ml of deionised water and 10 ml of glycerol which stabilises urea. The stock is stored at 4 °C.
   Working EDTA buffer: 27 millimolar pH 6.5. 1.0 g of sodium-EDTA (Na_2-EDTA) was dissolved in 90 ml of deionised water and adjusted to pH 6.5 using 30% sodium hydroxide. The solution was then made up to 100 ml with deionised water.
   Working urease: 1 ml of stock urease was diluted with 100 ml of EDTA buffer and stored in a plastic bottle at 4 °C.
4. 4 ml of PNP was added to each tube followed by 5 ml of alkaline hypochlorite solution and mixed well. The tube was then incubated in a water bath at 37 °C for 20 minutes.
5. The intensity of the blue indophenol dye in the test tube was then read in a spectrophometer at 560 nm.

6. Each urine sample was measured in duplicate and the average used for calculation.

Calculation

A graph was plotted using the results of the standards. The concentration of urinary ammonia nitrogen of each sample was calculated from the standard graph. Urea-N of each sample was then calculated by subtracting the ammonia nitrogen obtained in section 5.5.6 from the results obtained in the assay in this section.

Repeatability

The analyses of urinary urea nitrogen were conducted by Dr Chandrasekar Persaud. The analytical precision of this assay was determined by within assay repeatability and a CoV of 1.50% was achieved. The CoV for between assays repeatability was 1.40%.

5.5.8 Isolation of urea from urine

Urea was isolated from urine using ion exchange resin in preparation for Isotope Ratio Mass Spectrometry (Jackson et al 1980). The urine is separated in the resin on the basis of its mass and charge, hence urea is trapped within the hydrogen ions of the resin. This method was developed to isolate urea free from ammonia, as on reaction with lithium hypobromite (LiOBr), ammonia forms nitric oxide (N_2O), which breaks down to nitrous oxide (NO), which has the same molecular weight as $^{15}N^{15}N$-urea. This assay was conducted by Dr Chandrasekar Persaud.

Method

1. A sample of urine containing approximately 3 mg of urea was adjusted to pH 2 using cresol red as an indicator.
2. The resin (Dowex 50WX8-200 mesh H^+ form) was loaded onto a column to a height of 2 cm and washed with deionised water to remove any impurities.
3. The urine sample was loaded onto the column and washed through with 2 ml of deionised water.
4. The urea was then eluted from the column with 5 ml volumes of disodium citrate buffer (21.01 g of citric acid and 8.0 g of NaOH made up to 1 litre at pH 3.41).

The first 5 ml of the eluate was discarded and the subsequent 15 ml were collected into a conical flask.

5. Using cresol red indicator, the contents of the flask were adjusted to pH 12 with 40% NaOH.
6. After adding anti-bumping granules, the eluate was reduced to 1 ml on a hot plate and then transferred to LP-3 tubes. The eluate was frozen at –20 °C until ready it was analysed on the mass spectrometer.

5.5.9 Liberation of nitrogen gas

Urea reacts with LiOBr in a monomolecular reaction to generate nitrogen gas ie. the two nitrogen atoms from one urea molecule is liberated as a single molecule of nitrogen gas. The liberation was conducted by Dr Chandrasekar Persaud.

Method

1. The samples in which urea was isolated from urine was frozen in a dry ice / propanol mixture.
2. 1 ml of LiOBr (4 ml bromine added to 120 ml 10% lithium hydroxide) was added to the frozen sample and was the frozen immediately with the dry ice / propanol mixture to stop any reaction from occurring. This assumes that 1 ml of LiOBr solution is sufficient to liberate 5 mg of nitrogen gas.
3. The frozen sample was loaded onto a liquid nitrogen trap pressure pump system. Any unwanted gas in the glass tube containing the frozen sample was removed.
4. The dry ice / propanol mixture was removed and the glass tube was heated to start the reaction between urea and LiOBr. Once the urea / LiOBr mixture turned yellow, an indication that the reaction was completed, the glass tube was refrozen.
5. The liberated nitrogen gas was collected through the liquid nitrogen cold trap into a glass tube which was then sealed. The purpose of the liquid nitrogen cold trap was to ensure removal of contaminants such as ethylamine or methylamine.

5.5.10 Measurement of isotopic enrichment

The mass spectrometer measures the relative amounts of nitrogen gas of different mass and thus allows assessment of the enrichment of urea excreted in the urine. The nitrogen gas liberated are in the forms of $^{15}N^{15}N$ (molecular weight of 30), $^{15}N^{14}N$ (molecular weight of 29 and $^{14}N^{14}N$ (molecular weight of 28) and hence allows the relative proportions of $^{15}N^{15}N$-urea, $^{15}N^{14}N$-urea and $^{14}N^{14}N$-urea excreted in the urine to be determined. The abundance of 15N atoms in the samples is expressed in relation to total nitrogen, known as atom percent and represents the number of ^{15}N atoms in 100 atoms of the element. Enrichment of the gas sample was measured in a triple collector isotope ratio mass spectrometer (SIRA 10, VG Isogas, Winsford, Cheshire, UK) by Dr Chandrasekar Persaud.

$$\text{Atoms percent} = \frac{\text{number of } ^{15}N \text{ atoms}}{\text{Number of } ^{15}N \text{ atoms} + \text{number of } ^{14}N \text{ atoms}} \times 100$$

5.5.11 Statistical analysis

Statistical analysis was carried out using SPSS for Windows software. All the results were presented in frequencies, percentages and mean (sd) where appropriate and parametric tests (One-way ANOVA, Paired T-test, Pearson Correlation) were performed to detect statistical significance differences at 95% confidence intervals. Although the number of subjects was small and the data were generally skewed, the data were presented in mean and standard deviation to enable assessment of central tendencies and distribution of data from the mean. To avoid erroneous reporting of results, the mean and median of the data were analysed and these were generally similar. Both parametric and non-parametric tests were also applied to the data and the results were again mostly similar. The outcomes were not affected by the application of parametric statistics.

5.6 Results

5.6.1 Characteristics of subjects

12 subjects who have had total colectomy and permanent ileostomy for more than 12 months were recruited. 6 volunteers who did not have gastrointestinal disease or surgery

were also recruited as a reference population for this study. The characteristics of these 18 subjects are shown in Table 5.2.

Table 5.2 Characteristics of reference and ileostomy subjects

	Reference subjects	Ileostomy: NSBR	Ileostomy: SBR
Number	6	6	6
Sex	3 females, 3 males	2 females, 4 males	3 females, 3 males
Age (years): Range	25-57	45-66	34-67
Mean (sd)	43.0 (13.6)	54.8 (7.6)	50.3 (11.5)

Due to difficulties encountered in recruitment, the subjects in the 3 groups were not matched for age or sex. The ileostomy subjects were, in general older, than the reference subjects but the mean ages of the subjects in the 3 groups were not significantly different from one another. All the subjects in NSBR group had ulcerative colitis, while in the SBR group, 4 had Crohn's disease and 2 had ulcerative colitis. These 2 ulcerative colitis subjects had small bowel resection due to previous failure of ileal pouch surgery. The extent of small bowel resection and the length of remaining small bowel in SBR ileostomy subjects were not known.

5.6.2 Body mass index and body composition

The BMI and body composition of all the subjects on habitual and low protein diets are shown in Tables 5.3 and 5.4 respectively.

Table 5.3 BMI and body composition of all subjects on habitual diet [mean (sd)]

	Reference	NSBR	SBR
Weight (kg)	80.0 (16.8)	73.7 (12.4)	67.6 (12.7)
Height (m)	1.73 (0.10)	1.64 (0.07)	1.67 (0.10)
BMI (kg/m^2)	26.5 (4.9)	27.1 (3.1)	24.4 (4.3)
BF (kg)	20.8 (8.4)	22.0 (4.4)	19.1 (5.7)
FFM (kg)	59.1 (13.4)	51.7 (11.5)	48.5 (11.4)
BF%	25.7 (9.0)	30.3 (6.6)	28.5 (8.4)

Table 5.4 BMI and body composition of all subjects on low protein diet [mean (sd)]

	Reference	NSBR	SBR
Weight (kg)	80.3 (17.9)	73.2 (11.9)	68.1 (11.8)
Height (m)	1.74 (0.10)	1.65 (0.07)	1.67 (0.10)
BMI (kg/m^2)	26.4 (4.6)	26.8 (2.9)	24.7 (4.0)
BF (kg)	21.2 (6.6)	21.0 (4.2)	19.6 (5.4)
FFM (kg)	59.2 (14.4)	52.1 (12.2)	48.6 (11.2)
BF%	26.3 (6.9)	29.4 (7.4)	29.0 (8.6)

The BMI of SBR subjects was lower than reference and NSBR subjects but NSBR subjects have slightly higher BMI than reference subjects. However, both NSBR and SBR subjects have relatively higher adiposity and lower lean mass compared to reference subjects. In these small numbers, none of the differences were significant.

5.6.3 Resting metabolic rate

The RMR of all the subjects is shown in Table 5.5

Table 5.5 RMR of all subjects on habitual and low protein diets [mean (sd)]

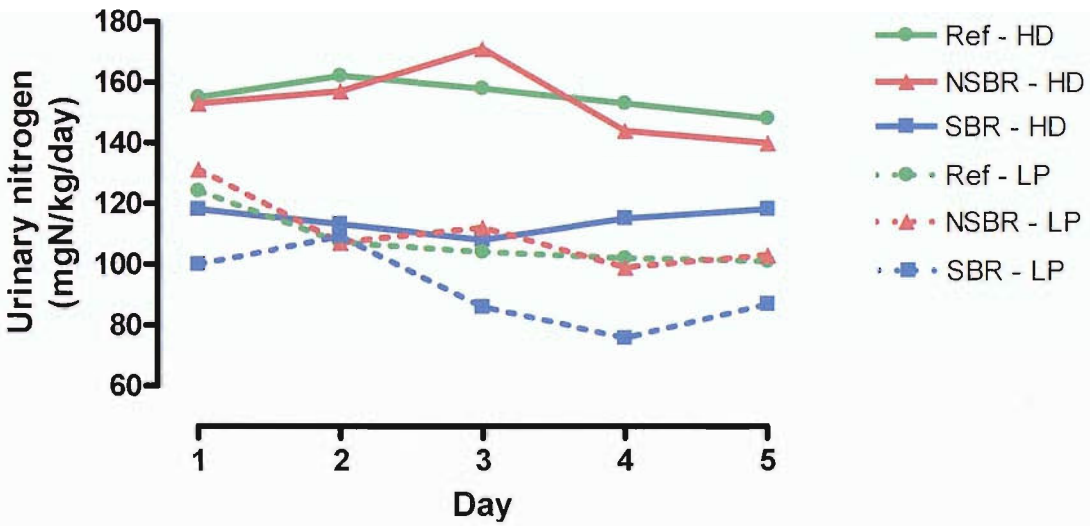
	Reference	NSBR	SBR
HD-RMR (kcal/day)	1335.5 (215.7)	1481.5 (382.4)	1252.7 (143.6)
HD-RMR/weight (kcal/kg/day)	18.1 (4.1)	19.9 (2.5)	18.8 (2.2)
LP-RMR (kcal/day)	1465.8 (328.2)	1346.3 (216.9)	1228.2 (164.1)
LP-RMR/weight (kcal/kg/day)	18.5 (2.9)	18.6 (2.6)	18.6 (4.6)

There were no significant differences in the RMR among the 3 groups of subjects or between NSBR and SBR subjects. The RMR per kilogramme body weight in all the three groups of subjects was also similar. A reduction in protein intake did not alter the RMR of the all the subjects.

5.6.4 Nitrogen and energy intakes

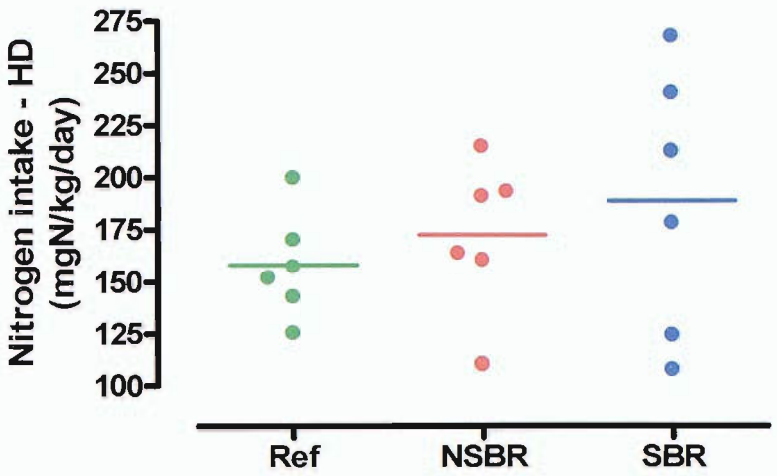
All 18 subjects completed both habitual and low protein studies satisfactorily. All the diets were recorded and analysed. Days 1 to 3 were taken as the adaptation period after which all the subjects were expected to be in a steady metabolic state. Therefore the average nitrogen and energy intakes for each study period were calculated from the mean intakes of Days 4 and 5. Individual average nitrogen intake on habitual diet is shown in Appendix 5A and on low protein diet is shown in Appendix 5B. Figure 5.2 shows the average urinary nitrogen excretions on habitual diet and the mean urinary nitrogen excretions on each of the five days on low protein diets for each group of subjects. The reduction in nitrogen intakes was reflected in the changes in urinary nitrogen excretions. The urinary nitrogen excretions for reference and NSBR subjects appeared to reach a plateau on Days 4 and 5 while for SBR subjects, it appears that a steady state was attained by Day 5.

Figure 5.2 Urinary nitrogen excretion of subjects on habitual and low protein diets



The average nitrogen intakes for the reference, NSBR and SBR subjects on habitual diets were 157.9 (25.3) mgN/kg/day, 172.4 (36.6) mgN/kg/day and 188.8 (63.8) mgN/kg/day respectively. Although there were no significant differences among the three groups and between NSBR and SBR subjects, nitrogen intakes of ileostomy subjects, particularly SBR subjects, tended to be higher and more widely distributed as illustrated in Figure 5.3.

Figure 5.3 Average nitrogen intake of subjects on habitual diets



On low protein diets, the average nitrogen intakes for the reference, NSBR and SBR subjects were 94.7 (9.0) mgN/kg/day, 101.8 (16.6) mgN/kg/day and 109.0 (41.1) mgN/kg/day respectively and no significant differences were seen among the three groups and between NSBR and SBR subjects. However, the mean reduction in nitrogen intakes from habitual to low protein diets was significant for each of the three groups. For reference subjects, the mean reduction in nitrogen intakes was 63.2 (18.7) mgN/kg/day (39.4%) ($p<0.001$), for NSBR subjects, the mean reduction was 70.7 (21.3) mgN/kg/day (40.3%) ($p<0.001$) and for SBR subjects, the mean reduction was 79.8 (29.2) mgN/kg/day (42.1%) ($p<0.001$).

The average energy intakes on habitual and low protein diets are summarized in Table 5.6. These intakes were not significantly different among the three groups or between NSBR and SBR subjects. For low protein diets, whilst nitrogen intakes were reduced by 40% in all subjects, energy intakes were kept at the same level as that of habitual diets. Using benchmarks of 1.4 times RMR for sedentary lifestyle, 1.7 times RMR for moderately active lifestyle and 1.9 times RMR for very active lifestyle to estimate energy requirements (Dietary Reference Values for Food and Energy and Nutrients for the United Kingdom 1999), the mean energy intakes for each group of subjects were adequate to provide for moderately and very active lifestyles. Individual average energy intake on habitual diet is shown in Appendix 5A and on low protein diet is shown in Appendix 5B.

Table 5.6 Average energy intake on habitual and low protein diets (mean (sd))

		Reference	NSBR	SBR
HD	kcal/day	2535 (567)	2280 (684)	2381 (607)
	kcal/kg/day	32.5 (8.7)	31.3 (9.7)	36.3 (10.2)
	energy requirement	1.8 x RMR	1.6 x RMR	2.0 x RMR
LP	kcal/day	2489 (479)	2305 (661)	2382 (420)
	kcal/kg/day	32.1 (9.5)	31.9 (9.1)	35.9 (8.2)
	energy requirement	1.7 x RMR	1.7 x RMR	2.0 x RMR

A comparison of energy and nitrogen intakes as measured by FFQ in the cross-sectional study and weighed food diary in this study is shown in Table 5.7. Energy intakes as measured by both methods were similar for reference subjects but in ileostomy subjects, it was 300 kcal lower using weighed food diaries. Nitrogen intakes of both reference and ileostomy subjects using weighed food diary were approximately 3.5 g lower than FFQ. This confirms that the FFQ over-estimates true daily nitrogen intakes.

Table 5.7 Comparison of mean energy and nitrogen intakes measured by FFQ and weighed food diary

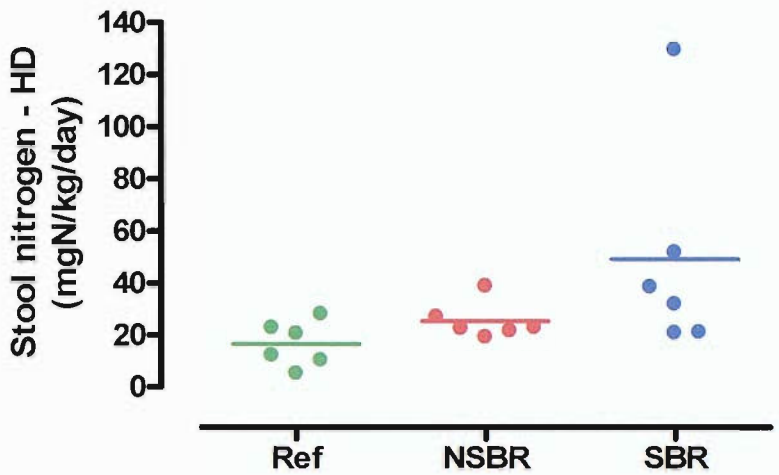
	Reference	Ileostomy
Energy intake (FFQ) – kcal/day	2464 (812)	2630 (961)
Nitrogen intake (FFQ) – g/day	16.0 (5.2)	16.3 (6.2)
Energy intake (HD) – kcal/day	2535 (565)	2330 (619)
Nitrogen intake (HD) – g/day	12.5 (3.1)	12.6 (3.8)

5.6.5 Stool nitrogen losses

All subjects collected stool for the entire 5-day study period on both habitual and low protein diets. Daily stool nitrogen content was quantified and the average stool nitrogen excreted for the each study period was calculated from the mean stool nitrogen of Days 4 and 5 with Days 1 to 3 taken as the adaptation period. Individual average stool nitrogen loss on habitual diet is shown in Appendix 5A and on low protein diet is shown in

Appendix 5B. Figure 5.4 illustrates the distribution of stool nitrogen losses on habitual diets in each group of subjects.

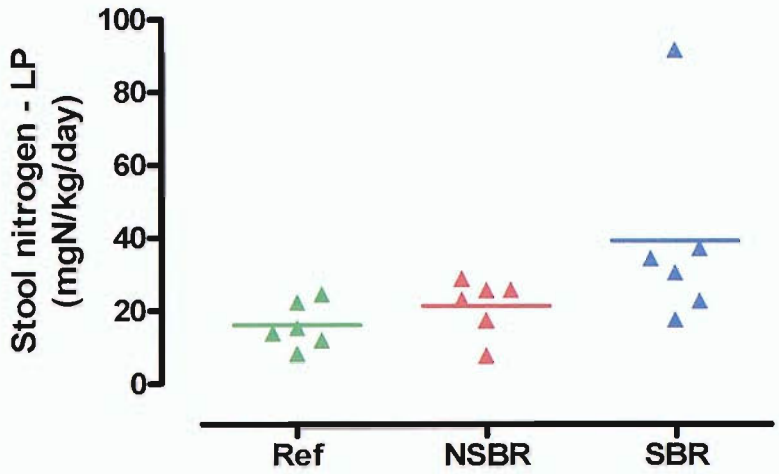
Figure 5.4 Average stool nitrogen loss of subjects on habitual diets



On habitual diets, the average stool nitrogen losses for reference, NSBR and SBR subjects were 16.83 (8.55) mgN/kg/day, 25.49 (6.91) mgN/kg/day and 49.08 (41.08) mgN/kg/day respectively. Although the mean stool nitrogen losses were higher in ileostomy subjects, particularly SBR subjects, no significant differences were found among the three groups and between NSBR and SBR subjects. In SBR group, there is an outlier (subject 11) whose stool nitrogen loss was very much higher and this individual also had the highest nitrogen intakes among all 18 subjects.

Stool nitrogen losses on low protein diets are shown in Figure 5.5. On low protein diets, the average stool nitrogen losses for reference, NSBR and SBR subjects were 16.12 (6.14) mgN/kg/day, 21.53 (7.66) mgN/kg/day and 39.21 (26.82) mgN/kg/day respectively but no significant differences were found among the three groups or between NSBR and SBR subjects. As with the habitual diets, the stool nitrogen loss of subject id 11 was very much higher than the other subjects.

Figure 5.5 Average stool nitrogen loss of subjects on low protein diets

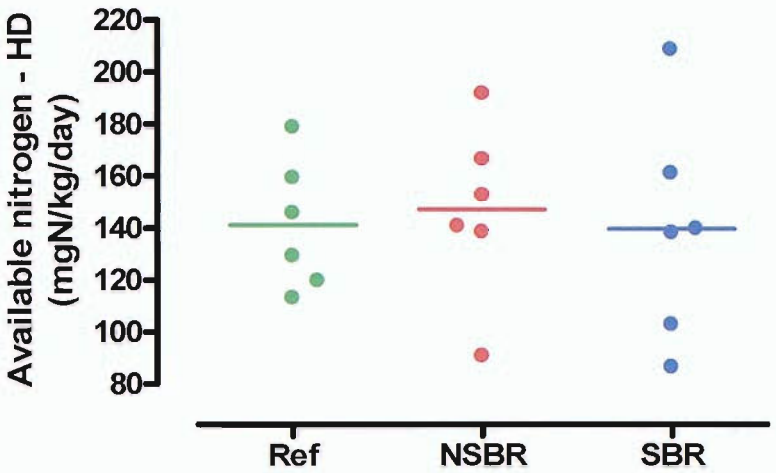


Despite a reduction in nitrogen intake by 40%, no significant differences were seen in stool nitrogen losses between the two diets for all three groups of subjects. The mean reduction in stool nitrogen losses for reference, NSBR and SBR subjects were only 0.7 mgN/kg/day (4.2%), 4.0 mgN/kg/day (15.7%) and 9.9 mgN/kg/day (20.2%) respectively.

5.6.6 Available nitrogen

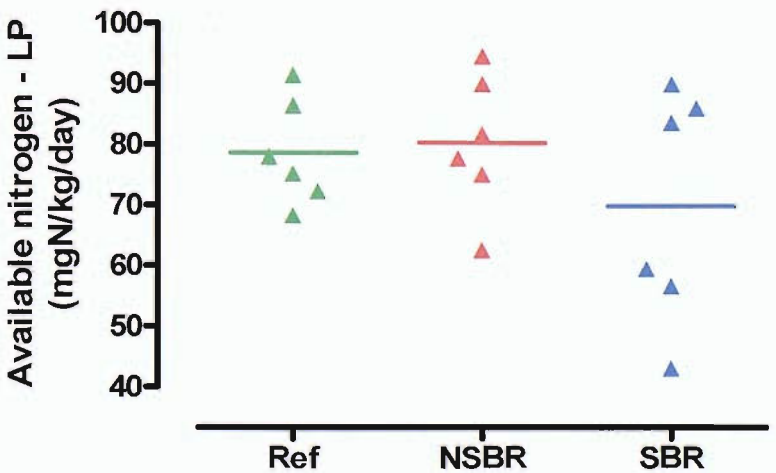
By subtracting average stool nitrogen losses (section 5.6.5) from average nitrogen intake (section 5.6.4), the average amount of nitrogen that is absorbed and hence, available to subjects for metabolic utilisation can be calculated. Individual average available nitrogen on habitual diet is shown in Appendix 5A and on low protein diet is shown in Appendix 5B. The distribution of average available nitrogen is illustrated in Figures 5.6. On habitual diets, the average available nitrogen for reference (141.07 (25.17) mgN/kg/day), NSBR (146.92 (33.77) mgN/kg/day) and SBR (139.74 (43.42) mgN/kg/day) subjects were similar but the results were widely distributed, especially for SBR subjects. It would appear that ileostomy subjects, especially those with SBR, maintain nitrogen availability by consuming more nitrogen to compensate for higher stool nitrogen losses.

Figure 5.6 Average available nitrogen to subjects on habitual intakes



On low protein diets, the average available nitrogen for reference, NSBR and SBR subjects were 78.61 (8.77) mgN/kg/day, 80.23 (11.35) mgN/kg/day and 69.77 (19.25) mgN/kg/day respectively and the distribution is illustrated in Figure 5.7. Although the mean available nitrogen appeared to be lower in SBR subjects, no significant differences were seen among the three groups or between NSBR and SBR subjects. This is probably due to the widely distributed results.

Figure 5.7 Average available nitrogen to subjects on low protein diets



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When protein intake was reduced, there was a significant decline in available nitrogen for all three groups of subjects as illustrated in Figure 5.8. The mean reduction in available nitrogen for reference, NSBR and SBR subjects were 62.5 mgN/kg/day (43.3%) ($p=0.001$), 66.7 mgN/kg/day (44.2%) ($p=0.001$) and 70.0 mgN/kg/day (49.2%) ($p=0.002$) respectively.

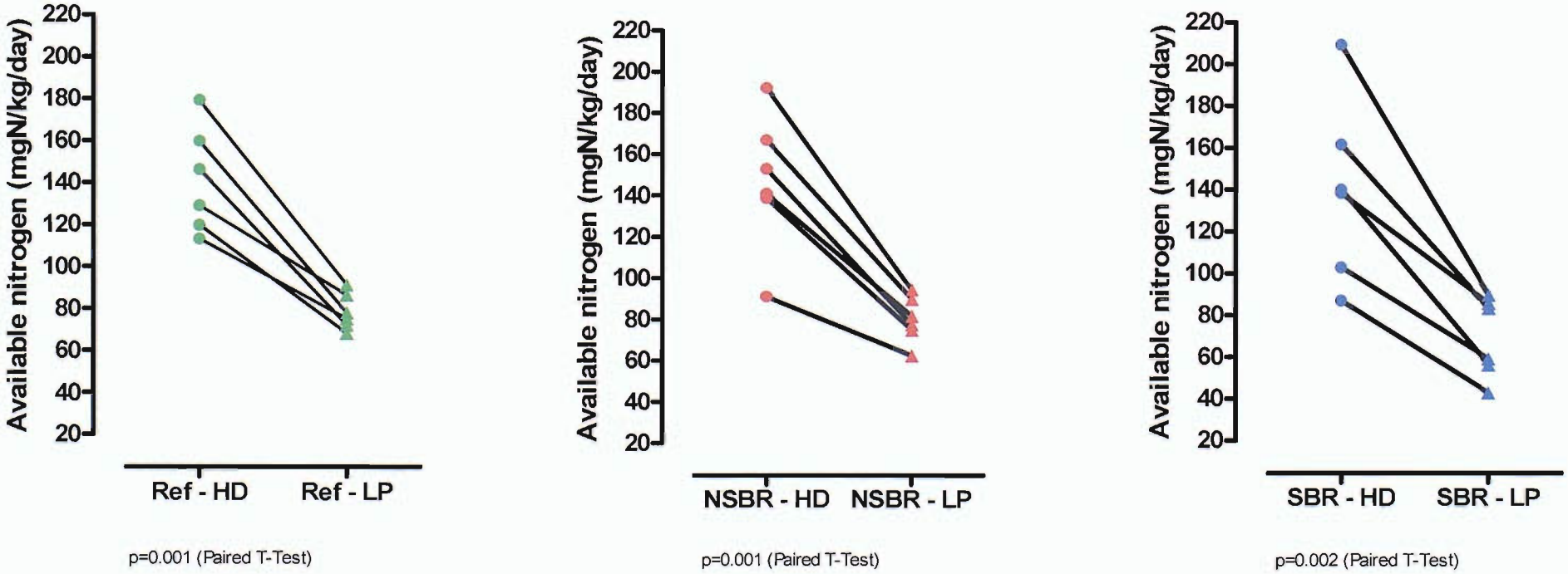
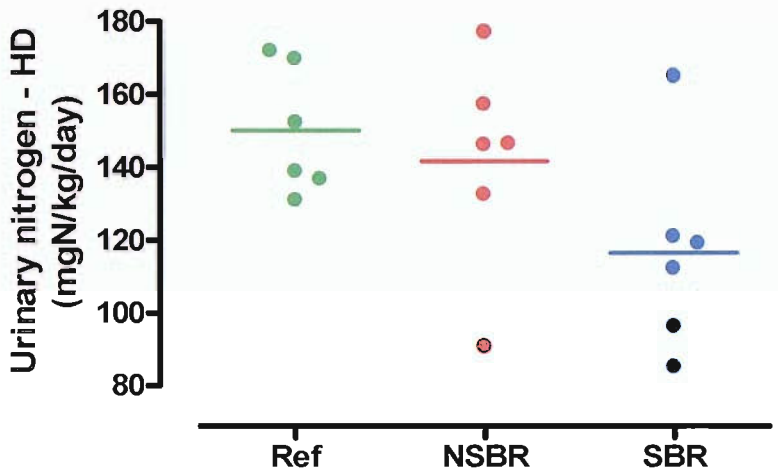


Figure 5.8 Changes to available nitrogen following a reduction in nitrogen intake

5.6.7 Urinary nitrogen excretion

All 18 subjects collected urine for the entire 5-day study period on both the habitual and low protein diets. Daily urinary nitrogen excretion was quantified and the average urinary nitrogen excreted for the each study period was calculated from the mean urinary nitrogen of Days 4 and 5 with Days 1 to 3 taken as the adaptation period. Individual average urinary nitrogen excretion on habitual diet is shown in Appendix 5A and on low protein diet is shown in Appendix 5B. Figure 5.9 shows the distribution of urinary nitrogen excretions on habitual diets for all three groups of subjects.

Figure 5.9 Average urinary nitrogen excretion of subjects on habitual diets

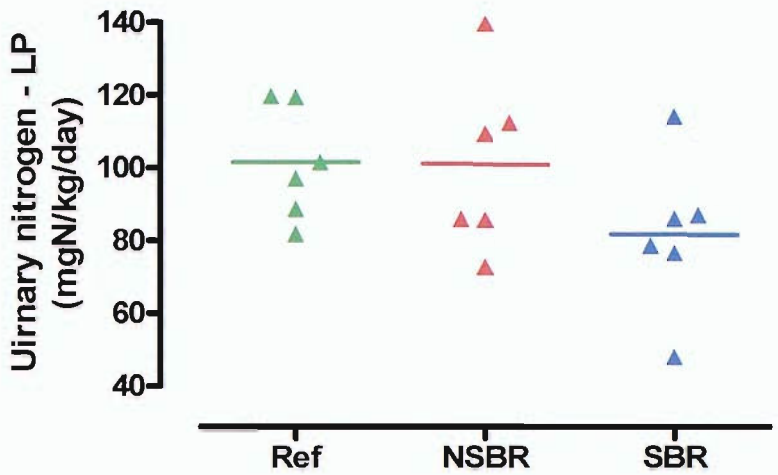


On habitual diets, the average urinary nitrogen excretion for reference, NSBR and SBR subjects were 150.27 (17.48) mgN/kg/day, 141.84 (28.98) mgN/kg/day and 116.58 (27.47) mgN/kg/day respectively. It is evident that the results were widely distributed for ileostomy subjects and although urinary nitrogen excretion appeared to be generally lower in SBR subjects, no significant differences were found among the three groups of subjects and between NSBR and SBR subjects.

On low protein diets, the average urinary nitrogen losses for reference, NSBR and SBR subjects were 101.46 (15.57) mgN/kg/day, 101.02 (24.20) mgN/kg/day and 81.76 (21.21) mgN/kg/day respectively with no significant differences found among the three groups of subjects or between NSBR and SBR subjects. As with the habitual diets, these results

were more widely distributed and appeared to be generally lower in SBR subjects as shown in Figure 5.10.

Figure 5.10 Average urinary nitrogen excretion of subjects on low protein diets



When protein intake was reduced by 40%, there was a significant decline in urinary nitrogen excretion for each group of subjects (Figure 5.11). The mean reduction in urinary nitrogen excretion for reference, NSBR and SBR subjects were 48.8 mgN/kg/day (32.7%) ($p<0.001$), 40.8 mgN/kg/day (28.9%) ($p=0.001$) and 34.8 mgN/kg/day (30.0%) ($p=0.001$) respectively.

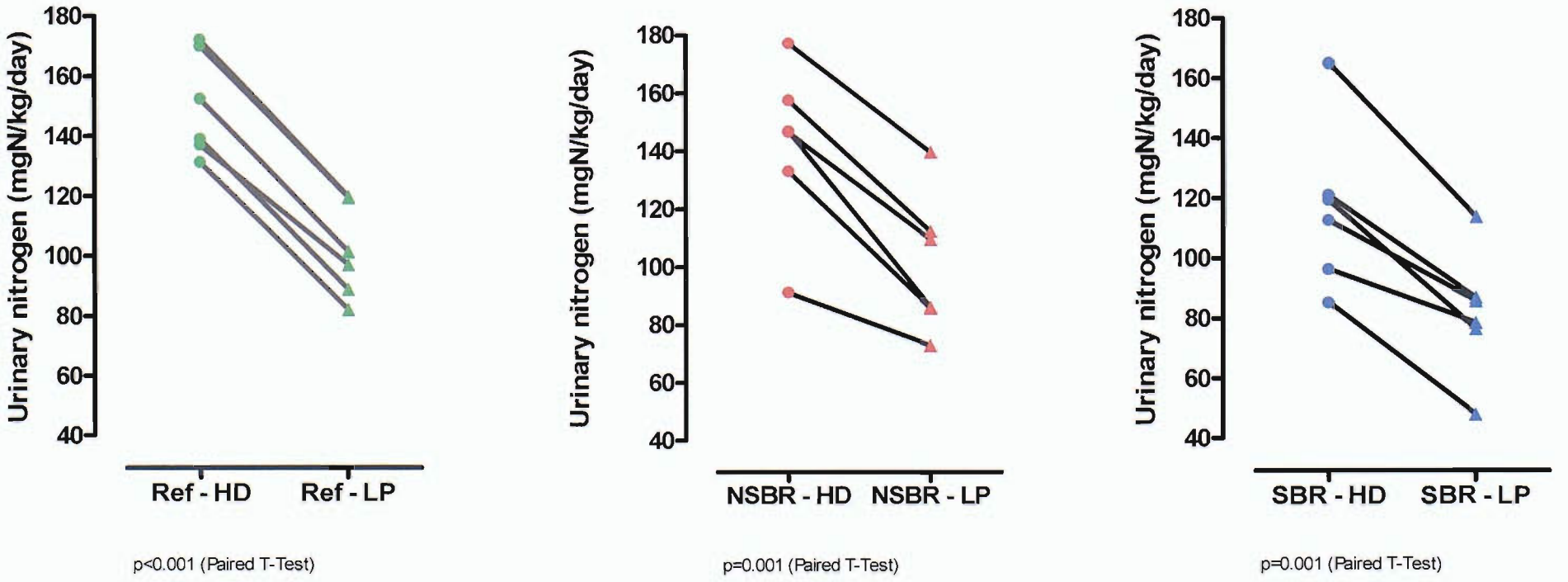
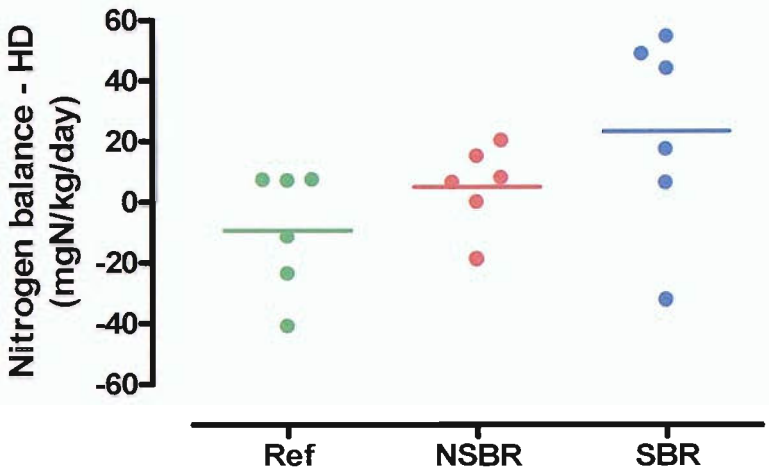


Figure 5.11 Changes to urinary nitrogen excretion following a reduction in nitrogen intake

5.6.8 Nitrogen balance

The average nitrogen balance attained by each subject was calculated by subtracting average urinary and stool nitrogen losses from average nitrogen intake. Individual data of nitrogen balance on habitual diet is shown in Appendix 5A and on low protein diet is shown in Appendix 5B. Figure 5.12 shows the distribution of nitrogen balance intakes for all three groups of subjects.

Figure 5.12 Average nitrogen balance of subjects on habitual diets



On habitual diets, 3 reference subjects were in positive nitrogen balance while 3 were in negative nitrogen balance. The mean nitrogen balance for the reference group was -9.20 (20.04) mgN/kg/day. For ileostomy subjects, only 1 subject in each of NSBR and SBR groups was in negative nitrogen balance while the rest were in positive balance. The mean nitrogen balances for NSBR and SBR subjects were 5.08 (13.62) mgN/kg/day and 23.16 (33.05) mgN/kg/day respectively. Therefore, both NSBR and SBR subjects were in overall positive nitrogen balances while the reference subjects were in slight negative nitrogen balances but these results were not significantly different among the three groups of subjects and between NSBR and SBR subjects.

On low protein diets, all the 6 reference subjects were in negative nitrogen balances with a mean nitrogen balance of -22.85 (7.13) mgN/kg/day. For ileostomy subjects, 5 subjects from each of the NSBR and SBR groups were in negative nitrogen balances while 1 from each group was in positive nitrogen balance and the mean nitrogen balances for NSBR

and SBR subjects were -20.78 (19.86) mgN/kg/day and -11.99 (16.05) mgN/kg/day respectively. There were no significant differences among the three groups of subjects or between NSBR and SBR subjects. The distribution of nitrogen balances on low protein diets is shown in Figure 5.13.

Figure 5.13 Average nitrogen balance of subjects on low protein diets

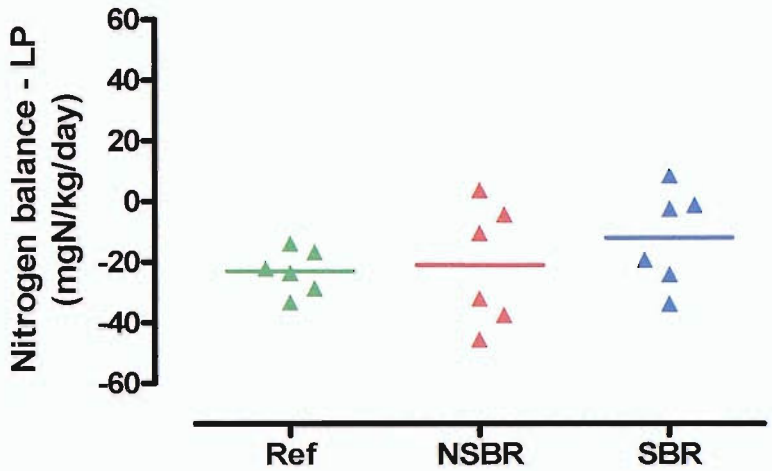


Figure 5.14 shows the changes in nitrogen balances for each group of subjects when protein intakes were reduced by 40%. In the reference group, although there was an overall fall in nitrogen balance, the mean reduction of 13.7 mgN/kg/day was not statistically significant. However, in the NSBR group, the reduction in protein intake resulted in a significant fall in nitrogen balance by an average of 25.9 mgN/kg/day ($p=0.023$) whereas the greatest reduction in overall nitrogen balance, by an average of 35.1 mgN/kg/day, was seen in SBR subjects ($p=0.017$).

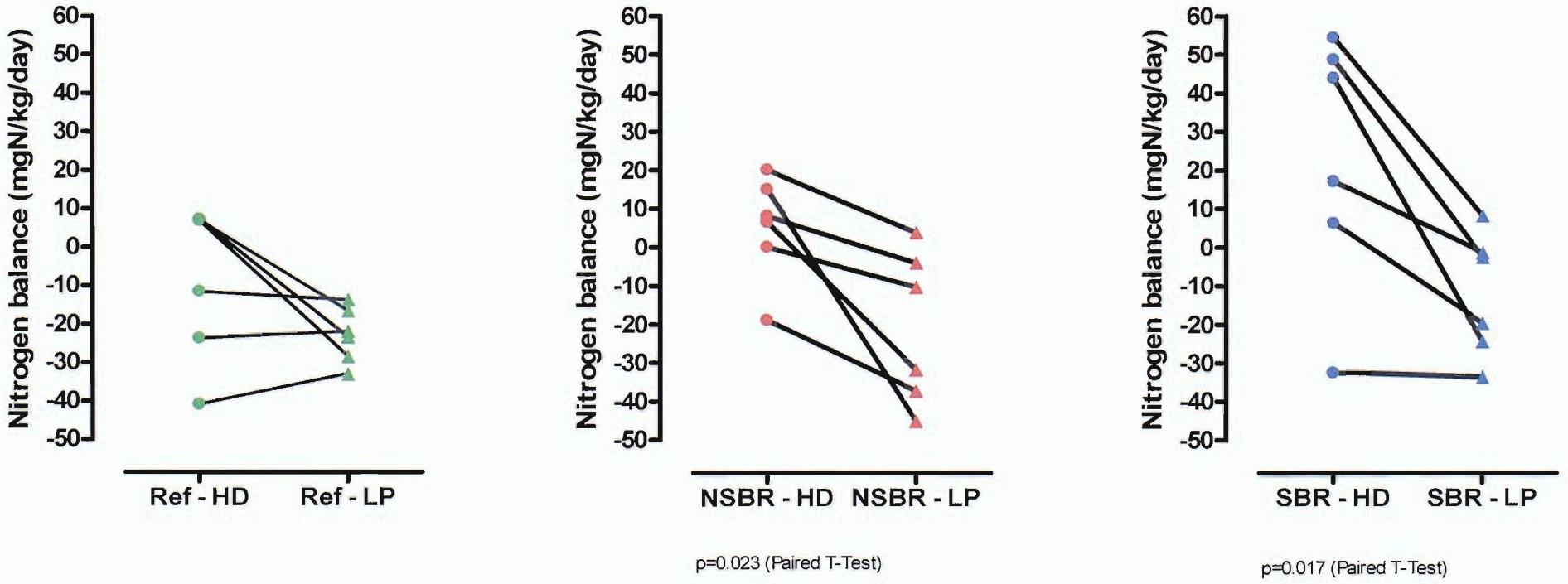
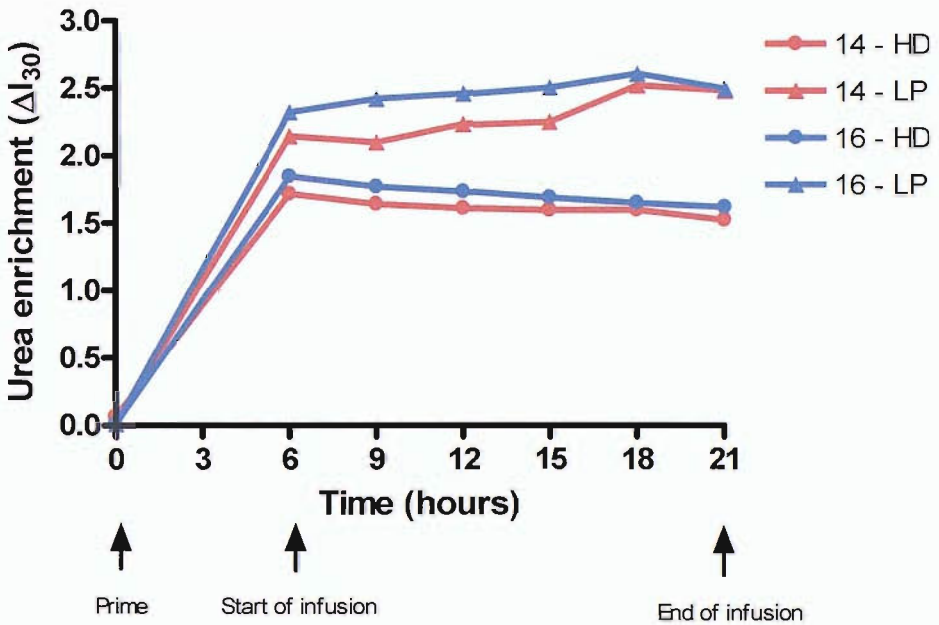


Figure 5.14 Changes to nitrogen balance following a reduction in nitrogen intake

5.6.9 Urea kinetics – enrichment of urinary urea

Studies of urea kinetics on habitual and low protein diets were conducted on Day 5 of each study period when all the subjects should have reached a steady metabolic state. As discussed above, the prime-constant intravenous infusion model was used and plateau enrichment in urinary urea was identified by visual inspection. Figure 5.15 illustrates examples of satisfactory plateau enrichment indicating steady isotopic state.

Figure 5.15 Rise to plateau of urinary $^{15}N^{15}N$-urea in subjects 14 and 16 in arbitrary units.



For both habitual and low protein diets, the calculation of the various aspects of urea kinetics was based on Day 5 nitrogen intakes which were not significantly different among the reference, NSBR and SBR subjects or between NSBR and SBR subjects (Table 5.8). However, the 40% reduction in nitrogen intakes when the diet was changed was significant in each of the three groups of subjects. Individual Day 5 nitrogen intake on habitual diet is shown in Appendix 6A and on low protein diet is shown in Appendix 6B.

Table 5.8 Day 5 nitrogen intakes on habitual and low protein diets [mean (sd)]

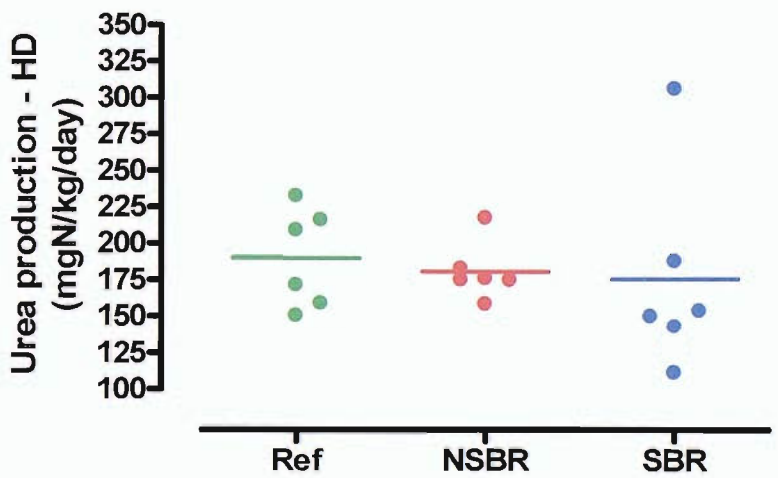
	Reference	NSBR	SBR
HD-Nitrogen intake(mgN/kg/day)	164.22 (14.71)	155.67 (25.33)	175.15 (71.23)
LP-Nitrogen intake (mgN/kg/day)	94.00 (8.56)*	101.60 (16.49)*	108.65 (41.06)*

Paired T-Test: *p<0.01, HD vs LP

5.6.10 Urea kinetics – urea production

Urea production (P) was derived from the relative amounts of $^{15}N^{15}N$-urea: $^{14}N^{14}N$-urea in the urine measured by the relative intensities using isotope mass spectrometry (section 5.5.10). Individual urea production on habitual diet is shown in Appendix 6A and on low protein diet is shown in Appendix 6B. Figure 5.16 shows the distribution of P on habitual diets for the three groups of subjects.

Figure 5.16 Urea production in subjects on habitual diets



On habitual diets, mean P was not significantly different among the reference, NSBR and SBR subjects (189.90 (33.69) mgN/kg/day, 180.98 (19.49) mgN/kg/day and 175.59 (68.18) mgN/kg/day respectively) or between NSBR and SBR subjects. However, P tended to be generally lower in the SBR group which was also widely distributed with an outlier (subjects 12).

On low protein diets, mean P was also not significantly different among the 3 groups of subjects (reference: 135 (38.42) mgN/kg/day, NSBR: 120.78 (18.20) mgN/kg/day and SBR: 115.94 (16.00) mgN/kg/day) and between NSBR and SBR subjects and the responses to reduced nitrogen intake were more variable in the reference and SBR subjects than in NSBR subjects (Figure 5.17)

Figure 5.17 Urea production in subjects on low protein diets

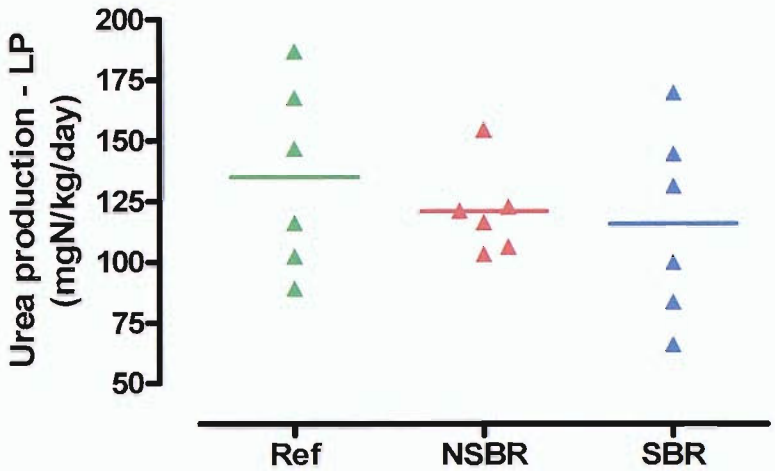


Figure 5.18 shows that when protein intake was reduced significantly, P also fell significantly in all three groups of subjects. This reduction in P was reflected in urinary urea enrichment which was higher when subjects were on low protein diets (Figure 5.15). For the reference subjects, P fell by 54.90 mgN/kg/day (28.9%) ($p=0.019$) while for NSBR and SBR subjects, the reductions were slightly higher than in the reference subjects at 60.20 mgN/kg/day (33.3%) ($p<0.001$) and 59.65 mgN/kg/day (34.0%) ($p=0.019$) respectively.

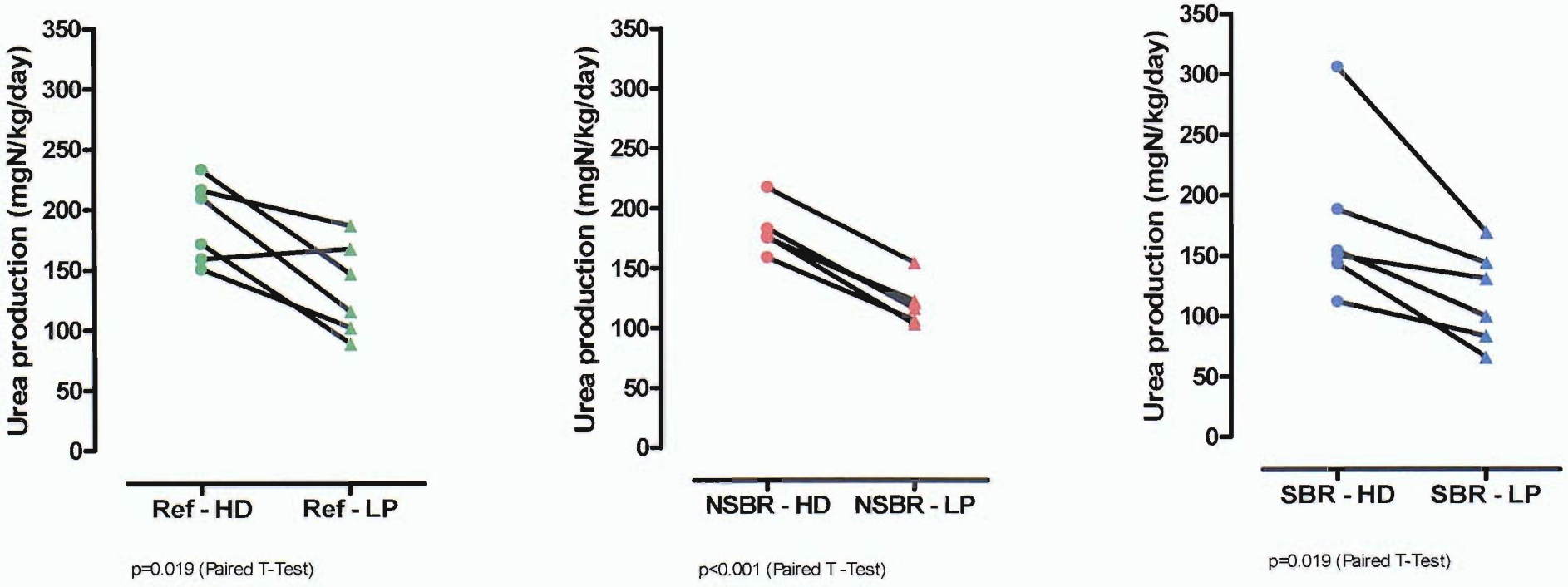
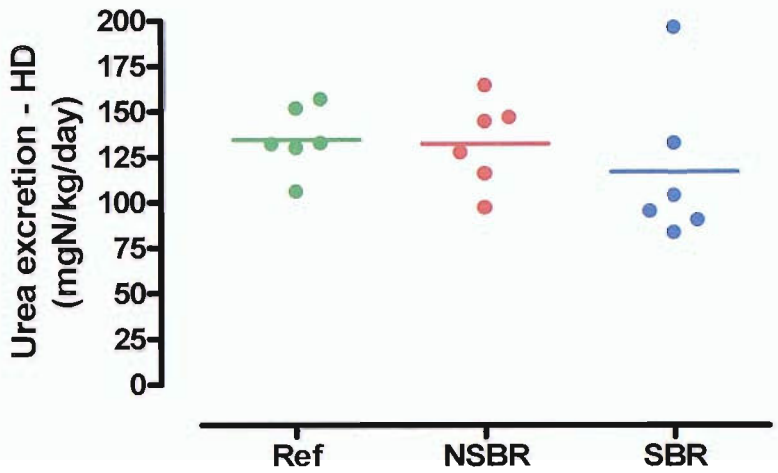


Figure 5.18 Changes to urea production following a reduction in nitrogen intake

5.6.11 Urea kinetics - urea excretion

The rates of urea excretion (Eu) were assessed by direct analysis of urinary urea content (section 5.5.7). Individual E_U on habitual diet is shown in Appendix 6A and on low protein diet is shown in Appendix 6B. Figure 5.19 shows the distribution of urinary urea excretion on habitual diets.

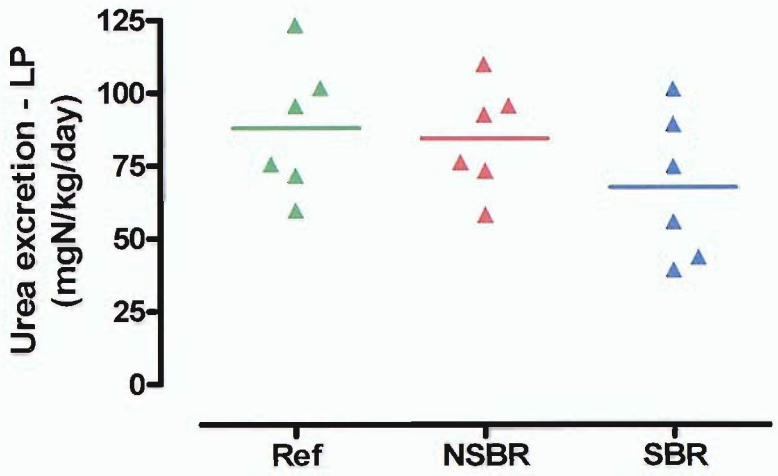
Figure 5.19 Urinary urea excretion in subjects on habitual diets



On habitual diets, mean E_U were 134.70 (17.86) mgN/kg/day, 132.61 (24.07) mgN/kg/day and 116.71 (42.19) mg/N/kg/day for reference, NSBR and SBR subjects respectively. These values were not significantly different among one another or between NSBR and SBR subjects but the results tended to be lower and were more widely distributed in SBR subjects with an outlier (subjects 12).

On low protein diets, mean E_U were also not significantly different among the reference (88.11 (23.25) mgN/kg/day), NSBR (84.44 (18.57) mgN/kg/day) and SBR (67.61 (25.19) mgN/kg/day) subjects or between NSBR and SBR subjects. Figure 5.20 shows the distribution of E_U on low protein diets.

Figure 5.20 Urinary urea excretion in subjects on low protein diets



The differences in E_U between habitual and low protein diets are shown in Figure 5.21. There was a significant reduction in the mean E_U for each group of subjects when nitrogen intake was reduced. The largest reduction in E_U was seen in the SBR subjects (42.1%, 49.10 mgN/kg/day, p=0.004), followed by NSBR subjects (36.3%, 48.18 mgN/kg/day, p<0.001) and than the reference subjects (34.6%, 46.59 mgN/kg/day, p=0.001).

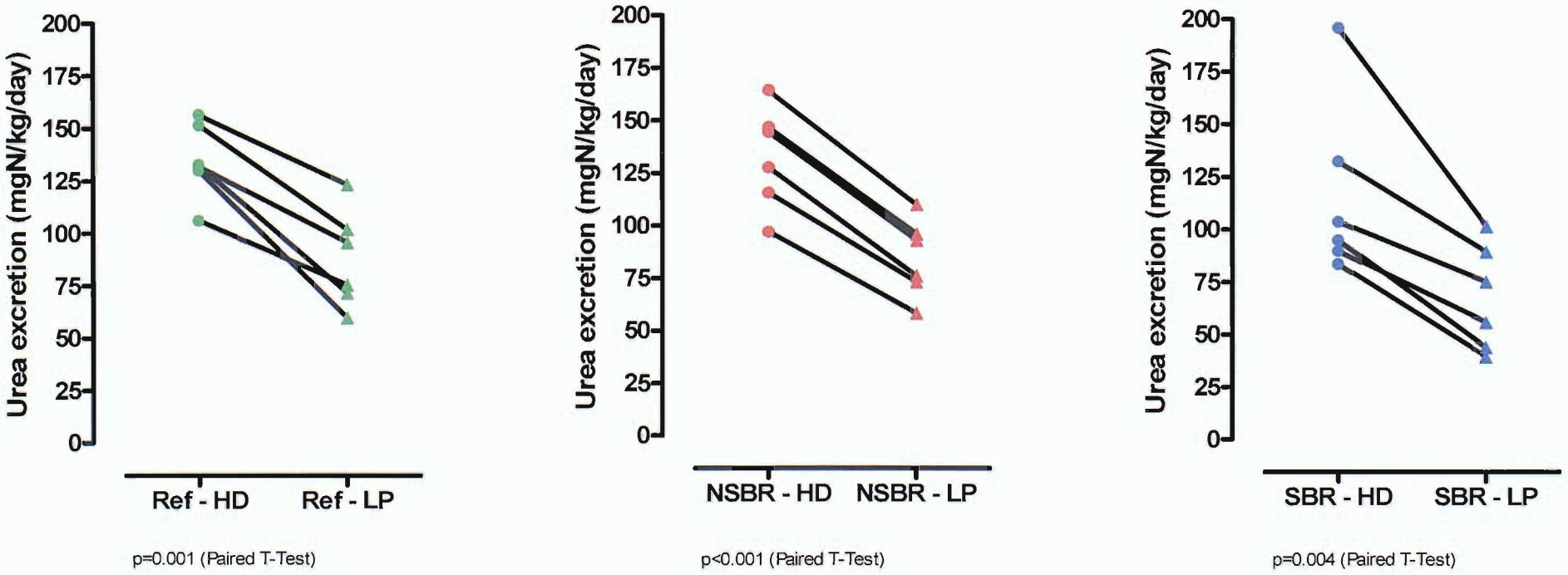
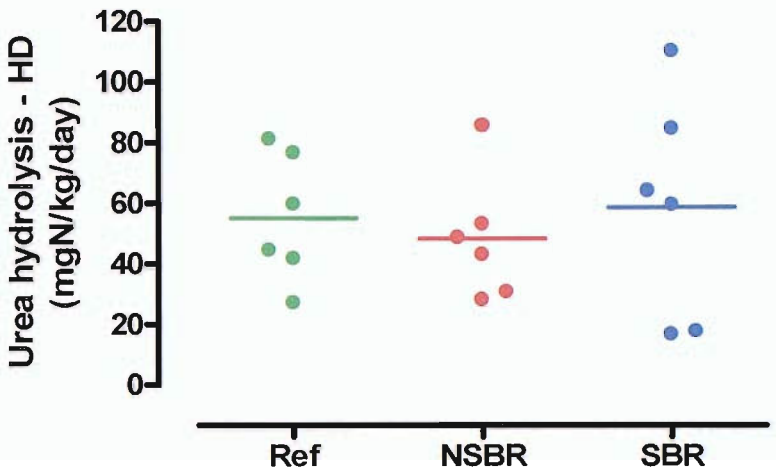


Figure 5.21 Changes to urinary urea excretion following a reduction in nitrogen intake

5.6.12 Urea kinetics - urea hydrolysis

From the assumption that the difference between urea production and that excreted in the urine has passed into the colon for nitrogen salvage, urea hydrolysis (T) was calculated. Individual T on habitual diet is shown in Appendix 6A and on low protein diet is shown in Appendix 6B. Figure 5.22 shows T for all three groups of subjects on habitual diets.

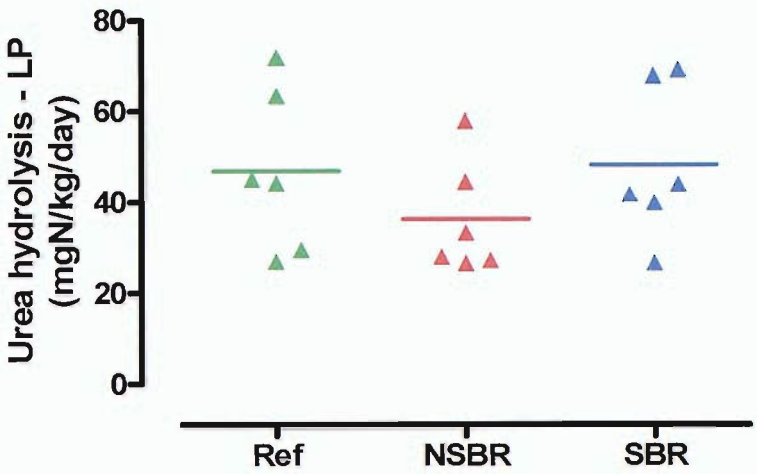
Figure 5.22 Urea hydrolysis in subjects on habitual diets



On habitual diets, mean T were similar amongst reference subjects (55.20 (21.09) mgN/kg/day), NSBR subjects (48.37 (20.73) mgN/kg/day) and SBR subjects (58.89 (36.70) mgN/kg/day) but the results for SBR subjects were more widely distributed than the other two groups. These results suggest that ileostomy subjects were capable of urea hydrolysis.

On low protein diets, mean T for reference, NSBR and SBR subjects were 46.89 (17.95) mgN/kg/day, 36.35 (12.55) mgN/kg/day and 48.33 (16.890 mgN/kg/day respectively. These values were also not significantly different among the 3 groups and between NSBR and SBR subjects although T appeared to be generally lower in NSBR subjects (Figure 5.23).

Figure 5.23 Urea hydrolysis in subjects on low protein diets



Although nitrogen intake was reduced to the same extent for every subject, the changes in T were, however, variable (Figure 5.24). There was a reduction in T in all NSBR subjects whereas in both the reference and SBR subjects, 4 out of 6 in each group showed reduced T and 2 subjects had increased T. Overall, the largest reduction was seen in NSBR group (24.9%, 12.01 mgN/kg/day, p=0.036) followed by SBR group (17.9%, 10.56 mgN/kg/day) and then the reference group (15.1%, 8.31 mgN/kg/day).

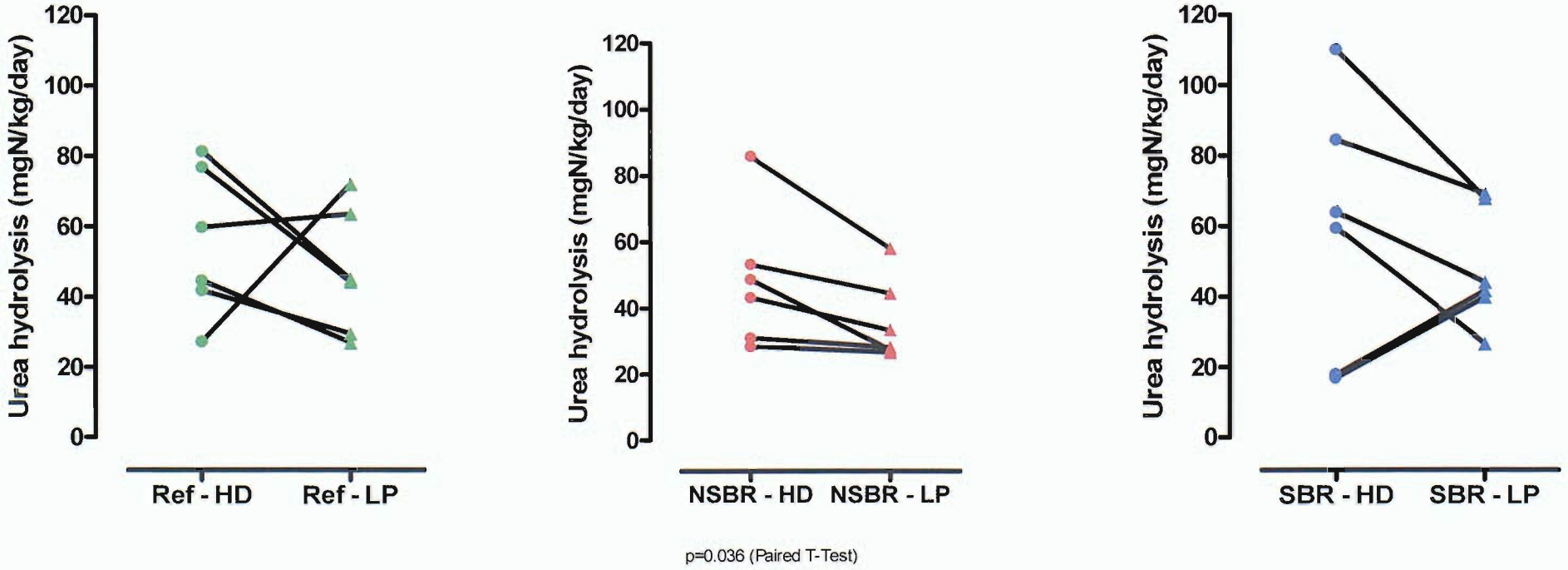


Figure 5.24 Changes to urea hydrolysis following a reduction in nitrogen intake

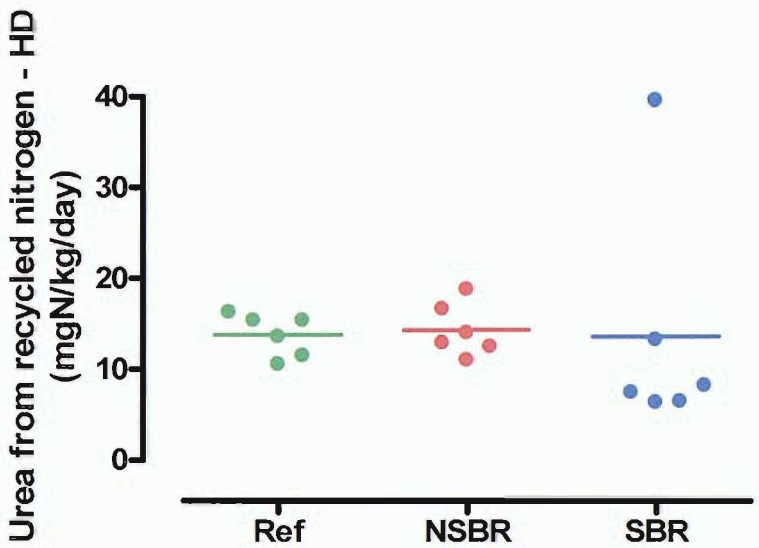
5.6.13 Urea kinetics - recycled nitrogen

Nitrogen derived from urea hydrolysis (T) enters the nitrogen pool where a proportion will return to urea synthesis (Pr) and the rest are retained for synthetic process (S). Pr was derived from the relative amounts of $^{15}N^{15}N$: $^{14}N^{14}N$ and $^{15}N^{14}N$: $^{14}N^{14}N$ and S was derived by subtracting Pr from T.

Urea produced from recycled nitrogen (Pr)

Individual Pr on habitual diet is shown in Appendix 6A and on low protein diet is shown in Appendix 6B. Figure 5.25 shows the distribution of Pr on habitual diets.

Figure 5.25 Urea produced from recycled nitrogen (Pr) in subjects on habitual diets



On habitual diets, mean Pr for reference subjects, NSBR subjects and SBR subjects were 13.78 (2.28) mgN/kg/day, 14.37 (2.88) mgN/kg/day and 13.60 (13.01) mgN/kg/day respectively. Although these values were not significantly different among the three groups of subjects and between NSBR and SBR subjects, Pr tended to be lower in SBR subjects than in reference and NSBR subjects and there is also an outlier (subjects 12) with a very high Pr.

On low protein diets, mean Pr for reference subjects, NSBR subjects and SBR subjects were 8.79 (1.40) mgN/kg/day, 8.77 (1.56) mgN/kg/day and 7.40 (2.83) mgN/kg/day respectively. These values were also not significantly different among the three groups of

subjects and between NSBR and SBR subjects but again, Pr were more widely distributed in the SBR group with an outlier (subject 7) who had a very low Pr (Figure 5.26).

Figure 5.26 Urea produced from recycled nitrogen (Pr) in subjects on low protein diets

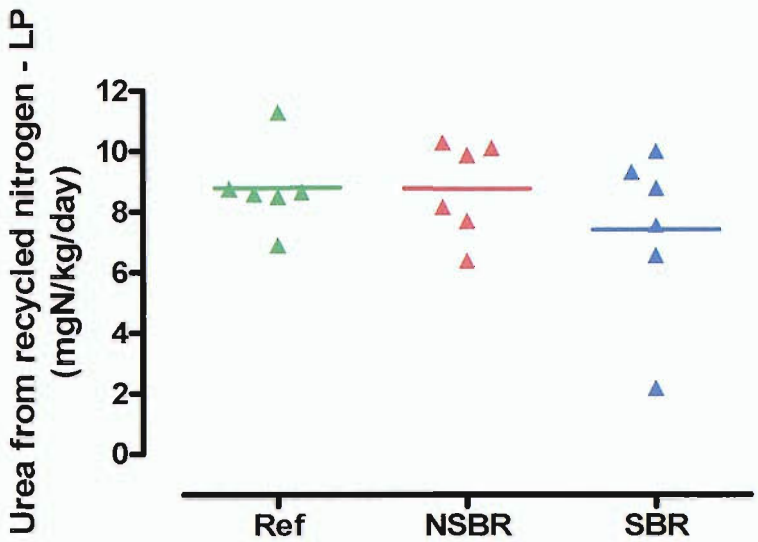


Figure 5.27 illustrates the changes in Pr in all the subjects when habitual diets were changed to low protein diets. Pr was significantly reduced in both the reference (36.2%, 4.99 mgN/kg/day, p=0.003) and NSBR (39.0%, 5.60 mgN/kg/day, p=0.004) groups but not in the SBR group despite the largest mean reduction in Pr (45.6%, 6.20 mgN/kg/day).

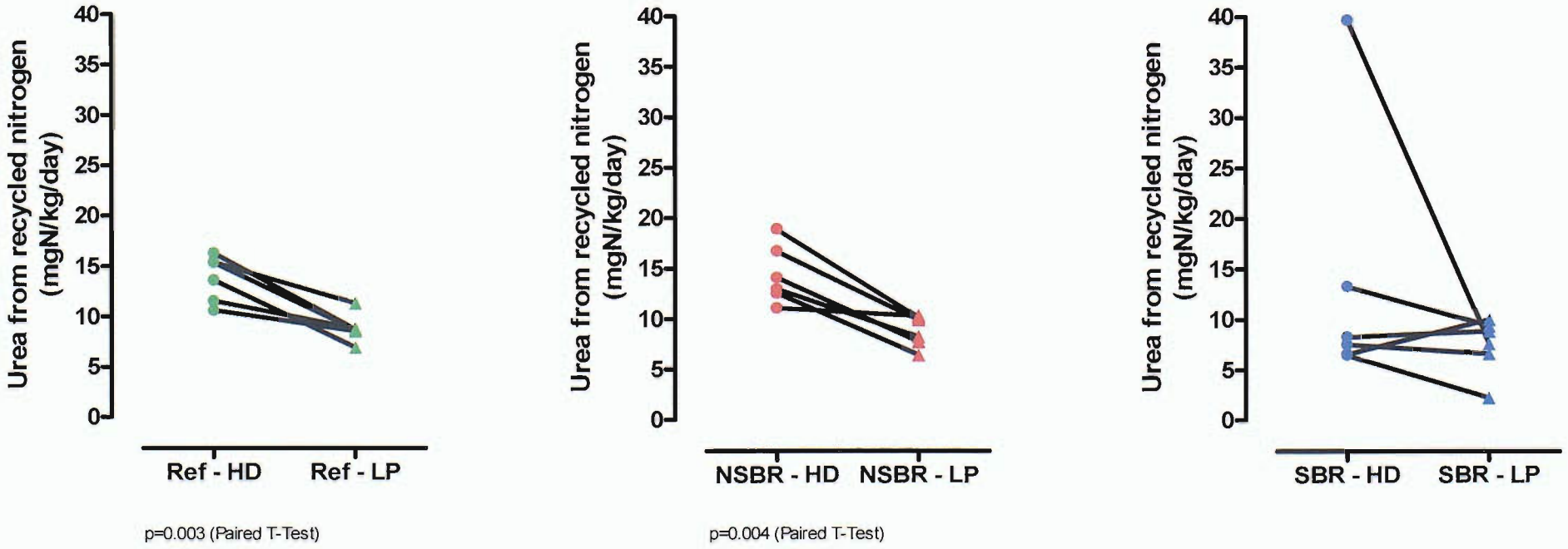
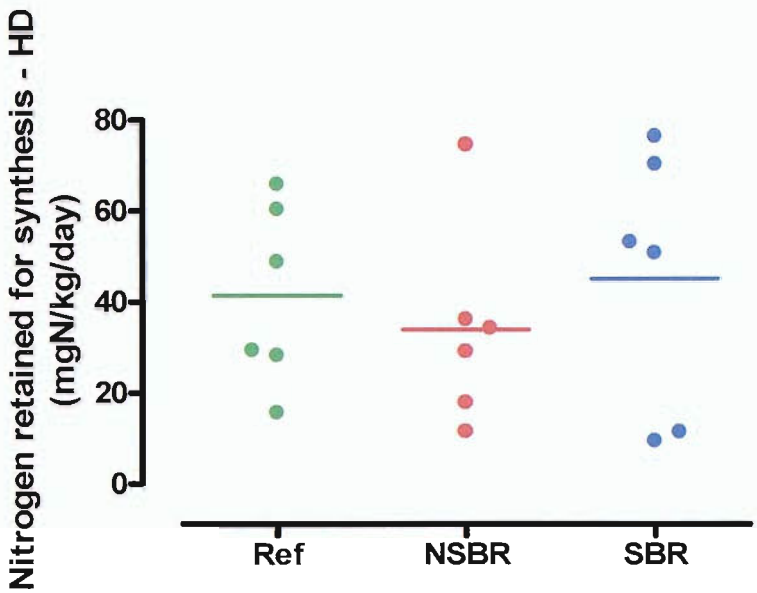


Figure 5.27 Changes to Pr following a reduction in nitrogen intake

Recycled nitrogen retained for synthetic processes (S)

The levels of recycled nitrogen contributing to synthetic processes on habitual and low protein diets for individual subject are shown in Appendix 6A and Appendix 6B respectively. Figure 5.28 shows the distribution of S on habitual diets.

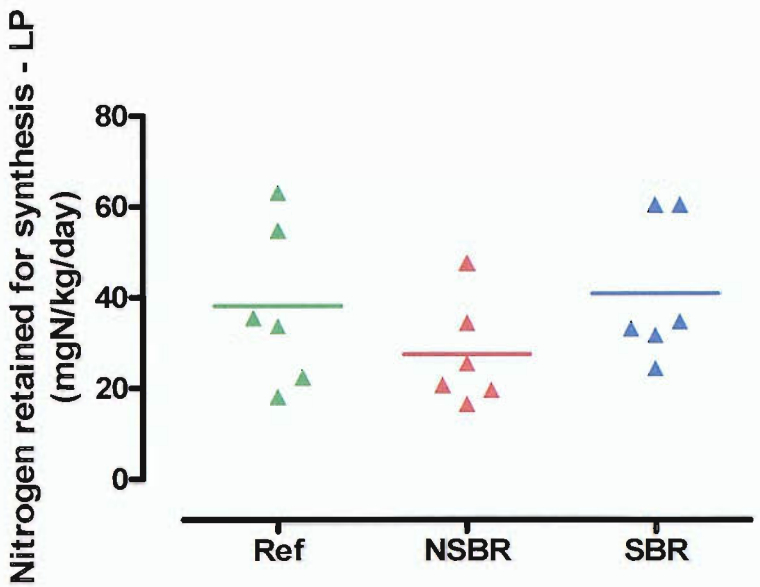
Figure 5.28 Nitrogen retained for synthetic processes (S) in subjects on habitual diets



On habitual diets, mean S for reference, NSBR and SBR subjects were 41.42 (19.97) mgN/kg/day, 34.00 (22.06) mgN/kg/day and 45.28 (28.69) mgN/kg/day respectively. These values were not significantly different among the three groups of subjects and between NSBR and SBR subjects but S were widely distributed within each group subjects. These results confirm that approximately 25% of dietary nitrogen is salvaged by bacteria and returns to the nitrogen pool for further metabolic interactions.

On low protein diets, mean S for reference, NSBR and SBR subjects were 38.10 (17.70) mgN/kg/day, 25.58 (11.63) mgN/kg/day and 40.93 (15.58) mgN/kg/day respectively. As in habitual intakes, S were widely distributed (Figure 5.29) and these values were not significantly different among the three groups of subjects and between NSBR and SBR subjects.

Figure 5.29 Nitrogen retained for synthetic processes (S) in subjects on low protein diets



Although nitrogen intakes were reduced to the same extent in all the subjects, the responses in urea kinetics with respect to S were variable in all three groups of subjects as illustrated in Figure 5.30. For reference subjects, S was reduced in 4 subjects and increased in 2 subjects but overall, there was no significant reduction in S (8.0%, 3.32 mgN/kg/day). For NSBR subjects, S was reduced in 3 subjects and marginally increased in 3 subjects with no significant reduction in overall S (18.9%, 6.42 mgN/kg/day). For SBR subjects, S was reduced in 4 subjects and increased in 2 subjects but there was no significant reduction in the overall S between the two diets (9.6%, 4.35 mgN/kg/day).

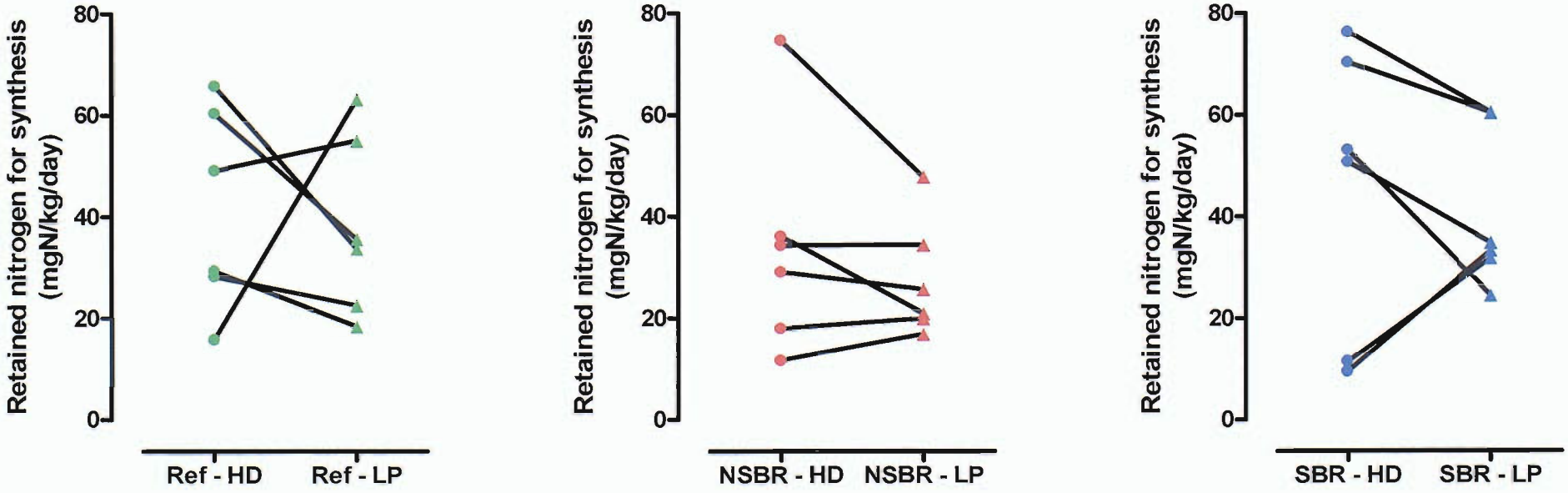
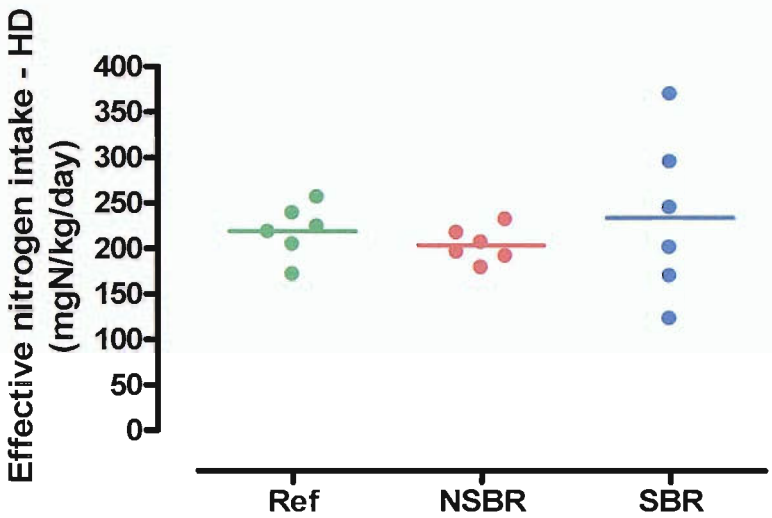


Figure 5.30 Changes to S following a reduction in nitrogen intake

5.6.14 Effective nitrogen supply

Since nitrogen recycled from urea hydrolysis in the colon (T) is returned to the nitrogen pool for further utilisation, ie to P and S, the total nitrogen supply going into the nitrogen pool is effectively the sum of nitrogen intake (I) and that derived from urea hydrolysis, ie I + T. The effective nitrogen supply for individual subject on habitual diet is shown in Appendix 6C and on low protein diet is shown in Appendix 6D. Figure 5.31 shows the distribution of I + T on habitual diets.

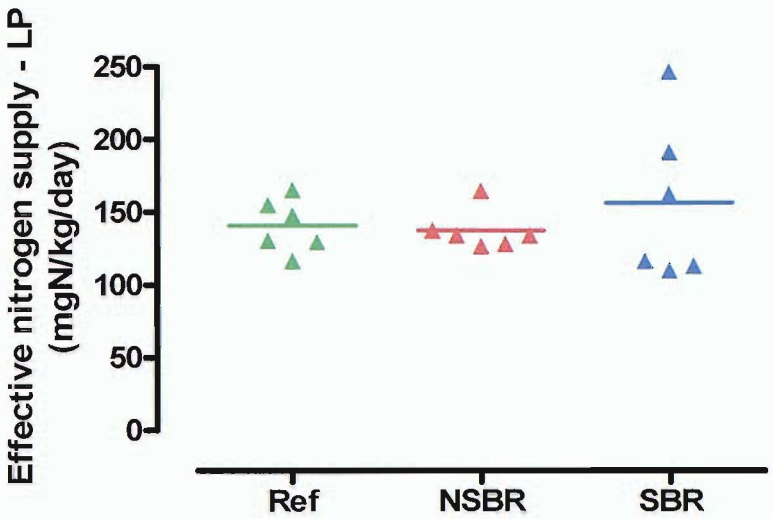
Figure 5.31 Effective nitrogen supply in subjects on habitual diet



On habitual diets, the mean I + T for reference, NSBR and SBR subjects were 219.42 (29.06) mgN/kg/day, 204.04 (18.57) mgN/kg/day and 234.03 (88.86) mgN/kg/day respectively. These values were similar among one another and between NSBR and SBR subjects but the results were widely distributed in SBR subjects.

On low protein diets, mean I + T for reference, NSBR and SBR subjects were 140.88 (18.18) mgN/kg/day, 137.93 (13.70) mgN/kg/day and 156.98 (54.84) mgN/kg/day respectively. These values were also similar among one another and between NSBR and SBR subjects but the results were widely distributed in SBR subjects compared to NSBR and reference subjects (Figure 5.32).

Figure 5.32 Effective nitrogen supply in subjects on low protein diets



The effective nitrogen supply declined significantly in all three groups of subjects when nitrogen intake was reduced (Figure 5.33). The mean reductions for reference, NSBR and SBR subjects were 78.54 mgN/kg/day (35.8%) (p=0.002), 66.11 mgN/kg/day (32.4%) (p=0.001) and 77.05 mgN/kg/day (32.9%) (p=0.005). It would appear that there was no significant up-regulation of urea-nitrogen salvage to compensate for the reduction in nitrogen intake.

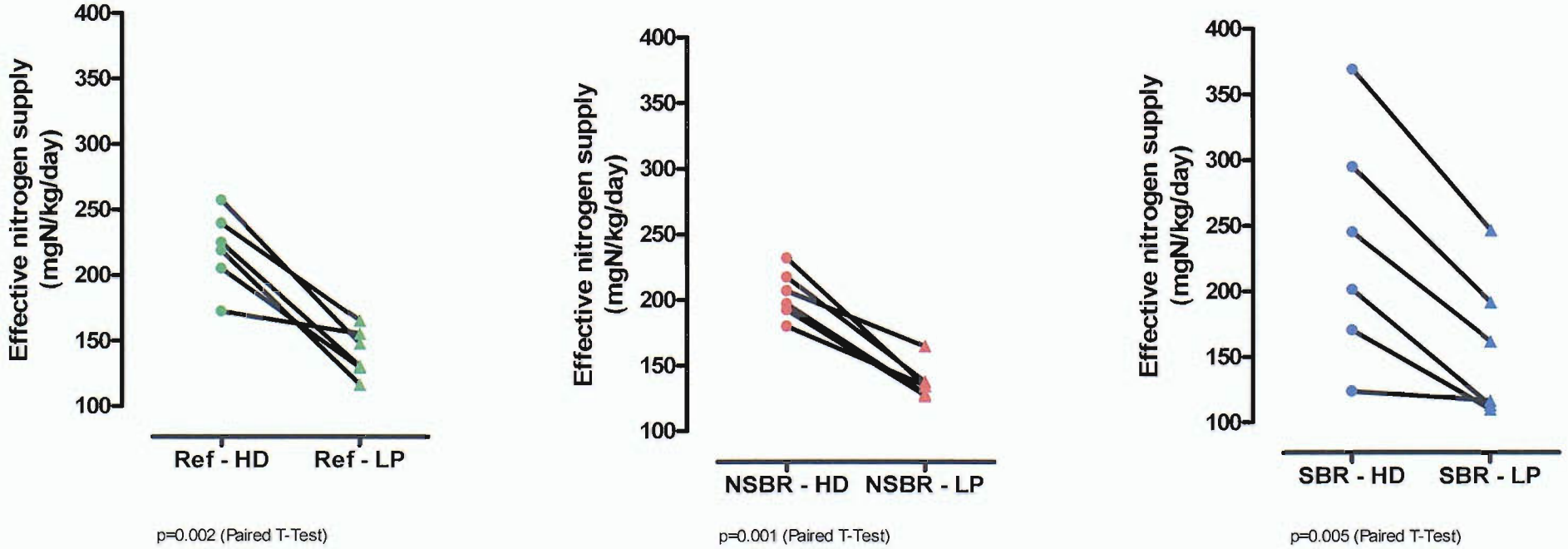


Figure 5.33 Changes to effective nitrogen supply following a reduction in nitrogen intake

5.6.15 Urea kinetics in relation to nitrogen intake

The various aspects of urea kinetics relative to nitrogen intake on both habitual and low protein diets were examined and the results for each of the three groups of subjects are shown in Table 5.9. Individual results for urea kinetics relative to intake are shown in Appendix 6C and Appendix 6D for habitual and low protein diets respectively.

Table 5.9 Urea kinetics relative to intake in all subjects [mean (sd)]

	Reference		NSBR		SBR	
%	HD	LP	HD	LP	HD	LP
P/I	116.0(19.7)	144.5(42.1)	119.8(28.5)	121.3(24.4)	108.2(40.5)	110.7(29.6)
Eu/I	82.4(11.2)	93.9(22.8)	86.2(15.6)	83.0(10.6)	72.2(25.3)	64.3(18.5)*
T/I	33.6(12.9)	50.6(21.6)	33.6(22.2)	38.3(21.9)	35.9(22.0)	46.4(13.8)
S/I	25.1(12.1)	41.2(21.1)	24.3(21.8)	29.3(19.2)	27.4(17.2)	38.9(11.0)
Pr/I	8.5(1.6)	9.4(1.5)	9.4(1.9)	9.0(3.0)	8.5(3.0)	7.4(4.0)

One-way ANOVA: *p=0.037, LP: Ref v NSBR v SBR

On habitual diets, P/I, Eu/I, T/I, S/I and Pr/I were similar among all three groups of subjects and between NSBR and SBR subjects. On low protein diets, Eu/I was significantly lower in SBR while P/I, T/I, S/I and Pr/I were similar among the three groups of subjects and between NSBR and SBR subjects. The differences in urea kinetics relative to intake between the two diets are shown in Table 5.10

Table 5.10 Differences in urea kinetics between low protein and habitual diets

LP-HD (%)	Reference	NSBR	SBR
P/I	28.5	1.5	2.5
Eu/I	11.5	-3.2	-7.9
T/I	17.0	4.7	10.5
S/I	16.1	5.0	11.5
Pr/I	0.9	-0.4	-1.1

Compared to NSBR and SBR subjects, the reference subjects demonstrated the largest increase in P/I and despite an increase in Eu/I, the increase in T/I and S/I were also higher than the other two groups. For NSBR subjects, the various aspects of urea kinetics relative to intake did not appear to change very much when nitrogen intake was reduced whereas for SBR subjects, a very modest increase in P/I was perhaps compensated by an 8% reduction in Eu/I resulting in a slight increase in T/I and S/I.

5.6.16 Urea kinetics relative to urea production

Potential changes in the disposal of urea to excretion and hydrolysis and hence, the impact on Pr and S on habitual and low protein diets was also examined. The various aspects of urea kinetics relative to urea production on habitual and low protein intakes are shown in Table 5.11. Individual results for urea kinetics relative to intake are shown in Appendix 6E and Appendix 6F for habitual and low protein diets respectively.

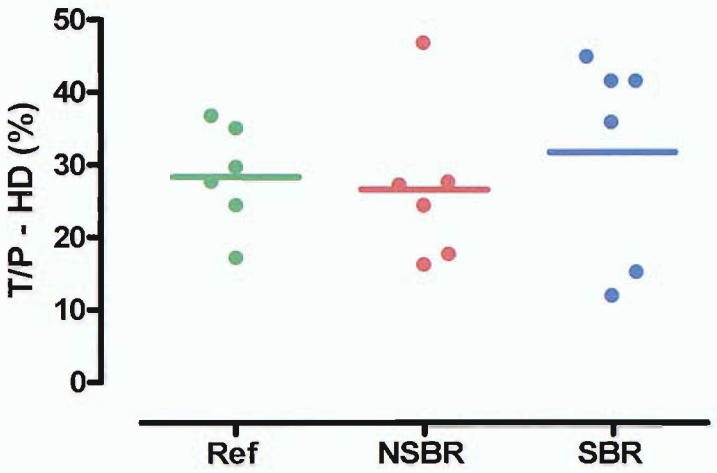
Table 5.11 Urea kinetics relative to urea production in all subjects [mean (sd)]

	Reference		NSBR		SBR	
%	HD	LP	HD	LP	HD	LP
Eu/P	71.6(7.1)	65.4 (5.8)	73.3(11.0)	69.8 (10.2)	68.1 (14.5)	58.0 (6.0)*‡
T/P	28.4(7.1)	34.2 (5.8)	26.7(11.0)	30.2 (10.2)	31.9 (14.5)	42.2 (6.0)*‡
Pr/P	7.4(1.7)	6.8 (1.6)	7.9(1.3)	7.3 (1.0)	6.9 (3.4)	6.4 (2.3)
S/P	20.9(7.3)	27.3 (6.8)	18.7(12.0)	23.0 (9.6)	25.0 (14.4)	35.6 (6.1)*‡

One-way ANOVA: * $p<0.05$, Ref v NSBR v SBR; T-Test: ‡$p<0.05$, NSBR v SBR

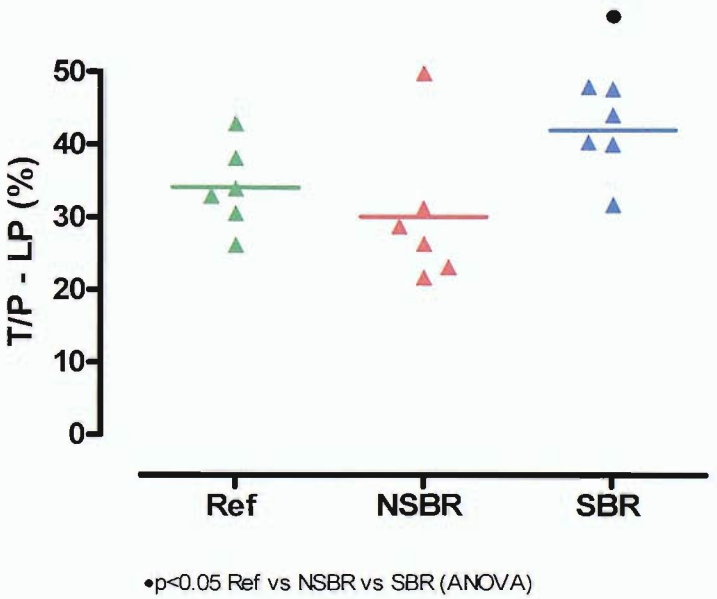
On habitual intakes, Eu/P, T/P, S/P and Pr/P were similar among all three groups of subjects and between NSBR and SBR subjects. The results of T/P were widely distributed in SBR and NSBR subjects but in general, SBR subjects tended to have higher T/P than the reference and NSBR subjects (Figure 5.34).

Figure 5.34 Distribution of T/P of subjects on habitual diets



On low protein diets, Eu/P was significantly lower but T/P and S/P were significantly higher in SBR subjects than in reference and NSBR subjects. T/P was widely distributed in NSBR subjects (Figure 5.35)

Figure 5.35 Distribution of T/P of subjects on low protein diets



For all three groups of subjects, although no significant differences were seen in Eu/P, T/P, S/P and Pr/P when these values were compared between low protein and habitual diets, however, the trend indicated that on low protein diets, Eu/P was reduced, T/P and S/P were increased while Pr/P remained relatively unchanged. Therefore in all three groups of subjects, while Pr was maintained when protein intake was reduced, there was a compensatory reduction in urinary urea excretion allowing for higher rates of urea hydrolysis and hence an increased in the amount of nitrogen retained for other synthetic processes.

5.7 Discussion

In this section, habitual nitrogen intakes using weighed food diary were measured along with stool nitrogen losses and urinary nitrogen excretions. Using stable isotope $^{15}N^{15}N$-urea, urea kinetic studies were also conducted. The aim of the studies was to clarify issues raised in the cross-sectional survey by obtaining a better estimate of nitrogen balance in ileostomy subjects and examining the capacity for urea-nitrogen salvage in the absence of the colon. The ability of ileostomy subjects to maintain nitrogen balance and the associated dynamics of urea kinetics in response to metabolic stress created by reducing protein intake were also investigated.

Before discussing the results, it is important to note that amongst the three groups of subjects, none of the differences observed in the measurements of nitrogen balance and urea kinetics reached statistical significance. The most likely explanations for this were the small numbers of subjects and the variability which reflected the heterogeneity of all the subjects who were unmatched for age and sex and, in the SBR group, the varying small bowel lengths in individual subjects. However, despite the lack of statistical differences, trends in the data permit interpretation.

In terms of the protocol used in this thesis, it was important that nitrogen balance and urea kinetics studies were conducted under steady metabolic state, especially when the second study involved a reduction in protein intake which required a period for adaptation. In this and previous short-term balance studies (Langran et al 1992, Danielsen and Jackson 1992), 5 days was sufficient for a new steady state to be reached

with little difference in urinary nitrogen excretions between Days 4 and 5 (Figure 5.2). Four to five days is the average period of time taken to re-establish a new steady state on low protein diets, with 95% of individuals reaching a new steady state within 8 days (Rand et al 1976). Therefore, nitrogen balance for each subject was calculated using the average result of Days 4 and 5 and urea kinetic studies were conducted on Day 5. As we also recognised that fluctuations in urea excretion and hydrolysis have previously been demonstrated and this phenomenon was ascribed to diurnal feeding habit (Price et al 1994, Quevedo et al 1994, Jackson 1994a, Meakins 1996b), we conducted urea kinetics while the subjects were being fed orally with whole foods at 3-hourly interval so that a steady intake of nitrogen was maintained. However, one might argue that the best way to achieve this was to use continuous feeding rather than intermittent oral feeding but that would have required the use of formula liquid feed administered via a nasogastric tube. As our primary aim was to gain an understanding of nitrogen metabolism in free-living subjects under habitual conditions and hence the need to minimise the invasiveness of our study, we opted for intermittent oral feeding which we felt was also more physiological than tube feeding. Furthermore, it has been known that placement of a nasogastric tube can abolish cephalic response to feeding leading to an increase in gastrointestinal transit which may reduce nutrient absorption (Duncan et al 1998). With regard to the other aspects in the protocol like collection, processing and analyses of the specimens and data calculation where potential errors in our results could arise, we have confidence that all necessary steps were taken to ensure that procedures were followed diligently. All our subjects were very motivated and completed the studies successfully with no difficulties. They were compliant on low protein diets as demonstrated by a corresponding reduction in urinary nitrogen excretion.

Nitrogen balance studies on habitual diets

Overall, our results showed that under habitual conditions, all our subjects were essentially in nitrogen balance. SBR ileostomy subjects were in more positive nitrogen balance (23.16 (33.05) mgN/kg/day) than the NSBR (5.08 (13.62 mgN/kg/day) and reference (-9.20 (20.04) mgN/kg/day) subjects indicating a higher degree of nitrogen retention. Despite higher nitrogen intakes in NSBR (172.4 (36.6 mgN/kg/day) and SBR (188.8 (63.8 mgN/kg/day) subjects compared to the reference subjects (157.9 (25.3) mgN/kg/day), the amount of nitrogen available for metabolic engagement was similar among the three groups (reference: 141.1 (25.2) mgN/kg/day; NSBR: 146.9 (33.8) mgN;

SBR: 139.7 (43.4) mgN/kg/day). These results contrast with those of the dietary survey from the cross-sectional study which indicated that nitrogen intakes of ileostomy subjects were similar to reference subjects. In this more detailed study, ileostomy subjects, especially those who had SBR, ate more protein, probably to compensate for higher stool nitrogen losses. With the higher nitrogen intakes, energy intakes per kilogramme body weight also tended to be higher in SBR subjects. Although stool energy losses were not quantified, total stool lipid losses measured in a separate study on the same subjects showed that whereas NSBR subjects had similar losses to reference subjects, SBR subjects had exceptionally high lipid losses via the stoma which would make them vulnerable to energy deficit.

The more positive nitrogen balance seen in SBR subjects appears to be due to lower urinary nitrogen excretions. The results of this study also supported our findings in the cross-sectional survey where we reported lower urinary nitrogen excretions and positive apparent nitrogen balances in ileostomy subjects. They also supported our expectations of higher stool nitrogen losses in ileostomy subjects.

Assessment of dietary intakes using weighed food diaries also confirmed our suspicion that FFQ over estimated nitrogen intakes by approximately 3.5 g in ileostomy and reference subjects. Notwithstanding these findings, the choice to use FFQ was appropriate in the given setting (cross-sectional survey involving 120 subjects) although its shortcomings must be considered when data were interpreted.

Urea kinetic studies in habitual diets

When urea kinetics were measured under habitual conditions, we demonstrated that ileostomy subjects were capable of urea-nitrogen salvage in the absence of the colon. As individual groups, despite similar mean values, both urea production (P) (Figures 5.16) and renal urea excretion (Eu) (Figure 5.19) tended to be generally higher in reference subjects and generally lower in SBR subjects. The proportion of urea which was hydrolysed in the bowel (T/P) also tended to be higher in SBR subjects than in reference and NSBR subjects (Figure 5.34) while conversely, the proportion of urea excreted by the kidney (Eu/P) tended to be lower in SBR subjects than in reference and NSBR subjects. Consequently, the amount of salvaged nitrogen retained for utilisation (S) also tended to be higher in SBR subjects than in the reference and NSBR subjects (Figure

5.28). Although T/P was lowest in the NSBR group, the overall mean value of 26.7% was still compatible to the mean value for the reference group (28.4%) and the 25% reported in the literature for normal individuals consuming 70-75 g protein (Jackson et al 1984, Hibbert and Jackson 1991, Jackson 1995). Overall, these results indicate that under habitual conditions, total colectomy does not appear to have a detrimental effect on ileostomist's ability to salvage nitrogen from urea hydrolysis and indeed, ileostomists who have additional SBR operate higher levels of urea-nitrogen salvage which might represent adaptive up-regulation of the system. The urea-nitrogen salvage that occurs in the absence of the colon probably takes place in the terminal small bowel of the ileostomy (Wheeler et al 1993) which is known to contain bacteria (Percy-Robb et al 1969, Gorbach et al 1967, Finegold et al 1970).

Nitrogen balance studies on low protein diets

To assess whether ileostomy subjects can respond and adapt to a reduction in nitrogen availability, we assess nitrogen balance and urea kinetics after a significant reduction in dietary protein intake of 40%. Energy intakes were maintained at the same level as the habitual diets. On low protein intakes, all three groups of subjects went into negative nitrogen balances. However, the reduction in overall nitrogen balance was most significant in SBR group (35.15 mgN/kg/day, $p=0.017$), followed by NSBR group (25.86 mgN/kg/day, $p=0.023$) while the reduction in overall nitrogen balance for reference subjects was not statistically significant (13.65 mgN/kg/day). These observations may in part be explained by the fact that despite similar reductions, stool nitrogen losses remained proportionately higher for SBR and NSBR subjects resulting in greater reductions in the amount of nitrogen available for metabolic engagement. However, they might also be due to the inability of ileostomy subjects to increase urea-nitrogen salvage further than the level at which they were already operating. This probability is also supported by the observations that although urinary excretions were significantly reduced in all three groups of subjects when nitrogen intakes were reduced, the reference subjects showed somewhat greater reductions in urinary nitrogen loss (reduced by 32.7%) than NSBR (reduced by 28.9%) and SBR (reduced by 30.3%) subjects, a change that might also reflect up-regulation of active urea hydrolysis.

Urea kinetic studies on low protein diets

When urea kinetics on low protein diets were compared to habitual diets, there was a significant reduction in urea production in all three groups of subjects but to a lesser degree in reference subjects (28.9%) than in NSBR subjects (33.3%) and SBR subjects (34%). Urea production relative to nitrogen intake (P/I) was, however, increased in all three groups of subjects but again, to a much greater extent in reference subjects (28.5%) than in NSBR (1.5%) and SBR (2.5%) subjects. In the partitioning of urea, there was a marginal reduction in Eu/P and marginal rise in T/P in all the three groups. Overall hydrolysis and hence, nitrogen salvaged were reduced in all the three groups but to a much greater extent in NSBR subjects (T reduced by 24.9% and S reduced by 18.9%) than in SBR (T reduced by 17.9% and S reduced by 9.6%) subjects and reference (T reduced by 15.1% and S reduced by 8%) subjects. Hydrolysis relative to intake (T/I) rose by 17% in reference subjects, 4.7% in NSBR subjects and 10.5% in SBR subjects and the amount of nitrogen retained relative to intake (S/I) rose by 16.1% in reference subjects, 5% in NSBR subjects and 11.5% in SBR subjects. From these results, it would appear that although urea-nitrogen salvage was up-regulated, the increase in salvage was not sufficient to compensate for the much greater reduction in nitrogen intakes and as a result, all three groups of subjects could not sustain nitrogen balance. On the whole, the reference subjects faired better than ileostomy subjects by maintaining a higher level of urea production so that more urea could be hydrolysed and salvaged in order that the fall in nitrogen balance was minimised. Overall, these studies suggest that ileostomy patients may not have the reserve capacity for nitrogen salvage and are therefore more vulnerable in situations where nitrogen availability is reduced.

Validity of results

Before any conclusions can be drawn from these studies, the validity of the data should be examined by comparing them with the findings of previous reported studies. On habitual diets, stool nitrogen loss for the reference subjects was approximately 1.35 g/day which was compatible to the daily loss of 1-2 g reported for normal individuals (Gibson et al 1976b, Stephen and Cummings 1980b, Jackson 1995). On intakes similar to other studies (Langran et al 1992, Danielsen and Jackson 1992, Meakins and Jackson 1996a), our reference subjects were also in nitrogen balance. When protein intake was reduced by 40% to 47.4 g, stool nitrogen loss fell slightly (by 4.2%) in reference subjects to 1.29 g/day which was similar to the findings reported by Gibson et al (1976b) where stool

nitrogen loss of subjects on 40 g protein diets was 1.21 g/day. The marginal fall in stool nitrogen despite a significant reduction in protein intake was probably due to the maintenance of stool bacterial mass resulting from the relative increase in vegetable protein in replacement of meat protein in low protein diets. For NSBR ileostomy subjects, stool nitrogen loss on habitual intakes was 1.88 g/day which was compatible to the daily loss of 1.9-2.6 g reported in other studies (Gibson et al 1976b, Langkilde et al 1990, Hill 1998) while stool nitrogen loss for SBR subjects was much higher at 3.32 g/day. On low protein diets, stool nitrogen losses fell by 15.7% to 1.59 g/day in NSBR subjects and by 20.2% to 2.65 g/day in SBR subjects.

Direct comparisons of our urea kinetics results with the findings of previous reported studies were more difficult due to differences in experimental approaches (Table 5.12).

Table 5.12 Urea kinetics of normal individuals on varying nitrogen intakes in different studies with $^{15}N^{15}N$-urea isotope administered by oral prime-intermittent dosing (OPI), intravenous prime-intermittent dosing (IPI) and intravenous prime-constant infusion (IPC)

	Danielsen	**Langran**	**Meakins**	**Forrester**	**Hibbert**	**Our study**
No.	6	5	6	8	5	6
Sex	Male	Male	Male	Female	Mixed	Mixed
Isotope	OPI	IPI	OPI	IPI	IPI	IPC
Type of diet	Fixed Whole	Fixed Liquid	Fixed Whole	Habitual Whole	Fixed Liquid	Habitual Whole
Intake (mgN/kg/day)	165	149	147	167	220	158
Pu (mgN/kg/day)	199	194	209	150	205	190
T (mgN/kg/day)	80	92	83	40	73	55
Eu (mgN/kg/day)	118	101	126	110	129	135
T/P (%)	40	46	40	26	37	28
Eu/P (%)	60	54	60	74	63	72

From Table 5.12, it can be seen that for intakes of compatible magnitudes, urea production in our reference subjects was consistent with other studies but the disposal of urea to urinary excretion and hydrolysis in the bowel differed. However, different levels of disposal between normal individuals are well described with variability in urea-nitrogen salvage following hydrolysis reported to range from 25 to 75% of urea production (Hibbert and Jackson 1991, Jackson 1993b). Our results in our reference subjects are, therefore, compatible with published figures with the differences between studies likely to be due to a combination of differences in methodology, dietary manipulation, gender of subjects and experimental conditions, along with a true inherent variability in urea metabolism which is not fully understood. Furthermore, the inter-individual variability observed within each group of subjects in our study may also be due to different habitual nitrogen intakes and for SBR subjects, the variable length of remaining small bowel.

The principles of urea kinetics assume the presence of a metabolic steady state during administration of $^{15N}N^{15}N$-urea isotope with constant input into and output from the urea pool and therefore constant pool size. In our study, to ensure that we achieved this with greater confidence, we used the prime-constant intravenous infusion protocol rather than prime-intermittent dosing of isotope described in previous studies (Hibbert et al 1992, Langran et al 1992, Danielsen and Jackson 1992, Forrester et al 1994, Meakins and Jackson 1996a). The intravenous administration of isotope, as opposed to oral dosing, also eliminated potential errors caused by variable absorption of isotope and hydrolysis of isotope by Helicobactor Pylori prior to absorption (Graham et al 1987, Hibbert et al 1992). Therefore, at the expense of a relatively more invasive approach, our protocol was arguably superior. Another difference in our protocol was that the dose of isotope administered was ten times the dose given in previous studies (Hibbert et al 1992, Langran et al 1992, Danielsen and Jackson 1992, Forrester et al 1994, Meakins and Jackson 1996a). A larger dose was administered so that label enrichment in amino acids could be examined as part of another study. In theory, the administration of a large dose of isotope could potentially cause significant disturbances to the overall metabolic state of the subjects but from our calculations, the mean quantity of urea isotope entering the urea pool was only 1.68% of total urea production (range 1.04 - 2.73%). Therefore, the dose of urea isotope administered was relatively small compared to total urea production and would have behaved like a tracer as intended.

Urea-nitrogen salvage is a key component to the body's adaptive respond mechanism which aids in the maintenance of nitrogen balance when there is a reduction in nitrogen intake (Picou and Phillips 1972, Langran et al 1992). Higher rates of urea hydrolysis and nitrogen retention have been demonstrated in situations when the demands for nitrogen are high such as in pregnancy (Forrester et al 1994), in individuals with sickle cell anaemia (Jackson et al 1988) and in patients with intestinal failure (Moran et al 1991). For a fixed demand, salvage therefore increases as intakes falls and for a fixed intake, salvage increases as demands increases (Jackson 1994b). When nitrogen availability is limited, the response of urea metabolism is typically characterised by an increase in urea production, a reduction in urinary urea excretion and an increase in colonic urea hydrolysis relative to urea production (Jackson et al 1988, Langran et al 1992, Forrester et al 1994). However, there appears to be a limit to the capacity to which urea-nitrogen salvage can be up-regulated and studies have shown that if protein intake is below the minimum physiological requirement thought to be approximately 35 g per day, urea metabolism cannot be sustained resulting in a reduction of urea production and hydrolysis and hence failure to maintain nitrogen balance (Danielsen and Jackson 1992, Meakins and Jackson 1996a, Jackson 1998b). The urea kinetics of our subjects on low protein diets were compared to other similar studies and the results are illustrated in Table 5.13.

Table 5.13 Urea kinetics of individuals on low protein diets in different studies with $^{15}N^{15}N$-urea isotope administered by oral prime-intermittent dosing (OPI), intravenous prime-intermittent dosing (IPI) and intravenous prime-constant infusion (IPC)

	Langran	Danielsen	Meakins	Our study reference	Our study NSBR	Our study SBR
No.	5	6	6	6	6	6
Sex	Male	Male	Male	Mixed	Mixed	Mixed
Intake (mgN/kg/day)	76	68	62	95	102	109
Avail N (mgN/kg/day)				79	80	70
Pu (mgN/kg/day)	172	123	118	135	121	116
T (mgN/kg/day)	115	58	34	46.9	36.4	48
Eu (mgN/kg/day)	57	64	84	88.1	84.4	68
T/P (%)	64	46	29	34	30	42
Eu/P (%)	36	54	71	66	70	58
P/I (%)	229	181	190	145	121	111
T/I (%)	149	86	55	51	38	46
Eu/I (%)	79	95	135	94	83	64
Intake + T (Avail N + T) (mgN/kg/day)	191	124	96	141 (126)	138 (117)	157 (118)
Balance	+	-	-	-	-	-

In our low protein diet studies, despite higher nitrogen intakes as compared to the study of Langran et al (1992), there were significant reductions in urea production. Although urinary urea excretion was also significantly reduced, T/P was only modestly raised and

consequently, all three groups of subjects went into negative nitrogen balances. The net sums of intake and hydrolysis (I+T) were lower in our subjects especially when stool nitrogen losses were taken into account. The differences in our responses to the study of Langran et al (1992) could be due to different experimental approaches, but as our subjects behaved more like the subjects described in the studies of Danielsen and Jackson (1992) and Meakins and Jackson (1996a), we must accept the possibility that we might have over-estimated the amount of nitrogen intake in our subjects such that the intakes of our subjects were actually below the minimum physiological requirement necessary to maintain nitrogen balance. In order to mimic habitual conditions and improve compliance, we provided whole foods based on the subjects' preference and during the urea kinetics studies, we also provided different foods for the 3-hourly feeds as opposed to liquid formula feeds or fixed portions of jam or cheese sandwiches used in the other studies. This protocol invariably leads to a less accurate estimate of nitrogen content in the diets. Furthermore, apart from using a computerised food composition programme to calculate nutritional content of the diets, we also relied on the nutritional information on food packaging which might have contributed to any inaccuracy.

Regulation of nitrogen metabolism – urea availability

The identification of the factors which limit adaptation is of great importance to our understanding of nitrogen metabolism and protein requirement. From previous studies, it would appear that the control of urea metabolism might occur at both the level of hepatic production and the disposal of urea to either urinary excretion or colonic hydrolysis (Picou and Phillips 1972, Danielsen and Jackson 1992). We therefore need to examine the factors which might influence these aspects of urea metabolism and how these change in response to reduced nitrogen availability. We know from our study and previous reports that urea production does not show a linear relationship with intake (Danielsen and Jackson 1992, Child et al 1997, Jackson 1998a) but correlates significantly with I+T (Danielsen and Jackson 1992) (this study: habitual diets: $r=0.570$, $p=0.05$; low protein diets: $r=0.668$, $p=0.01$). The control of the partitioning of urea to renal excretion and colonic hydrolysis is less well defined but we do know that renal conservation of urea at the collecting ducts via urea transporters is under the influence of vasopressin (Gillin and Sands 1993, Olives et al 1996, Jackson 1998a). Furthermore, specific urea transporters have now been identified in the colon of rabbit (You et al 1993), the rumen of sheep (Rtizhaupt et al 1998) and the human colon (Ritzhaupt et al

1998) and the presence in both the kidney and colon of similar urea transporters suggests that the body may be able to provide a co-ordinated response to a low protein diet, by directly linking an increase in renal urea retention with an increase in the movement of urea into the colon for hydrolysis (Jackson 1998a). The studies of Meakins and Jackson (1996a) also provide other insights into the relationship between urea production and urea hydrolysis. When low protein diets were supplemented by urea, there was an increase in urea hydrolysis in the colon and individuals who were previously in negative balance came into balance. The enhanced salvage of urea-nitrogen was associated with a rise in the rate at which urea appeared in the urea pool and although urea production correlated with urea hydrolysis, it appeared that the movement of urea to the colon while consuming low protein diets could only be maintained when the rate of urea production was at least 150-170 mgN/kg/day (Meakins and Jackson 1996a, Jackson 1998a). In our studies, urea production also correlated with urea hydrolysis (habitual diets: $r=0.747$, $p<0.001$; low protein diets: $r=0.749$, $p<0.001$). On habitual intakes, the rates of urea production for all three groups of subjects were above 170 mgN/kg/day and the subjects were in nitrogen balance but on low protein diets, the rates of urea production for all three groups of subjects fell below 150 mgN/kg/day and consequently, the rates of urea hydrolysis were low and our subjects could not sustain nitrogen balance.

Regulation of nitrogen metabolism – energy availability

Apart from urea-nitrogen salvage, the influence of energy balance on nitrogen metabolism is well established. Energy is required for both protein synthesis and degradation and the full cost of maintaining protein turnover for the whole body is estimated to be in the region of 33% of resting energy expenditure (Jackson 1998b). Conversely, amino acid oxidation contributes approximately 15-20% towards our energy requirement and this pathway is thought to be a major drive for urea formation (Jackson 1994b). The work of Rose (1957) showed that inadequate energy intakes invariably lead to a failure to attain nitrogen balance even if protein intake was sufficient. Other studies have also demonstrated that at a given level of protein intake, the addition of energy to the diet improves nitrogen balance (Anderson et al 1969, Calloway 1975). Although the effects of varying energy intakes on urea kinetics has not been explored directly, studies conducted on children have illustrated the effects of varying dietary protein:energy ratios on urea kinetics (Jackson et al 1990b). At a protein:energy ratio of 10.6%, the pattern of urea kinetics was similar to that obtained on an adequate protein intake but when this

ratio was reduced to 8.8%, protein became limiting and the pattern shifted akin to that seen in the adapted state where E_U/P was decreased and T/P was increased (Jackson et al 1990b). From these results, it would appear the switch in urea kinetics takes place at an intake of about 9% protein energy and on this level of protein intake, a small increment in energy intake may have increased the metabolic demand for protein sufficiently to alter the pattern of urea kinetics such that urea-nitrogen salvage becomes more efficient (Jackson 1990c).

Table 5.14 shows the energy requirements and protein energy relative to total energy of habitual and low protein diets.

Table 5.14 Energy requirements (ER) and protein energy relative to total energy (P/E) of habitual and low protein diets

	Reference		NSBR		SBR	
	HD	LP	HD	LP	HD	LP
ER (x RMR)	1.8	1.7	1.6	1.7	2.0	2.0
P/E (%)	12.3	7.4	13.7	7.9	12.8	7.5

Using benchmarks of 1.4 times RMR for sedentary lifestyle, 1.7 times RMR for moderately active lifestyle and 1.9 times RMR for very active lifestyle to estimate energy requirements (Dietary Reference Values for Food and Energy and Nutrients for the United Kingdom 1999), the energy intakes of all three groups of subjects in our studies appeared to be adequate, although we need to bear in mind that stool energy losses in ileostomy subjects may be significant especially in SBR subjects. From our calculations, protein energy constituted 7.4-7.9% of total energy intake on low protein diets as opposed to 12.3-13.7% on habitual diets. At this level of protein:energy ratio on low protein diets, the switch in urea kinetics, seen in the study of Jackson et al (1990b), did not take place and all three groups of subjects went into negative nitrogen balance. This would suggest that the critical level of protein energy necessary to maintain urea hydrolysis is above 8% and below this level, the nitrogen requirements of this metabolic activity cannot be satisfied. In addition, it is also possible that on our low protein diets, the energy supplied was insufficient to support the higher rates of protein turnover

required as a result of reduced protein availability and hence urea was not being formed. An increment in energy intake could increase protein turnover (Golden et al 1977) which in turn would give rise to higher levels of amino acid oxidation and hence, urea production. The enhanced production in urea would drive urea hydrolysis in the bowel thereby enabling the subjects to sustain nitrogen balance. In SBR subjects, the possibility of excess stool energy losses, as indicated by the high stool lipid losses described earlier, could have led to the greatest energy deficit amongst the three groups resulting in the largest reduction in overall nitrogen balance.

Regulation of nitrogen metabolism – amino acid availability

The availability of individual amino acids in low protein diets may also influence the subjects' capability to attain nitrogen balance. When a switch is made from high protein to low protein intake, the amount of meat protein is invariably reduced with a relative increase in the amount of vegetable protein. This will significantly affect the quality of ingested proteins since the pattern of plant protein differs from that of human proteins (Jackson 1998b). Furthermore, apart from total nitrogen intake, the requirement for essential amino acids is also a major factor in determining nitrogen balance (Jackson 1998b). In order to achieve nitrogen balance at the lowest level of total nitrogen intake (3-4 g per day), essential amino acids must be supplied at 2-3 times the minimum level as determined by Rose (1957). Conversely, when essential amino acids are supplied at a minimum level, nitrogen balance can only be achieved if total nitrogen intake is doubled to 6-8 g per day (Rose 1957, Jackson 1995). Furthermore, there is also evidence to suggest that glycine, a conditionally essential amino acid is deficient in low protein diets. Meakins et al (1998) showed that levels of urinary 5-oxoproline were significantly higher when her subjects were on low protein diets (30 g) compared to habitual and 70 g protein diets. This indicates glycine deficiency and when low protein diets were supplemented with glycine, the negative nitrogen balances of her subjects were restored. Similarly, since non-essential nitrogen such as urea can substitute for non-essential amino acids (Jackson 1995), Meakins and Jackson (1996a) showed that the addition of urea to low protein diets consisting of 4.8 g total nitrogen enabled subjects to come into nitrogen balance but this was only achieved by the addition of 6.4 g of urea nitrogen (total nitrogen 11.2 g) as opposed to the addition of 3.2 g of urea nitrogen (total nitrogen 8 g). The amino acid profile of our low protein diets was not examined and we were not therefore able to assess the amount of essential amino acids in relation to total nitrogen

intake. Furthermore, we did not quantify the levels of urinary 5-oxoproline levels in our subjects and hence, could not relate glycine status to the observed negative nitrogen balance. It is therefore possible that at the level of total nitrogen intake of 7.4-7.6 g in our low protein diets, the quality of protein and hence, individual amino acid availability, might have been compromised such that metabolic demands could not be satisfied.

Effects of total colectomy and small bowel resection on nitrogen metabolism

When the effects of total colectomy on nitrogen balance and urea kinetics were examined, the responses of SBR subjects were found to be different from the NSBR and reference subjects. On habitual intakes, the behaviour of NSBR subjects was more akin to the reference subjects in that they had similar levels of nitrogen balance and their responses in terms of urea kinetics were also similar. This suggests that total colectomy alone has little impact on nitrogen balance and urea metabolism. Additional small bowel loss however appears to alter nitrogen metabolism and may represent a burden on the system. The nitrogen balance of SBR subjects was more positive on habitual intakes compared to reference and NSBR subjects and despite a slightly lower rate of urea production, urea hydrolysis in relation to urea production tended to be higher than the other two groups and more nitrogen was retained. There are several possible explanations for the above observations. For SBR subjects, apart from higher stool nitrogen losses, bile salt losses via the stoma are also known to be high (Percy-Robb et al 1971b, Fiasse et al 1983, Akerlund et al 1994). Furthermore, due to their underlying disease, which is usually Crohn's disease, their pattern of protein demand may also be different from NSBR and reference subjects. These factors could have given rise to a specific pattern of amino acid requirement which if not adequately met by dietary protein, would have led to the need for higher levels of amino acid turnover in order that demand was met. It has been known that for any given pattern of intake, the greater the amount of nitrogen exchange involved, the higher the amount of nitrogen required to achieve balance (Rose 1957, Jackson 1995). Therefore, the requirement for a higher level of total nitrogen in these subjects was possibly met by higher rates of urea hydrolysis and nitrogen retention. Besides increasing total nitrogen supply, there is a wealth of evidence indicating that urea-nitrogen salvage enhances the quality of nitrogen supply through the formation of essential and non-essential amino acids by colonic bacteria which are returned to the body and enter the amino acid pool for metabolic engagement (Giordano et al 1968, Tanaka et al 1980, Millward et al 2000, Jackson 1998a). Faced with a higher demand for

bile salt formation caused by excessive losses, non-essential amino acids like glycine, cysteine and serine may become conditionally essential in SBR subjects. Under such circumstances, the dependence on bacterial urea hydrolysis to improve the quality of nitrogen supply in order that demands are met becomes critical.

It is also of interest to note that despite a higher degree of nitrogen retention, the BMI and FFM of SBR subjects were lower compared to reference and NSBR subjects but BF% was higher than reference subjects. The body composition of NSBR subjects was also different from reference subjects with lower FFM and higher BF%. From these results, it would appear that ileostomy subjects might have difficulty in laying down lean tissue with a preponderance of fat deposition. It has been noted previously that in both children recovering from malnutrition and adults recovering from severe weight loss, there was a relative excess of adipose over lean tissue deposition irrespective of dietary protein intake (Jackson 1990c). The cause for this tendency towards a limitation of lean tissue deposition is not entirely clear but the presence of other limiting nutrients might be a factor. Reeds (1990) has discussed in some detail the extent to which glycine may act as the first limiting nutrient for protein deposition and the studies of Golden and Golden (1981), which demonstrated an increase in the rate of weight gain with greater lean tissue deposition following oral supplementation of zinc in the diets of children who had recovered from malnutrition, also suggested that zinc may be one of the factors that limit lean tissue growth. In our cross-sectional survey, both NSBR and SBR subjects were found to have significantly lower urinary excretions of sodium, calcium and magnesium (Ng et al 2004) and a relatively higher urinary excretion of potassium compared to reference subjects suggesting that ileostomy subjects may be depleted of these nutrients. As discussed previously in section 4.7, deficiencies in sodium and potassium have separately been shown to interrupt lean tissue deposition with a preponderance of fat deposition (Rudman et al 1975). Therefore, in ileostomy subjects, depletion in these nutrients might be limiting their abilities to assimilate lean tissue mass. For SBR subjects in particular, sodium, calcium and magnesium were all significantly lower compared to NSBR subjects and in addition, glycine could also be a limiting factor due to high demands.

The extent to which ileostomy subjects could respond to metabolic stress, created by limiting nitrogen availability, was examined in our studies. Our results suggest that

although all three groups of subjects were unable to sustain nitrogen balance on low protein diets, ileostomy subjects, especially SBR subjects, appeared to be more vulnerable. Although the response in urea kinetics were not as expected in all three groups of subjects, the main difference between reference and ileostomy subjects was that urea-nitrogen salvage was more efficient in reference subjects. Both urea production relative to nitrogen intake and urea hydrolysis relative to nitrogen intake were higher in reference subjects than in ileostomy subjects, and hence the amount of nitrogen retained for metabolic engagement was higher. The net reduction in total nitrogen availability (habitual nitrogen intake minus effective nitrogen supply (I+T) on low protein diets) was therefore only 17 mgN/kg/day in reference subjects while the figures were twice the amount in NSBR subjects (34 mgN/kg/day) and SBR subjects (32 mgN/kg/day). The reason for this difference is not clear but since urea-nitrogen salvage is a bacteria dependent metabolic activity, the quality of the microflora in the terminal small bowel of ileostomy subjects may be an issue. The bacterial flora in ileostomy subjects, which is known to be different from that of colonic microflora in terms of species (Percy-Robb et al 1971a, Natori et al 1992, Sandborn et al 1995) and quantity (Gorbach et al 1967, Percy-Robb et al 1969, Finegold et al 1970), may be limited in their capacity to up-regulate urea-nitrogen salvage significantly in response to reduced protein intake. Another possible explanation for this difference between reference and ileostomy subjects could be the higher amount of stool losses in ileostomy subjects which will, inevitably, have a more pronounced effect on both energy supply and the quality of protein available for utilisation, thereby resulting in the failure to maintain an adequate level of urea production that is necessary to drive urea hydrolysis.

Despite a poorer response in urea kinetics to low protein diets seen in ileostomy subjects compared to reference subjects, the response in SBR subjects appeared to be more effective than in NSBR subjects. Although urea production and urea production relative to nitrogen intake were lower in SBR subjects, urea hydrolysis, urea hydrolysis relative to urea production and urea hydrolysis relative to nitrogen intake were higher resulting in a higher level of nitrogen retention. However, this level of nitrogen salvage was insufficient and SBR subjects suffered the largest fall in nitrogen balance. As explained previously, a limited capacity for up-regulation in urea kinetics may be an issue, especially when urea-nitrogen salvage in SBR subjects was already operating at a higher level while on habitual intakes. Furthermore, the effects on energy and amino acid supply

caused by high stool losses would affect SBR subjects more than NSBR subjects and SBR subjects also face the additional demands on conditionally essential amino acids. All these factors will no doubt have contributed to the largest fall in nitrogen balance when protein intake was reduced and therefore, SBR subjects will be particularly vulnerable to metabolic stress.

5.8 Summary

In summary, the findings of our nitrogen balance and urea kinetic studies are:

1. Ileostomy subjects, especially those with additional small bowel resection, had higher stool nitrogen losses compared to reference subjects.
2. Ileostomy subjects, especially those with additional small bowel resection, had higher habitual nitrogen intakes compared to reference subjects, probably to compensate for higher stool nitrogen losses.
3. Ileostomy subjects were capable of operating urea-nitrogen salvage in the absence of the colon but under habitual conditions, ileostomy subjects with additional small bowel resection appeared to be operating urea-nitrogen salvage at a higher level compared to those who had total colectomy only and reference subjects. This is probably due to the higher stomal nitrogen losses and hence, a greater reliance on urea-nitrogen salvage to improve nitrogen supply.
4. When nitrogen intake was reduced, ileostomy subjects were not able to up-regulate urea-nitrogen salvage. Consequently, ileostomy subjects, particularly those with additional small bowel resection, suffered a significant reduction in nitrogen balance.

We can therefore conclude that our second hypothesis, as set out in Chapter 3, is partially supported and partially refuted by the above results. Following total colectomy, ileostomy patients are able to operate urea-nitrogen salvage under habitual free-living conditions but when nitrogen availability is reduced, they do not have the reserve capacity to up-regulate urea-nitrogen salvage and are therefore vulnerable to marked negative nitrogen balance. Ileostomy patients who had additional small bowel resection are particularly vulnerable to the effects of reduced nitrogen availability.

6

CONCLUSION

There is a host of evidence indicating the importance of colonic functions in the maintenance of our physiology and metabolism, especially the metabolism of nitrogen. The aim of this thesis was, therefore, to examine the impact of the loss of colon on the health of people with ileostomy and particularly, the changes that may occur in the handling of nitrogen. Our hypotheses were

1. following the removal of the colon, ileostomy patients may have compromised nitrogen status and relatively poor overall health; and
2. without the colon, ileostomy patients are incapable of operating urea-nitrogen salvage and hence, will be particularly prone to negative nitrogen balance if nitrogen intake is reduced.

Through a comprehensive programme of assessment which included a multi-dimensional cross-sectional health survey and studies of both nitrogen balance and urea kinetics under habitual free-living conditions and metabolic stress created by reducing nitrogen availability, we demonstrated that ileostomy patients have compromised nitrogen status and significant impairment of overall health. We also demonstrated that although ileostomy patients are able to maintain nitrogen balance and operate urea-nitrogen salvage in free-living conditions, they are, however, unable to up-regulate urea-nitrogen salvage and maintain nitrogen equilibrium when nitrogen availability is reduced and are, therefore, vulnerable to metabolic stress. These effects, which are associated with total colectomy, are increased in ileostomy patients who have had additional small bowel resection.

By simply taking a clinical history, we elicited that a significant proportion of ileostomy patients complained of lethargy and reduced level of activity. These symptoms were more common in those who had high output stoma and liquid stool. Using SF-36 to formally evaluate general health status, ileostomy patients had significantly lower health

scores and the health of the most unwell being was substantially worst compared to age and sex matched reference subjects. Furthermore, ileostomy patients who reported lethargy and reduced activity also had significantly lower health scores compared to those who did not have these symptoms. In clinical practice, symptoms of reduced physical function and capacity and the presence of high output stoma and liquid stools may, therefore, be used to identify ileostomy patients who have poor health and alert clinicians to conduct further assessment of their nutritional status. Ileostomy patients are also more likely to complain of gastrointestinal and nutritional symptoms which may contribute to their poor health.

To put the above subjective symptoms into objective context, ileostomy patients were also found to have significantly lower BMI and FFM and they were more likely to be underweight compared to people with intact colon. Furthermore, ileostomy patients who reported lethargy and reduced activity also tended to be underweight. These findings can account for impairment in physical, psychological and social well being. Stool nitrogen and energy (unpublished data) losses via the stoma were also higher in ileostomy patients compared to normal individuals and this could explain their lower BMI and FFM. With high stoma output, other nutrients like electrolytes, minerals and micronutrients are likely to be lost too. Although measurements of these nutrients in the blood did not indicate widespread deficiency, assessments of urinary electrolytes and minerals suggest that ileostomy patients may have depleted body stores of sodium, calcium and magnesium. Their total body potassium stores may also be diminished. Our findings illustrate that blood nutrient levels do not always reflect body stores as it would appear that organ synthetic functions are being maintained at the expense of other biological processes like lean tissue deposition, muscle function and bone mineralization which are more difficult to measure in clinical practice.

Sodium depletion is probably an important factor associated with our various findings. Ileostomy patients who are depleted in sodium are not only at risk of chronic dehydration, renal stone disease and renal impairment, they are also at risk of low body weight, reduced lean tissue mass and bone demineralization. Since urinary sodium concentration can be easily measured, it's use in the clinical setting to identify ileostomy patients who are asymptomatic but nonetheless at risk of subclinical malnutrition may be of value. From our anecdotal experience with a few ileostomy patients who have clinical

symptoms and signs of sodium and mineral deficiencies, parenteral replacement of sodium have led to weight gain, improvement in mineral balances and a better sense of well being. An interventional study involving a larger number of asymptomatic sodium depleted ileostomy subjects is necessary to examine the benefits of sodium replacement.

Under habitual free-living conditions, our ileostomy patients were able to maintain nitrogen balance but they had higher nitrogen intakes, possibly to compensate for higher stomal nitrogen losses. Without the colon, our ileostomy patients were also capable of urea-nitrogen salvage, presumed to occur at the terminal end of the small bowel which is colonised by bacteria. However, the situation was very different under metabolic stress. When habitual nitrogen intake was reduced by 40%, ileostomy patients suffered a significant fall in nitrogen balance. This was probably due to the continual high stomal nitrogen losses coupled with the inability to up-regulate urea-nitrogen salvage sufficiently to compensate for the nitrogen deficit. Ileostomy patients are, therefore, very vulnerable and early intervention with nutritional support should be instituted when these people are under metabolic stress, from either reduced nitrogen intake and/or excess nitrogen demands, both of which are commonly seen during illness or surgery. Under these circumstances, particular attention should also be paid to ensure that deficiencies in other nutrients like electrolytes, minerals and micronutrients are corrected so that nitrogen and other macronutrients can be utilised efficiently.

The behaviour of avoiding specific food groups amongst ileostomy patients was reported three decades ago and despite the lack of any scientific or physiological basis for this practice, a large proportion of these people continue to refrain from eating fruits and vegetables of various kinds. Health care professionals like the stoma care nurses and dieticians may have continued to give ileostomy patients this advice while others may have avoided these foods simply out of fear or from personal experience of indigestion and stomal blockage. On the whole, the intake of NSP in ileostomy patients is reduced, probably leading to lower intakes of magnesium, vitamin C and carotene. This is clearly detrimental as these people are already at risk of micronutrient deficiencies through excess stomal losses. In addition, reduced NSP intakes can adversely affect bacterial mass in the terminal small bowel and hence, potentially hamper the capacity for urea-nitrogen salvage as mentioned previously. It is important, therefore, to discourage ileostomy patients from avoiding specific food groups unless it is well documented that

the food causes a specific problem and also encourage health professionals to review the evidence and their practice.

The adverse effects of total colectomy on health and nitrogen metabolism are exacerbated by additional small bowel loss. Ileostomy patients with additional small bowel resection had reported poorer health compared to those with total colectomy only. In general, nutrient absorption in these people is compromised due to the additional loss of small bowel and they suffer higher stomal losses. Their urinary excretions of sodium, calcium, magnesium and nitrogen were found to be significantly lower than ileostomy subjects who had total colectomy only, indicating a higher degree of deficit in these nutrients. They may also have deficiencies in conditionally essential amino acids such as glycine, serine and cysteine generated by higher demands for bile salt synthesis due to the loss of enterohepatic recycling of bile acids. Hence, under habitual free-living conditions, their ability to maintain nitrogen balance is probably achieved partly by eating more to compensate for higher losses but also partly by operating urea-nitrogen salvage at a higher level to ensure that nitrogen demands are met both quantitatively and qualitatively. However, under metabolic stress, they are particularly vulnerable with little reserve to cope and are liable to a large fall in nitrogen balance. Early intervention with nutritional support in this group of patients is therefore vital. In addition, ileostomy patients with small bowel resection should also be screened for micronutrient deficiencies like vitamin B_{12} which can often be neglected. In our study, although ileostomy patients with Crohn's disease did not appear to be at greater risk compared to those who had ulcerative colitis, it probably reflects the absence of significant small bowel Crohn's disease in our cohort. However, ileostomy patients with significant small bowel Crohn's disease are likely to face similar problems described above.

In conclusion, our hypotheses are supported by the results of our findings. The importance of colonic functions can no longer be ignored as the loss of colon may lead to compromised nitrogen status, impairment of overall health and increased vulnerability during metabolic stress. People with ileostomy who have had additional small bowel resection appear to be particularly susceptible to the metabolic and clinical effects associated with total colectomy. In the absence of clinical guidelines on how ileostomy patients should be managed, we propose a care pathway based on our findings (Figure 5.36) and recommend a multi-disciplinary approach involving clinicians, nutrition nurse

specialists, stoma care nurses and dieticians so that the care of these people can be optimised. With a systematic approach to assessment and follow-up, the aims are to prevent the development of health problems and to timely detect and treat existing health problems so that associated morbidity and mortality can be reduced. We also hope that the care pathway will serve as a platform through which the care of these people can commence without delay while further clinical research takes place to determine the best approach.

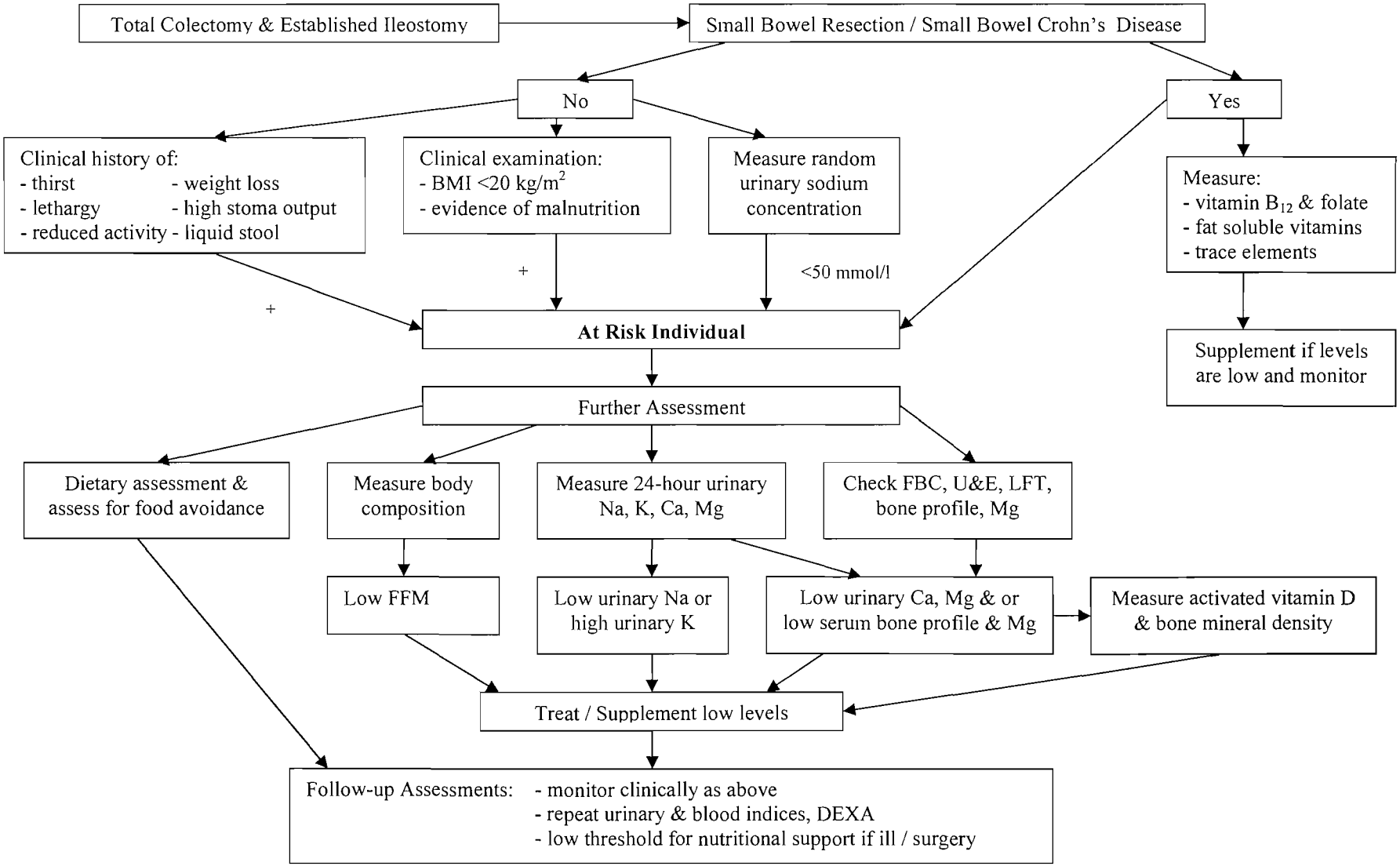


Figure 5.36 Care pathway for the clinical management of people who have had total colectomy and ileostomy

APPENDICES

APPENDIX 1 Body mass index and body composition data

1A BMI and body composition of NSBR and SBR ileostomy subjects

		NSBR (n=46)	SBR (n=14)
Height (m)	Median	1.68	1.65
	25th percentile	1.62	1.60
	75th percentile	1.73	1.74
Weight (kg)	Median	69.4	65.3
	25th percentile	63.2	57.3
	75th percentile	86.4	86.9
BMI (kg/m^2)	Median	25.1	24.8
	25th percentile	21.9	21.8
	75th percentile	27.9	30.9
Body fat (kg)	Median	20.0	21.6
	25th percentile	16.5	17.3
	75th percentile	25.2	26.2
BF%	Median	28.2	31.4
	25th percentile	23.6	27.5
	75th percentile	34.2	37.7
FFM (kg)	Median	50.4	44.5
	25th percentile	41.5	37.1
	75th percentile	61.3	56.1

1B BMI and body composition of subjects with ulcerative colitis and Crohn's disease

		Ulcerative colitis (n=39)	Crohn's disease (n=18)
Height (m)	Median	1.69	1.65
	25th percentile	1.63	1.60
	75th percentile	1.74	1.70
Weight (kg)	Median	72.5	65.3
	25th percentile	63.3	58.9
	75th percentile	86.3	96.2
BMI (kg/m^2)	Median	26.1	24.8
	25th percentile	22.4	21.7
	75th percentile	28.6	32.0
Body fat (kg)	Median	20.6	20.8
	25th percentile	17.1	15.6
	75th percentile	25.2	28.6
BF%	Median	28.5	31.8
	25th percentile	23.8	24.2
	75th percentile	33.8	39.1
FFM (kg)	Median	51.7	45.6
	25th percentile	41.2	39.9
	75th percentile	61.9	54.5

1C BMI of reference subjects compared to NDNS by age group [mean (sd)]

Age (years)		Reference		NDNS	
		Male	Female	Male	Female
25-34	N	0	5	227	213
	Weight (kg)		60.9 (11.2)	83.0 (13.1)	67.0 (13.3)
	BMI (kg/m^2)		24.0 (3.99)	26.4 (4.06)	25.4 (5.00)
35-49	N	10	7	263	331
	Weight (kg)	87.9 (8.4)	69.4 (9.1)	85.0 (14.4)	70.0 (15.7)
	BMI (kg/m^2)	28.6 (2.00)	26.2 (3.3)	27.4 (4.23)	26.7 (5.75)
50-64	N	13	9	264	269
	Weight (kg)	87.9 (10.2)	73.2 (8.9)	87.0 (14.9)	71.0 (14.0)
	BMI (kg/m^2)	28.0 (2.41)	27.2 (2.88)	28.4 (4.83)	27.4 (5.50)
65-74	N	6	2	311	338
	Weight (kg)	81.7 (6.9)	70.7 (4.7)	78.2 (12.6)	66.5 (12.6)
	BMI (kg/m^2)	27.4 (1.89)	28.5 (1.63)	26.8 (3.80)	27.0 (5.00)
75-84	N	6	2	131	205
	Weight (kg)	75.1 (4.8)	62.4 (3.5)	74.1 (11.1)	64.1 (11.0)
	BMI (kg/m^2)	26.5 (1.62)	25.7 (2.83)	26.2 (3.40)	26.7 (4.40)

1D Body composition of reference subjects and NHANES III by age [mean (sd)]

Age (years)	Body composition	Reference		NHANES III	
		Male	Female	Male	Female
20-29.9	N	0	3	384	426
	Weight (kg)		61.9 (14.9)	79.2 (16.6)	63.2 (14.3)
	FFM (kg)		42.7 (5.3)	61.3 (9.5)	42.8 (5.9)
	BF (kg)		19.2 (10.9)	17.9 (8.7)	20.5 (9.6)
	BF%		29.5 (9.7)	21.8 (6.2)	31.0 (7.5)
30-39.9	N	2	3	436	543
	Weight (kg)	82.7 (3.0)	61.3 (5.7)	84.0 (17.1)	69.1 (18.0)
	FFM (kg)	62.5 (3.0)	44.5 (2.2)	63.6 (10.5)	45.0 (6.9)
	BF (kg)	20.2 (6.0)	16.8 (3.6)	20.4 (8.5)	24.1 (12.3)
	BF%	24.3 (6.4)	27.2 (3.5)	23.6 (5.8)	33.0 (8.5)
40.49.9	N	8	6	410	454
	Weight (kg)	89.2 (8.9)	70.1 (9.7)	86.0 (17.0)	70.7 (16.8)
	FFM (kg)	67.0 (5.1)	44.9 (4.1)	64.6 (10.6)	44.8 (6.9)
	BF (kg)	22.2 (4.6)	25.2 (7.1)	21.3 (8.5)	25.9 (10.9)
	BF%	24.7 (3.1)	35.4 (6.2)	24.2 (5.7)	35.4 (6.9)
50-59.9	N	10	6	396	454
	Weight (kg)	88.2 (8.6)	70.4 (9.5)	86.9 (15.0)	73.9 (17.4)
	FFM (kg)	66.2 (5.8)	44.4 (4.7)	64.6 (8.8)	45.4 (6.7)
	BF (kg)	22.0 (3.5)	25.9 (6.5)	22.3 (8.3)	28.6 (11.6)
	BF%	24.9 (2.1)	36.5 (5.4)	25.1 (6.0)	37.3 (7.1)
60-69.9	N	5	5	465	447
	Weight (kg)	84.3 (13.7)	75.6 (6.0)	84.9 (14.7)	70.3 (15.1)
	FFM (kg)	60.6 (9.0)	43.6 (5.0)	62.3 (8.9)	43.6 (6.3)
	BF (kg)	23.7 (4.8)	32.0 (1.9)	22.7 (7.7)	26.7 (9.9)
	BF%	28.0 (1.4)	42.5 (2.6)	26.2 (5.5)	36.9 (6.9)
70 and over	N	10	2	447	538
	Weight (kg)	78.0 (6.2)	62.4 (3.5)	79.3 (13.3)	67.1 (14.5)
	FFM (kg)	55.3 (1.7)	34.8 (1.1)	59.1 (8.6)	42.3 (6.5)
	BF (kg)	22.6 (2.6)	27.6 (2.3)	20.3 (6.8)	24.8 (9.3)
	BF%	29.1 (3.0)	44.2 (1.3)	25.1 (5.5)	35.9 (6.9)

APPENDIX 2 Clinical history data

2A Clinical history of NSBR and SBR ileostomy subjects

	NSBR: n=46 (%)	SBR: n=14 (%)
Perception of general health:		
Good	40 (87.0%)	4 (28.6%)
Fair	4 (8.7%)	6 (42.9%)
Poor	2 (4.3%)	4 (28.6%)†
Lethargy:		
No	22 (47.8%)	2 (14.3.0%)
Yes	24 (52.2%)	12 (85.7%)†
Activity level:		
Active	43 (93.5%)	5 (35.7%)
Not active	3 (6.5%)	9 (64.3%)*
Appetite:		
Good / Normal	43 (93.5%)	13 (92.9%)
Poor	3 (6.5%)	1 (7.1%)
Weight in last 6 months:		
Gain	9 (19.6%)	1 (7.1%)
Stable	31 (67.4%)	11 (78.6%)
Lost	6 (13.0%)	2 (14.3%)
Renal stone – No	45 (97.8%)	12 (85.7%)
- Yes	1 (2.2%)	2 (14.3%)
Gall stone – No	44 (95.7%)	11 (78.6%)
- Yes	2 (4.3%)	3 (21.4%)
Gastrointestinal symptoms:		
Nausea – No	45 (97.8%)	12 (85.7%)
- Yes	1 (2.2%)	2 (21.4%)
Vomiting – No	45 (97.8%)	14 (100%)
- Yes	1 (2.2%)	0
Abdominal pain / discomfort – No	41 (89.1%)	9 (64.3%)
- Yes	5 (10.9%)	5 (35.7%)*
Bloating – No	38 (82.6%)	8 (57.1%)
- Yes	8 (17.4%)	6 (42.9%)
Blood loss / Malaena – No	46 (100%)	13 (100%)
- Yes	0	1 (7.1%)
Nutritional symptoms:		
Loss of taste – No	46 (100%)	12 (85.7%)
- Yes	0	2 (14.3%)
Skin – Normal	26 (56.5%)	11 (78.6%)
- Dry	20 (43.5%)	3 (21.4%)
Hair – Normal	45 (97.8%)	12 (85.7%)
- Hair loss	1 (2.2%)	2 (14.3%)
Nail – Normal	41 (89.1%)	13 (92.9%)
- Brittle	5 (10.9%)	1 (7.1%)
Tendency for infection – No	43 (93.5%)	9 (64.3%)
- Yes	3 (6.5%)	5 (35.7%)*

Fisher Exact Test: * $p<0.05$, Chi-square: † $p<0.05$

2B Clinical history of ileostomy subjects with ulcerative colitis and Crohn's disease

	Ucerative colitis n=38 (%)	Crohn's disease n=19 (%)
Perception of general health:		
Good	29 (76.3%)	12 (63.1%)
Fair	6 (15.8%)	4 (21.1%)
Poor	3 (7.9%)	3 (15.8%)
Lethargy:		
No	21 (55.3%)	2 (10.5%)
Yes	17 (44.7%)	17 (89.5%)†
Activity level:		
Active	32 (84.2%)	13 (68.4%)
Not active	6 (15.8%)	6 (31.6%)
Appetite:		
Good / Normal	36 (94.7%)	17 (89.5%)
Poor	2 (5.3%)	2 (10.5%)
Weight in last 6 months:		
Gain	7 (18.4%)	1 (5.4%)
Stable	26 (68.4%)	15 (78.9%)
Lost	5 (13.2%)	3 (15.7%)
Renal stone – No	36 (94.7%)	18 (94.7%)
- Yes	2 (5.3%)	1 (5.3%)
Gall stone – No	37 (97.4%)	16 (84.2%)
- Yes	1 (2.6%)	3 (15.8%)
Gastrointestinal symptoms:		
Nausea – No	37 (97.4%)	17 (89.5%)
- Yes	1 (2.6%)	2 (10.5%)
Vomiting – No	37 (97.4%)	19 (100%)
- Yes	1 (2.6%)	0
Abdominal pain / discomfort – No	34 (89.5%)	13 (68.4%)
- Yes	4 (10.5%)	6 (31.6%)
Bloating – No	34 (89.5%)	10 (52.6%)
- Yes	4 (10.5%)	9 (47.4%)*
Blood loss / Malaena – No	38 (100%)	18 (94.7%)
- Yes	0	1 (5.3%)
Nutritional symptoms:		
Loss of taste – No	37 (97.4%)	18 (94.7%)
- Yes	1 (2.6%)	1 (5.3%)
Skin – Normal	25 (65.8%)	11 (57.9%)
- Dry	13 (34.2%)	8 (42.1%)
Hair – Normal	37 (96.4%)	17 (89.5%)
- Hair loss	1 (2.6%)	2 (10.5%)
Nail – Normal	34 (89.5%)	17 (89.59%)
- Brittle	4 (10.5%)	2 (10.5%)
Tendency for infection – No	35 (92.3%)	14 (63.7%)
- Yes	3 (7.9%)	5 (26.3%)

Chi-square: * p=0.001, Fisher Exact Test: † p=0.005

APPENDIX 3 SF-36 data

3A Health scores of female and male reference subjects

	Female (n=25) Median (25^{th}-75^{th} percentile)	Male (n=35) Median (25^{th}-75^{th} percentile)
Physical function	100 (90.00-100)	95.00 (85.00-100)*
Role - Physical	100 (100-100)	100 (100-100)
Role – Mental	100 (100-100)	100 (100-100)
Social function	100 (100-100)	100 (100-100)
Mental health	84.00 (76.00-90.00)	84.00 (76.00-92.00)
Energy	75.00 (65.00-80.00)	70.00 (50.00-80.00)
Pain	88.89 (83.33-100)	88.89 (77.78-100)
Health perception	82.00 (72.00-91.00)	77.00 (62.00-85.00)

Mann-Whitney U Test: * p=0.014

3B Health scores of female and male ileostomy subjects

	Female (n=25) Median (25^{th}-75^{th} percentile)	Male (n=35) Median (25^{th}-75^{th} percentile)
Physical function	75.00 (47.50-97.50)	80.00 (50.00-90.00)
Role - Physical	100 (50.00-100)	100 (25.00-100)
Role – Mental	100 (66.67-100)	100 (33.33-100)
Social function	100 (61.11-100)	77.78 (66.67-100)
Mental health	68.00 (52.00-88.00)	76.00 (64.00-92.00)
Energy	50.00 (32.50-65.00)	55.00 (35.00-75.00)
Pain	88.89 (50.00-100)	88.89 (44.44-88.89)
Health perception	67.00 (37.50-84.50)	62.00 (42.00-77.00)

3C Health scores of reference subjects by age groups (median & 25th-75th percentile)

	Age 25-44 (n=10)	Age 45-64 (n=35)	Age 65-87 (n=15)
Physical function	100 (95.00-100)	95.00 (90.00-100)	90.00 (80.00-95.00)*
Role - Physical	100 (100-100)	100 (100-100)	100 (100-100)
Role – Mental	100 (100-100)	100 (100-100)	100 (100-100)
Social function	100 (94.44-100)	100 (100-100)	100 (100-100)
Mental health	82.00 (67.00-88.00)	84.00 (76.00-88.00)	88.00 (76.00-92.00)
Energy	67.50 (50.00-76.25)	70.00 (60.00-75.00)	80.00 (60.00-80.00)
Pain	88.89 (77.78-100)	88.89 (77.78-100)	88.89 (77.78-100)
Health perception	82.00 (63.25-87.50)	77.00 (67.00-87.00)	77.00 (62.00-82.00)

Kruskal-Wallis Test: * p=0.003

3D Health scores of ileostomy subjects by age groups (median & 25th-75th percentile)

	Age 25-44 (n=10)	Age 45-64 (n=32)	Age 65-87 (n=18)
Physical function	82.50 (41.25-96.25)	85.00 (61.25-95.00)	65.00 (50.00-81.25)
Role - Physical	62.50 (0-100)	100 (75.00-100)	50.00 (0-100)
Role – Mental	66.67 (0-100)	100 (100-100)	100 (33.33-100)
Social function	72.22 (44.44-100)	100 (100-100)	100 (66.67-100)
Mental health	62.00 (45.00-74.00)	78.00 (64.00-92.00)	76.00 (63.00-89.00)
Energy	*37.50(11.25-52.50)	62.50 (45.00-75.00)	42.50 (23.75-67.50)
Pain	77.78 (33.33-91.67)	88.89 (33.33-100)	88.89 (66.67-100)
Health perception	59.50 (35.25-67.75)	64.50 (42.75-82.00)	62.00 (43.75-83.25)

Kruskal-Wallis Test: * p=0.016

3E Health scores of ileostomy subjects with Crohn's disease and ulcerative colitis

	Crohn's disease (n=19) Median (25^{th}-75^{th} percentile)	Ulcerative colitis (n=38) Median (25^{th}-75^{th} percentile)
Physical function	75.00 (45.00-90.00)	80.00 (53.75-95.00)
Role - Physical	75.00 (0-100)	100 (43.75-100)
Role – Mental	100 (0-100)	100 (58.33-100)
Social function	77.78 (55.56-100)	100 (66.67-100)
Mental health	64.00 (52.00-80.00)	78.00 (67.00-89.00)
Energy	45.00 (35.00-65.00)	60.00 (35.00-75.00)
Pain	88.89 (33.33-100)	88.89 (55.56-100)
Health perception	62.00 (30.00-72.00)	62.00 (44.25-82.00)

3F Health scores of ileostomy subjects who reported and did not report lethargy

	Lethargy (n=36) Median (25^{th}-75^{th} percentile)	No lethargy (n=24) Median (25^{th}-75^{th} percentile)
Physical function	62.50 (41.25-90.00)†	92.50 (80.00-98.75)
Role - Physical	50.00 (0-100)*	100 (100-100)
Role – Mental	100 (0-100)‡	100 (100-100)
Social function	77.78 (55.56-100)*	100 (100-100)
Mental health	64.00 (52.00-83.00)†	82.00 (76.00-92.00)
Energy	40.00 (20.00-58.75)*	70.00 (61.25-80.00)
Pain	72.22 (33.33-97.22)‡	88.89 (88.89-100)
Health perception	52.00 (30.00-67.00)*	77.00 (62.00-87.00)

Mann-Whitney Test: *$p<0.001$, †$p=0.001$, ‡$p<0.05$

3G Health scores of ileostomy subjects who reported and did not report reduced activity level

	Activity reduced (n=12) Median (25^{th}-75^{th} percentile)	Activity not reduced (n=48) Median (25^{th}-75^{th} percentile)
Physical function	32.50 (12.50-53.75)*	82.50 (66.25-95.00)
Role - Physical	0 (0-68.75)*	100 (56.25-100)
Role – Mental	16.67 (0-100) ‡	100 (100-100)
Social function	55.56 (36.11-75.00)*	100 (77.78-100)
Mental health	50.00 (26.00-64.00)*	80.00 (68.00-92.00)
Energy	20.00 (15.00-38.75)*	62.50 (45.00-75.00)
Pain	27.78 (13.89-88.89) †	88.89 (66.67-100)
Health perception	18.50 (6.25-35.00)*	67.00 (53.25-82.00)

Mann-Whitney Test: *p<0.001, †p=0.005, ‡p=0.007

APPENDIX 4 Nutrient intakes data

4A Nutrient intakes of ileostomy and reference subjects

Nutrient	Ileostomy: n=59 median (range)	Reference: n=60 median (range)
Energy (kcal/day)	2443 (1151 - 5203)	2282 (1043 – 6255)
Protein (g/day)	99.5 (42.7 - 212.9)	91.3 (38.9 -231.8)
Fat (g/day)	100.6 (34.4 - 213.8)	83.2 (41.0 – 263.3)
Carbohydrate (g/day)	318.6 (36.4 – 748.5)	297.7 (134.4 – 761.6)
Non-starch polysaccharide (g/day)	17.7 (5.6 – 40.4)*	23.0 (6.8 -50.7)
Starch (g/day)	157.4 (13.1 – 289.8)†	131.3 (38.0 – 559.2)
Calcium (mg/day)	1143.0 (334.0 – 2266.4)	1085.9 (505.2 – 3852.5)
Magnesium (mg/day)	343.2 (142.3 – 667.6)†	388.8 (150.4 – 829.8)
Iron (mg/day)	13.7 (4.0 – 24.2)*	15.4 (4.5 – 37.1)
Zinc (mg/day)	12.4 (4.85 – 29.4)	12.2 (4.6 – 28.7)
Selenium (mg/day)	63.0 (25.0 – 153.9)	68.0 (28.8 – 482.8)
Copper (mg/day)	1.4 (0.56 – 5.12)	1.6 (0.6 – 5.9)
Carotene (µg/day)	2710 (571.6 – 6631.6)*	3277 (1332.8 – 41024.2)
Retinol (µg/day)	607.9 (98.9 – 5311.7)	453.0 (96.6 – 5317.0)
Niacin (mg/day)	21.9 (8.5 – 48.0)	22.1 (8.1- 48.2)
Riboflavin (mg/day)	2.6 (0.62 – 4.53)	2.3 (0.9 – 5.1)
Thiamin (mg/day)	2.0 (0.9 – 5.4)	2.0 (0.7 – 11.7)
Vitamin B_6 (mg/day)	2.7 (1.1 – 4.9)	2.8 (1.0 – 5.3)
Vitamin B_{12} (mg/day)	7.5 (2.0 – 36.8)	6.3 (1.6 – 34.9)
Vitamin C (mg/day)	126.7 (42.7 – 476.2)*	178.5 (55.0 – 398.2)
Vitamin D (µg/day)	3.0 (0.7 – 10.3)	3.4 (1.0 – 12.1)
Vitamin E (mg/day)	8.0 (2.8 – 20.2)	9.1 (2.5 – 24.9)
Folate (µg/day)	365.2 (144.3 – 594.8)	371.6 (146.3 – 1249.6)

Mann-Whitney: * $p<0.01$, † $p<0.05$

4B Nutrient intakes of NSBR and SBR ileostomy subjects

Nutrient	NSBR: n=45 median (range)	SBR: n=14 median (range)
Energy (kcal/day)	2536 (1201 - 5203)	2336 (1151 – 4167)
Protein (g/day)	101.1 (47.5 - 212.9)	88.4 (42.7 -177.8)
Fat (g/day)	103.0 (34.4 - 213.8)	93.5 (36.0 – 175.6)
Carbohydrate (g/day)	295.5 (36.4 – 748.5)	239.2 (158.8 – 499.5)
Non-starch polysaccharide (g/day)	18.4 (5.6 – 40.4)	13.0 (9.3 -32.6)
Starch (g/day)	159.4 (13.1 – 289.8)	149.8 (80.2 – 284.8)
Calcium (mg/day)	1154.2 (568.8 – 2266.4)	1102.6 (334.0 – 1720.7)
Magnesium (mg/day)	359.6 (181.1 – 667.6)	297.9 (142.3 – 496.6)
Iron (mg/day)	14.0 (4.0 – 24.2)	10.6 (6.4 – 20.5)
Zinc (mg/day)	12.4 (5.3 – 29.4)	10.6 (4.9 – 22.2)
Selenium (mg/day)	62.0 (25.0 – 153.9)	78.3 (25.4 – 135.6)
Copper (mg/day)	1.4 (0.6 – 5.1)	1.3 (0.7 – 2.9)
Carotene (μg/day)	2729 (571.6 – 6631.6)	2577 (1185.1 – 5040.4)
Retinol (μg/day)	607.9 (121.5 – 5311.7)	646.5 (98.3 – 2514.3)
Niacin (mg/day)	22.2 (8.5 – 48.0)	19.8 (10.3- 44.9)
Riboflavin (mg/day)	2.6 (1.1 – 4.5)	2.0 (0.6 – 3.9)
Thiamin (mg/day)	2.1 (0.9 – 5.4)	1.6 (1.0 – 3.2)
Vitamin B_6 (mg/day)	3.0 (1.1 – 4.3)	2.3 (1.3 – 4.9)
Vitamin B_{12} (mg/day)	7.7 (2.0 – 36.8)	7.1 (2.2 – 21.6)
Vitamin C (mg/day)	128.4 (46.6 – 476.2)	110.1 (42.7 – 269.5)
Vitamin D (μg/day)	3.0 (0.7 – 10.3)	2.4 (0.8 – 9.3)
Vitamin E (mg/day)	8.4 (2.8 – 20.2)	6.1 (3.7 – 16.7)
Folate (μg/day)	376.5 (182.0 – 594.8)	282.1 (144.3 – 573.6)

4C Nutrient intakes of ileostomy subjects with ulcerative colitis and Crohn's disease

Nutrient	Ulcerative colitis: n=37 median (range)	Crohn's disease: n=19 median (range)
Energy (kcal/day)	2613 (1151 - 5203)	2224 (1243 – 4167)
Protein (g/day)	102.5 (47.5 – 181.2)	88.5 (42.7 -212.9)
Fat (g/day)	103.4 (34.4 - 213.8)	86.9 (46.2 – 175.6)
Carbohydrate (g/day)	345.6 (130.8 – 748.5)	269.2 (36.4 – 499.5)*
Non-starch polysaccharide (g/day)	18.5 (5.6 – 29.4)	16.4 (7.7 -32.6)
Starch (g/day)	169.0 (30.2 – 289.8)	142.2 (13.1 – 289.3)
Calcium (mg/day)	1274.5 (568.8 – 2266.4)	930.8 (334.0 – 1720.7)*
Magnesium (mg/day)	359.6 (181.4 – 620.4)	303.2 (142.3 – 496.6)
Iron (mg/day)	14.7 (4.0 – 23.4)	11.4 (6.4 – 23.9)
Zinc (mg/day)	12.6 (5.3 – 24.6)	11.0 (4.9 – 29.4)
Selenium (mg/day)	63.0 (25.0 – 130.5)	55.3 (25.4 – 153.9)
Copper (mg/day)	1.4 (0.6 – 3.1)	1.3 (0.7 – 5.1)
Carotene (μg/day)	2847.8 (571.6 – 5101.7)	2556.3 (746.6 – 5040.4)
Retinol (μg/day)	607.9 (121.5 – 2452.8)	548.5 (98.9 – 5311.7)
Niacin (mg/day)	23.4 (8.5 – 35.2)	20.3 (10.3- 48.0)
Riboflavin (mg/day)	2.7 (1.1 – 4.5)	2.1 (0.6 – 4.7)
Thiamin (mg/day)	2.2 (0.9 – 5.4)	1.8 (1.0 – 5.0)
Vitamin B_6 (mg/day)	2.7 (1.1 – 4.9)	2.5 (1.3 – 4.5)
Vitamin B_{12} (mg/day)	7.5 (2.3 – 18.6)	7.2 (2.0 – 36.8)
Vitamin C (mg/day)	130.2 (46.6 – 476.2)	106.4 (42.7 – 269.5)
Vitamin D (μg/day)	3.0 (1.3 – 10.3)	2.7 (0.7 – 9.3)
Vitamin E (mg/day)	8.4 (2.8 – 20.2)	6.6 (3.7 – 18.1)
Folate (μg/day)	384.8 (182.0 – 594.8)	321.5 (144.3 – 573.6)

Mann-Whitney Test: * $p<0.05$

4D Nutrient intakes of ileostomy subjects who reported and did not report food avoidance

Nutrient	Food avoidance n=37 median (range)	No food avoidance n=22 median (range)
Energy (kcal/day)	2380 (1151 – 4167)	2619 (1243 – 5203)
Protein (g/day)	99.5 (47.5 – 212.9)	100.6 (42.7 -188.2)
Fat (g/day)	89.2 (34.4 – 175.6)	105.4 (52.8 – 213.8)
Carbohydrate (g/day)	335.4 (36.4 – 613.8)	306.6 (158.8 – 748.5)
Non-starch polysaccharide(g/day)	16.7 (5.6 – 40.4)	18.5 (9.9 -30.6)
Starch (g/day)	150.5 (13.1 – 284.8)	161.3 (94.2 – 289.8)
Calcium (mg/day)	1122.5 (568.8 – 1870.7)	1230.9 (334.0 – 2266.4)
Magnesium (mg/day)	343.2 (169.7 – 667.6)	331.7 (142.3 – 620.4)
Iron (mg/day)	13.7 (4.0 – 23.9)	14.3 (6.4 – 24.2)
Zinc (mg/day)	12.2 (5.3 – 29.4)	12.6 (4.9 – 25.2)
Selenium (mg/day)	57.7 (25.0 – 153.9)*	77.8 (30.0 – 132.3)
Copper (mg/day)	1.4 (0.6 – 5.1)	1.4 (0.8 – 3.1)
Carotene (μg/day)	2632 (571.6 – 6058.7)	2829 (746.6 – 6631.6)
Retinol (μg/day)	607.9 (98.3 – 5311.7)	604.5 (121.5 – 2514.3)
Niacin (mg/day)	21.9 (8.5 – 48.0)	23.3 (10.4- 44.9)
Riboflavin (mg/day)	2.4 (0.8 – 4.5)	2.6 (0.6 – 4.5)
Thiamin (mg/day)	2.0 (0.9 – 5.4)	2.1 (1.1 – 5.0)
Vitamin B_6 (mg/day)	2.9 (1.1 – 4.9)	2.5 (1.5 – 4.5)
Vitamin B_{12} (mg/day)	7.2 (2.2 – 36.8)	8.5 (2.0 – 21.6)
Vitamin C (mg/day)	128.4 (42.7 – 476.2)	113.3 (47.5 – 261.7)
Vitamin D (μg/day)	2.8 (0.8 – 10.3)	3.3 (0.7 – 9.3)
Vitamin E (mg/day)	8.2 (2.8 – 20.2)	7.7 (4.1 – 18.1)
Folate (μg/day)	362.0 (144.3 – 594.8)	375.0 (154.1 – 585.8)

Mann-Whitney Test: * $p<0.05$,

APPENDIX 5 Individual data for nitrogen balance studies

5A Individual data of all average values for nitrogen balance studies conducted on habitual diets

Group	Subject	Energy intake (kcal/day)	Nitrogen intake (mgN/kg/day)	Stool nitrogen (mgN/kg/day)	Available nitrogen (mgN/kg/day)	Urinary nitrogen (mgN/kg/day)	Nitrogen balance (mgN/kg/day)
Reference	1	3070	142.79	23.06	119.73	131.28	-11.56
	2	2660	199.66	20.81	178.85	172.04	6.81
	3	1879	151.71	5.69	146.02	139.00	7.01
	4	1933	157.25	28.24	129.01	169.90	-40.89
	5	2647	125.71	12.46	113.25	137.01	-23.76
	6	2806	170.23	10.70	159.54	152.37	7.16
SBR	7	2595	178.46	38.59	139.87	85.37	54.50
	8	1846	108.12	21.17	86.96	119.22	-32.26
	9	2340	213.10	51.66	161.44	112.59	48.85
	10	2197	124.29	21.43	102.86	96.36	6.50
	11	3440	267.86	129.63	138.23	120.93	17.30
	12	2245	241.10	32.03	209.07	165.04	44.04
NSBR	13	1244	110.39	19.48	90.91	90.91	0.00
	14	2127	191.40	38.65	152.74	146.32	6.42
	15	2647	215.07	23.09	191.98	177.08	14.90
	16	2140	160.30	21.83	138.47	157.30	-18.83
	17	2298	193.80	27.13	166.67	146.66	20.00
	18	3322	163.55	22.78	140.77	132.79	7.99

5B Individual data of all average values for nitrogen balance studies conducted on low protein diets

Group	Subject	Energy intake (kcal/day)	Nitrogen intake (mgN/kg/day)	Stool nitrogen (mgN/kg/day)	Available nitrogen (mgN/kg/day)	Urinary nitrogen (mgN/kg/day)	Nitrogen balance (mgN/kg/day)
Reference	1	3071	90.60	22.29	68.13	82.11	-13.80
	2	2610	103.39	12.04	91.36	119.77	-28.41
	3	1873	86.19	13.94	72.24	88.87	-16.62
	4	1933	102.01	15.46	86.55	119.47	-32.92
	5	2635	83.62	8.47	75.14	97.10	-21.96
	6	2802	102.54	24.50	78.04	101.45	-23.41
SBR	7	2650	87.24	30.69	56.54	48.18	8.37
	8	1846	66.17	23.01	43.15	76.62	-33.47
	9	2340	120.91	37.27	83.64	85.96	-2.32
	10	2196	77.25	17.88	59.37	78.73	-19.36
	11	3279	177.91	91.93	85.98	87.07	-1.09
	12	2245	124.37	34.45	89.92	113.99	-24.08
NSBR	13	1334	70.60	8.01	62.59	72.90	-10.31
	14	2127	106.52	28.82	77.69	109.43	-31.74
	15	2644	120.25	25.77	94.48	139.59	-45.12
	16	2143	100.95	25.92	75.03	112.23	-37.19
	17	2297	107.35	17.41	89.94	86.16	3.78
	18	3321	104.89	23.24	81.64	85.78	-4.13

APPENDIX 6 Individual data for urea kinetic studies

6A Individual data of all average values for urea kinetics studies conducted on habitual diets

Group	Subject	Day 5 Nitrogen intake (mgN/kg/day)	P (mgN/kg/day)	E_U (mgN/kg/day)	T (mgN/kg/day)	P_R (mgN/kg/day)	S (mgN/kg/day)
Reference	1	173.99	150.82	106.18	44.64	15.33	29.30
	2	175.48	232.50	151.33	81.17	15.36	65.81
	3	148.04	209.21	132.59	76.62	16.23	60.39
	4	179.54	216.06	156.35	59.71	10.60	49.10
	5	145.19	159.19	131.91	27.28	11.54	15.74
	6	163.10	171.62	129.82	41.80	13.60	28.20
SBR	7	141.48	143.28	83.65	59.63	6.44	53.20
	8	106.18	154.11	90.01	64.09	13.26	50.83
	9	226.94	150.30	132.32	17.98	6.55	11.43
	10	106.57	111.99	95.01	16.98	7.48	9.49
	11	284.66	188.31	103.73	84.58	8.24	76.35
	12	185.05	305.57	195.52	110.05	39.65	70.40
NSBR	13	110.82	182.91	97.16	85.75	11.09	74.67
	14	148.88	175.44	144.45	30.99	12.96	18.02
	15	153.58	217.27	164.08	53.19	18.84	34.35
	16	163.71	175.10	146.67	28.43	16.68	11.74
	17	182.95	176.37	127.67	48.69	12.54	36.15
	18	174.07	158.81	115.64	43.17	14.08	29.09

6B Individual data of all average values for urea kinetics studies conducted on low protein diets

Group	Subject	Day 5 Nitrogen intake (mgN/kg/day)	P (mgN/kg/day)	E_U (mgN/kg/day)	T (mgN/kg/day)	P_R (mgN/kg/day)	S (mgN/kg/day)
Reference	1	89.73	102.61	75.59	27.02	8.60	18.42
	2	107.74	147.09	102.05	45.04	11.27	33.77
	3	86.44	116.14	71.86	44.27	8.66	35.61
	4	101.70	186.83	123.29	63.54	8.51	55.03
	5	83.16	167.79	95.86	71.93	8.77	63.16
	6	100.18	89.53	60.00	29.53	6.93	22.60
SBR	7	86.59	66.59	39.52	26.77	2.20	24.58
	8	66.05	100.12	55.98	44.14	9.30	34.84
	9	120.73	131.30	89.51	41.79	9.98	31.81
	10	76.97	83.81	43.92	39.89	6.58	33.32
	11	177.51	144.52	75.21	69.30	8.78	60.52
	12	124.03	169.60	101.53	68.07	7.55	60.52
NSBR	13	70.74	116.44	58.41	58.03	10.28	47.74
	14	106.52	121.03	92.93	28.09	8.19	19.91
	15	120.13	154.46	109.93	44.53	10.11	34.43
	16	100.41	122.65	95.93	26.71	9.87	16.84
	17	107.16	103.49	76.15	27.34	6.43	20.91
	18	104.53	106.63	73.25	33.37	7.73	25.64

6C Individual data of all average values of urea kinetics relative to intake on habitual diets

Group	Subject	T + I (mgN/kg/day)	P/I (%)	E_U/I (%)	T/I (%)	P_R/I (%)	S/I (%)
Reference	1	218.63	86.68	61.03	25.65	8.81	16.84
	2	256.65	132.49	86.24	46.26	8.75	37.50
	3	224.66	141.32	89.56	51.76	10.97	40.79
	4	239.25	120.34	87.08	33.26	5.91	27.35
	5	172.46	109.65	90.86	18.79	7.95	10.84
	6	204.90	105.22	79.60	25.63	8.33	17.29
SBR	7	201.11	101.28	59.13	42.15	4.55	37.60
	8	170.27	145.14	84.77	60.36	12.49	47.87
	9	244.92	66.23	58.31	7.92	2.89	5.04
	10	123.55	105.09	89.16	15.93	7.02	8.91
	11	369.24	66.15	36.44	29.71	2.89	26.82
	12	295.10	165.12	105.65	59.47	21.43	38.04
NSBR	13	196.58	165.05	87.67	77.38	10.01	67.37
	14	179.87	117.84	97.03	20.81	8.71	12.11
	15	206.78	141.47	106.84	34.63	12.67	22.36
	16	192.14	106.96	89.59	17.36	10.19	7.17
	17	231.64	96.40	69.79	26.62	6.85	19.76
	18	217.23	91.24	66.44	24.80	8.09	16.71

6D Individual data of all average values of urea kinetics relative to intake on low protein diets

Group	Subject	T + I (mgN/kg/day)	P/I (%)	E_U/I (%)	T/I (%)	P_R/I (%)	S/I (%)
Reference	1	116.75	114.36	84.24	30.11	9.59	20.52
	2	147.78	143.17	99.33	43.84	10.97	32.87
	3	130.71	134.36	83.14	51.22	10.02	41.20
	4	165.24	183.71	121.23	62.48	8.37	54.11
	5	155.09	201.76	115.27	86.49	10.54	75.94
	6	129.71	89.37	59.89	29.48	6.91	22.56
SBR	7	113.36	76.55	45.63	30.92	2.54	28.38
	8	110.19	151.58	84.76	66.82	14.08	52.74
	9	162.51	108.75	74.14	34.61	8.27	26.35
	10	116.86	108.90	57.06	51.83	8.55	43.29
	11	246.82	81.41	42.37	39.04	4.95	34.09
	12	192.11	136.74	81.86	54.88	6.09	48.79
NSBR	13	128.77	164.60	82.57	82.03	14.54	67.49
	14	134.61	113.62	87.25	26.38	7.68	18.69
	15	164.66	128.58	91.51	37.07	8.41	28.66
	16	127.12	122.15	95.54	26.60	9.83	16.77
	17	134.50	96.58	71.06	25.51	6.00	19.52
	18	137.90	102.01	70.08	31.93	7.40	24.53

6E Individual data on all average values of urea kinetics relative to urea production on habitual diets

Group	Subject	E_U/P (%)	T/P (%)	P_R/P (%)	S/P (%)
Reference	1	70.40	29.60	10.17	19.43
	2	65.09	34.91	6.61	28.31
	3	63.38	36.62	7.76	28.86
	4	72.37	27.63	4.91	22.73
	5	82.87	17.13	7.25	9.89
	6	75.65	24.35	7.92	16.43
SBR	7	58.38	41.62	4.92	37.13
	8	58.41	41.59	8.61	32.99
	9	88.04	11.96	4.36	7.61
	10	84.84	15.16	6.68	8.48
	11	55.08	44.92	4.37	40.54
	12	63.98	36.02	12.98	23.04
NSBR	13	53.12	46.88	6.06	40.82
	14	82.34	17.66	7.39	10.27
	15	75.52	24.48	8.67	15.81
	16	83.76	16.24	9.53	6.71
	17	72.39	27.61	7.11	20.50
	18	72.82	27.18	8.86	18.32

6F Individual data of all average values of urea kinetics relative to urea production on low protein diets

Group	Subject	E_U/P (%)	T/P (%)	P_R/P (%)	S/P (%)
Reference	1	73.67	26.33	8.38	17.95
	2	69.38	30.62	7.66	22.96
	3	61.88	38.12	7.46	30.66
	4	65.99	34.01	4.55	29.46
	5	57.13	42.87	5.23	37.64
	6	67.02	32.98	7.74	25.45
SBR	7	59.61	40.39	3.31	37.08
	8	55.92	44.08	9.29	34.79
	9	68.17	31.83	7.60	24.23
	10	52.40	47.60	7.85	39.75
	11	52.04	47.96	6.08	41.88
	12	59.86	40.14	4.45	35.68
NSBR	13	50.17	49.83	8.83	41.00
	14	76.79	23.21	6.76	16.45
	15	71.17	28.83	6.54	22.29
	16	78.22	21.78	8.05	13.73
	17	73.58	26.42	6.21	20.21
	18	68.70	31.30	7.25	24.05

APPENDIX 7 Sample low protein diets for subject 2

Day 1

Breakfast	Jordan's country crisp – strawberry	100 g
	Semi skimmed milk	124 g (120 ml)
Lunch	Fresh white bread thick slice – 2 slices	88 g
	Margarine on each slice of bread	7 g x 2 = 14 g
	M&S ham – 6 slices	60 g
Dinner	M&S mini Cornish pastie	2
	M&S chunky chips	320 g
	Heinze tomatoe ketchup	40 g

Other food items – can be consumed any time of the day:

• Tea with 2 tablespoon of semi skimmed milk	3 mugs
• Apple medium size	2
• Banana medium size	1
• McVites digestive biscuits	3
• Jaffa cakes	3
• Yoghurt	100 g
• Coca-cola	325 g

Any amount of plain water or diet drinks can be consumed

All above items must be eaten within the day

Do not eat any item that is not on the list

Total energy = 2611 kcal

Total protein = 58.7 g (nitrogen = 9.4 g)

Day 2

Breakfast	Jordan's country crisp – strawberry	100 g
	Semi skimmed milk	124 g (120 ml)
Lunch	Fresh white bread thick slice – 2 slices	88 g
	Margarine on each slice of bread	7 g x 2 = 14 g
	M&S ham – 6 slices	60 g
Dinner	M&S cod cakes	2
	M&S bubble & squeak rosti cake (100 g)	1
	Heinze tomatoe ketchup	40 g

Other food items – can be consumed any time of the day:

• Tea with 2 tablespoon of semi skimmed milk	3 mugs
• Apple medium size	2
• Banana medium size	1
• Walker's crisp 25 g	1 packet
• McVites digestive biscuits	2
• Jaffa cakes	5
• Kit Kat 4-finger bar	1 packet
• Coca-cola	680 g

Any amount of plain water or diet drinks can be consumed

All above items must be eaten within the day

Do not eat any item that is not on the list

Total energy = 2611 kcal

Total protein = 59.0 g (nitrogen = 9.4 g)

Day 3

Breakfast	Jordan's country crisp – strawberry	100 g
	Semi skimmed milk	124 g (120 ml)
Lunch	Fresh white bread thick slice – 2 slices	88 g
	Margarine on each slice of bread	7 g x 2 = 14 g
	M&S ham – 6 slices	60 g
Dinner	M&S mini Cornish pastie	2
	M&S chunky chips	320 g
	Heinze tomatoe ketchup	40 g

Other food items – can be consumed any time of the day:

• Tea with 2 tablespoon of semi skimmed milk	3 mugs
• Apple medium size	2
• Banana medium size	1
• McVites digestive biscuits	3
• Jaffa cakes	3
• Yoghurt	100 g
• Coca-cola	325 ml

Any amount of plain water or diet drinks can be consumed

All above items must be eaten within the day

Do not eat any item that is not on the list

Total energy = 2611 kcal

Total protein = 58.7 g (nitrogen = 9.4 g)

Day 4

Breakfast	Jordan's country crisp – strawberry	100 g
	Semi skimmed milk	124 g (120 ml)
Lunch	Fresh white bread thick slice – 2 slices	88 g
	Margarine on each slice of bread	7 g x 2 = 14 g
	M&S ham – 6 slices	60 g
Dinner	M&S cod cakes	2
	M&S bubble & squeak rosti cake (100 g)	1
	Heinze tomatoe ketchup	40 g

Other food items – can be consumed any time of the day:

• Tea with 2 tablespoon of semi skimmed milk	3 mugs
• Apple medium size	2
• Banana medium size	1
• Walker's crisp 25 g	1 packet
• McVites digestive biscuits	2
• Jaffa cakes	5
• Kit Kat 4-finger bar	1 packet
• Coca-cola	680 g

Any amount of plain water or diet drinks can be consumed

All above items must be eaten within the day

Do not eat any item that is not on the list

Total energy = 2611 kcal

Total protein = 59.0 g (nitrogen = 9.4 g)

APPENDIX 8 **Sample diets of Day 5 (urea kinetic study) for subject 2**

Habitual diet **Total energy = 2722 kcal, Total protein = 97.5 g**

0600	Weetabix	2	547 kcal, 19.5 g protein
	Semi skimmed milk	200ml	
	McVites digestive biscuits	3	
	Mullerlite yoghurt	130 g	
	Lucozade	50 ml	
0900	^{13}C-palmitin emulsion		538 kcal, 19.5 g protein
	M&S egg & cress sandwich	half pack	
	Kit Kat 2-finger bar	1	
	Banana large size	1	
	Tea with 60 ml semi skimmed milk	1 mug	
	Enlive	95ml	
1200	M&S salmon & cucumber sandwich	1 pack	549 kcal, 19.4 g protein
	Jaffa cake	1	
	Apple medium size	1	
	Lucozade	170 ml	
1500	M&S egg & cress sandwich	half pack	540 kcal, 19.5 g protein
	Tea with 60 ml semi skimmed milk	1 mug	
	Kit Kat 2-finger bar	1	
	Banana large size	1	
	Enlive	110 ml	
1800	M&S prawn cakes	2	548 kcal, 19.6 g protein
	Mullerlite yoghurt	160 g	
	M&S potatoe croquette	2	
	Ketchup	40 g	
	Lucozade	30 ml	

Low protein diet **Total energy = 2608 kcal, Total protein = 58.7 g**

0600	Weetabix	2	522 kcal 11.7 g protein
	Semi skimmed milk	150ml	
	Tea with 20 ml semi skimmed milk	1 mug	
	Custard cream biscuits	3	
	Lucozade	177 ml	
0900	^{13}C-palmitin emulsion		522 kcal 11.6 g protein
	Fresh white bread medium slice	1	
	Margarine on bread	10 g	
	M&S honey roast lean ham	2 slices	
	Tea with 30 ml semi skimmed milk	1 mug	
	McVites chocolate digestive biscuits	3	
	Lucozade	88 ml	
1200	M&S mini cornish pastie	1	522 kcal 11.8 g protein
	M&S potatoe croquette	3	
	Ketchup	30 g	
	Mullerlite Yoghurt	80 g	
	Coca-cola	38 ml	
1500	Fresh white bread medium slice	1	520 kcal 11.8 g protein
	Margarine on bread	10 g	
	M&S honey roast lean ham	2 slices	
	Tea with 30 ml semi skimmed milk	1 mug	
	Banana large size	1	
	Jaffa cakes	2	
	Walkers's crisp 25 g	1 bag	
1800	M&S mini cornish pastie	1	522 kcal 11.8 g protein
	M&S potatoe croquette	3	
	Ketchup	30 g	
	Mullerlite yoghurt	80	
	Lucozade	30 ml	

APPENDIX 9 Calculations of urea kinetics

Urea Pool

In an isotopic steady state the enrichment of the urea pool is the same as the enrichment of any direct product of that pool, by definition:

$$\frac{(d_{30} + d_{29} + r_{29})}{P} = \frac{(t_{30} + t_{29})}{T} = \frac{(e_{30} + e_{29})}{E} \quad (1)$$

and $$\frac{d_{30}}{P} = \frac{t_{30}}{T} = \frac{e_{30}}{E} \quad (2)$$

Therefore from (2), $$P = \frac{E}{e_{30}} \cdot d_{30} \quad (3)$$

and from (1), $$r_{29} = (d_{30} \cdot \frac{e_{29}}{e_{30}}) - d_{29} \quad (4)$$

Nitrogen pool

In an isotopic steady state:

$$\frac{(t_{29} + t_{30})}{B + I + T} = \frac{r_{29}}{P} = \frac{s_{29}}{S} = \frac{x_{29}}{X} \quad (5)$$

and $$\frac{(t_{29} + t_{30})}{T} = \frac{r'}{P_R} = \frac{x'}{X_R} = \frac{s'}{S_R} \quad (6)$$

where P_R, X_R and S_R are those portions of metabolized urea, T, going to pathways P, X and S; and r', x' and s' are those molecules of $(t_{29} + t_{30})$ which will eventually give rise to r_{29}, s_{29} and x_{29}.

To derive P_R: $r' = r'_{29} + r'_{30}$ (7)

$$\frac{r'_{29}}{r'_{30}} = \frac{t_{29}}{t_{30}} = \frac{e_{29}}{e_{30}} \quad (8)$$

But each molecule of r'_{30} gives rise to two molecules of r_{29}.

Therefore, $r_{29} = r'_{29} + 2r'_{30}$ (9)

Hence $$r' = r_{29} \frac{(r'_{29} + r'_{30})}{(r'_{29} + 2r'_{30})} = r_{29} \frac{(e_{29} + e_{30})}{(e_{29} + 2e_{30})} \quad (10)$$

But from Equation (6), $P_R = r'. \frac{T}{(t_{29} + t_{30})}$

Substituting from Equation (1), $P_R = r'. \frac{E}{(e_{29} + e_{30})}$ (11)

By a similar process, $X_R = x'. \frac{E}{(e_{29} + e_{30})}$ (12)

where $x' = x_{29} . \frac{(e_{29} + e_{30})}{(e_{29} + 2e_{30})}$

and $S_R = T - (P_R + X_R)$ (13)

Flux $(Q_N) = \frac{\text{inflow of isotope}}{\text{enrichment in the pool}} = (t_{29} + t_{30})/\frac{(r_{29})}{P}$

From Equation (6), $(t_{29} + t_{30}) = r'. \frac{T}{P_R}$

Therefore, $Q_N = r'. \frac{T}{P_R} . \frac{P}{r_{29}}$ (14)

Substitution of values obtained by mass spectrometry

As explained in methods section 5.5.10, the mass spectrometer gives the relative intensities at m/e 28, 29 and 30, so that:

$$I_T = I_{28} + I_{29} + I_{30} \quad (15)$$

δI_{29} and δI_{30} are the increases in intensity at m/e 29 and m/e 30 which result from enrichment above natural abundance. The relative intensities are a measure of the relative amounts of the different isotopic species of nitrogen in the sample. Therefore in the formulae given above, I_T can be substituted for E, δI_{29} for e_{29} and δI_{30} for e_{30}.

Enrichment

The enrichment of specimens for the total nitrogen:

$$\text{atom per cent } {}^{15}N = \frac{100}{2(I_{28}/I_{29}) + 1}$$

and for urea: atom per cent $^{15}N = \frac{2.I_{30} + I_{29}}{2(I_{30} + I_{29} + I_{28})} \times 100$

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