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School of Medicine

Plasma Fatty Acid Composition of Patients with Colectomy and the influences on their Metabolism

by

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For Nan & Gramps

ABSTRACT

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PLASMA FATTY ACID COMPOSITION OF PATIENTS WITH COLECTOMY AND THE INFLUENCES ON THEIR METABOLISM

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Inflammatory Bowel Diseases affect tens of thousands of people in the UK. In severe cases, total removal of the colon (colectomy) and sometimes parts of the small intestine can become necessary. Consequences of such surgery often result in nutritional deficiencies, diarrhoea and interruption of the enterohepatic circulation. After an initial adaptation period, many colectomy patients do not appear to suffer significant ill health. However, the effects on lipid metabolism in such patients are not well described or understood.

The fatty acid compositions of plasma phosphatidylcholine (PC), triacylglycerol (TAG), non-esterified fatty acids (NEFA) and cholesteryl ester (CE) were determined in a healthy reference group and patients with established colectomy (CO), including a small subset of patients with additional small bowel resection (SB). Acute phase proteins were measured in the colectomy patients and these were not suggestive of any significant ongoing inflammation. Dietary intakes were also assessed in the reference and patient groups and were not indicative of any significant differences.

Colectomy patients exhibited significant alterations to their plasma fatty acid compositions. Data were presented as absolute concentration and relative (%) proportions. This highlighted the need to provide data in dual format as differences in concentrations often did not concur when expressed in relative terms. Significantly higher concentrations of PC and TAG fatty acids were seen and were more apparent in the SB subset. Several differences were also noted in CE and NEFA fatty acids in the colectomy group which were also more marked in SB patients. Concentrations and proportions of α-linolenic (ALNA) and docosahexaenoic acids (DHA) were consistently lower in all fractions and again to a greater magnitude in the SB subset.

The results suggested a potential involvement of lipid maldigestion and/or malabsorption processes in the pathogenesis of disturbed lipid profiles, particularly to *n*-3 PUFA. It is suggested that hepatic lipid export may be stimulated, potentially by low *n*-3 PUFA availability, resulting in raised plasma lipid concentrations and potential health risks associated with this. However, more research is needed to confirm these findings.

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ABBREVIATIONS

ALNA	α-linolenic acid
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARA	Arachidonic acid
BHT	Butyrated hydroxytoluene
BMI	Body mass index
BMR	Basal metabolic rate
CD	Crohn's disease
CDAI	Crohn's disease activity index
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein
CMC	Critical micellular concentration
CNMU	Clinical Nutrition and Metabolism Unit
CO	Colectomy only (no small bowel resection)
COX	Cyclo-oxygenase
CRP	C-reactive protein
CV	Coefficient of variation
DAG	Diacylglycerol
DGLA	Dihomo-γ-linoleic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DRV	Dietary reference value
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
ESR	Erythrocyte sedimentation rate
ETA	Eicostriaenoic acid
FABP	Fatty acid binding protein
FAME	Fatty acid methyl ester
FFQ	Food frequency questionnaire
FID	Flame ionisation detection

GC	Gas chromatography
GI	Gastrointestinal
GLA	γ-linoleic acid
HDL	High density lipoprotein
HSFE	Health Survey for England
HSL	Hormone sensitive lipase
IBD	Inflammatory bowel disease
IFN	interferon
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
LA	Linoleic acid
LCFA	Long-chain fatty acid
LC-PUFA	Long-chain polyunsaturated fatty acid
LDL	Low density lipoprotein
LOX	Lipoxygenase
LP	Lipoprotein
LPL	Lipoprotein lipase
LT	Leukotriene
MAG	Monoacylglycerol
MCFA	Medium-chain fatty acid
MUFA	Monounsaturated fatty acid
NEFA	Non-esterified fatty acid
OA	Oleic acid
PA	Palmitic acid
PBMC	Peripheral blood mononuclear cells
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Prostaglandin
PGE_2	Prostaglandin E_2
PI	Phosphatidylinositol
PL	Phospholipid
PLA_2	Phospholipase A_2
PS	Phosphatidyserine

PUFA	Polyunsaturated fatty acid
REE	Resting energy expenditure
SA	Stearic acid
SB	Colectomy patients with small bowel resection
SCFA	Short-chain fatty acid
SFA	Saturated fatty acid
SPE	Solid phase extraction
SPM	Spingomyelin
SUHT	Southampton University Hospitals Trust
TAG	Triacylglycerol
Tc	T cytotoxic cells
TEE	Total energy expenditure
TFE	Trifluroethanol
Th	T helper cells
TLC	Thin layer chromatography
TNF	Tumour-necrosis factor
TX	Thromboxane
UC	Ulcerative colitis
VLDL	Very low density lipoprotein

Chapter 1

Introduction

1.1 BACKGROUND TO RESEARCH

Inflammatory Bowel Diseases (IBD) affect tens of thousands of people in the UK, either as Crohn's Disease (CD) or Ulcerative Colitis (UC). In extreme cases, disease severity may be uncontrollable by conventional methods, such as steroid and immunosuppressive therapies. In some cases, the affected areas of the intestine may become so inflamed and damaged that surgical removal is required. In some instances the entire length of the large intestine (colon) may be removed, known as a total colectomy. Sometimes this may also be accompanied by removal of parts of the small intestine (ileum) if the disease also affects this area.

It is known that colectomy patients often suffer consequences of such radical surgery, and these can more obviously present as diarrhoea, vitamin and mineral deficiencies and disturbances to bile salt metabolism. Colon removal is not generally considered to play a significant role in fatty acid metabolism. However, it is known that the bacteria which normally colonise the large bowel do eventually relocate to the terminal ileum following colectomy (if indeed the terminal ileum was not also resected) and this has an impact on bile acid metabolism. As bile acids are important for the digestion and absorption of dietary lipids in the intestine, there is good reason to suggest that colectomy surgery may impact lipid digestion and absorption and therefore, fatty acid supply to the body may become compromised. It is not known whether some fatty acids may be affected by this to a greater extent than others.

Normal lipid metabolism in the body relies on the dietary supply of fatty acids (the component parts of dietary fat) via the intestine. The body is able to synthesise most fatty acids itself. However, there are two which cannot be synthesised so are dietarily essential. These essential fatty acids (EFA) are linoleic acid (LA, *n*-6 family) and α-linolenic acid (ALNA, *n*-3 family). These EFA are precursors in the synthesis of longer-chain polyunsaturated fatty acids (LC- PUFA) such as arachidonic acid (ARA) from LA and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from ALNA. As fatty acid supply may be limited in colectomy patients, it is also sensible to postulate that these patients may also have limited EFA availability. This in turn could also adversely affect the metabolism of other fatty acids in the body.

The inflammatory pathways prevalent in inflammatory disease are mediated by compounds called eicosanoids. These signalling molecules mediate many inflammatory responses and are synthesised from fatty acids contained in cell membranes. The nature of the fatty acid precursor can determine the magnitude and potency of the eicosanoids synthesised from them. Those synthesised from the *n*-3 precursor EPA are generally less potent and have more of an anti-inflammatory effect than those derived from the *n*-6 precursor ARA or dihomo-γ-linoleic acid (DGLA). Thus the fatty acid composition of the cell membrane has a role in the inflammatory response and it is widely believed that the balance of *n*-3 and *n*-6 fatty acids plays an important role in inflammatory conditions such as IBD.

Thus, there are two broad topics to consider; the supply of fatty acids to the body and the fatty acid demands of the body and how well these two processes are balanced. Although it is not generally considered problematic, there is reason to believe that colectomy patients may have disturbances to their lipid absorption and therefore, their whole body metabolism of this macronutrient. If the supply to the body is altered, it is sensible to hypothesise that the specific requirements (or demands) of the body may also be affected. However, there may be other factors to consider, such as the presence of active inflammation, which can also influence the body's demands for fatty acids.

There are few studies of fatty acids and IBD and of those published; many disparities exist between them in the patient populations, methods of analysis and reporting of results. The results are generally controversial but direct comparison between studies is often difficult. A further limitation of other available data is that many measure and/or present only the fatty acid composition of whole plasma (or serum). This limits the usefulness of such data and restricts our understanding of the topic as the fatty acids in whole plasma are derived from several sources, each with its own characteristic composition and function. Simply measuring plasma fatty acids potentially masks the variation in composition of the different lipid fractions and may lead to false conclusions.

The significant lack of research into fatty acids and IBD limits understanding in this area. There is even less understanding of the effect of colectomy on fatty acid metabolism and whether such patients may be at risk from altered profiles. It is also not known if resection of the small bowel may have a further impact on fatty acid metabolism.

With respect to the topics outlined above, the work presented in this thesis was performed to try to address some of these issues, in particular:

- To consider the fatty acid composition of individual plasma lipid fractions, rather than the composition of whole plasma alone.
- To characterise the fatty acid supply in patients with colectomy and a reference group of healthy adults.
- To consider the impact of an altered supply on the fatty acid requirements of the patient group.
- To consider possible influences on fatty acid metabolism and how each may affect the balance between fatty acid supply and demand.

1.2 STRUCTURE OF THESIS

This thesis is arranged into chapters, of which this introduction into the field of research is the first (Chapter 1). Chapter 2 reviews the current literature surrounding this topic; describing lipid metabolism, inflammation and inflammatory disease and how these areas overlap to form the basis of the research outlined in this thesis. Additionally, the limits of current knowledge and understanding are highlighted. The methods employed for the collection of the study data, analysis of biological samples, method validation work and data processing, are described in Chapter 3 along with details of statistical methods used for data analysis. This is followed by the results sections (Chapters 4 to 7) and a general discussion in Chapter 8. Finally, the appendices and references are provided in Chapters 9 and 10, respectively.

Chapter 2

Review of Literature

2.1 INTRODUCTION

This chapter aims to provide an introduction into the field of research around which the topic of this thesis is based. It begins by introducing lipids in general, including endogenous fatty acid synthesis. The processes of lipid digestion and absorption in the human gastrointestinal (GI) tract are then reviewed, including how fatty acids are transported around the body in the circulation in the form of lipoprotein (LP) molecules. Dietary intake of fatty acids is then discussed including methods for assessment of the diet. Next, ways for characterising fatty acid status are introduced, along with this thesis' concept of fatty acid pools and an appraisal of the principal methods for expressing fatty acid composition data. The review then discusses the topic of IBD, the definition of colectomy and the clinical consequences of this. An introduction to inflammatory processes is then presented, along with the significance of fatty acids in these pathways. Following this, other studies of fatty acid and inflammatory diseases are reviewed. Finally, the review is summarised and the purpose of the research topic given, including the study hypotheses.

2.2 TRIACYLGLYCEROLS & FATTY ACIDS

In the UK, a typical adult male consumes around 85 g of fat per day and a typical woman; 60 g, contributing roughly 35% of dietary energy (Henderson et al., 2003). Fats in the diet include triacylglycerols (TAG), phospholipids (PL), sterols and fat-soluble vitamins (such as vitamin A). The most abundant component of dietary fat (>95%) is TAG. The TAG molecule consists of 3 fatty acid molecules attached to a 3-carbon glycerol backbone. Dietary fats are mixed TAG, meaning that the three positions on the glycerol molecule are occupied by different fatty acids. The most abundant fatty acids in the diet have straight chains with an even number of carbon atoms (Calder, 1996).

Fatty acids are the most abundant constituents of dietary TAG, but rarely exist on their own in natural sources. Fatty acids are a chain of carbon atoms, varying in length from 4 (e.g. in milk) to 30 (e.g. in fish oils), with methyl and carboxyl termini. The carbon chain may contain one or more double bonds and as such, 3 types of fatty acid exist: saturated fatty acids (no double bonds,

SFAs) monounsaturated fatty acids (one double bond, MUFAs) or polyunsaturated fatty acids (two or more double bonds, PUFAs) (Figure 2.1). As fatty acids are aliphatic compounds, their properties depend largely on their chain lengths. Short-chain fatty acids (SCFAs, $n<6$) are polar molecules and soluble in water, medium-chain fatty acids (MCFAs, $n>6$ but <12) are also soluble in water. Fatty acids with a carbon chain of more than 12 atoms are referred to as long-chain fatty acids (LCFAs) and have limited water solubility.

2.2.1 NOMENCLATURE

A method of naming fatty acids according to their chemical structure exists universally. For example, ALNA has the systematic name 18:3*n*-3. The first digit indicates the number of carbon atoms in the molecule (18) and the second denotes the number of double bonds in the molecule (3). The position of the first double bond (from the methyl terminus) is indicated by *n*-3, *n*-6, *n*-7 or *n*-9; *n*-3 and *n*-6 sometimes referred to as ω-3 and ω-6 or omega-3 and omega-6 (Figure 2.1).

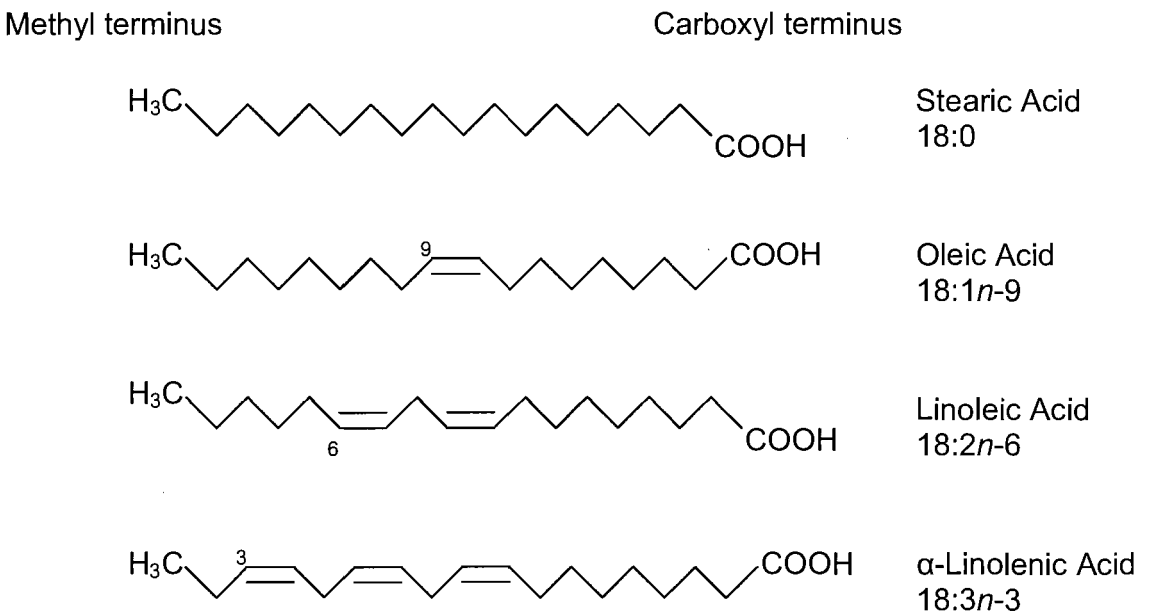


Figure 2.1 The structure of saturated, monounsaturated and polyunsaturated fatty acids (Calder and Field, 2002).

2.2.2 FATTY ACID SYNTHESIS

Mammals are able to synthesise SFAs *de novo* from non-fat precursors (e.g. glucose) via glycolysis and fatty acid synthase pathways, the end product of

which is palmitic acid (16:0, PA), which can be elongated to stearic acid (18:0, SA). The normal diet of a Western adult contains plenty of SFAs, so their synthesis by the body is not usually necessary. However, In order to maintain cell membrane structure, fluidity and function, unsaturated fatty acids are required, thus a mechanism exists for the introduction of a single double bond between carbon atoms 9 and 10. The reaction is catalysed by the enzyme Δ^9-desaturase, present in both plant and animal tissues, which converts SA to oleic acid (18:1*n*-9, OA). Additional double bonds can then be inserted into OA between the existing double bond and the methyl terminus of the carbon chain. A Δ^{12}-desaturase enzyme converts OA into LA (18:2*n*-6) and a Δ^{15}-desaturase enzyme converts LA into ALNA. However, only plant tissues contain the enzymes necessary for these additional conversions so these particular fatty acids must be consumed in the animal diet, and are therefore termed EFAs. Normally there is sufficient LA in the diet for the *n*-6 pathway to be predominant. Where there is little ALNA or LA in the diet the *n*-9 pathway from OA is the major pathway. The end product 20:3*n*-9 (eicosatrienoic acid, ETA) accumulates. The ratio of ETA to ARA (20:3*n*-9/20:4*n*-6) is used as a biochemical index of EFA deficiency; values above 0.4 being taken arbitrarily as indicative of deficiency (Gurr, 1988).

Following the pathway outlined in Figure 2.2, animals are able to convert dietary ALNA into EPA (20:5*n*-3), and similarly, dietary LA can be converted to ARA (20:4*n*-6), thus there is competition between the *n*-3 and *n*-6 fatty acid families for Δ^6 enzyme activity. Δ^6 desaturase has a preference for ALNA over LA, but is generally at a competitive disadvantage due to high intakes of LA in the diet (Gerster, 1998). EPA can be further elongated to docosapentaenoic acid (22:5*n*-3, DPA) and DHA (22:6*n*-3) by the Δ^5 and Δ^6 and desaturase enzymes, respectively, but there is controversy over the capability of humans to synthesise this fatty acid from ALNA (Burdge *et al.*, 2001) (Emken *et al.*, 2002) (Pawlosky *et al.*, 2001).

PUFA have important effects on the structure and properties of membranes and are also involved in the production of inflammatory mediators. As mentioned previously, LA and ALNA cannot be synthesised by the body so are therefore

dietarily essential. The average Western diet contains 10 to 20 times more *n*-6 than *n*-3 PUFA in the diet, and most tissues and the plasma reflect this as they also contain 10 to 20 times more *n*-6 PUFA (Spector, 1999). The only exceptions to this are the brain and retina which contain a high proportion of *n*-3 PUFA. For example, the aminophospholipids of neuronal membranes typically have a high concentration of PUFA, particularly DHA. Adult human cerebral cortex phosphatidylethanolamine (PE) contains 30.5% DHA and retina contains 22.2% DHA, suggesting an important role of this fatty acid for the proper visual and nervous system function (Burdge, 1998).

It can be stated that the body does not rely solely on dietary intake to provide it with the fatty acids it requires; it has a certain capacity to synthesise them from both from non-fat precursors and from fatty acids that are generally sufficient in the diet. The abundant storage depots of fat in the body must also be considered as they can play an important part in fatty acid supply. Therefore, an intrinsic "buffer" mechanism exists, acting to maintain a suitable supply of fatty acids. Thus, the body is not solely dependent on what is normally a highly variable dietary intake both in terms of quantity and composition.

This buffering mechanism may be important in certain diseases, such as IBD, when demands for fatty acids may be increased due to a high inflammatory burden. As described in later sections, fatty acids are direct precursors for several signalling molecules of the immune system, whose production increases substantially during inflammatory and infective episodes. The ability of an individual to up- or down-regulate certain parts of the synthetic pathways may be an important determinant of the body's management of inflammatory mechanisms, and may have a role in the magnitude or duration of the inflammatory event.

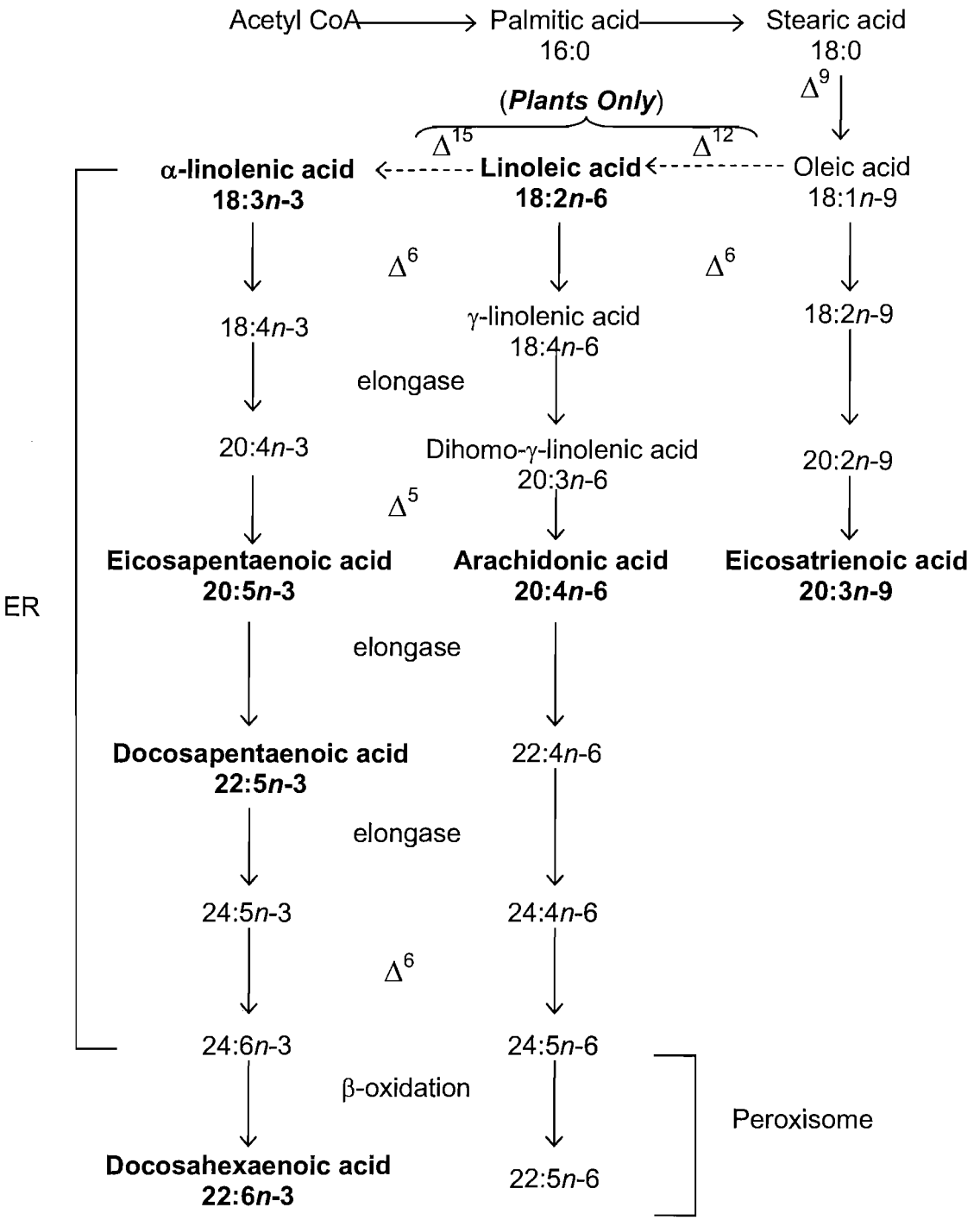


Figure 2.2 Metabolism of fatty acids from acetyl co-A. Δ^5, Δ^6, Δ^9, Δ^{12} & Δ^{15} represent the desaturase enzymes. ER; endoplasmic reticulum (Adapted from Calder, 1996).

2.3 PHOSPHOLIPIDS

PLs are a class of lipid and a major component of cell membranes. They consist of two fatty acid chains ("tails") and a phosphorylated alcohol head group, attached to a backbone of either glycerol (Figure 2.3) (when they are termed phosphoglycerides or glycerophospholipids) or sphingosine. PLs with only one fatty acid attached to the backbone are known as lysophospholipids. The amphiphilic nature of PLs allows them to form a bilayer structure in an aqueous environment, and this is the basis of the formation of cell membranes. They achieve this by orientating the polar groups of each layer outwards into the water, protecting the non-polar (hydrophobic) groups from the highly polar aqueous environment (Figure 2.4).

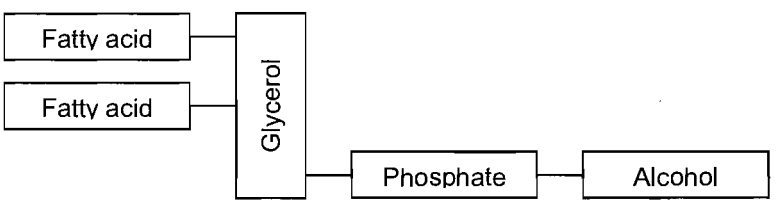


Figure 2.3 The basic structure of a phosphoglyceride (Stryer, 1995)

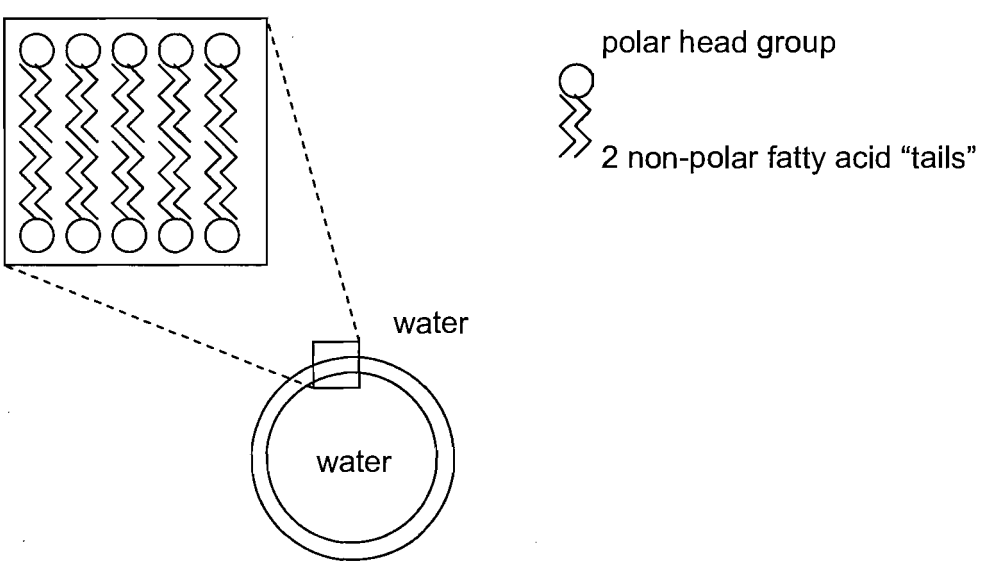


Figure 2.4 Diagram showing the arrangement of the polar and non-polar entities of a lipid in a bilayer.

The simplest phosphoglyceride is phosphatidic acid. Although there are only small amounts of this compound found in membranes, it is an important intermediate in the synthesis of other phosphoglycerides. The major phosphoglycerides are derived from phosphatidic acid, when its phosphate head group becomes esterified to the hydroxyl group of an alcohol such as serine, ethanolamine, choline, glycerol or inositol (Figure 2.5). The resultant phosphoglycerides are phosphatidylserine (PS), PE, phosphatidylcholine (PC), phosphatidylglycerol and phosphatidylinositol (PI), respectively. The structure of sphingomyelin (SPM) differs slightly to that of the other PLs, in that the backbone is sphingosine; an amino alcohol. Sphingosine contains a long hydrocarbon chain and is linked to a fatty acid by an amide bond.

Each of the PLs has a characteristic fatty acid composition. In the phosphoglycerides, such as PC or PE, a SFA is normally found in position 1 on the glycerol molecule, and an unsaturated fatty acid at position 2. SPM is characterised by SFAs with a chain length >20 carbons (Cooper, 1970). Usually PS and PE are more unsaturated than other PLs in eukaryotic membranes (Cullis et al., 1996).

Consumption of PLs in the diet amounts to approximately 4 to 8 g per day. However, there is also a significant amount of endogenous formation of PL in the liver, approximating 7 to 22 g per day, which is secreted into the intestinal lumen via the bile (Carey et al., 1983).

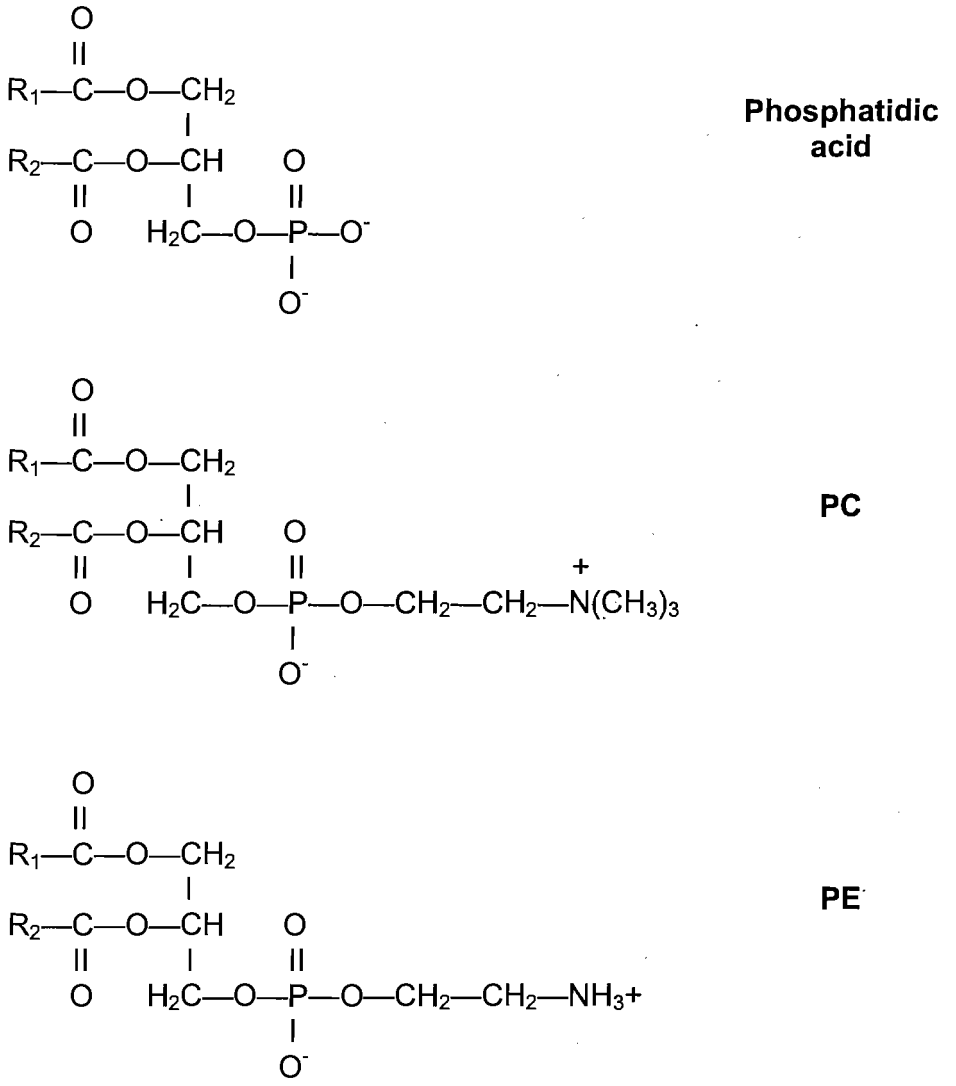


Figure 2.5 Formulas of some of the phosphoglycerides. PC and PE are both derived from phosphatidic acid. R and R^1 represent fatty acid groups (Stryer, 1995).

2.4 LIPID DIGESTION AND ABSORPTION

The processes of dietary lipid digestion and absorption must be appreciated to understand how these compounds are made available to the body and metabolised further to other classes of lipid, such as PLs and fatty acids. As lipids are insoluble in water, they cannot simply dissolve in the lumen of the gut for absorption. The lipids must be made accessible to the enzymes that break them down, and this is achieved by emulsification.

2.4.1 DIGESTION

TAG, the major component of dietary lipid, is insoluble in aqueous media; therefore it must be converted into other forms of lipid with increased abilities to interact with water. Lipase enzymes catalyse the hydrolysis of lipids and include lingual, gastric and pancreatic lipases. The intermediary and end products of TAG digestion are diacylglycerols (DAGs), monoacylglycerols (MAGs), fatty acids and glycerol.

The Mouth

Lipid digestion begins in the mouth, where chewing breaks down large pieces of food into smaller pieces, increasing the surface area enormously and permitting efficient activity of the digestive enzymes.

Although lingual lipase originates in the mouth, the majority of its activity takes place in the stomach. Lingual lipase is thought to account for approximately $^1/_3$ of digested lipid (Carey et al., 1983), the major end products being DAGs and fatty acids. Lingual lipase (sometimes called salivary lipase) is specific only for the 1 and 3 ester bonds of TAGs; PLs and cholesteryl esters (CEs) are resistant to its actions (Patton, 1981). Although the enzyme is much more active on TAGs with SCFAs than those with LCFAs, the enzyme appears to have a preference for acting at the 3 position, which seems to be independent of the fatty acid present (Staggers et al., 1981).

The Stomach

Once food is swallowed and enters the stomach, it continues to be ground down, yet further increasing surface area and mixing food particles with enzymes and other secretions derived both from the mouth and the stomach. The muscle contractions of the stomach also produce the shear forces required to coarsely emulsify lipids. Potential emulsifiers in the acidic environment of the stomach are digests of dietary proteins, polysaccharides and PLs. The partially digested contents of the stomach are now referred to as chyme. Further digestion of lipids in the stomach by lingual and gastric lipases yields partial glycerides and fatty acids, and these amphiphiles help to disperse the lipid in the stomach and intestine.

A circular muscle, called the pyloric sphincter, regulates emptying of the stomach contents into the duodenum. It opens approximately twice each minute; each time delivering approximately 3 ml of chyme, so coarsely emulsified lipid enters the upper small intestinal lumen as 0.5 μm droplets (Thomson *et al.*, 1988).

The Upper Small Intestine

The small intestine is said to be about 6 m long, although measurements in living humans have found lengths to vary between 3 and 4.5 m (Frayn, 1996a). Two important organs discharge into the small intestine. Firstly, the gall bladder, a store for bile synthesised in the liver, releases its contents via the common bile duct, and secondly, the exocrine pancreas, which discharges its secretions through the pancreatic duct. The common bile duct joins the pancreatic duct, and together they both release their secretions into the duodenum. The released bile is capable of further emulsification of the lipids released from the stomach. The pancreas releases pancreatic lipase (amongst other enzymes and juices), which further digests lipids.

Physical mixing of the emulsion continues in the small intestine as peristaltic movements in the duodenum continue to increase the surface area of the lipid droplets by decreasing their size.

Bile

Bile is formed in the hepatocytes and is made up of several substances, including bile acids, bile pigments, cholesterol, PLs and electrolytes. Bile acids are mostly in the form of taurine or glycine conjugates and are amphipathic molecules capable of forming micelles when they are above a specific concentration, called the critical micellular concentration (CMC) (Thomson *et al.*, 1988). Below the CMC, they exist as monomers. Physiological levels of bile acids are usually well above the CMC (Erlinger, 1987).

After entering the duodenum, some components of bile are irreversibly lost in the faeces e.g. bile pigments, whereas others, mainly bile acids, are reabsorbed

by active transport at the terminal ileum and return to the liver to be excreted once again. There is also some passive diffusion of bile acids in the jejunum, but this is poor. This recycling system is known as enterohepatic circulation. Although the majority of bile acids are reabsorbed, approximately 2 to 5% are lost in the faeces each day. Daily synthesis of bile acids in the human is around 600 mg, which allows the pool of around 3 to 5 g to be maintained efficiently (Erlinger, 1987).

The bile is a source of fatty acids because it contains PC from which fatty acids can be cleaved via the action of phospholipase A_2 (PLA_2). Approximately 90% of the PC entering the intestine is derived from bile (Thomson *et al.*, 1988). Interruption to the enterohepatic circulation (e.g. reduced bile resorption at the terminal ileum) could limit lipid absorption because depletion of PLs results in a decrease in the production of LP membrane components.

Pancreatic Lipase

Pancreatic lipase acts in a similar way to lingual lipase as it hydrolyses the 1 and 3 ester bonds of TAG to release 2-MAG and fatty acids. Further hydrolysis results in the formation of glycerol and fatty acid. Pancreatic lipase in inhibited by bile acid in concentrations above the CMC, but this inhibition can be overcome by the presence of colipase. Colipase, derived from pancreatic juice, is secreted in an inactive form, so requires activation via tryptic hydrolysis before it can attach itself to the ester bond region of the TAG molecule. Pancreatic lipase is then able to bind strongly to the colipase, allowing it to carry out hydrolysis of the TAG, even in the presence of bile acids. Pancreatic lipase and colipase, PLA_2, calcium and bile acids all act synergistically to hydrolyse lipids in the upper part of the small intestine (Carey et al., 1983).

2.4.2 ABSORPTION

Fatty acid distribution within TAG is important in the digestion and absorption of lipids. The lipids of most mammals (except for pigs) have the 2-position of glycerol occupied by unsaturated fatty acids. Similarly, in most plant oils SFAs occupy the 1- and 3-postitions and LA the 2-position of glycerol. There is also some diversity in the fatty acid structure of fish oils. For example in mackerel,

herring and cod oils, EPA and DHA are often found in the 2-position, whereas in fat from seal and polar bear these fatty acids occupy the 1- and 3-positions (Carlier et al., 1991).

Some researchers have suggested that the digestion and absorption of PUFA is not the same as that for SFA and MUFA and that a possible substantial pathway for absorption into the blood exists for these fatty acids (Carlier et al., 1991).

As both MAGs and fatty acids have amphipathic properties and their release by digestion from TAG works to further emulsify the lipids, thus digestion progresses further. Gradually, much smaller groups of molecules, called mixed micelles, form. They are named as such because they contain both bile acids (also capable of forming micelles alone) and other molecules, mainly fatty acids and MAG. In particular, ionised free fatty acids (constituting over 50% of the fatty acids at jejunal pH) and 2-MAGs form mixed micelles with PL. This enables non-polar lipids such as saturated and monounsaturated LCFAs to move easily through the aqueous environment of the intestinal lumen and present to the absorptive cells of the small intestine. This movement of lipid digestion products into the micellular phase appears to be a critical step in the absorption of dietary fatty acids (Carlier et al., 1991).

Lipid absorption from the intestine begins in the distal part of the duodenum and is usually complete beyond 100 cm of the jejunum. Lipid particles are transported to the absorptive cells as mixed micelles, and the lipid molecules are absorbed in the upper part of the small intestine. However, the bile salts which facilitate the emulsification process are not principally resorbed until reaching the terminal ileum, thus dissociation of the lipid molecules and bile salts must occur in the jejunum. A microclimate at a low pH at the brush border membrane appears to be an important factor in this process. A specific carrier-mediated energy-dependent process is then responsible for the movement of fatty acids through the microvillus membrane (Caspary, 1992).

Once they have passed through the microvillus membrane, LCFA are bound by one or more specific fatty acid binding proteins (FABP). High concentrations of FABP are found in areas where lipids are absorbed, i.e. the proximal jejunum has a higher concentration of FABP than the ileum. The protein has a higher affinity for unsaturated LCFA than for saturated equivalents (Shiau, 1987). The FABP act as transport proteins and direct fatty acids towards the endoplasmic reticulum (ER) of the mucosal epithelial cell where TAG are resynthesised. The incorporation of these resynthesised TAG with cholesterol, CE, fatty acids, fat-soluble vitamins and apolipoproteins in the Golgi apparatus results in the formation of chylomicrons (CM) and very-low-density lipoprotein (VLDL). The apolipoproteins form an integral part of the LP molecule and serve to direct it to its destination, hence a lack of LP synthesis can result in lipid accumulation in the cell (Shiau, 1987). From the Golgi apparatus, the CM migrate to and fuse with the plasma membrane and their contents are released by exocytosis. They then leave the enterocyte, move into the lymphatic system and finally appear in the circulation (Caspary, 1992).

TAG constituted of MCFA are absorbed much more easily than those comprising LCFA. MCFA are more soluble in water and more easily hydrolysed than their longer-chain counterparts. Additionally, their hydrolysis products do not need bile salts for emulsification and they are not esterified intracellularly and as such, do not need to be incorporated into CM. In contrast, they enter the plasma directly in the form of non-esterified fatty acids (NEFA, bound to plasma albumin) and are transported by the portal vein directly to the liver after release from the epithelial cells (Frayn, 1996a). This process of lipid digestion and absorption is represented pictorially in Figure 2.6.

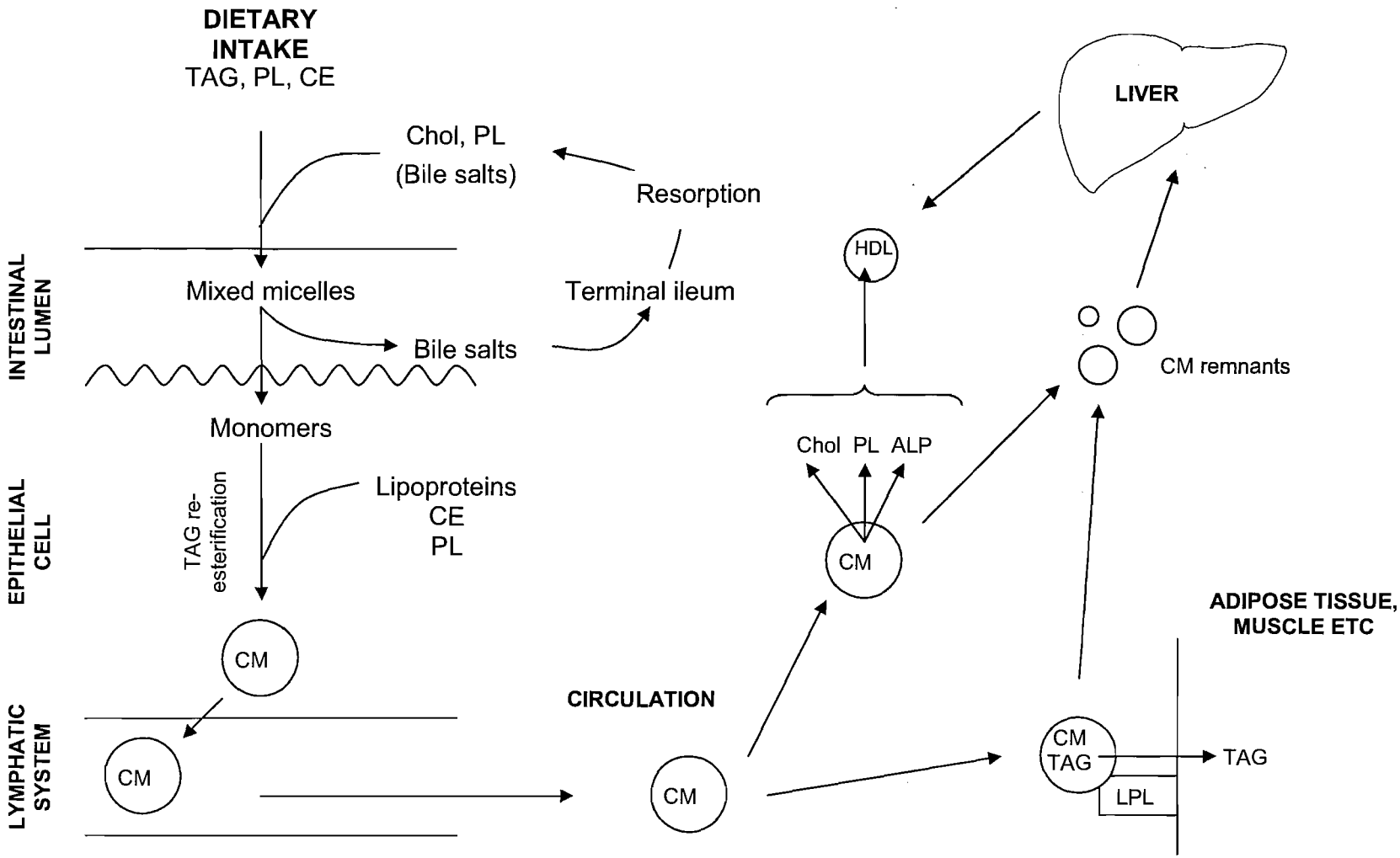


Figure 2.6 Diagrammatic representation of the processes of lipid digestion and absorption in the human intestine. LPL; lipoprotein lipase.

Other Processes in the Small Intestine

The absorption of fatty acids and MAG is completed during the passage of food through the duodenum and the jejunum. Further down the GI tract at the terminal ileum, absorption of further specific compounds takes place, such as that of vitamin B_{12} and bile salts. Bile salts are left behind when the other components of the mixed micelles are absorbed higher up the intestine. As well as maintaining body stores of bile (mentioned previously), resorption of bile is also important to maintain whole-body stores of cholesterol (Frayn, 1996a).

2.4.3 THE COLON

The colon (or large intestine) is approximately 1 ½ metres in length extending from the terminal ileum (the ileo-caecal valve) to the anus. The function of the colon is mainly that of water absorption, but there is also significant bacterial activity which plays a role in the digestion of carbohydrate which has escaped digestion further up the GI tract. The principle products of the fermentation of undigested carbohydrate are SCFA, namely acetate, butyrate and propionate. Acetate is absorbed by the intestinal cells of the colon (colonocytes). Upon entering the bloodstream, acetate can be converted to acetyl-CoA in the liver so can be used as a precursor for lipogenesis or as a substrate for oxidation. Very little butyrate enters the bloodstream from the colonocytes as it is mostly used as a fuel by the colon. It is thought that SCFA play an important role in protecting the colon from cancer (Frayn, 1996a).

2.4.4 LIPID STORAGE & MOBILISATION

The body has a specific demand for energy and this varies between individuals according to gender, race, age, height, weight, body composition and physical activity levels. Energy taken into the body in excess of these demands is taken up into fat cells (adipocytes) in adipose tissue. Adipose tissue plays a protective role in surrounding some of the internal organs, but certainly its main function is that of stored chemical energy in the form of TAG in lipid droplets (Saleh et al., 1999). When required, these TAG stores can be hydrolysed by hormone sensitive lipase (HSL) which releases NEFA into the circulation to be oxidised for energy by the tissues. Stored TAG represents a very large depot of stored

energy in humans and the flow of fatty acids in and out of adipose tissue represents a large proportion of the energy metabolism of the body that is under constant regulation. Adipose tissue stores of TAG are usually accumulated over many years and for an individual on a typical Western diet, represent the deposition of TAG from the plasma (as opposed to deposition of TAG from *de novo* lipogenesis).

Plasma TAG is present in the form of LP particles such as CM and VLDL. These are discussed in more detail in section 2.5. These large particles are too large to escape through the capillaries into the interstitial fluid so cannot be taken up directly by the adipocytes. They overcome this by the production of lipoprotein lipase (LPL), an enzyme which hydrolyses the TAG in LP particles, releasing fatty acids. These fatty acids can then diffuse into the interstitial space and reach the adipocytes, where they combine with glucose derivatives to form TAG. As LPL must act in the capillaries, it is exported from the adipocytes to the cells lining the capillaries of adipose tissue. It is attached to the cells by chains of a complex glycosaminoglycan called heparan sulphate. This molecule is a carbohydrate with highly negatively charged groups. The enzyme molecules attach to these via charge interactions. LPL activity in adipose tissue is stimulated by insulin whose concentration increases in response to raised blood glucose concentration (e.g. after a meal) (Frayn, 1996c).

Stores of TAG in adipose tissue can be mobilised into NEFA (bound to albumin) for energy at times of need. This process is known as lipolysis and is catalysed by HSL. It acts at the surface of the TAG droplet and catalyses the hydrolysis of the ester bonds of two fatty acids. A second enzyme, monoacylglycerol lipase removes the third fatty acid. The fatty acids leave the cell and enter the plasma pool of NEFA. In contrast to LPL, HSL is inhibited by high insulin concentrations (Frayn, 1996c). This balanced process of fatty acid storage and mobilisation is represented pictorially in Figure 2.7.

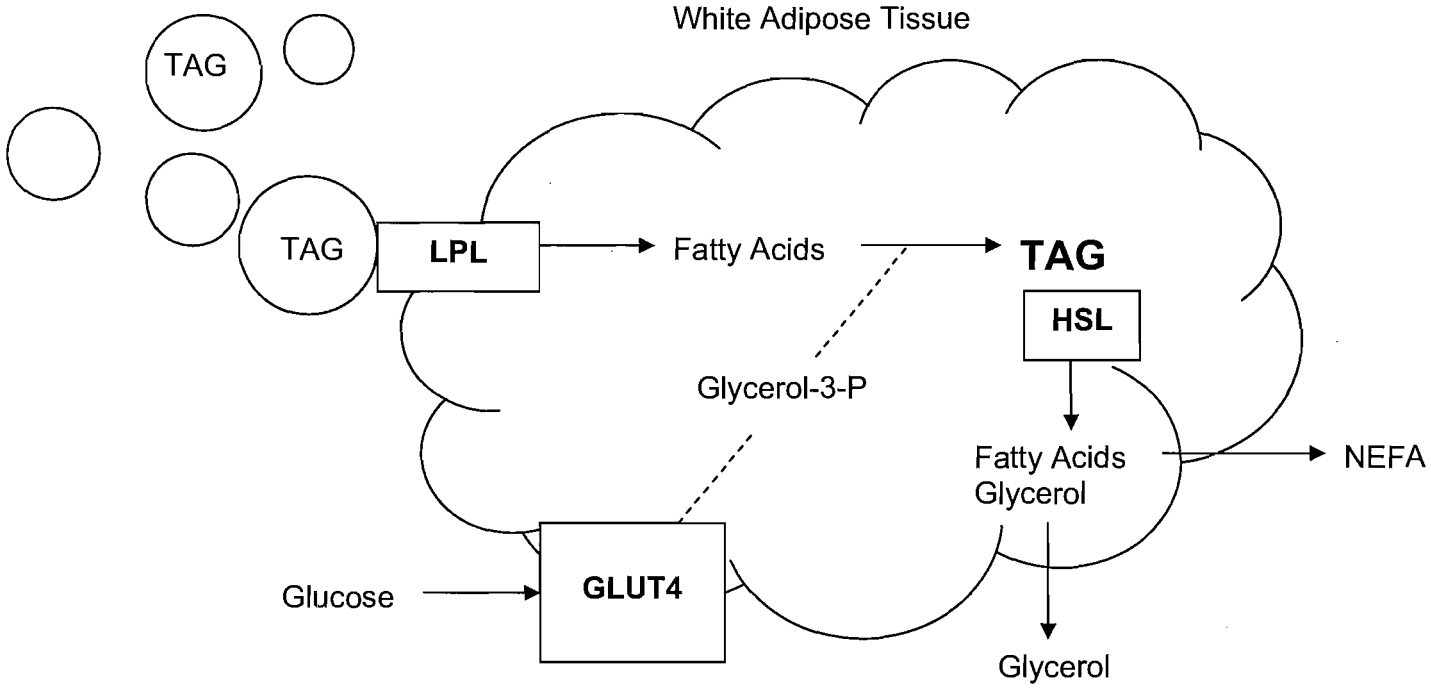


Figure 2.7 The deposition and mobilisation of fatty acids in white adipose tissue (Adapted from Frayn, 1996c). LPL, lipoprotein lipase; glycerol-3-P, glycerol-3-phosphate; HSL, hormone sensitive lipase.

2.5 THE TRANSPORT OF LIPIDS IN THE CIRCULATION

So far, only the presence of PLs in the form of CM in the circulation has been discussed. However, in times of fasting (such as waking in the morning) there are very few CM molecules in the circulation as it may be many hours since the last meal. There are several other lipid-rich molecules that are formed partly of PL, along with other lipid compounds such as TAG. PL are an intrinsic part of the LP molecules found in the blood stream, of which there are several different types. LP are, as the name suggests, molecules of lipids and protein and are specialised structures for the transport of cholesterol and TAG in the bloodstream (Frayn, 1996b).

LP are particles with a very hydrophobic lipid core and a hydrophilic coat, making them particularly suited for transport in the plasma. The core typically consists of TAG and CE, and the coat of PL and free cholesterol (CE are highly hydrophobic, but free or unesterified cholesterol has amphipathic properties because it has a hydroxyl group, Figure 2.8).

Each LP particle has one or more protein molecules associated with it, called apolipoproteins. These apolipoproteins have both hydrophobic and hydrophilic regions. The hydrophobic domain of the apolipoprotein is found associated with the middle of the LP molecule and serves to anchor it to the particle. The hydrophilic region is found at the surface of the particle in contact with the aqueous milieu of the plasma.

LP are commonly classified according to their density upon ultracentrifugation. This also separates the particles according to function, but this is not definitive and some continuum between the fractions exists, whereby each fraction contains a range of different particles with differing functions (Frayn, 1996b). The four major classes of LP are CM, VLDL, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). These classes of LP originate from varying sources and each has a characteristic balance of TAG in the core and amount of protein and PL in the coat. The characteristics of the major LP are summarised in Table 2.1.

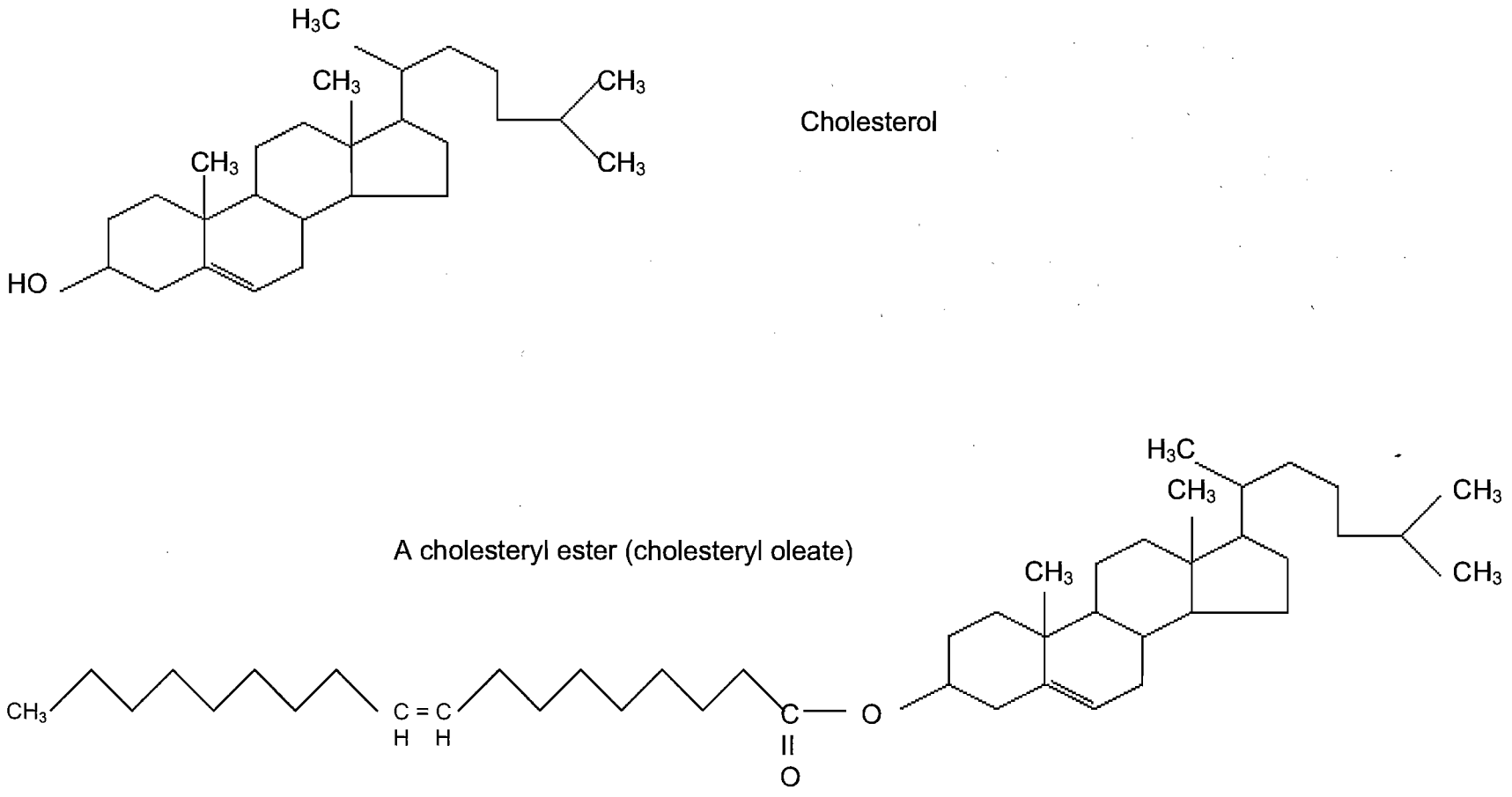


Figure 2.8 Structures of cholesterol and a typical cholesteryl ester (cholesteryl oleate) (Adapted from Frayn, 1996d).

Fraction	Density Range (g/ml)	Diameter (nm)	Major Lipids
CM	<0.950	80-1000	Dietary TAG
VLDL	0.950-1.006	30-80	Endogenous TAG (from liver)
LDL	1.019-1.063	20-22	Cholesterol and CE
HDL	1.063-1.090	9-15	CE and PL

Table 2.1 Characteristics of the major LP classes (Frayn, 1996b).

2.5.1 CHYLOMICRON METABOLISM

CM are the major source of exogenous TAG in the circulation as they transport dietary TAG from the epithelial cells of the intestine around the body and are finally taken up by the liver as CM remnants. As previously described, TAG and cholesterol absorbed from the intestinal lumen are re-esterified in the enterocyte and secreted as CM into the circulation via the lymphatic system. These CM particles consist mostly of a core dietary TAG (approx 90% by weight) and CE with a coat of cholesterol and PL (Figure 2.9).

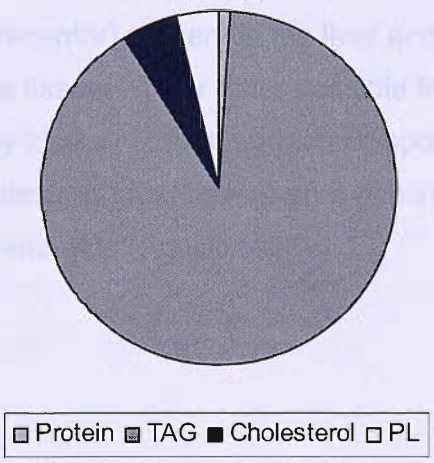


Figure 2.9 The composition (% by weight) of CM particles. Protein 1%, TAG 90%, Cholesterol 5% and PL 4%. (Data taken from Frayn, 1996b)

In the circulation, CM rapidly acquire the apolipoprotein Apo C2, making them substrates for LPL. Thus, as they pass through the capillary beds of tissues expressing this enzyme at their surface (such as muscle and adipose tissue), the TAG is hydrolysed and the particles shrink. The CM particles may also lose

some of their cholesterol and PL coat and some of their apolipoproteins which are taken up by other particles such as HDL.

These smaller CM particles are now termed CM remnants and because they have lost much of their TAG core, are rich in CE. These particles may be atherogenic. Once they shrink to a certain size they become ligands for specific receptors in the liver. Here they are taken up and therefore removed from the circulation (Frayn, 1996b).

2.5.2 VLDL METABOLISM

Like CM, VLDL particles are rich in TAG, but have more PL, protein and cholesterol than CM particles (Figure 2.10). They originate in the liver; hence they are an endogenous source of TAG. VLDL particles are also substrates for LPL expressed on the surface of cells in the capillary beds, so the VLDL particles deliver lipid energy in the form of TAG from the liver to other tissues in the body. As the TAG is hydrolysed from the VLDL, some of the surface coat becomes redundant and is passed on to mainly HDL particles. The resulting particles, rich in CE, have two fates. They can either be taken up directly by a receptor (the LDL receptor) present in the liver and other tissues, therefore delivering CE to the tissues. Their other possible fate is to remain in the circulation until they lose all of their surface components (except Apo B100, a shell of PL and cholesterol) and have a core rich in CE. They then become LDL particles or VLDL remnants (Frayn, 1996b).

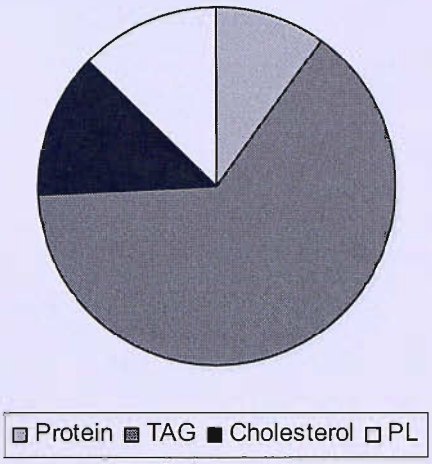


Figure 2.10 The composition (% by weight) of VLDL particles. Protein 10%, TAG 65%, Cholesterol 13% and PL 13%. (Data taken from Frayn, 1996b)

2.5.3 LDL METABOLISM

The newly formed LDL particles have lost a lot of their TAG content and are rich (approx 45%) in cholesterol (Figure 2.11). They have a fairly long half-life in the circulation (about 3 days) and as such are relatively metabolically stable. They deliver cholesterol to the tissues mainly through uptake via the LDL receptor. Intracellular content of cholesterol will therefore be higher in tissues expressing this receptor. Uptake of cholesterol into the cell has two effects. Firstly, biosynthesis of cholesterol in the cell is suppressed and secondly, synthesis of the LDL receptor is also inhibited, therefore reducing the number of receptors expressed on the cell surface. Thus, the uptake of LDL-cholesterol by the cell via the LDL receptor has a negative feedback effect on the cells ability to uptake more LDL-cholesterol (Frayn, 1996b).

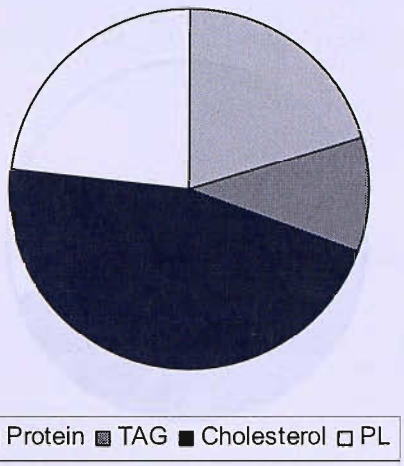


Figure 2.11 The composition (% by weight) of LDL particles. Protein 20%, TAG 10%, Cholesterol 45% and PL 23%. (Data taken from Frayn, 1996b)

The LDL receptor is expressed on most nucleated cells but uptake is particularly active in the liver and other tissues with a high demand for cholesterol for biosynthesis of other compounds (e.g. adrenal glands and ovaries use cholesterol as a precursor in the synthesis of steroid hormones). LDL binds to the receptor and is taken up into the cell where CE is hydrolysed, releasing cholesterol. This cholesterol is then used for incorporation into membranes and for steroid biosynthesis and in the liver, for the synthesis of bile acids and VLDL. Some cholesterol is re-esterified into cholesteryl esters which can be removed from the cell by incorporation into HDL (Frayn, 1996b).

2.5.4 HDL METABOLISM

HDL particles work in the opposite way to LDL particles as they remove cholesterol from cells and transport it to the liver for excretion. They are smaller particles rich in protein and PL but low in TAG (Figure 2.12). They are initially excreted by the liver as disc-shaped particles, consisting principally of PL and their apolipoprotein, Apo A1. They acquire unesterified cholesterol from the surface coat of CM and VLDL particles as they shrink due to the actions of LPL on their TAG core. They also pick up unesterified cholesterol by interaction with cells, although the mechanism is not clear. Unesterified cholesterol is esterified by the enzyme LCAT so the HDL particles acquire a core of CE and become more spherical in shape.

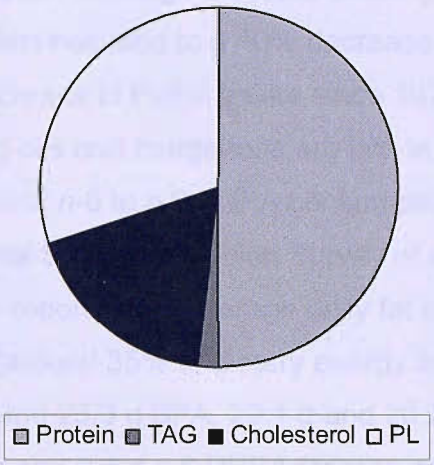


Figure 2.12 The composition (% by weight) of HDL particles. Protein 50%, TAG 2%, Cholesterol 18% and PL 30%. (Data taken from Frayn, 1996b)

A protein in the circulation called cholesteryl ester-transfer protein (CETP) is responsible for catalysing the exchange of CE and TAG between LP which seems to be by facilitated diffusion along concentration gradients. When the concentration of TAG in the plasma is high (e.g. after a meal when CM concentrations are high) CETP catalyses the exchange of CE from HDL to CM, whilst TAG moves in the opposite direction. The CE remain in the CM until uptake by the liver as CM remnants. This leaves a HDL particle rich in TAG which can then be hydrolysed by hepatic lipase. This results in smaller CE-depleted HDL particles which are then able to pick up further cholesterol from the tissues as described above.

The CE in the HDL particles can be taken up by the liver by several mechanisms. This may be by receptor-mediated uptake of large HDL particles containing Apo E or possibly by hydrolysis of CE by hepatic lipase. Some HDL CE is also transferred to the TAG-rich LP. These mechanisms all result in smaller HDL particles which are ready to accept more cholesterol from the tissues. Once arriving at the liver, cholesterol can then be excreted by the liver as cholesterol and as bile salts in the bile (Frayn, 1996b).

2.6 FATTY ACIDS AND THE DIET

Increased consumption of margarines and cooking oils in the place of butter and solid cooking fats has lead to a 40% decrease in SFA, 30% decrease in MUFA and 25% increase in PUFA intake since 1970 (Department of Health, 1994b). As cooking oils and margarines are rich in LA, there has also been an increase in the ratio of *n*-6 to *n*-3 PUFA consumption (Department of Health, 1994b). The National Diet and Nutrition Survey of adults living in the UK carried out during 2000/01 reported the average daily fat intake was 86.5 g for men and 61.4 g for women (around 35% of dietary energy in both cases). This constituted 32.5 g and 23.3 g SFA, 29.1 g and 20.2 g MUFA, 2.3 g and 1.7 g *n*-3 PUFA and 12.9 g and 9.4 g *n*-6 PUFA for men and women, respectively, thus a ratio of *n*-6 to *n*-3 PUFA intake of approximately 5.5:1 (Henderson et al., 2003).

2.6.1 ASSESSING DIETARY INTAKE

There are several ways to assess the dietary intake of an individual, group of individuals or a population. The method of assessment employed will depend on several factors and some aspects for consideration may be:

- Study population size
- Level of accuracy required
- Absolute intakes or changes over time
- Nutrient(s) under investigation
- Resources (e.g. time, costs, coding and/or input of response data)
- Compliancy/ability of study population
- Availability/necessity for trained interviewers

Dietary intake assessment can be administered via three different techniques. These are direct analysis techniques such as duplicate diet or aliquot sampling, record techniques such as weighed record or estimated record or interview techniques such as 24 hour recall or food frequency questionnaire (FFQ) (Ralph, 1993).

A common method often used for the assessment of larger study groups is the FFQ. The FFQ is a questionnaire containing a list of foods and requires the respondent to indicate generally how often each food is eaten (e.g. number of times per day, week, month or year). Foods chosen by the investigator to be included into the FFQ are usually chosen specifically for the study and may not necessarily assess the total diet (Burley *et al.*, 2000).

Each method of dietary intake assessment has its strengths and weaknesses and there is no method which can measure intake without error. It is therefore important that such sources of error are accounted for and the limitations of the method appreciated. Principal strengths of the FFQ are:

- An indication of usual intake of both foods and nutrients may be obtained
- Highly trained interviewers are not normally required
- Questionnaire can be investigator administered or self-administered
- Does not affect normal eating patterns
- Response rates are usually high as the questionnaire is fairly simple

However, the method obviously has its weaknesses and these are listed below:

- Relies on patient memory of previous intake
- Quantification may be inaccurate due to poor estimation or the use of average portion sizes
- May be difficult to administer or inappropriate to use for individuals consuming an atypical diet
- Difficult to validate and often costly
- Considerable programming time and expertise needed to convert food frequencies to specific intake of nutrients

It is therefore imperative that careful consideration of the purpose of the questionnaire be considered before designing or using the tool. Failure to do this may result in a FFQ that is inappropriate to generate the information that is required from it (Burley *et al.*, 2000).

2.7 CHARACTERISING FATTY ACID STATUS

As outlined in the previous sections, lipids enter the body via the food we eat in our diet. Digestion and absorption processes result in the ultimate delivery of exogenous fatty acids to muscle, adipose tissue or to the liver. From the liver, fatty acids are reassembled into new particles and released back into the circulation where they are delivered to the muscle, adipose tissue and other tissues requiring them. Fatty acids in the circulation can be present in several forms; PL, TAG, CE or NEFA. Other fatty acid sources in the body are in the various adipose tissue depots or the liver. In order to study and try to understand fatty acid metabolism and how individual or groups of fatty acids may play a role in disease mechanisms, it is important to consider that fatty acid 'status' is not solely what is found in the plasma or in cell membranes; but what is present in the whole body. It is essential that both the individual pieces as well as the completed jigsaw are considered together, rather than just any individual component in isolation.

Fatty acid status is a very generic term whose meaning varies between groups and individual researchers in this area. In the context of this thesis however, fatty acid status is considered to incorporate both what can be measured in the circulation for example, but also what cannot be accessed or analysed easily. It is practically and ethically impossible to measure all fatty acids in the body *in vivo* and without also destroying the active metabolic pathways which operate only in a living organism. However, it is possible to investigate certain parts of this metabolism and to hypothesise on the integration of those areas that cannot be easily studied. A good starting point and the most accessible and commonly studied aspect of fatty acid status and metabolism in inflammation is the plasma or serum composition. Although the plasma is constituted of fatty acids in PL, TAG, NEFA and CE, usually only the former fraction is considered, if indeed the plasma is partitioned at all. The break down of the plasma into these individual fractions is important when trying to establish fatty acid status, but many groups do not consider this. Therefore, when drawing conclusions, the different influences, inputs and demands which may have an important role in fatty acid metabolism are omitted from the models.

2.7.1 FATTY ACID POOLS

It is possible to analyse the fatty acid composition of several lipid fractions within plasma and these include TAG, PL, NEFA and CE. Each lipid fraction has its own characteristic composition and biological role. Each may have a different origin and be targeted to different locations in the body, performing different functions on the way. Certain lipid fractions may represent fatty acids with a transport or delivery role, whereas others may have more functional duties. Thus, the novel concept of fatty acid 'pools' is introduced in this thesis (Figure 2.13) in an attempt to understand what fatty acid status really is.

There is a supply of fatty acids to the body in the form of dietary fat. However, this supply is modest in comparison to the quantity of fatty acids stored in adipose tissue. These adipose tissue stores are believed to accurately reflect the fatty acid composition of the diet over a long period of time (van Staveren *et al.*, 1986) (Field and Clandinin, 1984). Therefore, determining the fatty acid composition of adipose tissue can provide information on the longer term fatty acid composition of the diet. However, this would require a tissue biopsy, which is not a straightforward procedure. Circulating NEFA are largely derived from adipose tissue so the fatty acid composition of NEFA is representative of that of adipose tissue, thus NEFA could be considered as a proxy measurement of the composition of a storage pool of fatty acids. Synthesis of fat is relatively rare if consumption of energy of fat is greater than 25% (Hellerstein, 1999). Therefore, the adipose tissue stores of such individuals tends to reflect dietary fat consumption (Arab, 2003). Arterial NEFA composition differs from that of venous NEFA as these are subject to utilisation by cells. In addition, the composition of adipose tissue varies between depots. Therefore, the composition of NEFA in plasma is not a perfect reflection of adipose tissue composition, but they may give an indication of this. However, it is important to remember that despite the limitations, the NEFA fraction is a fatty acid pool and should not be disregarded.

TAG and PL are present in the circulation in lipoprotein molecules, with PL forming the outer coat and TAG and CE constituting the core of the molecule. In the fasted state, these lipoproteins will be principally in the form of VLDL. It is

argued that PL are the more significant fraction in plasma because they are the most important source of circulating fatty acids, especially PUFA (Cabré et al., 1992; Holman et al., 1979). Therefore, plasma PL may represent a 'transport pool' of fatty acids (as opposed to a storage pool) as it is from this fraction that cells obtain their supply of fatty acids (Cawood et al., 2005).

It is important to consider the fatty acid composition of the individual plasma lipid fractions and this becomes more evident in the results sections of this thesis. Firstly, the four lipid fractions each have their own characteristic composition and some of these fractions exhibit alterations to fatty acid balance to a greater extent than others. In addition, when the four fractions are considered as one (i.e. whole plasma) some of the alterations to fatty acid composition detected in individual fractions are no longer apparent thus, masking valuable insights into the make up of these fractions. Characterising fatty acid 'status' involves considering not just one pool of fatty acids, but all of them. In addition to this, the supply into the pools and drain out of them must also be considered, as status is a sum of these two processes.

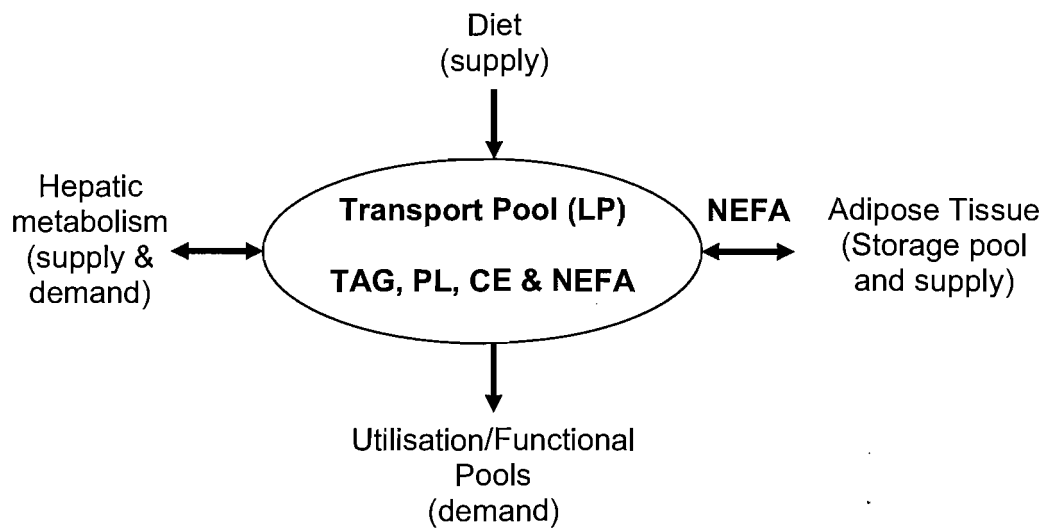


Figure 2.13 The concept of fatty acid pools.

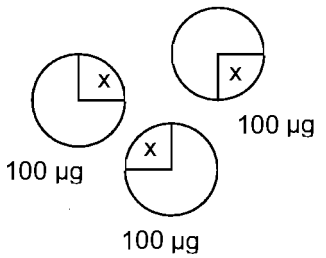
2.7.2 EXPRESSING FATTY ACID COMPOSITION

In addition to the issues highlighted above, the study of fatty acid composition of any given medium is also affected by what is measured and the way in which the data are expressed. Fatty acid composition data can be expressed in two main ways; either as an absolute concentration or as a percentage or proportionate value. Fatty acid data are commonly expressed in percentage terms, but the data are then influenced significantly by the number fatty acids measured and reported. As there is no universally accepted list of which fatty acids in a sample should be reported, the results reported by different groups vary widely making direct comparisons rarely possible.

The true fatty acid composition of a given lipid fraction in the body, such as plasma PC, is probably best described in both percentage and absolute terms. Although two types of comparison can often yield very similar results and conclusions, there is certainly merit in considering both types of data expression when trying to establish true fatty acid composition. As cells obtain their supply of fatty acids from the lipoproteins in the blood, it is fair to say that the ability of cell to uptake fatty acids will depend not only on how many lipoprotein molecules that can be processed or accessed by the cell in a given time, but also on the number of lipoproteins present (absolute concentration) and the composition of these molecules (relative proportion). The ability to uptake and the availability of EFA is probably of particular importance as cells are not able to synthesise these fatty acids *de novo*.

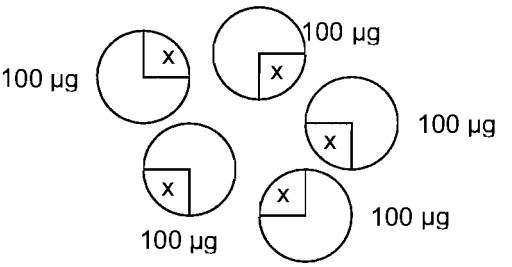
To illustrate: a given fatty acid (fatty acid X) may be present at a relative proportion of 25% in sample A and in sample B. Sample A contains a total of 300 µg of fatty acids, sample B contains 500 µg. Therefore, there are 75 µg of fatty acid X in sample A and 125 µg in sample B. Had this fatty acid only been measured in percentage terms, the samples would have appeared identical. However, with the added concentration data, we can see that although the relative make up is the same, there is 67% more fatty acid X in sample B than in sample A (Figure 2.14).

Frequently, the fatty acid concentration and proportion expressions can yield different results and therefore influence the conclusions. Hence, in this thesis, fatty acid composition data are expressed and compared using both an absolute concentration and a relative proportion. This is very important to gain a more accurate picture of real fatty acid composition of the plasma.



Sample A

Proportion fatty acid x = 25%
Total fatty acids = 300 µg
Fatty acid X = 75 µg



Sample B

Proportion fatty acid X = 25%
Total fatty acids = 500 µg
Fatty acid X = 125 µg

Figure 2.14 The importance of data expression in both concentration and proportion terms illustrated by the fatty acid composition of two theoretical lipid samples

2.8 INFLAMMATORY BOWEL DISEASE

The term IBD includes two diseases: UC and CD. The diseases are more common in Europe and North America than in tropical Africa, South America, Asia and Japan. Prevalence of the two diseases, especially CD, appears to have increased since the Second World War (Ekbom, 1999). UC affects approximately 120,000 people in the UK; around 1 in 500 people (NACC, 2006). It affects males and females equally. CD affects 1 in 1000 people in the UK, approximately 60,000 people (NACC, 2006). CD is commonly diagnosed between the ages of 15 and 40 years and fewer patients are diagnosed in older age groups (Rhodes et al., 1994a). UC is also rare in children; diagnosis is uncommon before the age of 10 and it is much less common than CD in this age group. Most patients are diagnosed aged between 20 and 40 years, although there is a further higher incidence rate around the age of 70 (Rhodes et al., 1994b).

UC and CD are characterised by chronic relapsing inflammation of the GI tract causing pain, severe and prolonged diarrhoea and leading to significant weight loss. In UC, the disease site is restricted to the colon, but in CD any part of the GI tract, from mouth to anus, can be affected.

2.8.1 RISK FACTORS

Although the precise causes of UC and CD are not properly understood, they appear to have both environmental and genetic influences.

Genetic

About 10% of CD patients have a first-degree relative with the disease and siblings are affected 30 times more often than the general population. However, there are only few reports of twins with the disease. Interestingly, there also appears to be a 3.5 fold increased risk of CD in people with first-degree relatives with UC. This has lead to speculation that UC and CD may represent a spectrum of polygenic disease rather than two individual conditions. Perhaps the inheritance of only a few of the relevant genes might confer an increased risk of developing UC, but inheritance of a more complete genotype would predispose to CD (Rhodes et al., 1994b). There are associations of other diseases with the prevalence of CD. Patients with ankylosing spondylitis (chronic inflammation of the spinal column) are nine times more likely to suffer from CD. Patients with CD also appear to be more likely to come from families with an increased prevalence of atopic disease. As both of these conditions are inherited, this somewhat supports the genetic component of CD (Rhodes et al., 1994a).

First-degree relatives of patients with UC are 15 times more likely to develop UC than the general population, and siblings of UC patients are estimated to be at a 19-fold increased risk of the disease. There appears to be a relatively high incidence of the disease in monozygotic twins, but the data may be misleading (Rhodes et al., 1994b).

Environmental

Contrary to many other diseases in man, an increased risk of UC is conferred in non- and ex-smokers (Rhodes et al., 1994b). The opposite is true in the case of CD where smoking is estimated to increase the risk of developing the disease three-fold (Rhodes et al., 1994a).

There has obviously been much interest in the role of the diet as a risk factor for CD, in particular, milk, fibre and sugar. Whereas the data regarding fibre intake and risk of CD is controversial, there does appear to be a link between high refined carbohydrate intake and risk of CD. This is one of the few consistently positive findings in patients with CD and is independent of any risk associated with smoking. There is little evidence implicating the diet as a risk factor for UC (Rhodes et al., 1994b).

2.8.2 CLINICAL FEATURES

Although CD can affect any part of the GI tract, it principally affects the small intestine as some 60% of cases affect the ileum, but roughly 30% of cases are restricted to the colon. It is rare that the mouth, oesophagus and stomach are affected. CD classically features focal areas of chronic inflammation which are discontinuous and clearly separated by areas of normal intestine. The intestinal wall becomes inflamed, ulcerated and thickened which narrows the lumen. This can cause obstruction of the intestine. A simple description of the presenting features of CD is difficult because of the considerable variations in the affected site or sites, extent and severity of disease. However, most patients present with general ill health and lack of energy. Other common features are abdominal pain, loss of appetite, nausea, diarrhoea and abdominal tenderness, of which the anorexia often results in significant weight loss (Rhodes et al., 1994a).

Inflammation in UC usually begins in the rectum and extends proximally to involve a variable length of the rest of the colon. Occasionally, the terminal ileum may also be inflamed along with the caecum. This may affect the function of the ileo-caecal valve. This usually resolves after colectomy. Diarrhoea is also often a presenting feature in UC, but this depends on the extent and severity of

the disease. A frequently occurring or constant feeling of needing to empty the bowels (tenesmus) is also a common symptom of UC. Anaemia, hypoalbuminaemia, fever and weight loss often accompany more severe disease (Rhodes et al., 1994b).

Occasionally, it may not be possible for a physician to make a firm diagnosis of either UC or CD as there is sometimes an overlap of clinical features that are commonly characteristic of one condition only. Medical therapy is similar for both conditions and the distinction only really becomes important with the consideration of surgery (Rhodes et al., 1994b).

2.8.3 MALNUTRITION IN CROHN'S DISEASE

Malnutrition is a common feature of CD. Patients often eat less because of the worsening of their symptoms associated with food intake. Malabsorption due to chronic diarrhoea or as a result of extensive small bowel resection also has an influence. There may also be continued inflammation (even after resective surgery) and enteric loss of nutrients. Many patients may suffer a range of disorders from deficiencies of one or several micronutrients. These are summarised in Table 2.2.

Nutrient	**Feature of Malnutrition**
Zinc	Impaired taste
Iron	Anaemia
Vitamin B_{12}	Pernicious anaemia
Vitamin K	Bruising
Potassium	Lethargy, ileus
Calcium	Ileus, tetany, osteoporosis (longer term deficiency)
Magnesium	Tetany
Vitamin C	Scurvy
Protein	Hypoproteinaemia, oedema

Table 2.2 A summary of nutritional deficiencies commonly featuring in patients suffering from Crohn's disease (Rhodes et al., 1994a). Ileus = loss of peristaltic movements of the GI tract.

Anaemia occurs in up to 70% of CD patients and is often due to a genuine deficiency of iron. However, the inflammatory processes associated with CD can sometimes account for abnormalities in serum iron and transferrin saturation and as such, iron deficiency is often over-diagnosed. Increased losses of protein in the GI tract can result in hypoproteinaemia and this can be assessed by the measurement of serum albumin concentration. Deficiencies of the fat soluble vitamins is also common and low plasma vitamin A concentrations are often found in patients with hypoproteinaemia but do not necessarily indicate a deficiency of the vitamin. Dark adaptation tests are often used to confirm true vitamin A deficiency requiring supplementation. Low plasma zinc concentrations often occur in CD, particularly in those patients who are malnourished. However, this is related to plasma albumin concentrations and may not necessarily reflect a true deficiency of zinc, especially as tissue zinc deficiency is rare. Vitamin D deficiency is also common in CD patients, which further contributes to the substantial number of these patients suffering from osteopenia and osteoporosis. This can be due to several factors such as early menopause, malnutrition due to active disease and intestinal resection. Osteoporosis is often more common in those patients with small intestinal involvement (Rhodes et al., 1994a).

2.8.4 SURGICAL INTERVENTION

Surgical intervention is not always necessary in the treatment of IBD as many patients are conservatively managed with steroid and immunosuppressive therapies. There may be extensive disease but with minimal symptoms which may persist for long periods of time. Patients may remain in remission for extended periods but severe active disease may relapse after many years of apparent good health. However, even after extensive surgery to remove diseased parts of the bowel, a high proportion of patients will suffer disease recurrence and this is more likely in patients with small bowel involvement (Rhodes et al., 1994a).

Patients with UC often undergo colectomy surgery which is the surgical removal of the entire colon. The overall risk of colectomy in UC patients is about 20% (Rhodes et al., 1994b). CD patients with large bowel disease may also undergo

colectomy which may be accompanied by additional resection of parts of the small intestine, e.g. the terminal ileum, caecum and appendix. This may only involve removal of 10 to 20cm of terminal ileum, but some patients with more significant involvement of the small bowel may require more extensive resection.

There are several different types of colectomy surgery. The proctocolectomy with ileostomy procedure offers several advantages as it is the simplest operation involving a single surgical procedure and incurs the lowest morbidity and mortality rate (Kamm, 1999b). This operation removes the entire colon and rectum and therefore requires the formation of an ileostomy. This is where the remaining end of the ileum is brought out through the front wall of the abdomen so that the contents of the bowel can discharge externally into a bag adhered to the skin. Another procedure is colectomy with ileorectal anastomosis. This operation involves the removal of the diseased colon where the remaining ileum is surgically connected to the rectum which has been left behind. This operation has an obvious advantage over the former as it does not require a stoma, but is only really suitable for those patients whose rectum is relatively free of disease. This operation confers an increased risk of rectal malignancy (Henry, 1994). An alternative procedure is the proctocolectomy with ileoanal reservoir ("pouch"). This procedure allows the patient to maintain continence but is a far more complicated procedure involving several operations. The colon is removed and the distal small intestine is looped and joined together, commonly in a 'J' or 'W' shape, forming the pouch. This is then attached to a small cuff of remaining rectum. Unfortunately, many patients opting for this procedure need to have the pouch removed due to sepsis, fistulae, incontinence or pouchitis (Kamm, 1999b).

2.8.5 CONSEQUENCES OF COLECTOMY

Discharged ileostomy fluid resembles the chymus that would normally pass through the ileo-caecal valve. Low concentrations of SCFA and secondary bile acids show that little or no bacterial fermentation occurs (Christl and Scheppach, 1997).

Salt and Water Absorption

Since a major function of the colon is to reabsorb water and salts, it is not surprising that colectomy patients often suffer from chronic salt and water depletion (Christl and Scheppach, 1997). Approximately 1 to 1.5 L of nearly isotonic fluid passes into the colon daily and the majority of this is absorbed. Sodium is actively absorbed and chloride is absorbed in exchange for bicarbonate. This is then secreted into the lumen to neutralise acid. Subsequent to colectomy and ileostomy, the resorption capacity is decreased but varies widely between patients. Some patients may have ileostomy outputs in excess of 1 L per day (Christl and Scheppach, 1997). As the ileostomy output is nearly isotonic, these patients are prone to dehydration and hypotension. Almost all ileostomy patients exhibit signs of chronic salt and water depletion and this is shown by low levels of urinary sodium excretion and elevated blood renin and aldosterone (Christl and Scheppach, 1997).

Nutrient Absorption

With normal function of the small intestine, nutrient absorption after colectomy is not impaired (Christl and Scheppach, 1997). However, as the terminal ileum is the site for vitamin B_{12} absorption, if this has also been resected there may be impaired absorption of this micronutrient. Absorption can also be decreased even if the terminal ileum is intact as a result of bacterial colonisation at this site. The bacteria incorporate the vitamin reducing its availability for absorption. It is thought that 10 to 30% of colectomy patients have abnormal vitamin B_{12} absorption with 3 to 9% being clinically deficient (Christl and Scheppach, 1997).

Bile Acid Malabsorption

Some degree of bile acid malabsorption is common in colectomy patients, even those with no small bowel resection. Similarly to vitamin B_{12} malabsorption, this may be due either to resection of the terminal ileum or bacterial relocalisation from the resected colon to the remaining terminal ileum. Bacterial overgrowth of the terminal ileum can result in bile acid deconjugation which reduces absorption. 10 to 40% of colectomy patients have higher bile acid excretions than normal (Christl and Scheppach, 1997).

2.8.6 CONSEQUENCES OF EXTENSIVE SMALL BOWEL RESECTION

The length of the small intestine varies between individuals but it is normally roughly four metres long. The small intestine has a considerable reserve capacity for resorption whereby most patients can tolerate resection of up to 50% of the small intestine and still maintain their nutritional status on a normal oral intake. Many complications arise as a result of surgery as opposed to the inflammatory processes themselves. Incidence of complications is closely related to the extent of small bowel resection. If more than 50% of the small intestine is removed there may be serious nutritional, fluid and electrolyte deficiencies. These problems and the associated diarrhoea are known as short bowel syndrome.

Following small intestinal resection, there is a degree of adaptation that takes place. This increases the absorptive capacity per unit length of the remaining ileum. Subsequent to removal of the ileum, villus epithelial cell height increases and enhanced segmental glucose and sodium absorption has also been shown. The ileum adapts to a greater extent than the jejunum and it is thought that adaptation may take up to one year to be complete (Griffin and Northfield, 1994).

Probably the most commonly reported and troublesome problem following small bowel resection is diarrhoea. However, this often improves over time as the body may adapt by increasing water resorption (Rhodes et al., 1994a).

As discussed in the previous sections, bile salts and vitamin B_{12} are normally resorbed at the terminal ileum, but in patients with colectomy and terminal ileal resection, these may be lost in the stomal output. Vitamin B_{12} malabsorption may take years to manifest itself as normal body stores may last for approximately four years (Griffin and Northfield, 1994). However, the consequences of bile acid malabsorption will be noticed immediately. Removal of the terminal ileum breaks the enterohepatic circulation, leading to excessive losses of bile acids and vitamin D. Patients with CD have an increased bile acid turnover and this is correlated to the length of ileal resection, they also have elevated bile acid secretion in the stool (Tougaard *et al.*, 1986). Increased bile

acid losses exceeding the capacity of the liver to up-regulate bile synthesis can greatly reduce the bile salt pool and can lead to gall-stones, indeed 32% of patients with ileal resection have gall-stones; three times the normal figure (Rhodes et al., 1994a). Decreased bile acids means that ingested lipids are not sufficiently converted into micelles during digestion, the result being that lipids are not able to be properly absorbed. This results in a condition called steatorrhoea (high lipid levels in the faeces/stomal output) which can compound the problems of diarrhoea in these patients (Griffin and Northfield, 1994).

There are a number of treatments employed in the management of patients with problems as a result of short bowel syndrome, these are summarised in Table 2.3.

Problem	Cause	Treatment
Malnutrition	No oral intake	Parenteral nutrition
Adaptation stage diarrhoea and steatorrhoea	Hyperosmolar drinks, lack of absorptive capacity, rapid transit	Gradual introduction of solids or isotonic liquid feeds. Avoidance of hyperosmolar drinks. Antimotility agent.
Chronic diarrhoea and steatorrhoea	Lack of absorptive capacity	Small frequent meals, low fat diet
Fluid and electrolyte deficiency	Lack of absorptive capacity, decreased sodium absorption	Oral isotonic glucose solution, parenteral magnesium sulphate, oral metabolic mineral mixture

Table 2.3 Problems, mechanisms and treatment of short bowel syndrome (Adapted from Griffin and Northfield, 1994).

2.9 FATTY ACIDS AND INFLAMMATION: THE IMMUNE SYSTEM

Altered fatty acid compositions in the plasma, erythrocytes, leukocytes and tissues have been implicated in a wide range of disease pathologies including IBD, Alzheimer's disease, rheumatoid arthritis, asthma, eczema and a variety of other diseases. Although many studies have investigated the effects of supplementation with *n*-3 PUFA, results have been controversial. To gain an understanding of how fatty acids may be exerting their actions in these diseases, it is necessary to have a basic understanding how the immune

system operates, as inappropriate immune responses are implicated in some inflammatory diseases. This section aims to provide an overview of the immune system.

The human body poses an extremely attractive host to pathogens: viruses, bacteria, fungi, protozoa and multi-cellular parasites capable of causing infection and disease. These pathogens can invade, infect and ultimately kill their host and are able to evolve much more quickly than humans. Consequently the body has developed a very sophisticated immune system with which to protect against these unwanted invaders. The effectiveness of the immune system becomes especially apparent in immunodeficiency diseases (e.g. HIV) when individuals become increasingly susceptible to infection and mortality often results from organisms that are not normally fatal (Roitt et al., 1998c).

The immune response is divided into two general components: innate and acquired immunity. Innate immunity is natural (i.e. it is not induced) and does not change or improve with repeated exposure to pathogens. It is a general response that is not directed at specific organisms, but works to create a hostile environment for the pathogen, e.g. fever, making replication and survival more difficult. The innate immune response is predominant during early infection, serving as a first line of defence against invading pathogens (Roitt et al., 1998c).

Acquired immunity (also known as adaptive or specific immunity) allows the body to recognise and selectively eliminate disease-causing organisms and unlike innate immune responses, acquired immune responses are specific reactions to antigens expressed by host organisms. The specificity of the immune system means that a distinction can be made between two foreign protein molecules that differ in only one amino acid and its diversity allows it to recognise billions of different structures on these foreign antigens. Once an antigen has been recognised and a specific response mounted, it is also capable of 'remembering', so that upon any subsequent invasion by the same pathogen, the immune responses are even faster and more effective than the

first time. The immune system is also capable of recognising the difference between 'self' and 'non-self' molecules, so that it only normally responds to foreign antigens (non-self) and does not launch these powerful responses against its own proteins (Goldsby et al., 2000b).

2.9.1 IMMUNOLOGICAL CELLS

The principal cells of the acquired immune response are lymphocytes and antigen-presenting cells. Lymphocytes are another subset of leukocytes arising from the bone marrow and found circulating in the blood and lymphatic systems, and in various lymphoid organs. One of the two major types of lymphocytes are T lymphocytes (or T cells) and these mediate the cell-mediated branch of the immune system (Roitt et al., 1998c).

The cell-mediated branch of the acquired immune system (or T cell immunity) is the formation of large numbers of activated T lymphocytes, designed to attack and destroy the pathogen. T cells originate in the bone marrow and then migrate to the thymus gland to mature. There are two sub-populations of T cells, called T helper (Th) and T cytotoxic (Tc) cells. They can be distinguished from each other by the presence of different glycoproteins on the cell surface. These glycoproteins act as markers and can be identified by monoclonal antibodies, and are named CD membrane glycoproteins. Th and Tc cells generally display the CD4 and CD8 markers, respectively (Goldsby et al., 2000b).

Activated Th cells are called effector cells and secrete a number of soluble chemical factors known as cytokines. These chemical mediators take part in the immune response and have an important role in the activation of B cells, Tc cells, macrophages and many other cells involved in the immune response (Goldsby et al., 2000b). Th cells can be broadly divided into two further subsets (Th1 and Th2) on the basis of their profiles of cytokine release, which results in different types of immune response (Roitt et al., 1998b).

2.9.2 CYTOKINES

Cytokines are low-molecular weight proteins or glycoproteins produced mainly by Th cells in response to various stimuli. Some cytokines facilitate immune effector cell development, whereas others may have direct functions of their own. They can act directly on the cell from which they were secreted (autocrine), on local cells (paracrine), or on cells some distance away (endocrine). However, most cytokines act over short distances in an autocrine or paracrine manner. Cytokines act by binding to specific receptors on the membrane of target cells which trigger signalling cascades and ultimately alter gene expression of the target cells. Generally, cytokines and their receptors have a very high affinity for each other and it is because of this that even picomolar concentrations of cytokines can generate a biological effect (Goldsby et al., 2000a).

Many of the cytokines are called interleukins (IL). Other cytokines include the interferons (IFN), such as IFN-γ, and tumour necrosis factors (TNF), such as TNF-α (Goldsby et al., 2000a).

2.9.3 TH1 AND TH2 CELL SUBSETS

As mentioned previously, the Th cells can be divided into Th1 and Th2 subsets; determined by the pattern of cytokines released from them. Th1 cells secrete IFN-γ, IL-2 and TNF-α, and Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. There are in addition, some Th cells that secrete both typically Th1 and Th2 cytokines and these are known as Th0 cells. It is generally accepted that the clear cut distinction between Th1 and Th2 cells is oversimplified, as Th cells appear to form a continuum from those secreting typically Th1 or Th2 cytokines to Th0 cells secreting a mixture of both, of which the Th0 cells form the majority (Devereux, 2002).

Th1 cells are involved in cell mediated inflammatory reactions and several of the Th1 cytokines stimulate cytotoxic, inflammatory and delayed hypersensitivity reactions, whereas Th2 cells are associated with regulation of strong antibody and allergic responses. The two responses are mutually inhibitory. Thus, high

concentrations of Th1 cytokines will inhibit differentiation of Th cells into the Th2 subset and vice versa (Roitt et al., 1998a).

Imbalances between the Th1 and Th2-type responses are characteristic of many human diseases (Mossmann and Sad, 1996).

2.9.4 EICOSANOIDS

Eicosanoids are important mediators of the immune system and are a link between fatty acids, inflammation and immune function. Eicosanoids are a group of signalling molecules synthesised from 20-carbon fatty acids i.e. ARA, EPA and DGLA. There are several classes of eicosanoid, for example prostaglandins (PG), leukotrienes (LT) and thromboxanes (TX). In response to a stimulus, fatty acid precursors are cleaved from PL in the cell membrane usually via the action of PLA_2. ARA is usually the major precursor for eicosanoid synthesis because most membranes contain large amounts of this fatty acid compared to EPA and DGLA (Calder, 2001) (Figure 2.15).

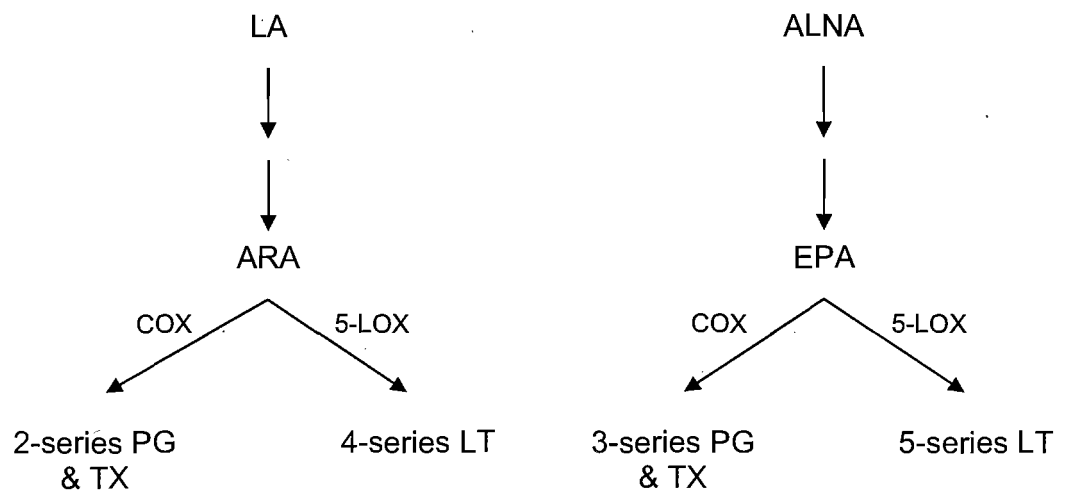


Figure 2.15 Synthesis of different eicosanoid classes from *n*-6 and *n*-3 PUFA precursors.

Eicosanoid synthesis is catalysed by two groups of enzymes: cyclo-oxygenase (COX) or lipoxygenase (LOX). Metabolism of ARA by COX results in the synthesis of the 2-series PG and TX, whereas metabolism by LOX synthesises enzymes results in the formation of 4-series LT (Figure 2.15). There are two

isoforms of COX, of which COX-2 is induced in immune cells as a result of stimulation and is responsible for the substantial increase in PG production that occurs when the cells are activated. Prostaglandin E_2 (PGE_2) is produced in large quantities and has a number of pro-inflammatory effects including fever and erythema induction, increasing vascular permeability and vasodilation and enhancing of pain and oedema caused by other agents (e.g. histamine). However, PGE_2 also suppresses lymphocyte proliferation and inhibits the production of several cytokines such as TNF-α, IL-1, IL-2, IL-6 and IFN-γ, so with respect to this, PGE_2 also has anti-inflammatory and immunosuppressive actions. PGE_2 also enhances immunoglobulin E (IgE) production by B cells but does not appear to have any effect on the production of the Th2 cytokines IL-4 and IL-10 (Calder, 2001).

One of the LT compounds, LTB_4 (found in high concentrations in inflamed bowel tissue (Kamm, 1999a)), is also released from membrane ARA and its actions include increasing vascular permeability and local blood flow, inhibits lymphocyte proliferation and also stimulates production of TNF-α, IL-1, IL-2, IL-6 and IFN-γ, thus the metabolism of ARA into these mediators with many opposing actions means that the overall effect will be influenced by the concentration and activity of these mediators, the timing of their production and the sensitivity of their target cells (Calder, 2001).

2.9.5 MODULATION OF THE IMMUNE SYSTEM

There are several mechanisms by which PUFA can modulate the immune system. One of these mechanisms is alteration of eicosanoid synthesis. ARA is the principal precursor of eicosanoid synthesis and those produced from this fatty acid (i.e. 2-series PG and 4-series LT) often have much more potency that those derived from EPA. There is a degree of competition between the PUFA as EPA and DHA competitively inhibit oxygenation of ARA by COX. In addition, EPA is also able to act as an alternative substrate to ARA for both the COX and LOX enzymes (Calder, 1997).

When humans ingest increased amounts of oily fish or fish oils, *n*-6 PUFA (especially ARA) are replaced by *n*-3 fatty acids in cell membranes, including

those of the immune system. As a result of this, production of ARA derived eicosanoids is decreased, including PGE_2 and LTB_4, and the production of *n*-3 equivalents from EPA is increased. The metabolism of EPA by COX and LOX results in the production of 3-series PG (such as PGE_3) and 5-series LT (such as LTB_5), respectively. EPA-derived eicosanoids are considered to be less potent than their ARA-derived counterparts but the activities of these compounds have not been fully elucidated. However, LTB_5 is around ten times less potent than LTB_4 as a neutrophil chemoattractant so could therefore be considered to be less pro-inflammatory (Calder, 2001).

Thus, *n*-3 replacement of ARA in cell membranes modulates the immune cell functions by eicosanoid-mediated effects (Teitelbaum and Walker, 2001). It is from this that much interest in the role of *n*-3 and *n*-6 PUFA in inflammatory processes has developed and it is widely believed that the balance of these fatty acids may have important influences in inflammatory diseases such as CD and UC. Despite a wide range of study designs employed to investigate the effects of dietary *n*-3 PUFA on immune function and many inconsistencies, it is still clear that addition of high doses of *n*-3 PUFA to the diet for several weeks leads to substantial decreases in ARA-derived eicosanoids (Caughey et al., 1996; Endres et al., 1989; Kelley et al., 1999; Lee et al., 1985; Meydani et al., 1993; Sperling et al., 1993) and a variety of immunological effects that are generally considered as anti-inflammatory (Caughey et al., 1996; Gallai et al., 1993; Kelley et al., 1999; Meydani et al., 1991; Meydani et al., 1993).

Many *n*-3 PUFA supplementation studies have involved patients suffering from chronic inflammatory diseases, including CD and UC, and which are often characterised by an inappropriate production of eicosanoids derived from ARA. Many of these studies report benefits of fish oil supplementation including improved disease symptoms or activity and decreased demands for anti-inflammatory drugs (Belluzzi et al., 1996; Hawthorne et al., 1992; Lorenz et al., 1989; McCall et al., 1989; Salomon et al., 1990).

2.9.6 IMMUNOLOGICAL FEATURES OF ULCERATIVE COLITIS AND CROHN'S DISEASE

The main feature of CD and UC is inflarnmation of the GI tract. This takes the form of many immunological changes, including altered populations of inflammatory cells and the activation of many inflammatory pathways.

The GI tract has its own specialised immune compartments. The most specialised being the Peyer's patches which are found in the ileum. These consist of both B and T cells. When an antigen crosses the epithelium of the gut from the lumen, specialised cells break the antigenic protein into fragments and present it to the T cells. The T cells recognise and interact with the antigen and become activated. They then become effector cells and secrete a number of soluble chemical mediators called cytokines. Cytokines take part in the immune response and play an important role in the activation of many cells involved in the immune response. B cells also react to the antigen by producing antibodies against it. These processes result in inflammation.

Activated T cells are normally found in the wall of the gut, but in IBD the processes which regulate them are disturbed. It is not known whether this is a result of a normal specific response to an unknown antigen or due to an inability to self-regulate the response to normal antigens (Kamm, 1999a).

In terms of immunology, CD and UC are largely similar, and most differences are quantitative rather than qualitative. Decreased total lyrnphocyte count, higher rate of immunoglobulin G (IgG) turnover, higher levels of serum antibodies to anaerobic intestinal bacteria and decrease immigration of neutrophils into skin windows are more common in CD than UC. The major difference is the presence of chronic inflammatory cells in the deeper layers of the bowel wall found in CD but not UC. One explanation is that the diseases follow a common pathway but are initiated by different stimuli (Elson, 1988).

IL-1β, 2, 6 and 8 and IFN-γ are produced in increased amounts in CD and UC. TNF-α is also produced during active IBD. TNF levels IL-4, 5, 10 and 13 are immunoregulatory and may have a repair role in IBD (Kamm, 1999a).

2.9.7 FATTY ACIDS AND INFLAMMATION

Inflammation is a complicated process which has many local and systemic effects on the body. Thus, within the context of this thesis the effects of inflammation on lipid metabolism should be considered.

Substrate Oxidation

A study by Mingrone *et al.* assessed the resting energy expenditure (REE) and substrate oxidation of a group of CD patients in remission and compared them to an age, sex and height-matched healthy control group (Mingrone *et al.*, 1996). They found that the CD patients had a significantly lower body weight than the controls and this was due to both lower fat and lean body masses, although the decrease was more pronounced in the former. Unsurprisingly, they also described a higher REE in the control group. However, when differences in body weight were allowed for, the CD patients showed a higher REE. The group ascribed this difference to a higher lean body mass proportion in these patients. The group also calculated the non-protein respiratory quotient and this showed that the CD patients had a higher lipid oxidation than the control group. As PGE_2 synthesis is raised in inflammation, this will increase ARA (and to a lesser extent, EPA) cleavage from membranes, thus increasing the demands for these fatty acids during inflammation.

Müller *et al.* also showed that fat (as opposed to carbohydrate) was the major energy substrate in patients with CD and these patients continued to oxidise fat even when there was no evidence for depletion of liver glycogen stores (Müller *et al.*, 1993). Thus increased lipid oxidation was not a result of feedback mechanisms due to depleted liver glycogen. The group considered the possible confounding effect of steroid therapy on lipid oxidation and suggested that cytokines would also contribute to altered metabolic responses in their patients.

Zinc

Zinc has an important role in fatty acid metabolism and lower tissue and blood leukocyte zinc content in patients with CD has been described (Ainley et al., 1983). There is some evidence for altered EFA metabolism in CD and this may

be related to zinc depletion (Cunnane *et al.*, 1986). Cunnane's group reported that *in vitro* human leukocyte cultures had reduced incorporation of both labelled LA and ARA into membrane PC and this incorporation was positively associated with zinc levels.

This evidence gives rise to the hypothesis that altered fatty acid balance may exist in diseases such as CD. This could result from reduced dietary supply either from a simple deficiency in the diet or perhaps more likely in colectomy patients, a reduced digestive and absorptive capacity. This, coupled with alterations to the EFA elongation and desaturation pathways and with an increased demand for ARA and EPA for eicosanoid synthesis, could exacerbate the condition.

Fatty Acids and Immune Function

There are many published papers reporting the influence of fatty acids on immune function. This has resulted from the knowledge that long chain *n*-3 PUFA can inhibit the metabolism of ARA to produce eicosanoids (Endres et al., 1989; Lee et al., 1985; Lokesh et al., 1986). There are many in vitro cell culture studies published, in addition to numerous animal feeding experiments but these will not be discussed in detail here. However, it has been shown that the addition of fish oils to animal feeds can exert a variety of immunomodulatory effects (Billiar et al., 1988; Lokesh et al., 1990; Yaqoob and Calder, 1995).

There is less information regarding the effects of fish oil feeding in humans, but the evidence base is growing. However, despite a number of studies, the field remains controversial, although many reviews have attributed many differences in findings to study methods and design. Many human studies have aimed to show that increasing the amount of fish oil in the diet will also increase the proportion of EPA in immune cells with a concurrent decrease in ARA content of these cells. Indeed, most studies have consistently reported a decrease in the ARA content of immune cells, although this does depend on EPA dose, duration of intake and the cell type studied (Caughey et al., 1996; Endres et al., 1989; Fisher et al., 1990; Healy et al., 2000; Kelley et al., 1999; Schmidt et al., 1996; Sperling et al., 1993; Yaqoob et al., 2000).

A decrease in the proportion of ARA in immune cell membranes from *n*-3 PUFA supplementation will decrease the amount of precursor available for the production of 2-series PG and 4-series LT. Long chain *n*-3 PUFA also appear to inhibit ARA release from membranes, possibly via the inhibition of phospholipases (Caughey et al., 1996; Endres et al., 1989; Kelley et al., 1999; Lee et al., 1985; Meydani et al., 1993; Sperling et al., 1993). EPA is also able decrease the metabolism of ARA by COX and LOX enzymes via competitive inhibition. Therefore, an increase in the amount of EPA in immune cells will result in a decreased ability of immune cells to synthesis ARA-derived eicosanoids.

This has been shown in several studies, including a more recent study by Trebble *et al* who investigated the effects of fish oil supplementation on the fatty acid composition of plasma and concurrent assessment of markers of immune cell function (Trebble *et al.*, 2003a). They found that increased dietary *n*-3 PUFA intake increased the incorporation of EPA and DHA into plasma PC and erythrocyte PE although this tended to plateau at higher intakes. They also noted that TNF-α and IL-6 production by peripheral blood mononuclear cells (PBMC) decreased with increasing *n*-3 intakes, but there was a tendency for a "U-shaped" response to increased *n*-3 PUFA intake (Trebble *et al.*, 2003a). A further study by this group also assessed plasma and erythrocyte fatty acid composition and the production of PGE_2 by PBMC. The group found that PGE_2 production decreased significantly with *n*-3 PUFA supplementation and this tended towards a dose-dependant relationship. In addition, PGE_2 production was negatively associated with EPA and positively associated with ARA composition of the plasma (Trebble *et al.*, 2003b).

Fatty Acid Supplementation in IBD

There is growing interest in the use of *n*-3 PUFA supplements as possible therapies for the prevention and or treatment of chronic inflammatory diseases, such as IBD. However, there is only a modest amount of published papers that have investigated the potential therapeutic benefits of fish-oil supplementation in these diseases.

Several studies have shown a potential benefit of *n*-3 PUFA supplementation in UC, (Aslan and Triadafilopoulos, 1992; Hawthorne et al., 1992; Loeschke et al., 1996; Lorenz et al., 1989; McCall et al., 1989; Salomon et al., 1990). Fewer studies have investigated the effects of fish oil in CD and results seem controversial. A study by Belluzzi *et al* compared the effect of an enteric-coated free fatty acid fish oil capsule with a placebo in CD patients with a high risk of relapse. They found that after 1 year, 59% of patients in the fish oil group were still in remission, compared to only 26% of patients in the placebo group (Belluzzi *et al.*, 1996). However, a study by Lorenz-Meyer *et al* did not demonstrate any differences between fish oil and placebo patients in terms of remission rates or markers of disease activity (Lorenz-Meyer et al., 1996).

A recent paper reported a comprehensive systematic review of literature surrounding this topic which included many of the studies mentioned above (MacLean *et al.*, 2005). The paper reviewed 13 controlled trials assessing the effects of *n*-3 fatty acids on clinical, sigmoidoscopic, or histologic scores; rates of induced remission or relapse or requirements for steroids and other immunosuppressive agents in CD or UC. The data presented suggested that *n*-3 fatty acids may reduce the need for corticosteroid treatment in IBD. However, as the authors concluded, there was insufficient evidence to conclude about the effects of *n*-3 PUFA on clinical measures or outcomes.

Overall, it would seem that there may be potential benefits of *n*-3 PUFA supplementation in the therapeutic treatment of IBD, but the evidence to date is not fully adequate and is inconclusive. The few studies published show disparities in study design as well as employment of different forms of fatty acids and these may have significant confounding influences on results.

2.10 FATTY ACIDS AND INFLAMMATORY BOWEL DISEASE

There has been some interest in the area of fatty acid intake in IBD, especially PUFA as both the *n*-3 and *n*-6 parent fatty acids are essential in the diet. Therefore the body relies solely on the diet for its source of these fatty acids. It

is also known that inflammation alters fatty acid metabolism due to increased demands for LA and ALNA for tissue repair and membrane formation (Siguel and Lerman, 1996). This may result therefore, in abnormal fatty acid profiles, abnormal eicosanoid precursor availability and alterations to cell function.

2.10.1 STUDIES OF FATTY ACIDS AND INFLAMMATION IN INFLAMMATORY BOWEL DISEASE

Despite this interest however, fatty acid profiles in patients with IBD have not been well investigated. Of the few studies which have been undertaken involving such patients, results are generally controversial making overall conclusions difficult. Heterogeneity between studies in terms of patient features, disease characteristics and activity, methods of analysis and data expression must have some degree of influence on the overall conclusions made. However, this section aims to give a brief overview of such studies and to outline the findings of these. This aims to try to establish an overall view of what happens to fatty acid profiles in IBD.

Esteve-Comas *et al.* (1992) investigated the fatty acid profiles of 73 patients with active IBD and compared them to those of 107 healthy adults. The patients consisted of 41 UC and 32 CD patients, all with active disease based on a clinical disease scoring system. The patients were also classified as having mild, moderate or severe active disease using this score. They found the proportion of DGLA and ARA in whole plasma to be lower in both disease groups when compared to the controls. They also found significantly higher proportions of ALNA and DHA in the IBD patients. Stepwise decreases in *n*-6 PUFA proportions with increasing disease severity were also noted. The *n*-3 PUFA concentrations followed a similar pattern although proportions still remained higher in the IBD groups than in controls. They suggested that active IBD leads to an increase in fatty acid utilisation by the body and therefore upregulates PUFA biosynthetic pathways, possibly for increased synthesis of ARA-derived eicosanoids. They also postulated that in the absence of active disease (and therefore increased fatty acid utilisation) *n*-3 PUFA concentrations would be further increased and suggested that *n*-3 PUFA supplementation in IBD may not be appropriate.

In a later study, Esteve-Comas *et al.* investigated the fatty acid patterns in the plasma of IBD patients in remission and compared them again to a healthy control group (1993). The 66 IBD patients (27 CD and 24 UC) were a subgroup of the patients studied in the active disease state in the previous study. Blood samples were taken three months after the patients had been classified as inactive (again based on a clinical disease scoring system). The study reported both relative proportions and absolute concentrations of fatty acids in plasma lipids. Results showed higher concentrations of DGLA and ARA and a higher relative proportion of ARA in the IBD patients compared to the healthy reference group. The group also reported higher concentrations of ALNA, EPA and DHA and higher relative proportions of ALNA and DHA in the IBD group. They concluded that the results of this study further supported their hypothesis of upregulated PUFA biosynthesis proposed in their previous paper. They stated that in the absence of active inflammation, fatty acid utilisation (with respect to PUFA biosynthesis) would be lower, resulting in the higher levels of both *n*-6 and *n*-3 PUFAs, rather than just *n*-3 PUFA (as in active IBD). They suggested a metabolic defect was responsible for these changes observed in IBD patients. The group also further expressed their reluctance to supplement IBD patients with *n*-3 PUFAs as they found *n*-3 PUFAs to be higher in these patients.

The study of Sigeul & Lerman (1996) investigated 47 patients with chronic intestinal disease or resection and compared them to healthy controls. 25 patients had CD, 11 had UC, 4 had coeliac disease (with malabsorption) and 7 had short bowel syndrome. They reported lower proportions of plasma LA and ARA and of EPA, DPA and DHA. They also reported significantly lower total plasma EFA proportion and a lower *n*-3:*n*-6 PUFA ratio associated with gastrointestinal disease. These findings starkly contrasted with those of Esteve-Comas *et al.* and the authors ascribed these differences to widely differing methods of analysis. Esteve-Comas *et al.* used 1 ml plasma in their extractions, whereas Siguel & Lerman employed micro-extraction methods, stating that the macro methods cause small (but significant) losses of LA and ALNA. Esteve-Comas *et al.* used a 30 m column and a fast (short) gas chromatography (GC) method for peak separation. Siguel & Lerman believed this method to be

insufficient to properly separate fatty acids of interest from other contaminants. They suggested that these differences may have resulted in Esteve-Comas *et al.* improperly measuring key fatty acids and therefore affecting the proportionate profile of all other reported fatty acids. Indeed, Esteve-Comas *et al.* reported 0.0% ALNA in the reference patients in both papers, a finding which is not supported by the results of Siguel & Lerman or the results from the studies detailed in this thesis. Other discrepancies in fatty acid proportions were also noted (Siguel and Lerman, 1996). However, Esteve-Comas *et al.* did find increased total fatty acid concentrations in their IBD patients, the opposite of what was reported by Siguel & Lerman. Higher concentrations of total fatty acids in colectomy patients are also described in this thesis (Chapters 6 to 8). Esteve-Comas *et al.* found an increased conversion of parent *n*-3 and *n*-6 PUFAs to longer-chain derivatives and attributed their findings to a metabolic feature of IBD. However, Siguel & Lerman challenged that hypothesis and postulated that increased conversion of precursors to derivatives is a characteristic of EFA deficiency and stated that this should always be treated with supplementation (Siguel and Lerman, 1996).

Geerling *et al.* (1999) investigated the fat intake and fatty acid profiles in plasma PL of CD patients, stating that most other studies have not concurrently assessed dietary intake of fat although it is known that this significantly affects fatty acid profile (Bjerve *et al.*, 1993). They aimed to assess the relationship between changes in fatty acid composition and disease duration by studying CD patients with both recent (less than 6 months) and long-term (more than 10 years) history of the disease. The two CD groups were compared to two age and sex matched control groups. However, the two CD groups were substantially different in age and gender ratio so were not comparable with each other. Dietary intake of fat was assessed using the cross-check dietary history method, specifically designed to assess fat and antioxidant intake. This revealed slightly lower % PUFA and LA intake in the CD patients but this was not significant. Disease activity was assessed using the Crohn's Disease Activity Index (CDAI) with concurrent biochemical assessment of inflammatory status by C-reactive protein (CRP). Patients in the newly-diagnosed CD group showed considerably higher mean and range CRP values than those with long-

term CD. This suggests more active disease state in the newly-diagnosed patients, although the differences were not statistically significant. Patients in the long-term CD group were regarded as being clinically in remission but 15 of the 32 patients in this group scored a CDAI in excess of 150, which is considered to be active disease. Geerling *et al.* attributed this to a high liquid stool frequency in many of these patients as 27 patients had previously undergone small bowel resection. It could therefore be suggested that the newly-diagnosed and long-term CD groups were more representative of active and inactive disease, respectively. There were also considerably more patients in the long-term group who were taking steroids at the time of the study (13 of 32 compared to 3 of 20 in the newly-diagnosed CD group) but the confounding influence of this was not taken into consideration.

Geerling *et al.* reported similar proportions of LA and DGLA between the 2 CD groups and their respective reference groups, but lower ARA proportions (significantly different between long-term patients and controls). They also noted marginally higher ALNA (not significant) and higher DPA but lower DHA proportions in the CD groups. The sum of *n*-3 PUFAs in CD groups was lower than that of the reference groups and this was significant in the long-term patients. The fatty acid profiles were not correlated with the length of remaining ileum, lifetime prednisolone dose, CDAI score, CRP concentrations or zinc status. Although serum zinc concentrations were significantly lower in the long-term CD group, they noted no differences in fatty acid profile between patients with or without zinc deficiency. Geerling *et al.* concluded that disturbances in fatty acid profile must be a result of altered hepatic plasma PL metabolism, rather than EFA malabsorption as the proportion of EFA were not different.

The findings of lower DHA proportions in both groups again contrasted with the findings of Esteve-Comas *et al.* and Geerling *et al.* suggested several reasons for these differences. Esteve-Comas *et al.* determined the fatty acid profile of total plasma lipids (incorporating TAG, CE, NEFA and PL), whereas Geerling isolated and measured only the fatty acids of the PL fraction, arguing that these are the most important quantitative source of both circulating and membrane PUFA. They also raised methodological disparities, stating that extraction

techniques were improved in their study and made similar comments to that of Siguel & Lerman with regards to the GC method employed by Esteve-Comas *et al.* Indeed, the time difference of several years between the studies may have some relevance. Geerling *et al.* also commented that the studies of Esteve-Comas *et al.* had not assessed the dietary intake of fats in their patients and could therefore not exclude that dietary intake (with respect to fish) was not different between their patient and reference groups.

Geerling *et al.* noted that the changes in fatty acid profiles they observed could be explained by alterations in the activities of the desaturase or elongation enzymes brought about by steroid therapy, as Δ^6 desaturase activity is decreased by steroids (Brenner, 1981). This could explain the higher proportions of DPA accompanied by relatively lower DHA proportions observed in these patients. They concluded that the altered fatty acid profiles were a result of altered metabolism and not EFA malabsorption.

A further study by Geerling *et al.* (2000) investigated the effects of a liquid supplement, containing either antioxidants or antioxidants and *n*-3 PUFA, on long-term CD patients in remission. The group assessed antioxidant status and fatty acid composition of plasma PL before and after the 3 month supplementation period. 37 patients and 70 controls were randomly assigned (double blind) to receive a placebo formula, antioxidant formula (AO) or antioxidant and *n*-3 PUFA formula (AO/*n*-3). Disease activity of the CD group was assessed by the CDAI and measurement of CRP. They found that several antioxidants (i.e. β-carotene, vitamin C & E, selenium, zinc, copper, glutathione peroxidase and superoxidase dismutase) were lower in the CD group than the controls at the start of the study. After supplementation with either the AO or AO/*n*-3 formula, antioxidant status was improved in the CD patients such that there were no differences between them and the control group. They also noted a significant increase in the proportion of EPA and DHA in plasma PLs in the AO/*n*-3 group. This also led to an increase in the total proportion of *n*-3 PUFAs in the plasma PLs of this group. These changes were accompanied by a decrease in the proportion of ARA. Thus, *n*-3 PUFAs (i.e. EPA and DHA) were incorporated into plasma PLs at the expense of *n*-6 PUFAs, especially ARA.

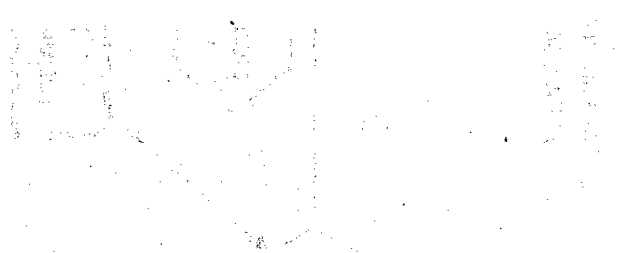
They suggested that fish oil is anti-inflammatory principally through its ability to decrease the amount of ARA in PLs and may be beneficial in changing the eicosanoid pattern in these patients.

More recently, a study by Trebble *et al.* (2004) described the plasma PC fatty acid profiles of CD patients with either active or quiescent disease. This is one of the few studies which also made a concurrent assessment of habitual dietary intake by administering a FFQ. The intake of dietary fat between the two patient groups and the control group was not found to be different. The CD patients were stratified to the active or quiescent disease group using a combination of laboratory markers (CRP and Erythrocyte Sedimentation Rate, ESR) and by CDAI. The group employed lipid extraction and separation techniques very similar to those described in this thesis. The fatty acid composition of plasma PC was presented using concentration (g/100g fatty acids) rather than the more commonly used percentage terms. The authors noted trends for lower ARA and DHA concentration and higher EPA concentration in the quiescent group when compared to the control group, although these were not statistically significant. However, comparison of fatty acid data between the active CD group and controls revealed significantly lower concentrations of LA, DPA and DHA and a higher concentration of DGLA. The lower DHA concentration was consistent with the findings reported by Geerling, but again conflicted with the higher DHA levels reported in Esteve-Comas' patients. The author suggested a possible reason for this could be the analysis of different plasma lipid pools and varying disease severity between the studies.

From the few studies outlined above it can be clearly seen that there is considerable disagreement of results between them. Esteve-Comas *et al.* twice reported higher *n*-3 PUFA percentages in their IBD patients, whereas Geerling *et al.* and Trebble *et al.* noted almost the opposite. There appears to be much disparity between studies in terms of study design and patient characteristics, methods of analysis and other factors (i.e. assessment of dietary intake and biochemical markers of inflammatory status) all of which may contribute to the variation in results and the subsequent conclusions of the investigative groups. The differing results from these groups have lead each to postulate a different

causal mechanism for altered fatty acid profiles in patients with inflammatory disease (Figure 2.16). In addition, Geerling clearly believes there is evidence to support *n*-3 PUFA supplementation in IBD patients, where Esteve-Comas strongly refutes such suggestions.

It seems that the same basic hypothesis underlies all of these studies: altered fatty acid profiles are a result of inflammation and maintenance of remission or severity of disease are somehow linked to *n*-3 and *n*-6 PUFA metabolism. However, which of these mechanisms (if any) actually exist *in vivo* still remains to be elucidated. It may be that all of the proposed metabolic changes interact in varying degrees. Perhaps what is more likely is that different patients exhibit different metabolic disturbances which alter their PUFA metabolism to varying extents. This could partly explain the apparent contrasting findings of several of these studies. However, it is also possible that fatty acid profiles could also influence the initial development of the disease and perhaps this is supported by the increasing incidence of inflammatory diseases in populations where *n*-3 PUFA intakes are falling. It is possible that lower intakes of *n*-3 PUFA could be one of the risk factors for developing such diseases.



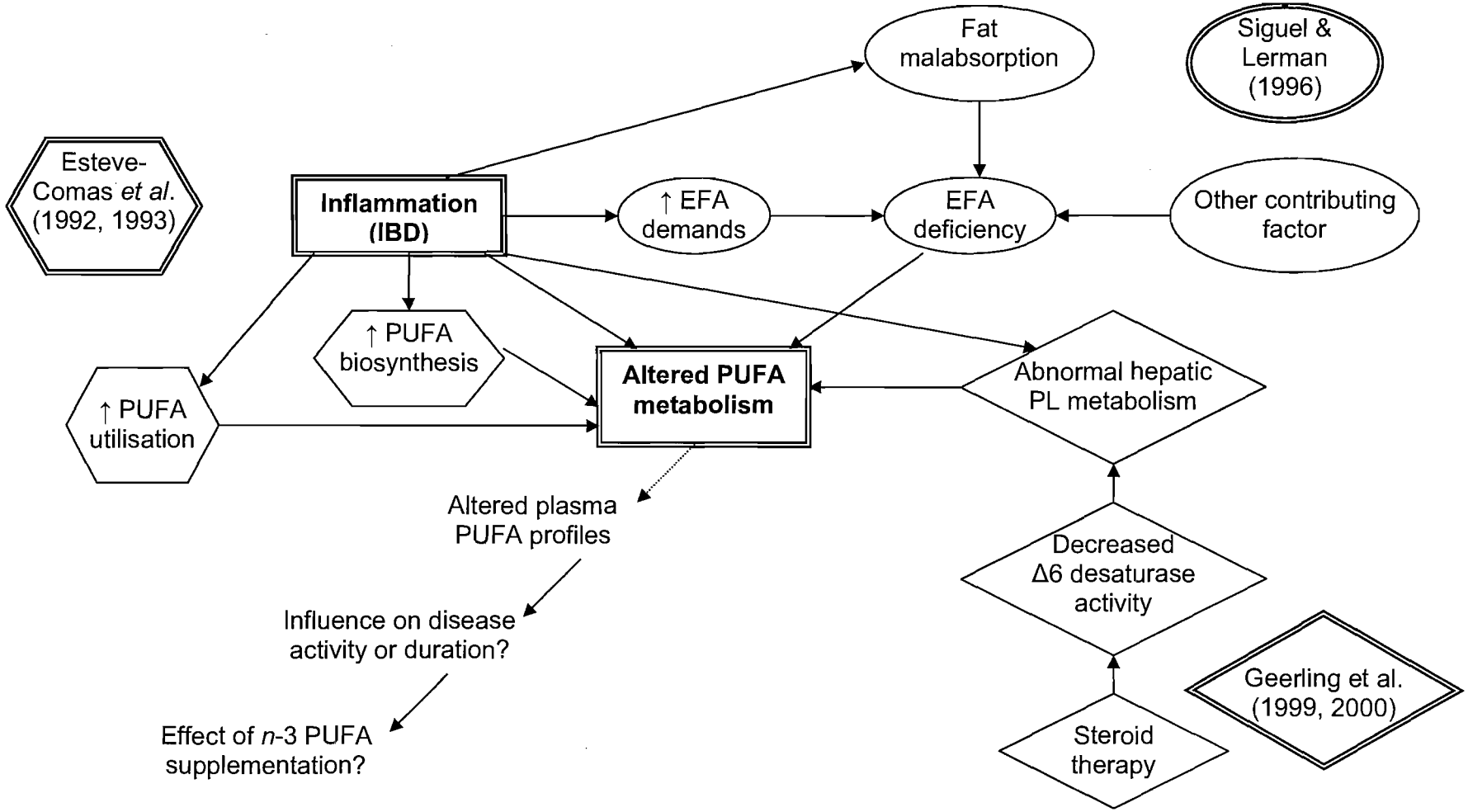


Figure 2.16 Hypothesised mechanisms of metabolic alterations observed in inflammatory bowel disease (Esteve-Comas et al., 1992; Esteve-Comas et al., 1993; Geerling et al., 2000; Geerling et al., 1999) and (Siguel and Lerman, 1996).

2.10.2 STUDIES OF FATTY ACID PROFILES IN IBD PATIENTS WITH COLECTOMY

Although there are only few studies that have investigated fatty acid profiles in active and dormant UC and CD, even fewer have studied patients with a previous history of the disease who have undergone radical bowel resections as curative treatment for their disease. In fact there appear to be just two papers reporting the fatty acid profiles of colectomy patients.

The study of Esteve-Comas *et al.* (1993) which investigated IBD patients in remission also included a small group of UC patients with colectomy (n=15). Blood samples were taken three to six months after colectomy surgery. They reported higher (but not significant) concentrations of LA and ARA and of ALNA and EPA. They also noted significantly higher DHA concentrations in the colectomy patients compared to controls. Interestingly, DHA concentration in the colectomy patients was lower than that observed in the UC patients in remission. Proportionate values for the fatty acids followed a similar pattern. This pattern of fatty acid profiles was similar to that seen in the inactive IBD patients studied at the same time, but the changes were less marked in the colectomy group. They concluded that the persistence of higher DHA concentrations, even after colectomy surgery, was an outcome of a metabolic defect in IBD patients. But the possibility that plasma PUFA profiles could normalise over a period of time greater than 3 to 6 months was not ruled out.

A later study performed by the same group investigated the plasma fatty acid pattern in long term colectomy patients (Esteve *et al.*, 1998). They studied a group of 63 UC patients who had undergone colectomy surgery between 2 and 16 years before the study, and compared them to 30 healthy controls. The colectomy group were 32 with ileoanal reservoir (pouch) with and without pouchitis and 31 patients with ileostomy. Dietary intake of the study patients was not assessed. They determined the fatty acid profile in both total plasma lipids and in plasma PL (separated by thin layer chromatography, TLC). They reported significant increases in SFA concentration in total plasma lipids. These increases were more marked when the data were expressed as %s. There was also a slight (non significant) increase in DGLA concentration and proportion

and slight decreases in ALNA and EPA concentrations. The proportion of ALNA was also lower in the colectomy patients and this was significant in those with a pouch. A small but insignificant increase in DHA concentration was also noted, but no changes in proportion were observed. Esteve *et al.* reported the addition of an internal standard for the quantification of absolute fatty acid concentrations and it appears that this was added at the time of GC analysis rather than during lipid extraction. But despite the use of this standard to allow quantification of fatty acid, fatty acid composition data for the plasma PL fraction were only provided in percentage terms. They reported significantly higher LA and ALNA proportions, slightly lower ARA proportion in the colectomy patients compared to the controls. There were no differences between the two groups in DHA proportion. It is interesting to note the influence of the methodological improvements in this study. Esteve-Comas *et al.* had previously reported almost non-detectable proportions of ALNA in their reference patients, but in this study, the reference value for ALNA in plasma lipids was approximately 0.5%. This was consistent with the findings and comments of Siguel & Lerman (1996). It is also interesting to refer back to the comments of Geerling *et al.* (1999) who argued that the composition analysis of total plasma lipids was less useful than that of plasma PLs, which they believed to be the functionally more relevant fraction. Esteve *et al.* observed a significantly lower ALNA proportion in the colectomy patients and no difference in LA proportion. However, in the PL fraction, ALNA and LA proportions were significantly higher in the colectomy patients. The group concluded that the fatty acid profile previously described in IBD was not observed in long-term colectomy patients, suggesting that it related to the presence of inflamed intestine.

Although not involved with fatty acid composition investigations, it is interesting to note the findings of a study by Nissinen et al. (2004). This group investigated the effects of different colectomy constructions on cholesterol metabolism. One of the three colectomy patient groups studied included patients with "conventional" ileostomy; such patients could be considered similar to the colectomy patients studied in this thesis. The group noted slightly lower plasma cholesterol concentrations in patients with colectomy. This was accompanied by higher TAG concentrations, although large variations within a small group (n=6)

of patients meant that the increase was not significant. The group also noted significantly lower % cholesterol absorption from the diet, accompanied by significantly higher cholesterol synthesis and an almost four-fold higher loss of bile acids when compared to normal controls. Although this study was limited only to cholesterol metabolism, it highlights the significant consequences in the handling of this compound in patients with colectomy who are otherwise considered to be well.

From all of these reported studies, one thing is clear: there is no accepted definition of 'fatty acid status' if indeed this concept has ever been fully considered. No groups studying IBD have attempted to establish a global picture of fatty acids (such as the fatty acid pools concept) which considers all of the sources and drains on circulating fatty acids. Using solely the fatty acid composition of whole plasma as a marker for whole body fatty acid status does not provide sufficient information, as the plasma really only represents a transport pool of fatty acids; it does not incorporate the storage and functional pools. Neither is it fully established what the functional significance of the composition of the fatty acid transport pool is. It is clear that further research is needed to establish the fatty acid composition of the different components of the transport pool in addition to considerations of other sources of fatty acids in the body.

2.11 SUMMARY OF LITERATURE AND AIMS OF RESEARCH

The review of the literature has attempted to outline the normal physiological processes involved in the digestion and absorption of dietary lipids, their subsequent metabolism and the effects of inflammatory bowel disease on these processes.

In order to satisfy the metabolic demands for fatty acids, in particular EFA, the following are required:

- An adequate dietary supply,
- A sufficient digestive and absorptive capacity of the intestine,

- The effective delivery of absorbed fatty acids to the liver or adipose tissue,
- The capability to form longer chain fatty acids from their EFA precursors (which in turn requires enzymatic machinery and appropriate cofactors),
- The effective mobilisation and transportation of fatty acids from the liver/adipose tissue to target cells and tissues

This assumes also that there are:

- No unusual fatty acid losses,
- No unusual fatty acid demands (e.g. for excessive PG synthesis or unusually high requirements for tissue maintenance and repair)

If there is an adequate supply of fatty acids with respect to the demands, then the balance of fatty acids and proper cell function are maintained.

It has been concluded that although the normal processes of digestion and absorption are well documented, fatty acid metabolism and the many metabolic pathways involved in maintaining normal fatty acid balance are less well understood. Further question surrounds the topic of fatty acid metabolism in inflammatory diseases, and especially those that have a direct effect on the functions of the GI tract.

It has already been reasonably well described that patients with IBD have perturbations to their fatty acid profiles, although some controversy surrounds the exact nature of this as some studies appear to contradict one another. However, it is conceded that methodological disparities may have some bearing on this, in addition to a degree of heterogeneity between the groups of patients investigated in different studies. Some groups argue that the PL fraction of the plasma is functionally the most relevant, but most groups report only the fatty acid composition of total plasma lipids. It was discussed earlier that the various lipid fractions each represent a different fatty acid pool of the body, each with its characteristic composition and function and no single fraction represents all pools. However, it is clear from the literature that other groups have not

considered the importance of studying the fatty acid composition of the plasma in its component parts because each fraction represents a different fatty acid pool with a specific function.

Current thinking is principally based upon a common assumption that perturbations in fatty acid profiles are a result of inflammatory disease and not a cause. Inflammation and malabsorption have both been suggested as contributors to EFA deficiency and subsequent alterations to PUFA metabolism. Conversely, steroid therapy and hepatic changes have also been implicated. However, it is also possible that altered fatty acid profiles may pre-exist the inflammatory condition rather than result from it.

However, it is very clear that there is a significant lack of research into fatty acids and IBD and this limits current understanding. Even less research has investigated patients who have undergone colectomy for severe IBD. There is evidence stating that colectomy patients often have disturbances to their lipid digestion and absorption capabilities, there has been very little research into the actual implications of this, not least with respect to fatty acid metabolism.

It is also not known whether patients with UC and CD present with similar features of fatty acid abnormality or if a different profile is exhibited by the two pathologies. Neither is it known whether patients with additional small bowel resection have a different pattern of fatty acids compared to those with no small bowel resection. It is not understood which other factors may also have an influence on fatty acid profiles in such patients.

To conclude, our understanding of the interaction between fatty acids and inflammatory bowel disease is not well understood. Many issues need to be addressed to help construct a better understanding of the role that fatty acids may play in the health and disease of IBD patients. From the limitations in the literature, it is clear there are many questions needing to be explored and answered in order to clarify some of the issues surrounding this topic. Some of these questions are those to be explored and discussed in this thesis, including:

- How does fatty acid composition vary between plasma lipid fractions?
- How does the way in which fatty acid composition data are expressed (i.e. as concentrations or relative proportions) affect the results?
- Is there an effect of resection of the terminal ileum on fatty acid metabolism?

2.11.1 HYPOTHESES

- Despite assumed and apparent good health, colectomy patients will exhibit alterations to the fatty acid composition of the plasma when compared to a reference group.

These disturbances may be due (wholly or in part) to:

- Altered exogenous supply of fatty acids to the body (i.e. altered dietary habits due to colectomy or stoma).

- Lipid maldigestion and/or malabsorption in the intestine due to colon removal (i.e. loss of terminal ileum or its function, decreased transit time).

- Altered metabolic handling of fatty acids, either due to altered hepatic metabolism or abnormal demands due to active inflammation.

These hypotheses are illustrated in Figure 2.17 showing the potential influences of these factors on fatty acid metabolism in the body.

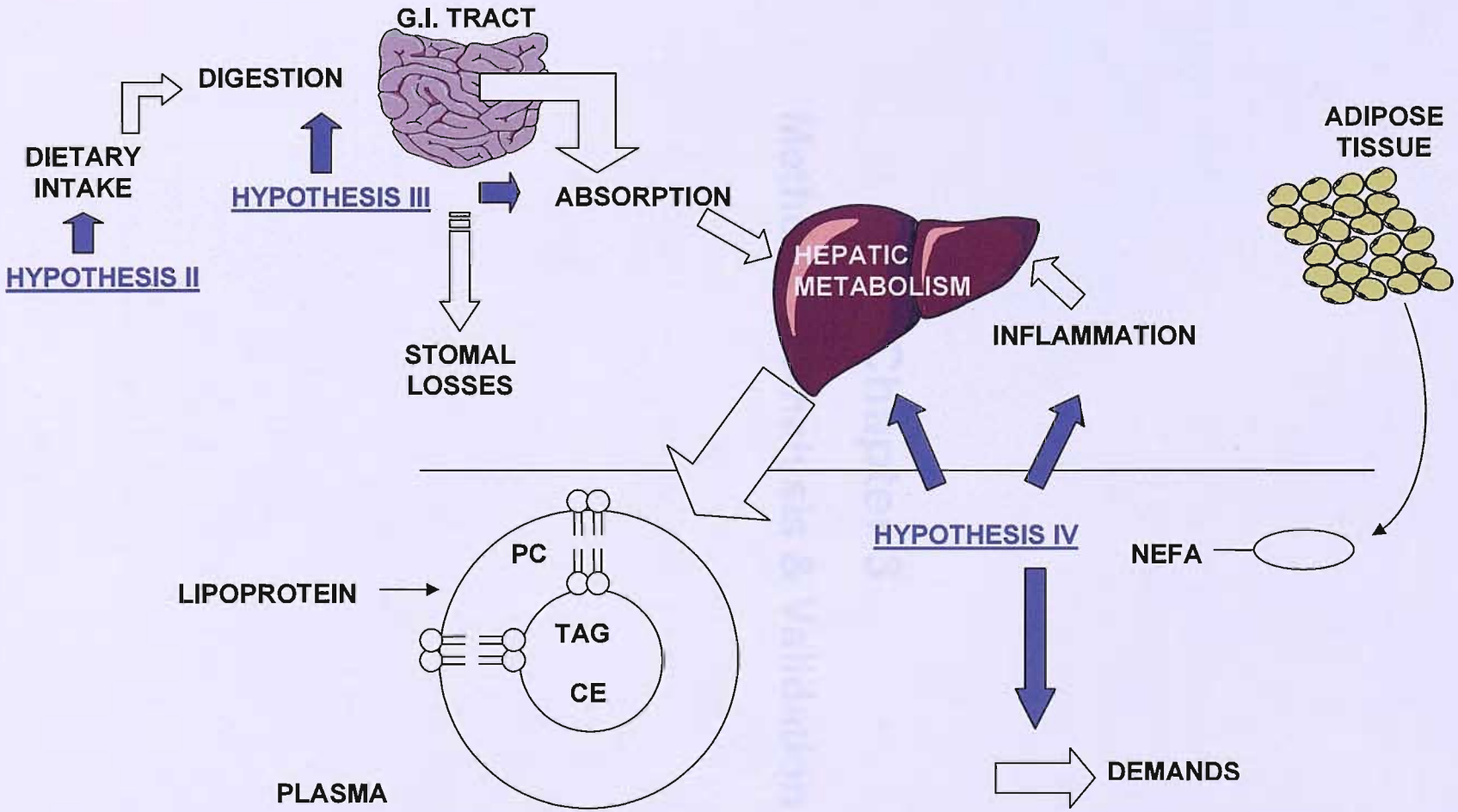


Figure 2.17 Hypothetical influences of colectomy on whole body fatty acid metabolism to be explored in this thesis.

Chapter 3

Methods of Analysis & Validation

3.1 INTRODUCTION

This chapter describes the methods employed for the recruitment of patients, the collection of information and samples from them and the analyses of these data. Firstly, an overview is given of the overall purpose of the study presented in this thesis, including the primary and secondary objectives. Following this, the methods of identification and recruitment of patients into the study are described, detailing the information and samples collected from both the reference and colectomy populations. Next, the methods of data and statistical analysis are presented, followed by a detailed account of the laboratory techniques employed for plasma fatty acid composition analysis by gas chromatography. Finally, evidence is presented to support the validity of the methods and the reproducibility of results.

3.2 STUDY OVERVIEW AND DESIGN

The overall purpose of the study was to identify and recruit a group of patients with colectomy, along with a corresponding group of healthy adults to act as a reference group. The main aim was to then take blood samples from all subjects and to perform fatty acid composition analyses.

3.3 OJBECTIVES

3.3.1 PRIMARY

- To determine the fatty acid composition in plasma PC, TAG, NEFA and CE of reference and colectomy groups.
- To compare the composition data with that of other published works.

3.3.2 SECONDARY

- To establish the dietary intake of reference and colectomy subjects.
- To investigate a possible effect of inflammation on fatty acid profiles in colectomy patients.

- To establish if stomal losses may influence fatty acid profiles.
- To consider the influence of small bowel resection on fatty acid composition in colectomy patients.

3.4 METHODS

3.4.1 PATIENT IDENTIFICATION AND RECRUITMENT

The patients selected for investigation in this study were recruited from a population available through Southampton General Hospital.

161 patients with who had undergone total colectomy with ileostomy within the six years prior to enrolment were identified from a surgical database and gastroenterology outpatient clinic at Southampton University Hospitals Trust (SUHT) (Figure 3.1). From these, 121 sets of medical notes were reviewed by a clinical research physician and 76 patients were identified as suitable for inclusion into the study. Exclusion criteria were:

- Patients with ileostomy reversal with pouch formation, ileorectal or ileoanal anastomosis.
- Patients with insulin-dependent diabetes mellitus.
- Patients who had moved out of the county (Hampshire).

Those patients who were identified as suitable for inclusion were invited to participate in the study by letter followed by a phone call. 60 patients agreed to take part and mutually convenient appointments were arranged for them to attend the Clinical Metabolism and Nutrition Unit (CMNU) at Southampton General Hospital. Participants were provided with patient information sheets explaining the purpose of the study and informed consent was obtained once procedures had been explained and any patient questions had been answered.

This study received ethics approval (086/00) from the Southampton and South West Hampshire Research Ethics Committee on 4th April 2000.

Of the 60 patients recruited onto the study, 36 blood samples were randomly selected for laboratory fatty acid analysis. Details of how blinding and anonymity were achieved are described later (Section 3.4.6).

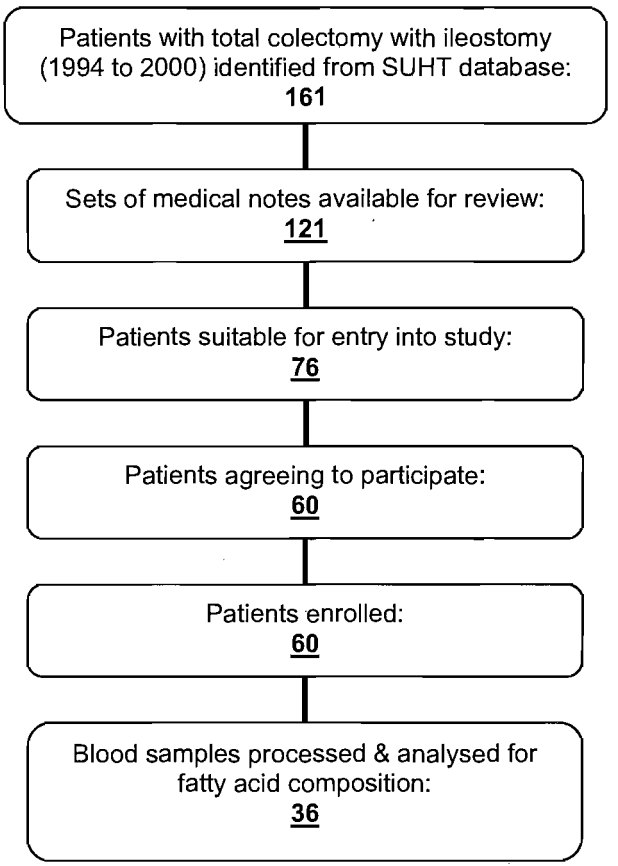


Figure 3.1 Flow process showing the availability and selection of colectomy patients for entry into the study.

3.4.2 PATIENT INTERVIEW AND DATA COLLECTION

All colectomy patients were sent a FFQ with instructions for completion prior to attendance at the CNMU. Completed questionnaires were collected and the data entered into a database. Patients were also requested to fast from 10:00pm on the night prior to the study.

Upon inclusion into the study, each subject was assigned a unique identification number that provided strict anonymity. A confidential master patient

identification list was maintained securely within the Human Nutrition Department at Southampton General Hospital.

The patients were asked about their general health and well-being including basic information regarding their weight and diet.

A physical assessment was also made and included measurement of height using a stadiometer and weight using digital scales (details for both in appendix). The scales were calibrated using a range of weights of known mass. The stadiometer was self-calibrating. The same equipment was used for the duration of the study period to ensure consistency of measurement. Body mass index (BMI) was determined as the Quetelet's Index (kg/m^2).

3.4.3 REFERENCE GROUP

The reference subjects were a group of 54 healthy adults recruited from within the Southampton area. The reference subjects were asked to complete a FFQ and asked to fast from the evening before blood samples were taken as described in Section 3.4.4. The reference subjects did not undergo clinical assessment but a physical assessment was performed as previously described. As per the colectomy patients, each subject in the reference group was assigned an identification number to maintain confidentiality.

3.4.4 SAMPLE COLLECTION

Reference Group

A fresh blood sample was collected by venepuncture using the vacutainer system and using lithium heparin as the anticoagulant. Samples were processed immediately for isolation and composition analysis of plasma PC, NEFA, CE and TAG as described in 3.4.8.

Colectomy Group

Fresh blood samples were collected from the colectomy patients by venepuncture using the vacutainer system. Samples were collected for fatty

acid analysis as per the reference group. Additional samples were also collected (as above) and immediately delivered to the hospital pathology laboratory where the following assessments were made:

- CRP
- ESR
- Cholesterol
- TAG
- HDL
- LDL

3.4.5 DATA ANALYSIS

Data captured from the patients were entered into a Microsoft Access database or an SPSS spreadsheet, both designed by the author. Statistical analyses were performed also using SPSS for Windows. Graphical images were created using either Excel or GraphPad Prism. Details for all software used are included in the appendices.

3.4.6 RANDOM SAMPLE SELECTION

Constraints of both resources and time unfortunately did not permit the analysis of all 60 colectomy and 54 reference patient samples. Therefore, a blinded random selection of 36 subjects from each group was made.

Variability in plasma lipids in response to hormonal changes during the menstrual cycle have been reported by several groups (Reed *et al.*, 2000). This generally means that non-pregnant and pre-menopausal women are excluded from research trials investigating fatty acid metabolism due to the influence of hormones on fatty acid concentrations. It would not have been possible to recruit sufficient patients into the trial if post-menopause was an inclusion criterion. Neither would it have been appropriate to exclude all female patients of childbearing potential. Therefore arbitrary age limits were applied to aid selection of patients from the two groups; patients younger than 25 years or

older than 75 years were not included in the analysis so as to attempt selection of the most representative group. This excluded 6 of 60 colectomy patients and 8 of 54 reference patients.

The next step was to randomly select 36 patients from each group in such a way that all subject samples had an equal chance of being chosen for laboratory analysis. Subjects were initially grouped by population (i.e. reference or colectomy) and by gender. The author was then blinded to all patient information other than the unique identification numbers, which were subsequently randomly ordered in the Access database. Using the 'RANDBETWEEN' function in Excel, a list of 36 random numbers between (and including) 1 and 54 and 1 and 46 was generated for the selection of subjects from the colectomy and reference groups, respectively. This ensured full blinding and no bias in the selection of subjects from both the colectomy and reference populations.

3.4.7 STATISTICAL METHODS

All statistical processing was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

The fatty acid composition data obtained from this study were tested for normality before any other statistical tests were selected. The Kolmogorov-Smirnov test was performed on the data for the Reference and Colectomy groups for all fatty acid concentrations and proportions for plasma PC, TAG, NEFA and CE (proportions only). These tests showed that the plasma PC data were largely normally distributed. However, for the TAG data approximately half of the variables for the proportionate data were found to be not normally distributed, along with the majority of the absolute concentration data. Tests on the NEFA data showed that approximately half of the variables for both the absolute and proportionate data were not normally distributed. Lastly, the majority of the proportionate fatty acid data for CE were also found to be not normally distributed.

In view of these findings, comparisons of two data sets (i.e. reference versus colectomy group) were by Mann-Whitney U as opposed to the unpaired *t* test as normal distributions were not forced by transformation of the raw data.

For the three-way analyses (Chapter 7) the one-way analysis of variance (ANOVA) was used with Bonferroni's *post hoc* test for multiple comparisons.

Power calculations were not performed as this was primarily regarded as a pilot study. This was due to several considerations; firstly, that only a relatively small number of colectomy patients were identified by means of the search of the hospital database and this was without any consideration of suitability for participation and wiliness of the individual patients to enter into the study. In addition, constraints of both finances and time were of considerable bearing and were of some limitation to the final number of samples which could be processed and analysed. Finally, the paucity of the data available in this area in the published literature also limited the ability to perform a confident and useful power analyses.

All results in this thesis are presented as mean ± standard deviation or median (range) as indicated. For all statistical comparisons, differences were considered to be significant at $P<0.05$. Symbols representing P values are used consistently throughout to denote levels of significance: $P<0.05$ is denoted by *, $P<0.01$ by ** and $P<0.001$ by †.

3.4.8 PROCESSING OF HUMAN PLASMA LIPIDS FOR GAS CHROMATOGRAPHY - FLAME IONISATION DETECTOR ANALYSIS OF PHOSPATIDYLCHOLINE, TRIACYLGLYCEROL, NON-ESTERIFIED FATTY ACID AND CHOLESTERYL ESTER

Materials

Full details of the manufacturers and suppliers of the materials used are provided in the appendices.

Sample Collection and Storage

A fresh blood sample (10 ml) was collected by venepuncture from each patient entering this study, using the vacutainer system with lithium heparin as the anti-coagulant. Plasma was separated immediately or whole blood stored for a maximum of ten minutes at 4°C.Samples were processed immediately for isolation and composition analysis of plasma PC, NEFA, CE and TAG.

Preparation of Plasma

Plasma was separated from erythrocytes by centrifugation at 1125 *g*, for 15 minutes at 4°C. Plasma was collected by aspiration and lipid extraction was performed within one hour, or the sample stored at -20°C.

Plasma Lipid Extraction

Plasma lipids were extracted by a modified method of Folch *et al* (1957).

Internal standards di-pentadecanoyl PC (PC 15:0/15:0) (100 μg) in trifluoroethanol (TFE, 100 μl), triheptadecanoin (100 μg in chloroform-methanol, 2:1 v/v) and heneicosanoic acid (30 μg chloroform-methanol, 2:1 v/v) were added to plasma (1.0 ml) and mixed briefly by vortexing. Addition of these standards permitted quantification of fatty acid concentrations in the PC, TAG and NEFA fractions, respectively. No internal standard for the cholesterol ester fraction was added. Chloroform-methanol (2:1 v/v) (5.0 ml) containing butyrated hydroxytoluene (BHT, 50 μg/ml) as antioxidant was added. The preparation was mixed vigorously by vortexing and then shaken at room temperature for 15 minutes. 1 M NaCl (aq) was added (1.0 ml) and the organic and aqueous phases separated by centrifugation at 1620 *g*, for ten minutes at 14°C. The aqueous phase was removed, the organic phase collected by aspiration and then dried under N_2 at 40°C. The interfacial protein disc was homogenised in 0.9% (w/v) NaCl (aq) (1.0 ml) and chloroform-methanol (2:1 v/v) (5.0 ml). The organic and aqueous phases were separated and collected as before, combined with the organic phase from the previous extraction, and dried under N_2 at 40°C. Dried lipid extract was either stored at -20°C, or Solid Phase Extraction (SPE) was performed immediately.

Solid Phase Extraction of Phosphatidylcholine, Non-Esterified Fatty Acids, Triacylglycerol and Cholesteryl Ester

The isolated plasma lipids were separated by SPE by a method described by Burdge *et al* (2000).

Plasma lipid extracts containing appropriate internal standard were dissolved in chloroform (1.0 ml) by vortex mixing and applied to an aminopropylsilica column (100 mg packed silica per 1.0 ml cartridge) under gravity. The column was washed with chloroform (2 x 1.0 ml) and drawn through under vacuum. The void fractions were then combined and dried under N_2 at 40°C. PC was eluted with chloroform-methanol (3:2 v/v, 1.0 ml) under vacuum (Caesar *et al.*, 1988). The column was washed with methanol (1.0 ml) to remove residual PL and the NEFA fraction eluted with chloroform-methanol-acetic acid (50:1:1, by vol., 2.0 ml) under vacuum.

TAG and CE fractions were isolated in the void fraction from the first stage of SPE (above). A fresh column was pre-conditioned with hexane (4 x 1.0 ml). The void fraction was dissolved in hexane (1.0 ml) and applied to the column under gravity. The column was then washed with hexane under vacuum to elute the CE fraction. TAG was eluted with hexane-chloroform-ethylacetate (20:1:1 by vol., 2 x 1.0 ml) under vacuum.

The isolated lipid fractions dissolved in solvent were dried under N_2 at 40°C and either stored at -20°C or immediately trans-esterified to form fatty acid methyl esters (FAME).

Preparation of Fatty Acid Methyl Esters

FAME were prepared by incubation with acidified methanol.

Lipids isolated by SPE were dissolved in dry toluene (1.0 ml) by vortex mixing. Freshly prepared methanol, containing 20 ml H_2SO_4/L, (2.0 ml) was added, the preparation mixed well and incubated at 50°C for 18 hours (Burdge *et al.*, 2000). The mixture was cooled and neutralised with a $KHCO_3$ (0.25 M) and

K_2CO_3 (0.5 M) solution (2.0 ml), then mixed well by vortexing. FAME were isolated by adding hexane (2.0 ml) and the organic and aqueous phases separated by centrifugation at 1125 *g* for 10 minutes at 14°C. The hexane layer was collected by repeated wash steps, transferred to gas chromatography autosampler vials and dried under N_2 at room temperature. The samples were then temporarily stored at -20°C or prepared immediately for analysis.

Preparation for Gas Chromatography Analysis

FAME were prepared for GC analysis by dissolving in hexane.

Dried FAME samples were redissolved in dry hexane (100 µl, 100 µl, 30 µl or 500 µl for PC, TAG, NEFA and CE fractions, respectively) and mixed briefly by vortexing. For samples used to determine lipid recovery, tricosanoic acid methyl ester recovery reference standard (ME 23:0) in hexane was added in equal mass to the internal recovery standard and the samples mixed by vortexing. FAME were resolved on a Gas Chromatograph (with an autosampler) with Flame Ionisation Detection (FID). Peaks were identified by comparison of retention times with known FAME standards and areas defined by drawing baselines under the peaks. Peak areas were used to calculate recovery of internal standards and the concentration of individual fatty acids in each sample (methods described in appendices). The concentration of each individually measured fatty acid in relation to the sum of concentrations for all measured fatty acids calculated the relative proportion of each (%).

3.5 VALIDATION OF METHODS

3.5.1 INTRODUCTION

As with any analytical procedure, it is good practise to establish the degree of analytical variability that may be expected, prior to the processing of study samples. It was not possible to measure true accuracy using this particular analytical method. Therefore, analysis yielding high internal standard recovery and a low coefficient of variation was assumed to reflect a high level of accuracy.

There are two principal components to the methods used for this analysis; laboratory analysis (addition of internal standards, extraction, isolation and derivatisation of fatty acids) prior to separation and analysis by GC, and post-processing analysis (manual integration of fatty acid peaks on chromatograms after GC-FID analysis).

This validation study consisted of two main aims. Firstly, to establish that post-processing techniques were internally consistent. Secondly, that consistency of results existed for the analysis of several identical samples. This served to establish confidence that any differences detected between study samples existed because of real differences *in vivo* and were not an artefact of operator or mechanical variability in processing. The methods validated were those described in the previous section (3.4.8).

3.5.2 AIMS

1. To assess competence in i) post-processing and ii) laboratory analysis.
2. To establish ability and repeatability when recovering internal standards and fatty acids from a series of identical plasma samples.

3.5.3 DESIGN

Post-Processing Analysis

The ability of the author to perform consistent integration of fatty acid peaks on a single chromatogram needed to be established prior to the assessment of laboratory analysis techniques.

A single plasma PC chromatogram was selected for this assessment. Three fatty acid peaks (PA, DPA and DHA) were manually integrated and peak area calculated on six different days by the author. These fatty acid peaks were selected because of the peak size and area they represent on a typical plasma PC chromatogram; PA being very large, DPA quite small in comparison and DHA yet smaller. Small differences in the integration of larger peaks would impact operator variability to a lesser extent than variation in the integration of

smaller peaks. Values for the coefficient of variation (CV) were calculated for the integration of these three fatty acid peaks. Results are provided in Table 3.1.

Laboratory Analysis

A plasma sample was taken from a single source, divided into eight aliquots and processed in parallel immediately using the methods described in Section 3.4.8. PL standards were each obtained from a single batch to ensure consistent quality and concentration. PL standard recovery, plasma concentration and relative proportion (%) of fatty acids and CV of these values were calculated. Results are presented in Table 3.2, Table 3.3 and Table 3.4, respectively.

3.5.4 RESULTS: POST-PROCESSING ANALYSIS

Table 3.1 summarises the CV for repeated integration of fatty acid peaks PA, DPA and DHA. CV values were <0.01, 0.21 and 0.24% for repeated integration of PA, DPA and DHA peaks, respectively (raw data provided in appendices).

	FATTY ACID PEAKS INTEGRATED		
	PA	**DPA**	**DHA**
Peak Area Count (Mean±SD)	644613±44	25520±54	76006±185
Coefficient of Variation (%)	<0.01	0.21	0.24

Table 3.1 Summary of results for the integration of three plasma PC fatty acid peaks on six different occasions

As expected, the degree of variation increased with decreasing peak area, as variations in the integration of smaller peaks represent a larger proportion of overall peak area. Overall, repeatability of the integration of the three peaks was high as CV were less than 0.3%. It was therefore assumed that any differences that may exist between samples in subsequent analyses would be due either to variations in laboratory analysis, or true differences between samples and not due to poor operator post-processing techniques.

3.5.5 RESULTS: LABORATORY ANALYSIS

Recovery of Internal Standard From Plasma Phosphatidylcholine, Triacylglycerol and Non-Esterified Fatty Acid

Table 3.2 summarises the recovery of PC, TAG and NEFA internal standard from their respective fractions, originating from eight aliquots of a single plasma sample (raw data provided in appendices). Mean internal standard recovery was 81.6, 65.4 and 82.2% with CV of 2.4, 5.5 and 10.9% from the PC, TAG and NEFA fractions, respectively. Internal standard recovery less than roughly 50% can cause an unfavourable reduction in chromatogram peak area and height. This sometimes results in small fatty acid peaks being indistinguishable from 'noise' in the baseline trace, making confident integration of such peaks impossible. However, it was observed that recovery of internal standard from all fractions was high, and in all cases, even the smallest peaks (ALNA, for example) were confidently identified and integrated. The recovery values obtained were also comparable to those of other laboratory technicians within the department.

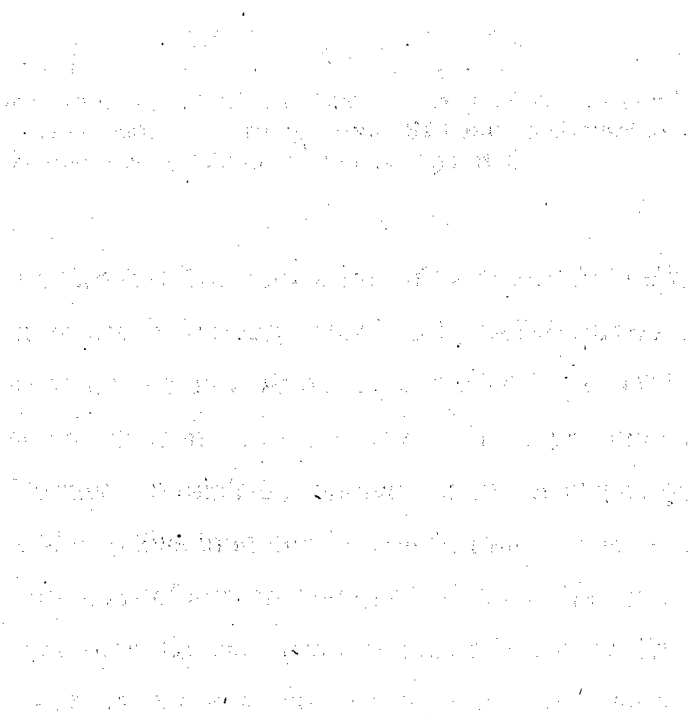
Internal standard recovery is assumed to be a good representation of true fatty acid recovery from the plasma sample, as a definitive measurement of fatty acid recovery is not possible. It could be argued that internal standards are more readily recovered from plasma than intrinsic lipids. However, this method is currently considered to be an appropriate proxy of fatty acid recovery from plasma lipids.

	Recovery of Internal Standard (%)		
	15:0 (PC)	**17:0 (TAG)**	**21:0 (NEFA)**
Mean±SD (Range)	81.6±2.0 (79.0-84.9)	65.4±3.6 (58.0-68.6)	82.2±9.0 (66.6-92.2)
CV (%)	2.4	5.5	10.9

Table 3.2 Internal standard recovery from plasma eight identical PC, TAG and NEFA samples.

Concentration of Fatty Acids in Plasma Phosphatidylcholine, Triacylglycerol and Non-Esterified Fatty Acid

The concentrations (µg/ml) of fatty acids in the TAG, PC and NEFA validation samples are summarised in Table 3.3, (raw data are provided in the appendices). Variations in fatty acid concentration were generally less than 5%, with higher variations seen for the smaller fatty acid peaks and in the NEFA fraction. This is because small differences in the integration of smaller peaks have a greater impact on overall variation than such differences in the integration of larger peaks. There was greater overall variation seen for the NEFA fraction because NEFA are found at a much lower concentration than TAG and PC in plasma.



	Concentration of Fatty Acid (µg/ml) in Lipid Fraction					
	PC		TAG		NEFA	
Fatty Acid	Mean ±SD	CV (%)	Mean ±SD	CV (%)	Mean ±SD	CV (%)
PA	753.1 ±15.6	2.1	428 ±10.4	2.4	15.0 ±1.1	7.2
SA	351.4 ±11.1	3.2	55.9 ±1.5	2.7	7.0 ±0.4	6.1
OA	243.1 ±9.1	3.7	639.4 ±13.5	2.1	28.2 ±1.1	4.0
LA	586.7 ±18.3	3.1	362.1 ±7.7	2.1	12.5 ±0.6	5.1
GLA	ND	ND	12.7 ±0.3	2.7	ND	ND
ALNA	6.6 ±0.3	4.1	18.8 ±0.5	2.4	2.5 ±0.1	4.4
DGLA	59.2 ±2.3	3.9	8.4 ±0.2	2.9	ND	ND
ARA	258.6 ±9.1	3.5	21.9 ±0.7	3.3	ND	ND
EPA	71.7 ±2.6	3.7	3.4 ±0.1	2.6	ND	ND
DPA	30.2 ±1.4	4.6	7.9 ±0.4	5.6	ND	ND
DHA	157.6 ±7.2	4.6	8.0 ±0.3	3.3	2.3 ±0.1	4.5
TOTAL	2518.3 ±76.5	3.0	1566.7 ±34.6	2.2	68.4 ±3.6	5.3

Table 3.3 Concentration (µg/ml) of fatty acids in plasma PC, TAG and NEFA validation samples. Values are mean, standard deviation (SD) and coefficient of variation (CV). Some fatty acids were not detectable in all fractions (ND).

Relative Proportion (%) Concentration of Fatty Acids in Plasma Phosphatidylcholine, Triacylglycerol and Non-Esterified Fatty Acid

The relative proportions of fatty acids (%) in the TAG, PC, NEFA and CE fractions are summarised in Table 3.4, (raw data are provided in the appendices). Variation in relative proportion of fatty acid was generally low (roughly 2%) and was less than that for concentration. This is likely to be due to differences in the mass of internal standard added to the samples, used to calculate concentration. CV was lower for fatty acids in the TAG and PC fractions, and is probably due to higher concentrations of these fractions in the plasma, making individual fatty acid peaks larger and easier to integrate.

	Relative Proportion of Fatty Acid (%) in Lipid Fraction							
	PC		TAG		NEFA		CE	
Fatty Acid	Mean ±SD	CV (%)	Mean ±SD	CV (%)	Mean ±SD	CV (%)	Mean ±SD	CV (%)
PA	29.9 0.3	1.0	27.3 ±0.1	0.5	22.2 ±0.5	2.4	12.6 ±0.1	0.4
SA	14.0 ±0.0	0.3	3.6 ±0.0	0.8	10.4 ±0.2	2.2	1.2 ±0.0	3.8
OA	9.7 ±0.1	0.8	40.8 ±0.1	0.3	41.7 ±0.5	1.2	20.4 ±0.2	1.0
LA	23.3 ±0.0	0.1	23.1 ±0.0	0.2	18.5 ±0.3	1.9	56.4 ±0.2	0.3
GLA	ND		0.8 ±0.0	1.7	ND		0.7 ±0.0	6.1
ALNA	0.3 ±0.0	1.4	1.2 ±0.0	1.0	3.8 ±0.1	3.1	0.5 ±0.0	1.5
DGLA	2.3 ±0.0	1.2	0.5 ±0.0	1.8	ND		0.7 ±0.0	3.5
ARA	10.3 ±0.1	0.5	1.4 ±0.0	1.7	ND		6.5 ±0.0	0.5
EPA	2.8 ±0.0	0.7	0.2 ±0.0	1.8	ND		0.6 ±0.0	1.7
DPA	1.2 ±0.0	1.7	0.5 ±0.0	6.0	ND		ND	
DHA	6.3 ±0.1	1.6	0.5 ±0.0	1.8	3.4 ±0.2	6.1	0.4 ±0.0	5.1

Table 3.4 Relative proportions (%) of fatty acids in plasma PC, TAG, NEFA and CE validation samples. Values are mean, standard deviation (SD) and coefficient of variation (CV). Some fatty acids were not detectable in all fractions (ND).

Processing of eight identical plasma samples for TAG, PC, NEFA and CE fatty acid analysis showed that overall there was a small degree of variability in the composition of the fractions. There was greater variability in the calculation of fatty acid concentrations than for relative proportions. This was most likely due to small variations in the mass of internal standard added to each sample, as the area of the internal standard peak was used directly to calculate fatty acid concentrations (μg/ml). This variation was impossible to quantify, but the pipette used to administer the internal standard into the sample was validated, thus variations in the volume of solvent administered (containing dissolved standard) was minimised, and the standard was thoroughly mixed with solvent to homogenise the solution as fully as possible.

3.6 CONCLUSION

From the results presented above, the overall variation that can be seen in the results for fatty acid composition data was considered small enough for there to be confidence that differences observed between study populations would be due to true differences and not analytical or operator variability.

Chapter 4

Results I: Characteristics of the Study Subject Groups

4.1 INTRODUCTION

The purpose of this chapter is to describe the patients studied in this thesis. The patients were a group of adults with a previous history of UC or CD who had undergone surgical removal of the large bowel (colectomy). Some patients had also endured varying degrees of additional resection of parts of the small bowel. This chapter describes and compares the basic demographics of the two study groups followed by reporting the general health and blood measurements results for the colectomy patients.

4.2 QUESTIONS

- Is the colectomy group comparable to the reference group in terms of age, BMI and gender?
- What is the general physical status of the colectomy patients; is there any obvious concern for their health?
- Is there any evidence of active inflammation in the colectomy patients?

4.3 AIMS

- To describe the demographics and basic physical characteristics of both patient groups.
- To describe the general state of health of the colectomy patients.
- To measure and describe blood inflammatory markers and circulating lipid concentrations.

4.4 METHODS

Patients were identified and recruited as described in Chapter 3. Assessments of health and physical status were made and blood samples taken and analysed for inflammatory markers and blood lipid concentrations.

Subsequent to blinded random selection of 36 subjects from both the colectomy and reference groups, subject information was revealed and the characteristics of the populations analysed.

4.5 RESULTS

4.5.1 BASIC CHARACTERISTICS OF THE REFERENCE GROUP

The reference group consisted of 36 subjects, with an approximate 50:50 ratio of males to females (Table 4.1). Ages ranged from 27 to 64 years and BMI measured from a minimum of 19.6 kg/m^2 (healthy) to a maximum of 36.6 kg/m^2 (obese). According to the standard categories, none of the reference patients were underweight (Figure 4.1). Over 60% of patients were in the healthy BMI range and 12 patients were overweight. Two patients had a BMI greater than 30 kg/m^2, placing them into the obese category. Overall, nearly 40% of the patients in this group had a BMI greater than 25 kg/m^2.

Variable		
Number of subjects		36
Males/Females		19/17
Age range (years)		27-64
Mean (±SD) age		42.7±11.3
BMI range (kg/m^2)		19.6-36.6
Mean (±SD) BMI		25.4±4.0
BMI group (kg/m^2)	Underweight (<19)	0
	Healthy (19-25)	22 (61%)
	Overweight (25-30)	12 (33%)
	Obese (>30)	2 (6%)

Table 4.1 The basic demographic and anthropometric characteristics of the reference group.

4.5.2 BASIC CHARACTERISTICS OF THE COLECTOMY GROUP

The colectomy group consisted also of 36 patients, again with an approximate 50:50 ratio of males to females (Table 4.2). This group had a larger age range than the reference group and was significantly older ($p<0.001$). BMI range was also much wider in this group, with measurement from 14.3 (very underweight)

to 43.0 kg/m2 (very obese), but mean BMI was not significantly different from that of the reference group. The BMI measurements were divided into the standard categories (Figure 4.1). Only 3 of the 36 patients were classified as underweight with a BMI of less than 19 kg/m^2 and nearly 40% of patients had a BMI within the healthy range of 19-25 kg/m^2. 9 patients had a BMI which classified them as overweight and 10 patients had a BMI of more than 30 kg/m^2, classifying them as obese. Therefore, over half of the patients in the colectomy group had a BMI of over 25. It is clear that although mean BMI does not differ significantly between the reference and colectomy groups, the distribution of BMI measurements in each group is very different (Figure 4.1). A possible confounding influence of BMI is explored in more detail in Chapter 6.

Variable		Number of Patients
Number of subjects		36
Males/Females		19/17
Age range (years)		26-74
Mean (±SD) age		52.3±13.9†
BMI range (kg/m^2)		14.3-43.0
Mean (±SD) BMI		26.3±6.3
BMI group (kg/m^2)	Underweight (<19)	3 (8%)
	Healthy (19-25)	14 (39%)
	Overweight (25-30)	9 (25%)
	Obese (>30)	10 (28%)
Clinical reason for colectomy	Ulcerative colitis	21
	Crohn's Disease	15
Additional small bowel resection (>20cm)		13 (36%)
Months post colectomy (mean±SD, range)		15-252 85.7±54.7

Table 4.2 The basic demographic and anthropometric characteristics of the colectomy group. Mean age of the colectomy group was significantly different to that of the reference group, † = p<0.001.

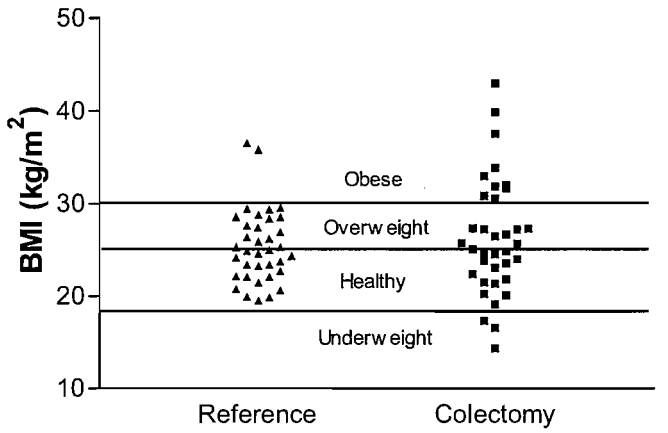


Figure 4.1 Categorised BMI measurements of the reference and colectomy groups; underweight = <19, Healthy = 19-25, Overweight = 26-30 and obese = >30.

The colectomy patients had all previously suffered severe IBD, necessitating the removal of the colon, and in some cases, additional resection of the small intestine. 21 colectomy patients (58%) were previous UC sufferers, and 15 (42%) had suffered from CD. 13 patients (36%) in the colectomy group had undergone additional small bowel resection (SB subset) in addition to their colectomy and ileostomy, the remaining 23 patients (64%) had undergone a straightforward colectomy with no significant small bowel involvement (Colectomy-Only, CO subset) (Table 4.2). When subdivided into UC and CD groups, 6 (29%) of the UC patients had undergone small bowel resection (>20 cm in length) in addition to colectomy as had 7 (47%) of the 15 CD patients (Figure 4.2). A higher percentage of patients in the CD group with small bowel resection was not surprising as CD often affects the terminal ileum. The ages and BMI of the 4 groups did not differ significantly from each other (Table 4.3).

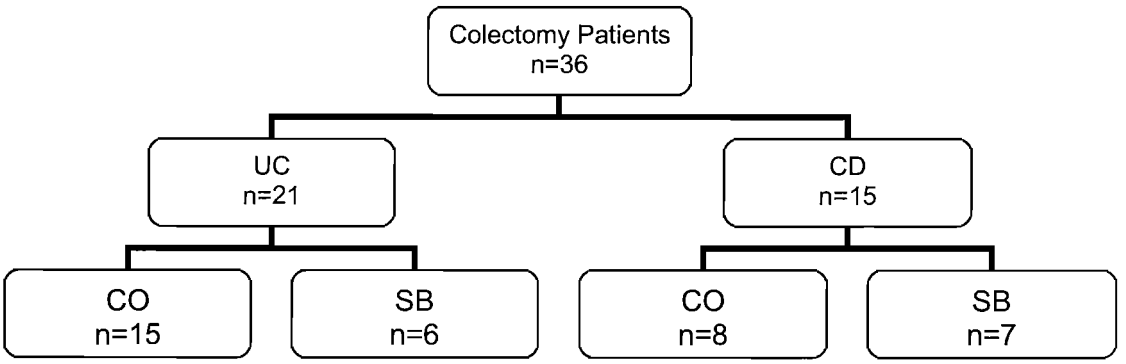


Figure 4.2 Distribution of colectomy patients showing previous IBD pathology and presence or absence of SBR in addition to colectomy.

	Subset	Age	BMI	Gender
UC	CO	55.0±14.9	26.0±5.0	9M, 6F
	SB	52.7±14.4	25.8±4.5	3M, 3F
CD	CO	52.6±15.2	26.8±9.5	4M, 4F
	SB	45.6±9.7	27.0±7.2	3M, 4F

Table 4.3 Age, BMI and gender characteristics of the sub-sets of the colectomy group (mean±SD). The age and BMI of the 4 sub-groups were not significantly different to each other (analysed by ANOVA with Bonferroni *post-hoc* analysis).

Inclusion criteria meant that all patients in the group were at least 12 months post-operative. Months since surgery ranged from 15 to 252 months (21 years), the mean was roughly 84 months (7 years) (Table 4.2). The majority of patients in the group had well-established colectomies over 50 months old (5 years) (Figure 4.3).

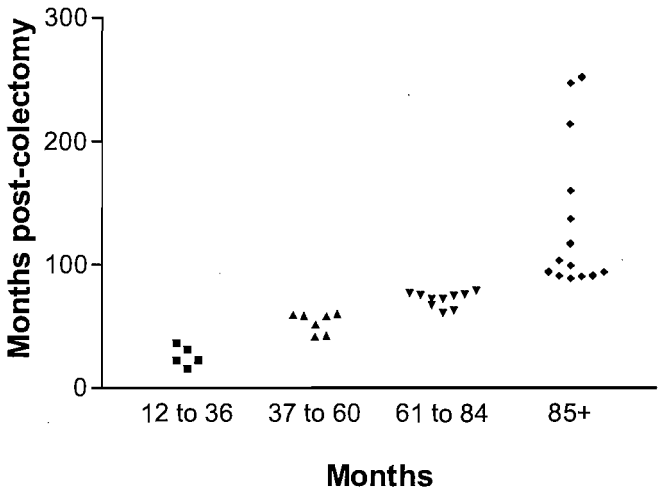


Figure 4.3 Period of time since colectomy surgery completion.

4.5.3 GENERAL HEALTH AND WELL-BEING REPORTED BY THE COLECTOMY GROUP

The patients were questioned on various aspects of their general health and well-being, the results of this are summarised in Table 4.4.

When asked about their diet, the majority of patients (94%) reported a good appetite for food; only 2 patients described their appetite as poor. Over half of the patients questioned (64%) stated that they did avoid specific foods because of their ileostomy (the most common reason was to minimise flatus production). Foods most commonly avoided included nuts and certain fruits and vegetables.

27 (75%) patients said that their recent weight pattern was stable. 6 patients reported recent weight loss and 3 reported recent weight gain. 6 patients in the group reported that they were currently on a weight-loss diet and not surprisingly, 5 of the six patients reporting recent weight loss were patients who were dieting. The single remaining patient on a diet described their recent weight pattern as stable.

Variable		Number of Patients
Appetite	Good/normal	34 (94%)
	Poor	2 (6%)
Specific food avoidance	Yes	23 (64%)
	Nuts	17 (47%)
	Some fruit/veg	13 (36%)
Recent weight pattern	Stable	27 (75%)
	Loss	6 (17%)
	Gain	3 (8%)
Dieting	Yes	6 (17%)
	No	30 (83%)
General health post-operatively	Poor	6 (17%)
	Fair	7 (19%)
	Good	23 (64%)

Table 4.4 Colectomy patients' perception of general health and well-being since colectomy surgery.

The patients in the colectomy group were questioned on their own perception of their general state of health (Table 4.4). 23 patients (64%) in the group said that they believed their general state of health since their colectomy and ileostomy operations was good. 7 patients (19%) said their health was fair and 6 reported poor health (17%).

4.5.4 BLOOD ANALYSES OF THE COLECTOMY GROUP

Some additional blood samples were collected from the colectomy patients in this study to be analysed for inflammatory markers and blood lipids. These samples were analysed by the hospital pathology laboratory. Due to financial constraints of the study, collection and processing of counterpart samples from the reference group subjects was not possible. Therefore, a reference data source was required. To provide sensible comparisons, the measurements of

TAG, cholesterol, HDL, LDL and CRP taken from the colectomy patients were compared against published data in the Health Survey for England (HSFE), 2003 (Blake et al., 2004). This survey collected non-fasting (for total cholesterol, HDL and CRP) and fasting (for LDL and TAG) blood samples from around 12,000 and 1,500 adults, respectively, living in private households in England.

Inflammatory Markers - CRP

CRP is an acute phase protein produced in response to injury or infection and is a good indication of general inflammation. In healthy people, CRP is usually <1 mg/L, but this can rise to as much as 1000 mg/ml during the acute phase response to injury or infection (Blake et al., 2004). CRP concentrations measured in the colectomy patients were compared against data published in the HSFE as described above. Statistical analyses were of course not possible as the raw data from the HSFE were not available.

On the contrary to what may have been expected for patients with a history of severe inflammation, the results from this comparison indicated that the CRP concentrations in the colectomy patients were indeed lower than those of the HSFE cohort (Table 4.5 and Figure 4.4). It was noted that the standard errors of the mean for the colectomy data were larger than that for the HSFE data and this was most likely due to the small sample size in the colectomy group.

Blood Assessment	**Men**		**Women**	
	HSFE	Colectomy	HSFE	Colectomy
CRP (mg/L)	3.1±0.12	1.3±0.46	3.8±0.24	3.3±1.37

Table 4.5 The CRP concentration in the blood of the colectomy patients and a reference population (HSFE) (Blake et al., 2004). Figures presented are mean±SEM.

Figure 4.4 The CRP concentration in the blood of the colectomy patients and a reference population (Blake et al., 2004). Bars represent the mean; error bars are SEM. Grey bars are the reference population; black bars are the colectomy group.

Inflammatory Markers - ESR

ESR can also be used as an indicator of non-specific inflammation. Unfortunately, a suitable reference group against which to compare the ESR measurements obtained from the colectomy patients could not be identified. Therefore the results are compared to the normal range as determined by the analysing laboratory. 8 patients in the colectomy group had an ESR measurement that was above the upper limit of normal.

Measurement	Normal Range (SUHT)	Range	Mean±SD	Patients Outside Normal Range
ESR	0-19	2.0-62.0	13.1±12.6	>19 = 8 (22%)

Table 4.6 The ESR measurement from blood samples collected from the colectomy patients. The normal range quoted is the SUHT normal range applicable at the time of sample collection and processing.

Lipids

Blood samples collected from the colectomy patients were analysed for concentrations of cholesterol, TAG, HDL and LDL. As stated earlier, these measurements could not be taken from the reference group, so the results obtained from the colectomy patients were compared to data reported in the

HSFE (Blake et al., 2004) (data provided in appendix). As the HSFE data are reported according to specific age groups (25-34, 35-44, 45-54, 55-64 and 64-74 years), the colectomy data were divided into the same categories to aid comparison. It is pertinent to mention that by doing this, the individual sub-sets of the colectomy group contained very small numbers (Table 4.7) resulting in larger standard errors in this group. Due to the small number of colectomy patients in each sub-set, results should be interpreted with caution.

Age Group (years)	25-34	35-44	45-54	55-64	65-74
Males	0	3	4	5	6
Females	5	1	5	4	2

Table 4.7 Table showing the number of colectomy patients in each category when the group is divided according to the age groups published in the HSFE (Blake et al., 2004).

Total cholesterol concentrations of the colectomy group males were compared to the gender-matched HSFE reference population (Figure 4.5). This showed that the male colectomy patients in the 35-44 yr and 55-64 yr age groups had a lower average cholesterol concentration than the reference population. In the 45-54 yr and 65-74 yr age brackets, the cholesterol values between the groups were very similar.

When the comparison was repeated for females, similar findings were evident (Figure 4.6). In the majority of the age groups, cholesterol concentrations were similar, although in the colectomy group the values tended to be slightly lower. Only in the 55-64 yr age group was the cholesterol concentration in the colectomy patients slightly higher than in the reference population.

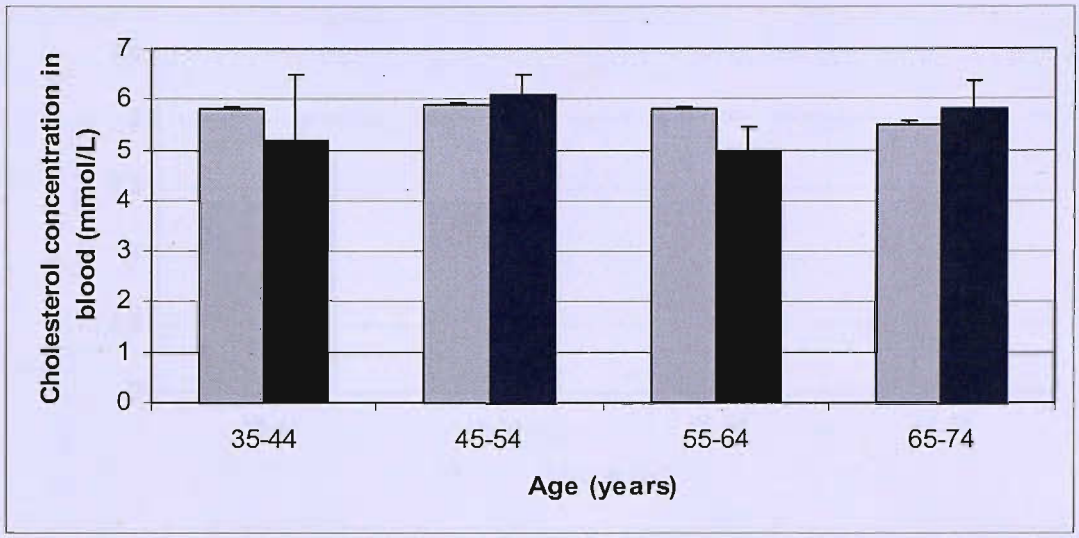


Figure 4.5 The total cholesterol concentration in the blood of the male colectomy patients (black bars) and a male reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

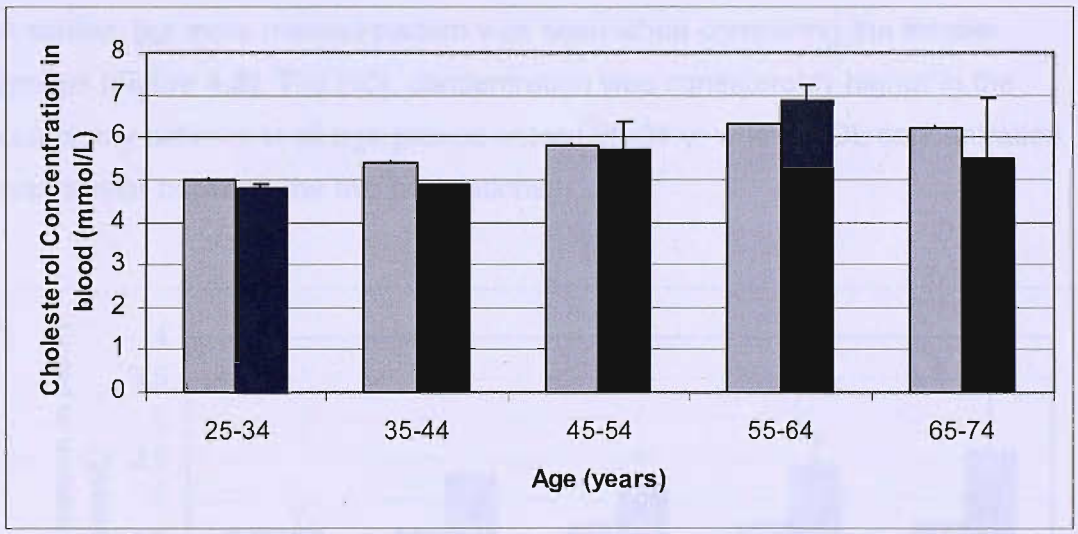


Figure 4.6 The total cholesterol concentration in the blood of the female colectomy patients (black bars) and a female reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

HDL concentrations in male colectomy patients were again compared to the HSFE reference population (Figure 4.7). This clearly showed a tendency for higher HDL concentrations in the colectomy group in all age groups except 65-74 yr where concentrations were similar.

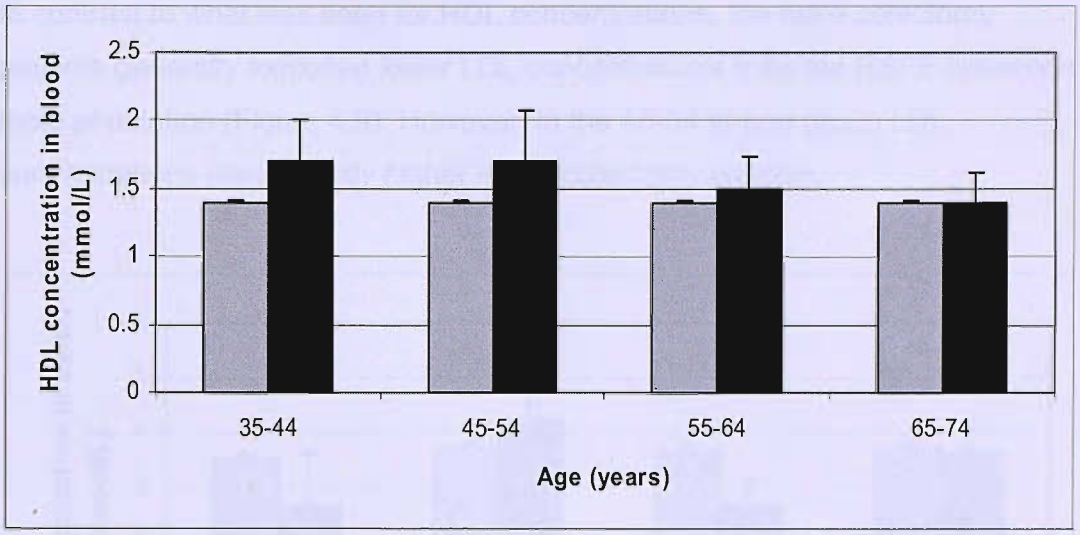


Figure 4.7 HDL concentration in the blood of the male colectomy patients (black bars) and a male reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

A similar, but more marked pattern was seen when comparing the female groups (Figure 4.8). The HDL concentration was considerably higher in the colectomy patients in all age groups except 25-34 yr where HDL concentration was similar between the two populations.

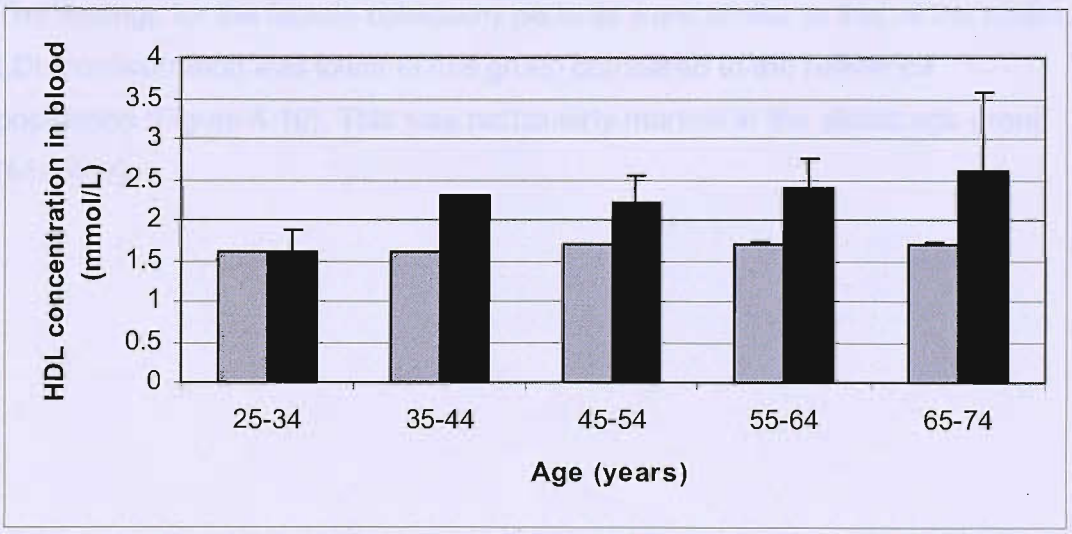


Figure 4.8 HDL concentration in the blood of the female colectomy patients (black bars) and a female reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

In contrast to what was seen for HDL concentrations, the male colectomy patients generally exhibited lower LDL concentrations than the HSFE reference male population (Figure 4.9). However, in the 45-54 yr age group LDL concentrations were slightly higher in the colectomy patients.

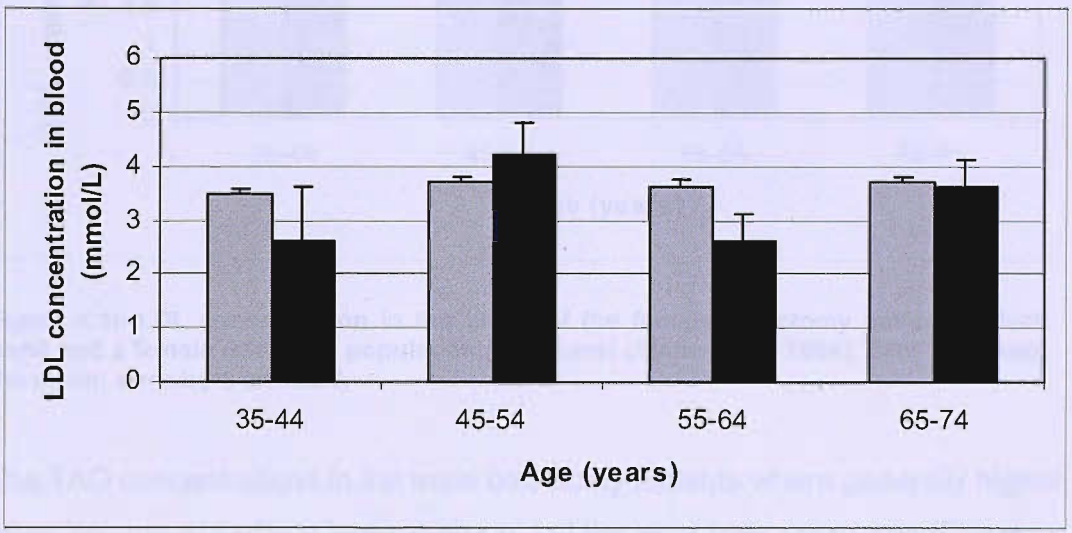


Figure 4.9 LDL concentration in the blood of the male colectomy patients (black bars) and a male reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

The findings for the female colectomy patients were similar to that of the males; LDL concentration was lower in this group compared to the reference population (Figure 4.10). This was particularly marked in the eldest age group (65-74 yr).

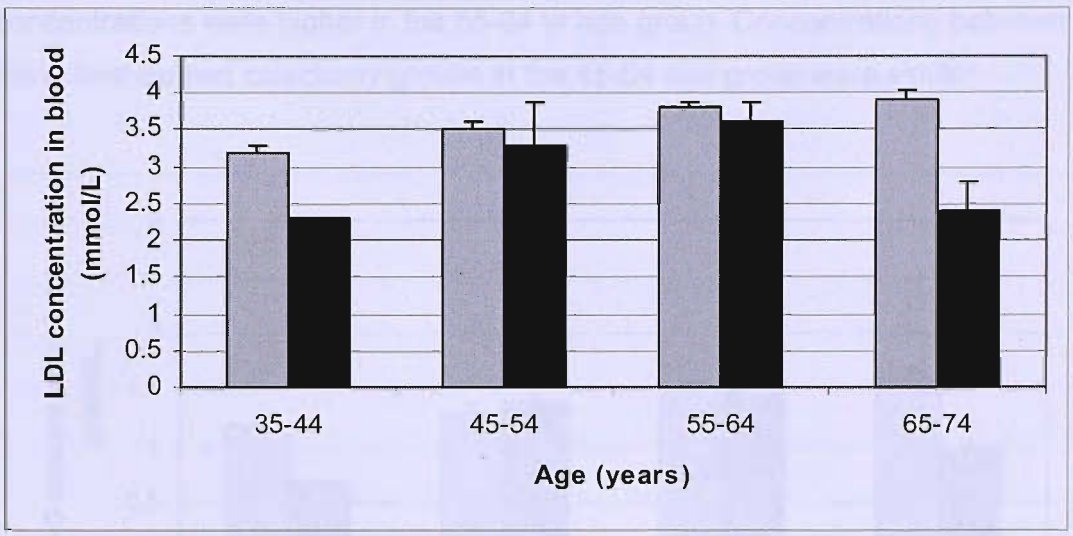


Figure 4.10 LDL concentration in the blood of the female colectomy patients (black bars) and a female reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

The TAG concentrations in the male colectomy patients where generally higher when compared to the reference group, but the error bars were large (Figure 4.11). This was particularly noticeable in the 45-54 yr age group.

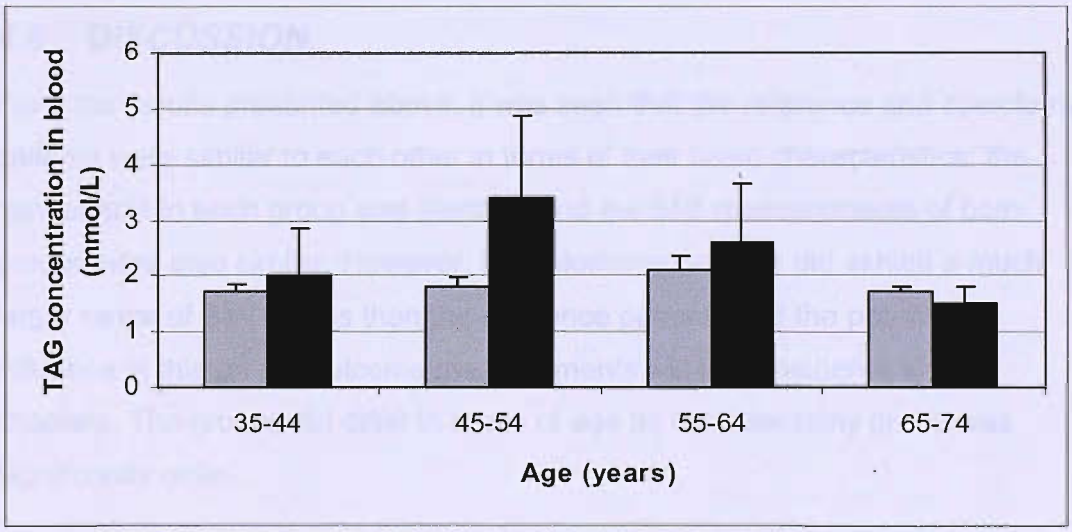


Figure 4.11 TAG concentration in the blood of the male colectomy patients (black bars) and a male reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

In the females, TAG concentrations of the colectomy patients were markedly lower in the 35-44 and 65-74 yr age groups (Figure 4.12). In contrast, TAG

concentrations were higher in the 55-64 yr age group. Concentrations between the reference and colectomy groups in the 45-54 age group were similar.

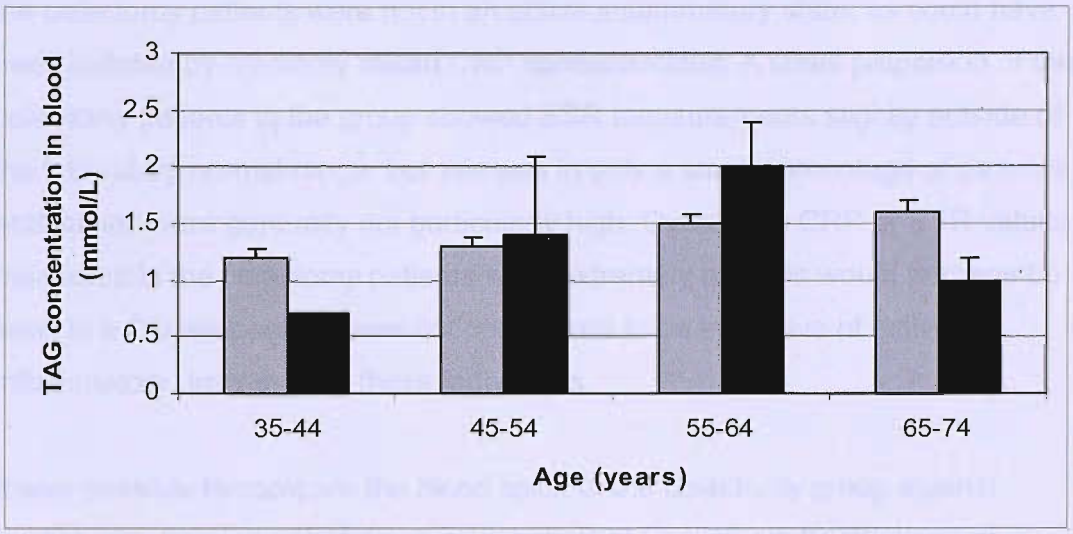


Figure 4.12 TAG concentration in the blood of the female colectomy patients (black bars) and a female reference population (grey bars) (Blake et al., 2004). Bars represent the mean; error bars are SEM.

4.6 DISCUSSION

From the results presented above, it was seen that the reference and colectomy patients were similar to each other in terms of their basic characteristics; the gender split in each group was identical and the BMI measurements of both groups were also similar. However, the colectomy patients did exhibit a much larger range of BMI values than the reference patients and the potential influence of this on the outcome measurements will be considered in later chapters. The groups did differ in terms of age as the colectomy group was significantly older.

Overall, the colectomy patients appeared to be in a generally good state of health; the vast majority of them reporting good health post-operatively, good appetite and stable weight patterns (other than those who were dieting). Few patients were very thin; almost a third had a BMI measurement classification of obese.

Comparison of CRP concentrations against the HSFE reference data showed that the mean values were lower in the colectomy group. This suggested that the colectomy patients were not in an active inflammatory state, as could have been indicted by markedly raised CRP concentrations. A small proportion of the colectomy patients in the group showed ESR measurements slightly outside of the laboratory normal range, but this was in only a small percentage of patients and values were generally not particularly high. Overall, no CRP or ESR values measured in the colectomy patients were extremely high, as would perhaps be seen in a CD relapse, so were not considered to be indicative of active inflammatory processes in these individuals.

It was possible to compare the blood lipids of the colectomy group against published reference data from a large cohort of English adults (Blake et al., 2004). In general, the results suggested that cholesterol concentrations of the colectomy patients were comparable to that of normal adults, although there was possibly a tendency for slightly lower concentrations in the colectomy group. These results were not suggestive of any major deviation in cholesterol concentration in the colectomy patients from what would be expected in adults of the same age.

Comparison of HDL and LDL concentrations between the two groups did show differences between the colectomy group and the reference population. Generally HDL concentrations in the colectomy patients were higher than those of the reference patients. In contrast, LDL concentrations were generally lower in the colectomy patients. Higher LDL and lower HDL concentrations in the population are generally accepted to convey a higher risk of cardiovascular disease (Assmann et al., 1996) so it is interesting to note that the colectomy patients do not appear to exhibit this more atherogenic lipid profile.

When considering plasma TAG concentrations, the male colectomy patients showed a slightly higher concentration than the HSFE reference group, although with this measurement, the standard errors seen in the colectomy group data were particularly large due to the small number of patients in the

group and large variation in TAG concentrations. It is possible that with a larger number of patients in the colectomy group that these data could be comparable to the HSFE figures and could therefore suggest that the TAG concentrations in the colectomy group are indeed representative of the general population.

Assessment of TAG concentrations between the female colectomy patients and the HSFE reference group was not so straight forward; both higher and lower figures were seen across the age groups in comparison to the HSFE reference data. It is possible that with a larger number of subjects in the colectomy group that TAG values may have been found to be similar between the two groups.

When interpreting the blood lipid results shown earlier in this chapter, it was important to consider that the reference data were taken from a very large number of subjects. However, the number of colectomy patients studied in this thesis is comparatively small. Larger standard error bars for the colectomy group data were noted in the graphs presented earlier and this was due to a very small number of subjects in each individual age group. However, these were the best data available which would allow some useful comparisons. Although definitive conclusions from the comparison of these two data sets must be made with caution, nevertheless, the results did provide a good indication of how representative the colectomy patients may be of a 'normal' or 'average' adult population. Overall, the cholesterol concentrations did generally seem to be comparable to the general population, but some potential differences in LDL and HDL were likely.

Of the four blood lipid measurement comparisons made, it was particularly difficult to compare the colectomy patients' TAG concentrations with the HSFE data. Firstly, because raw data for the HSFE data set were not available but also because of the very small number of colectomy patients in each age bracket. In addition, TAG concentrations in particular were very variable in the colectomy group leading to large standard errors. However, in the main results sections of this thesis (see later chapters), direct comparisons of plasma TAG concentration have been made between the colectomy patients and the matched reference group. Therefore, comparison with the HSFE data set for

this particular measurement just serves here as a rough indicator of how the colectomy patients' data compare to a large cross-section of the population.

4.7 CONCLUSIONS

Overall it was concluded that:

- The reference and colectomy groups were suitably similar to each other to allow direct comparison of the two groups' fatty acid data.
- Overall the colectomy patients reported being in a good state of health and appeared to be generally healthy.
- The colectomy patients did not show any signs of severe or significant inflammation.
- Some potential disturbances in blood lipids may be evident, but data were insufficient to draw definitive conclusions.

The next question to be answered following these results is whether the dietary intake of the colectomy patients is different to that of the references patients and indeed if the plasma fatty acid composition is different between the groups, despite the apparent well being of the colectomy patients.

Chapter 5

Results II: Dietary Intake

5.1 INTRODUCTION

The previous chapter described the basic health of the colectomy patients and it was noted that despite some indication of differing HDL and LDL concentrations in the blood, the colectomy patients did otherwise appear healthy. Almost all of the patients in the colectomy group reported a good appetite, although more than half said that they did actively avoid certain types of food as a result of their ileostomy. As the principal aim of this study was to investigate possible differences in the circulating fatty acid composition between a reference and colectomy group, it was necessary to attempt to first rule out the possibility that differences could be attributed to disparities in the dietary intake. In terms of fatty acid pools, analysing the diet will help to determine the exogenous supply to the body. Therefore, this chapter describes the dietary intake of the reference and colectomy groups

5.2 METHODS

Dietary intakes of energy, protein, carbohydrate, fat and micronutrients were assessed using a FFQ which was administered as described in 3.4.2 (blank questionnaire provided in appendices). The questionnaire included over 200 items of food and drink and had previously been used in another study where repeatability and validity against a 7-day weighed record were assessed (Shaheen et al., 2001).

The raw data were entered into a Microsoft Access database then converted into dietary intake values. The database was designed (and frequency data converted) by Dr Rachel Thompson, Public Health Nutrition, Institute of Human Nutrition, University of Southampton. The complete data for the reference and colectomy groups were split into males and females to eliminate variations due to gender differences. The results for reference and colectomy groups were analysed using t-test and compared males and females separately.

5.3 RESULTS

A summary of the results is shown in Table 5.1. As expected, the males of both groups had a higher total energy intake than their counterpart females. The

males of both groups had a very similar intake of approximately 12 MJ/day. The females in the colectomy group had a tendency for a lower energy intake than the reference females, but this was not significant. Both groups had a higher average total intake than the Dietary Reference Value (DRV) given by the Department of Health. However, this value is derived for males weighing 70 kg and females weighing 65 kg. Many of the patients in both groups were heavier than this so would therefore have a higher energy demand.

Protein intake expressed both as a total and as a percentage of total energy intake for the males was similar between the groups. Again, the figures for intake were much higher than the DRV, for the same reason stated above. The females of the colectomy group tended to have a lower total absolute intake of protein, but this contributed a higher percentage towards total energy intake than the reference females but this was not significant.

Both the total absolute carbohydrate intake and the percentage of this of total energy intake were slightly lower in the males of the colectomy group, but this was not significant. The percentage of absolute carbohydrate intake for both male groups was slightly higher than the DRV. The females in the colectomy group tended to have a lower total and a lower percentage of carbohydrate in the diet than the reference females but differences were not significant. Both groups of females had a percentage of carbohydrate that was higher than the DRV, but for the female colectomy patients, this was only marginally higher.

Total fat intake and percentage of total intake provided by fat were both similar between the reference and colectomy males. The percentage of fat in the diet was also similar to the DRV for these groups. The females in the colectomy group had a lower total fat intake compared to the reference females, but this was not significant. However, fat expressed as a percentage of total energy intake was significantly higher in the colectomy group ($p<0.05$) and was also slightly higher than the DRV of 33%.

Dietary SFA as both a total and as a percentage of total energy intake tended to be higher in the males in the colectomy group, but this was not significant. The

females in the colectomy group also had a higher SFA intake, but this was not significant. However, expressed as a percentage of total energy intake, the value for the colectomy females was significantly higher ($p<0.05$) and was also marginally higher than the DRV.

Total intake of MUFA was slightly higher in the colectomy males but slightly lower in the colectomy males when compared to their respective reference groups, but these differences were not significant. MUFA intake as a percentage of total energy consumed was similar between male groups. This value was slightly higher in the females of the colectomy group when compared to the reference group but this was not significant. All groups had an percentage intake of MUFA slightly below the DRV.

Both the males and females in the colectomy group had a lower intake of PUFA in comparison to their reference group counterparts. However, these differences were not significant. The percentage of energy intake that is PUFA was very similar between the male groups. The females in the colectomy group had a slightly higher percentage intake of PUFA in comparison to the reference females, but this was not significant. All groups had a percentage intake of PUFA slightly below the DRV.

Total intake of *n*-6 PUFA was slightly lower in both the males and the females of the colectomy groups when compared to the reference groups, but these differences were not significant. Intakes of *n*-3 PUFA were similar between both male groups. However, the colectomy females had a slightly lower intake than their reference group counterparts, but this was not significant. Therefore the slightly lower PUFA intake in the colectomy groups was mainly attributed to lower *n*-6 PUFA consumption in these groups, rather than lower *n*-3 intake. The ratio of *n*-6 PUFA intake to *n*-3 PUFA intake was similar for all groups.

Gender (M/F)	Nutrient Intake/day	Reference Group	Colectomy Group	DRV
M	Energy (MJ)	12.1±4.6	12.0±3.8	10.9[1]
F		11.3±5.1	9.4±3.2	7.8[2]
M	Protein (g)	107.7±40.3	111.7±42.0	55.5g/day
F		105.5±48.7	93.8±32.4	45.0g/day
M	Protein as % of Total Energy Intake	15.1±1.8	15.9±4.1	
F		14.8±3.3	17.0±4.2	
M	Carbohydrate (g)	363.8±129.3	345.9±108.8	
F		343.9±166.9	274.4±111.7	
M	Carbohydrate as % of Total Energy Intake	51.3±6.0	49.3±8.5	47%
F		51.4±6.9	48.4±11.8	
M	Fat (g/day)	112.7±55.8	112.9±44.9	
F		100.3±53.4	90.8±33.2	
M	Fat as % of Total Energy Intake	34.3±6.1	34.9±5.6	33%
F		32.9±5.1	36.8±7.1*	
M	SFA (g)	30.0±14.8	34.0±17.9	
F		26.7±13.5	28.2±12.9	
M	SFA as % of Total Energy Intake (%)	9.3±2.8	10.3±2.9	10%
F		8.8±2.5	11.2±3.1*	
M	MUFA (g)	29.7±12.9	30.0±12.8	
F		26.0±15.0	23.8±9.4	
M	MUFA as % of Total Energy Intake (%)	9.3±2.2	9.3±1.9	12%
F		8.5±1.7	9.7±2.4	
M	PUFA (g)	15.7±8.4	14.6±6.6	
F		13.1±7.1	11.2±5.2	
M	PUFA as % of Total Energy Intake (%)	4.7±1.2	4.6±1.4	6%
F		4.3±0.9	4.6±1.5	

Gender (M/F)	Nutrient Intake/day	Reference Group	Colectomy Group	DRV
M	*n*-6 PUFA (g)	13.3±7.8	12.5±5.8	Min 1% from LA
F		11.1±6.5	9.6±4.7	
M	*n*-3 PUFA (g)	1.8±0.9	1.8±0.8	Min 0.2% from ALNA
F		1.7±0.9	1.5±0.6	
M	*n*-6:*n*-3 ratio	7:1±3.0	7:1±2.8	
F		7:1±2	6:1±1	

Table 5.1 Summary of dietary intakes of the reference and colectomy groups. [1]Applies to male, 30-59 years of age, 75kg with Physical Activity Level of 1.5 times BMR, [2]applies to female, 30-59 years of age, 60kg with Physical Activity Level of 1.4 times BMR (Department of Health, 1994a). * $p<0.05$.

5.4 DISCUSSION

Despite a few small differences, the dietary intake of the colectomy group was largely similar to that of the reference group in terms of energy, carbohydrate, protein and fat. Quantitatively, the females in the colectomy group had a higher fat intake than the females in the reference group, but qualitatively these intakes were similar. In particular, there were no significant differences found between the reference and colectomy groups in the consumption of *n*-3 or *n*-6 PUFA.

5.4.1 SELECTION OF THE FFQ FOR DIETARY INTAKE ASSESSMENT

Despite limitations of every type of tool that could have been employed on this study, the utilisation of the FFQ was deemed to be the most appropriate. This was mainly because the interest was not in absolute quantification of intake but the need to compare the intakes of two subject groups, both believed to be consuming a broadly typical Western diet. This was largely supported by the recommendations presented in a consensus document prepared by experts in this field (Burley et al., 2000) (Table 5.2). By using such a tool, a large number of individuals can be evaluated and a better approximation of the usual diet of the population may be obtained (Margetts *et al.*, 1989). In addition, comparison studies with a 24-hour recall and weighed food diary have demonstrated reasonable agreement in nutrient intakes (Margetts *et al.*, 1989).

STRENGTHS	WEAKNESSES
Indication of usual dietary intake can be obtained	Memory of past eating patterns required
Questionnaire can be self-administered	Recall period may be imprecise
Does not affect normal eating patterns	Quantification may be inaccurate
High response rate	Questionnaire may not be suitable for individuals with atypical diets (e.g. ethnic minorities)

Table 5.2 Some of the strengths and weaknesses of the Food Frequency Questionnaire as a tool to assess dietary intake. (Adapted from Burley *et al.*, 2000)

5.4.2 VALIDATION OF THE FFQ

In this study, the intakes of all major food groups were similar, suggesting no major differences between the groups in fatty acid intake. However, this FFQ has not been validated for use in colectomy patients so the data were compared to data from a study of similar patients (Bingham *et al.*, 1982), to the National Food Survey 2000 (2001) and to the predicted Basal Metabolic Rate (BMR) (Table 5.3). Total Energy Expenditure (TEE) values were derived using predictive equations for BMR according to age, weight and gender (Department of Health, 1991), then multiplied by 1.4 or 1.5 for males and females, respectively, to account for physical activity (Department of Health, 1994a).

Study	Type of Survey	Energy (MJ/day)	Fat (g/day)
Current – Reference group	FFQ	11.4	106
Current – Reference group	Predicted TEE	9.8	N/A
Current – Colectomy patients	FFQ	10.8	103
Current – Colectomy patients	Predicted TEE	9.5	N/A
Bingham 1982	Weighed record	10.0	106
National Food Survey 2000	Questionnaire	7.3	74

Table 5.3 Comparison of mean energy and fat intakes of patients in this study with other published data.

Compared to the data published by Bingham *et al.*, both energy and fat intakes of colectomy patients in this study, as estimated by FFQ, were comparable. However, the estimates for energy intake in both the colectomy patients in the present study and those investigated by Bingham *et al.* were slightly less than the estimated energy intake of the reference group in the present study. Fat intakes across all three groups were similar. When compared to the National Food Survey, the energy and fat intakes for both colectomy groups and the reference group were substantially higher.

In contrast, the energy intakes of the reference and colectomy groups from the present study in comparison to the predicted TEE values seem to suggest that the FFQ may overestimate energy intake by around 1.5 MJ/day. Furthermore, 75% of the colectomy patients reported a stable weight pattern, suggesting that energy intake and expenditure in these patients should be similar. However, it is

important to consider that the TEE (and BMR) values are only a prediction, and based on an assumption of 'average' physical and occupational activity levels. Despite this potential overestimation of the FFQ, there was a reasonable correlation between predicted TEE and estimated energy intake as shown in Figure 5.1 and Figure 5.2. However, this was significant only in the reference group ($p<0.05$).

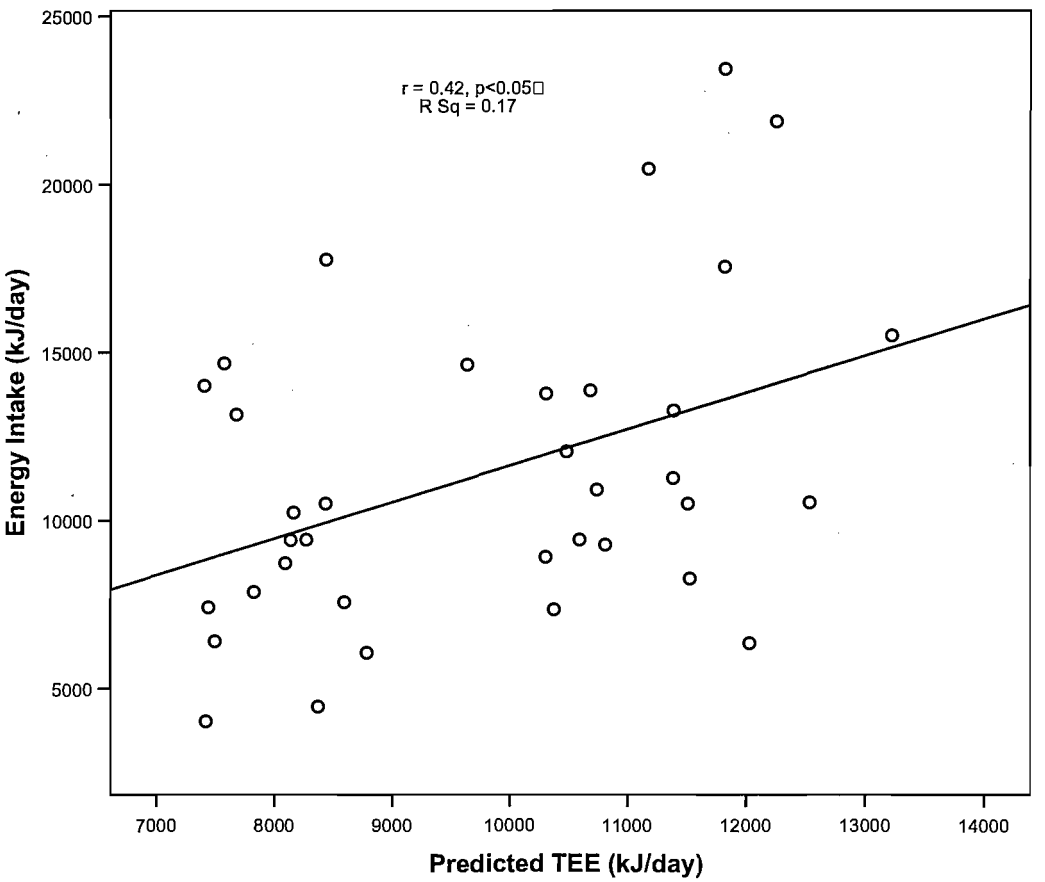


Figure 5.1 Relationship between predicted TEE (kJ/day) and energy intake (kJ/day) as estimated by FFQ questionnaire in the reference group.

Although there is good agreement between this and the study of Bingham *et al.*, and some reasonable correlation exists between estimated energy intake and predicted TEE, ideally, the FFQ utilised in this study would be fully validated for fat intake as this was really the dietary component of interest. Unfortunately, constraints of both finances and time did not permit any more sophisticated

validation work to be performed. In addition, validation of dietary lipid intake is not straightforward due to the many different types of fatty acid present in dietary lipids. Some groups have attempted to validate individual fatty acid intakes estimated by one or more intake measures (such as FFQ, diet history or 24-hour recall) by comparing with the fatty acid composition of adipose tissue samples (Baylin *et al.*, 2002; Cantwell *et al.*, 2005; Garland *et al.*, 1998; Knutsen *et al.*, 2003). Overall, the relationships between dietary intake assessments of fatty acid intake and adipose tissue compositions in these studies showed reasonable agreement, supporting the validity of such tools to estimate dietary lipid intake.

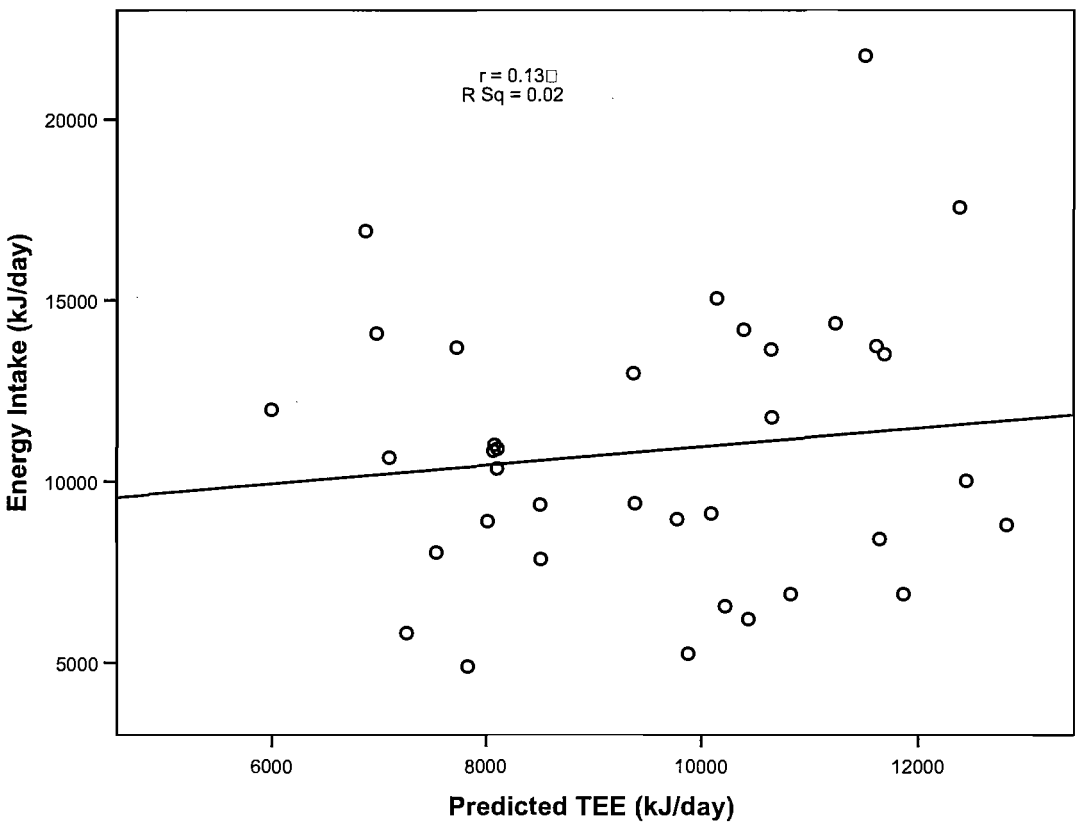


Figure 5.2 Relationship between predicted TEE (kJ/day) and energy intake (kJ/day) as estimated by FFQ questionnaire in the colectomy patients.

To be able to firmly conclude that differences in dietary intake could not account for any variations between the populations in plasma fatty acid composition, it would be necessary to assess the dietary intake of specific individual fatty acids

(such as EPA and DHA), rather than simple estimation of total *n*-3 or *n*-6 PUFA intake. Alternatively, a comparison of the consumption of the principal sources of *n*-3 PUFA, such as oily fish intake (rather than individual nutrients), could have provided valuable evidence to support the hypothesis that disparities in dietary intakes of lipid could not explain differences in plasma fatty acid composition. Unfortunately, the data available from the dietary intake questionnaires did not permit such detailed analyses, as the raw data which were originally entered into the database from the questionnaires were no longer available. The resources and finances required to obtain the archived original FFQ, as completed by the study subjects, and to re-enter and re-analyse these data for 72 individuals was prohibitive.

From the results available regarding dietary intakes of the two study groups, it is not possible to draw firm and definitive conclusions as the data are insufficient to support this. However, for a study such as this, it is important that dietary intake is assessed and despite the limitations, at least this was attempted and the results are able to provide some supportive evidence.

5.5 CONCLUSION

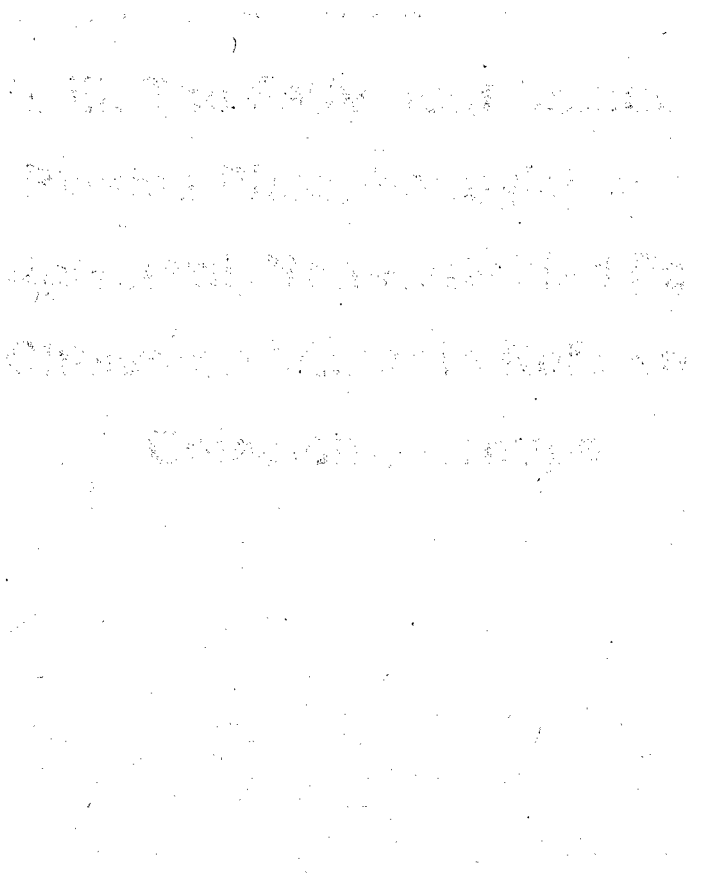
Overall, the results from the dietary intake analysis of the reference and colectomy groups indicated that intakes did not differ between them. However, the strength of the data available is limited and definitive conclusions were not appropriate on this basis. In general, the evidence available did not indicate significant variations between the two groups in qualitative lipid intake, in particular PUFA consumption. The results were not indicative of differences in PUFA intake of a magnitude which could potentially significantly influence plasma fatty acid composition. It is likely that the dietary intakes of the two groups were indeed similar and unlikely to account for potential differences in plasma fatty acid composition.

5.5.1 HYPOTHESIS II

- Colectomy patients will exhibit altered plasma fatty acid composition compared to a reference group and this will be due to: altered

exogenous supply of fatty acids to the body (i.e. altered dietary habits due to colectomy or stoma).

The evidence available suggested that differences in dietary intake between the two groups were unlikely. Therefore, it is also unlikely that the hypothesis is true.



Chapter 6

Results III: The Fatty Acid Composition of Plasma Phosphatidylcholine, Triacylglycerol, Non-Esterified Fatty Acid and Cholesteryl Ester in Reference and Colectomy Groups

6.1 INTRODUCTION

Alterations to plasma fatty acid profiles in inflammatory disease is well recognised, but considerably less well understood. There are several studies of patients with inactive and active IBD; both UC and CD, with colectomy or ileal resection or with other types of GI disease. However, patient cohorts vary widely, as do the criteria used for assessment and/or classification of disease activity. Some studies assess dietary intake, whereas others do not. Sample processing and analysis can differ considerably between groups and may have a substantial bearing on results and the subsequent conclusions drawn from them.

Rarely have studies measured fatty acids in terms of their concentration; most consider only the relative proportions (%) of their selected fatty acids. If purely the proportion of a fatty acid is given, the value is of course directly influenced by the number of other fatty acids also measured. This means that the results from a study measuring the proportion of 16 fatty acids are not directly comparable with a study which measures 20 fatty acids, especially if one or more of the 'omitted' fatty acids constitute a significant proportion of the total.

Frequently only the fatty acid composition of plasma or serum is measured; rarely are individual lipid fractions of the plasma considered. Lipids in the plasma are transported in the form of various types of LP particles. These particles have a core of TAG and CE surrounded by a coat of PL. In the fasted state, the principal LP present in the circulation is VLDL. NEFA are released from adipose tissue and are also present in the plasma in a relatively high concentration during fasting (compared to the post-prandial state). These different compartments for fatty acids are rarely separated or considered as individual fractions, although each has its own characteristic composition and function.

The fatty acid data taken from the subjects in this study have been expressed in both relative and absolute terms. In addition, the four major lipid fractions of the plasma have been separated and their composition considered individually.

6.2 AIM

The aim of this chapter was to characterise the fatty acid composition (both in absolute and relative terms) of plasma PC, TAG, NEFA and CE in colectomy and reference patients and to compare these results to other published works.

Questions to be addressed included:

- Is the total fatty acid concentration different between the groups; what is the size of the fatty acid transport pool?
- Do the colectomy patients have sufficient EFA?
- What is the availability of the eicosanoid precursor fatty acids; is there evidence of up-regulated synthesis?
- What is the availability of LC-PUFA?
- What is the overall *n*-6 and *n*-3 PUFA status in plasma; is total *n*-3 or *n*-6 PUFA status representative of what is seen for each individual fatty acid?
- How do the results from this study compare to that of others?

6.3 HYPOTHESES

Colectomy patients will exhibit altered plasma fatty acid composition in comparison to the reference group. Differences seen between the reference and colectomy groups will not be the same across all the lipid fractions, highlighting the importance of considering each fraction individually.

6.4 METHODS

The methods employed for subject identification and recruitment, for sample collection and processing and for statistical analysis are detailed fully in Chapter 3. Patients with a history of severe IBD resulting in colectomy surgery were identified and recruited into the trial. Pertinent assessments and measurements were made, including the collection of a blood sample used to isolate plasma lipids for fatty acid composition analysis.

6.5 STATISTICAL ANALYSIS

Details of statistical analyses and data processing are given in Chapter 3. Results are presented in scatter graphs, showing data points for both reference and colectomy groups. Statistical analyses were performed using Mann-Whitney U test, and significant differences between the two groups flagged using annotations as previously described. Differences were considered to be significant at $P<0.05$.

6.6 RESULTS

Fatty acids from plasma lipids were isolated and measured in PC, TAG, NEFA and CE fractions. The results are presented below, both in terms of concentration and as a percentage of the sum of fatty acids in that fraction (relative proportion). It should be noted that the absolute concentrations of fatty acids in the plasma CE fraction were not measured so are expressed only as a relative proportion; the total peak area count of all fatty acids measured taken as 100%.

6.6.1 TOTAL FATTY ACID CONCENTRATION

The total fatty acid concentration was calculated from the sum of the concentrations of PA, SA, OA, LA, ALNA, γ-linoleic acid (GLA), DGLA, ARA, EPA, DPA and DHA in each lipid fraction. The results are presented in Figure 6.1 to Figure 6.3.

There was roughly a 25% higher concentration of fatty acids in the plasma PC of the colectomy group. This was a significantly higher concentration than the reference group ($p<0.001$) (Figure 6.1).

Concentration of fatty acids in the TAG fraction was also significantly higher in the colectomy groups; the mean concentration of total fatty acid was almost 93% higher in this group ($p<0.01$) (Figure 6.2).

Total fatty acid concentration in the NEFA fraction was similar between the two groups (Figure 6.3). The range of values within the groups was also similar.

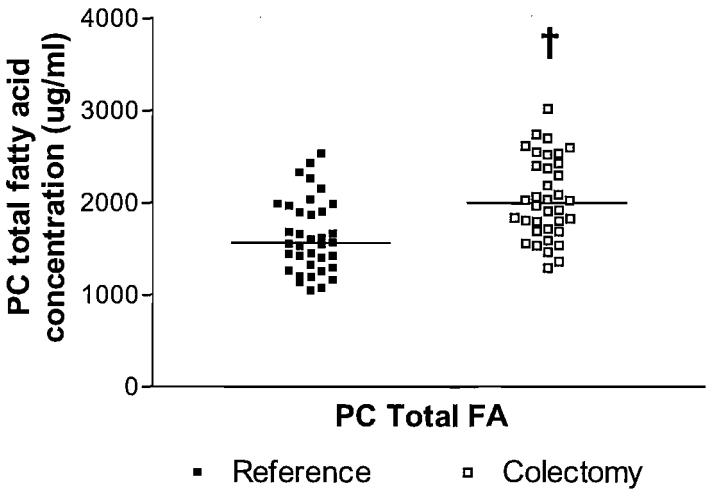


Figure 6.1
Total fatty acid concentration in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

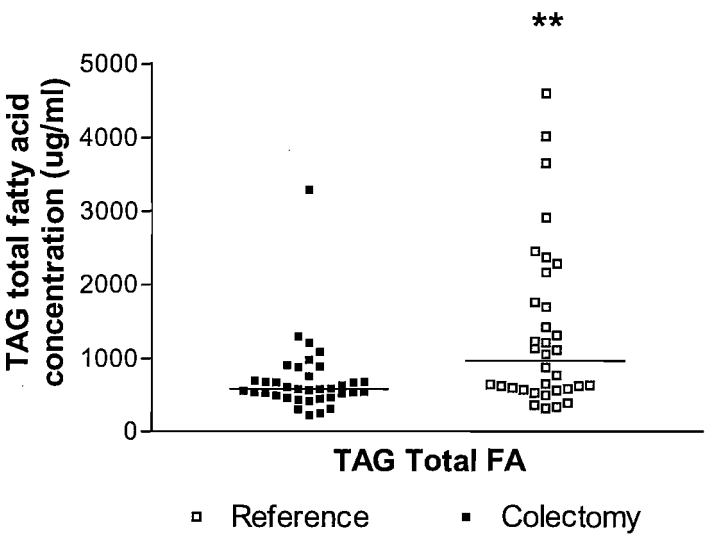


Figure 6.2
Total fatty acid concentration in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

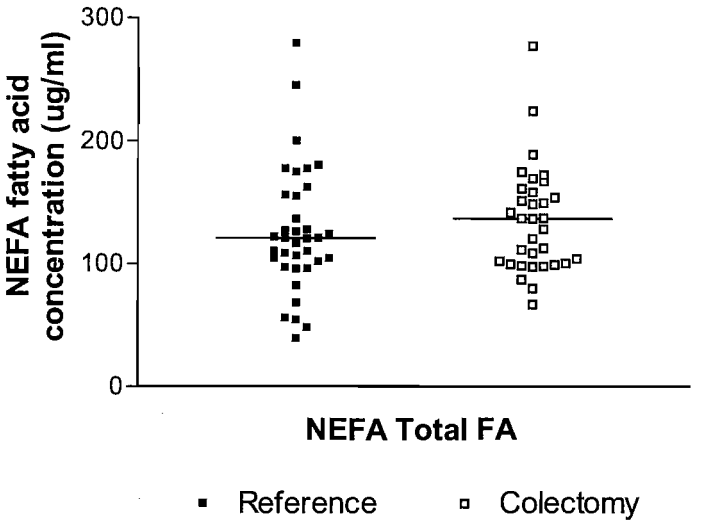


Figure 6.3
Total fatty acid concentration in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group.

6.6.2 ESSENTIAL FATTY ACID CONCENTRATION & PROPORTION

The essential fatty acids (LA and ALNA) were isolated from each of the four lipid fractions. These fatty acids are the dietarily essential *n*-3 and *n*-6 PUFA precursors of their respective longer chain PUFA derivatives such as EPA and ARA, respectively. The concentration and relative proportion of both ALNA and LA in each of the four plasma lipid fractions (a percentage of the total fatty acid concentration) are presented in Figure 6.4 to Figure 6.7 and Figure 6.8 to Figure 6.11, respectively.

α-Linolenic Acid

In the plasma PC fraction, no difference in concentration of *n*-3 precursor ALNA was seen between the two groups (Figure 6.4). However, the proportion of ALNA was approximately 23% lower the in colectomy group. This was a significant difference ($p<0.001$).

A similar pattern was observed in the plasma TAG fraction (Figure 6.5). The concentration of ALNA in the colectomy group was marginally higher, but the proportion of ALNA was roughly 20% lower. This difference in proportion of ALNA was significantly different ($p<0.001$).

The ALNA composition in the NEFA fraction was different to that of the PC and TAG fractions (Figure 6.6). It should be noted that in some cases the concentration of ALNA was very low and therefore undetectable. These cases are shown on the graph as zero. The colectomy patients exhibited approximately 65% more ALNA in plasma NEFA than the reference patients ($p<0.001$). The proportion of this fatty acid was also significantly higher in the colectomy group as ALNA was approximately 45% higher ($p<0.001$).

The concentration of fatty acids in the CE fraction was not measured. However, in this fraction, the proportion of ALNA was roughly 30% lower in the colectomy patients (Figure 6.7). This difference was highly significant ($p<0.001$).

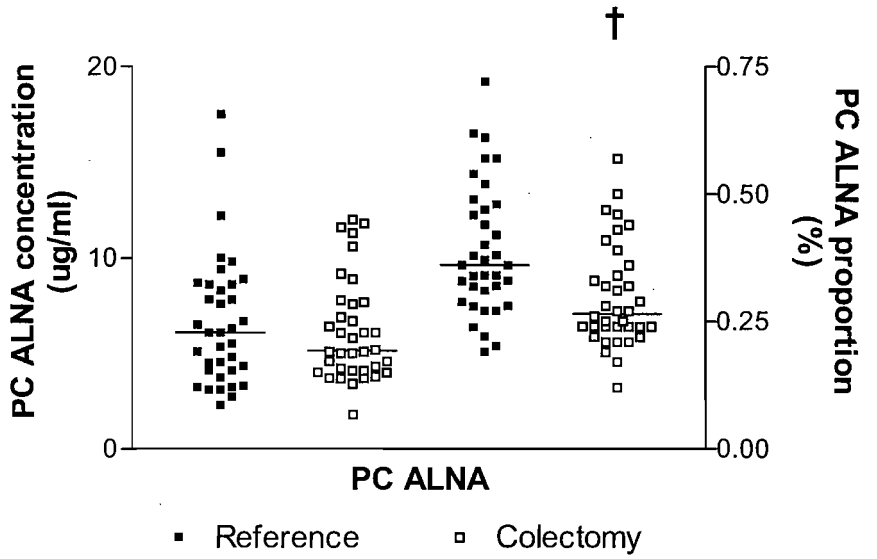


Figure 6.4 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of ALNA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

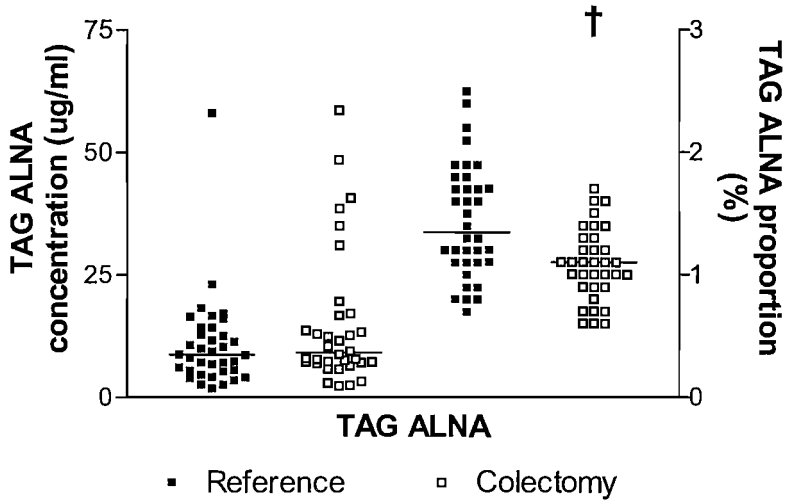


Figure 6.5 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of ALNA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

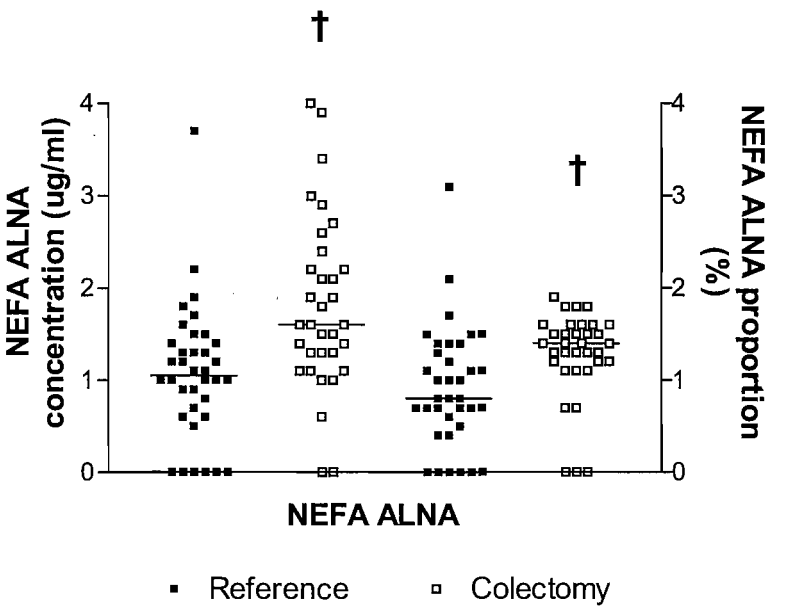


Figure 6.6 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of ALNA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

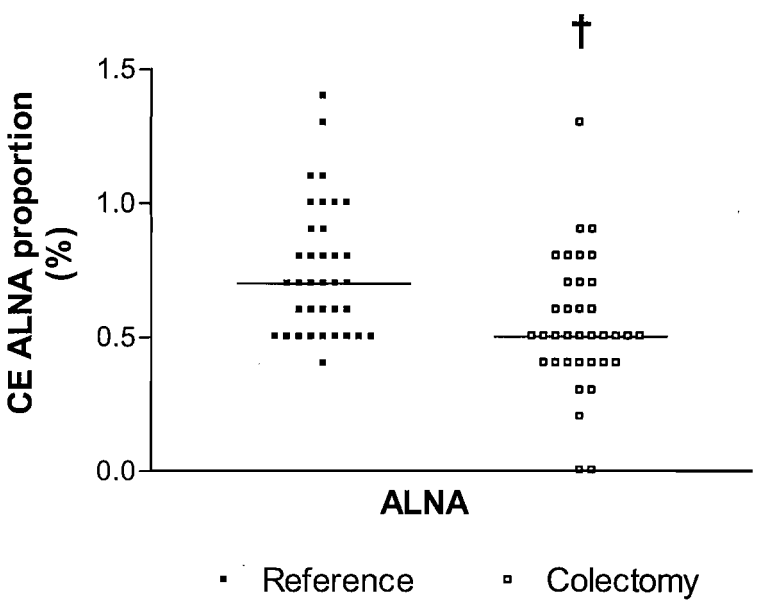


Figure 6.7 Relative proportion of ALNA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

Linoleic Acid

In the plasma PC fraction, the concentration of LA was approximately 20% higher in the colectomy patients (Figure 6.8). This was highly significant. In contrast, the relative proportion of LA was similar between the two groups.

A similar pattern was seen in the plasma TAG fraction (Figure 6.9). LA concentration was markedly higher in the colectomy group (70%) and this was significant ($p<0.01$). However, the proportion of this fatty acid was similar to the reference group.

In the NEFA fraction a different pattern was observed (Figure 6.10). The concentration of LA was similar in both groups. The proportion of LA was marginally lower in the colectomy group, but this was not significant.

In the CE fraction a markedly lower proportion of LA was observed (Figure 6.11); the proportion of LA in this fraction was approximately 15% lower in the colectomy patients and this was significant ($p<0.001$).

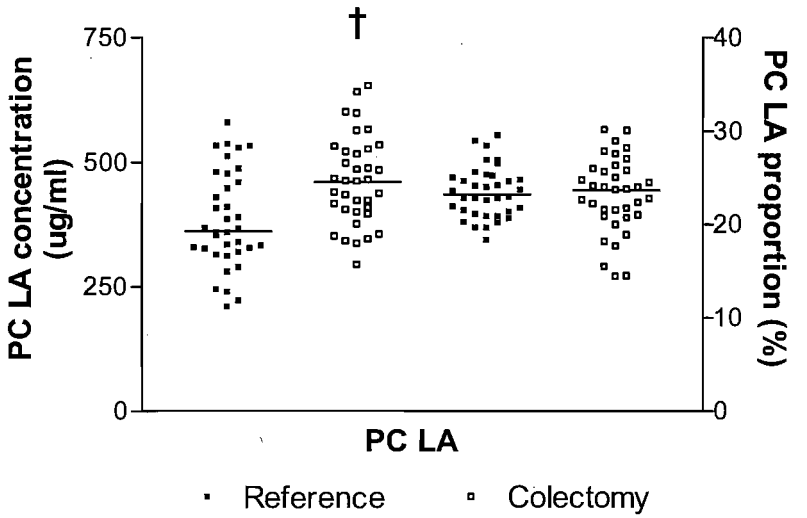


Figure 6.8 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of LA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

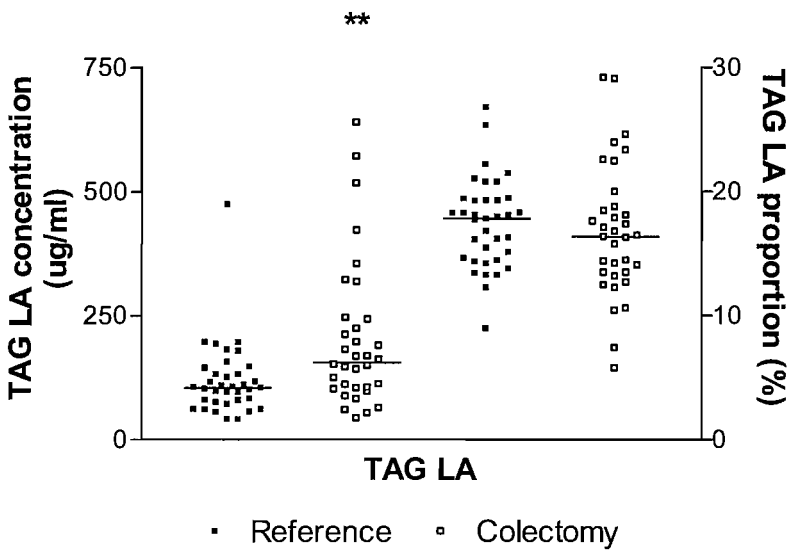


Figure 6.9 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of LA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

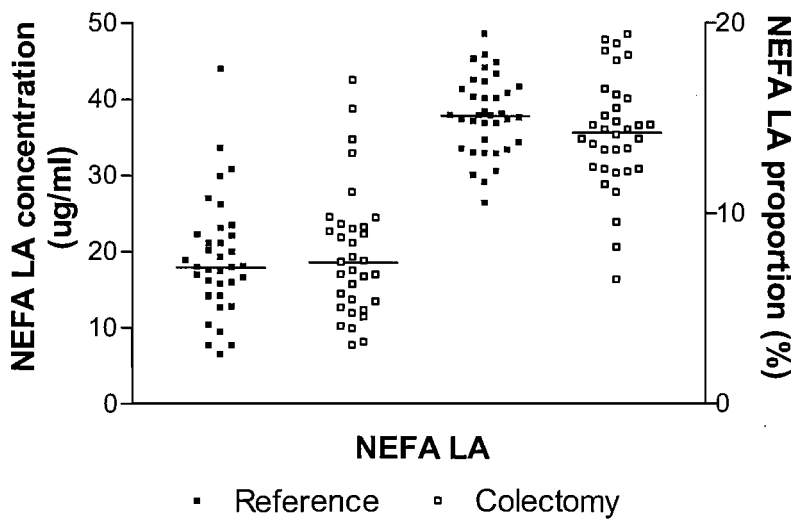


Figure 6.10 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of LA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

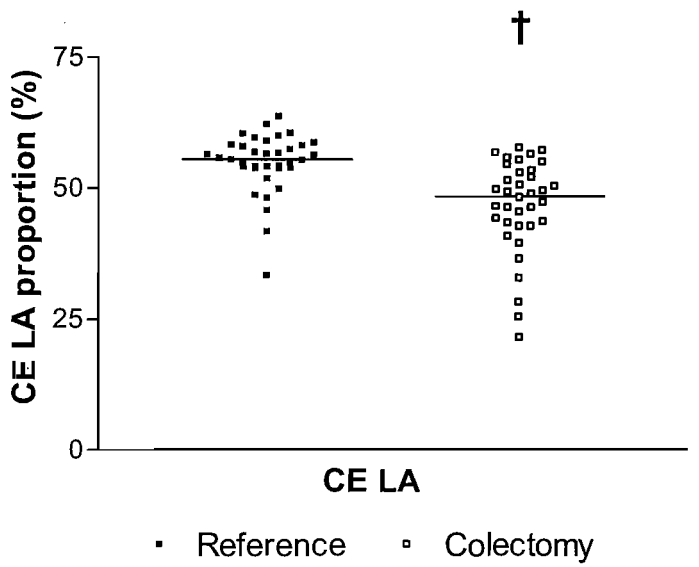


Figure 6.11 Relative proportion of LA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

6.6.3 ESSENTIAL FATTY ACID RATIO

The EFA ratio was expressed as a ratio of LA to ALNA in the PC, TAG, NEFA and CE fractions (Figure 6.12 to Figure 6.15).

In the plasma PC fraction, the ratio of LA to ALNA was approximately 30% higher in the colectomy group (Figure 6.12). This difference was significant ($p<0.01$).

A similar finding was observed in the ratio of LA:ALNA in plasma TAG (Figure 6.13). Again, this ratio was found to be significantly higher in the colectomy group than in the reference group ($p<0.05$).

In the NEFA fraction ALNA was not always detectable, so the calculation of the LA:ALNA ratio was not possible in these cases. However, the majority of patients did have a detectable amount of ALNA in the plasma NEFA fraction. The results are depicted in Figure 6.14 and show that the colectomy group had a significantly lower LA:ALNA ratio than the reference group ($p<0.001$).

In the plasma CE fraction, the LA:ALNA ratio was not different (Figure 6.15).

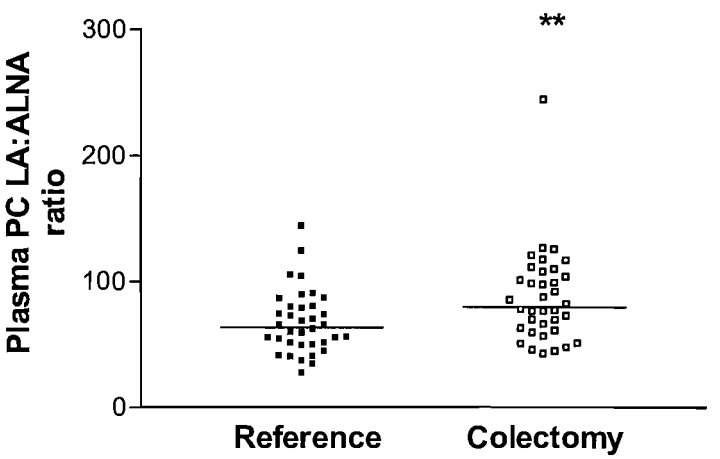


Figure 6.12 Ratio of LA:ALNA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

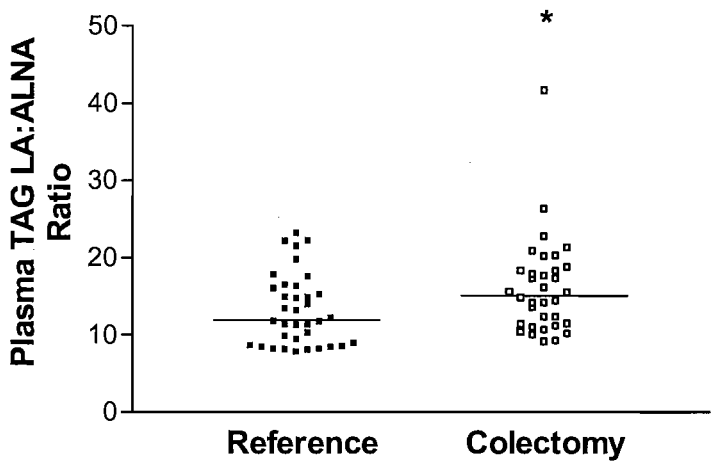


Figure 6.13 Ratio of LA:ALNA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. * = p<0.05 (Mann-Whitney U).

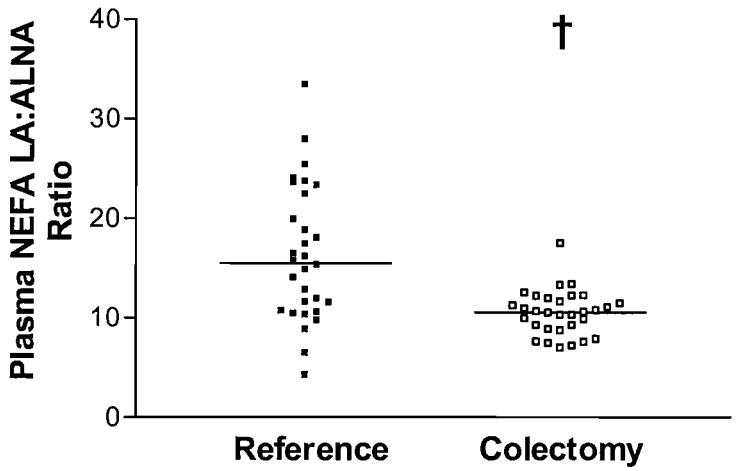


Figure 6.14 Ratio of LA:ALNA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

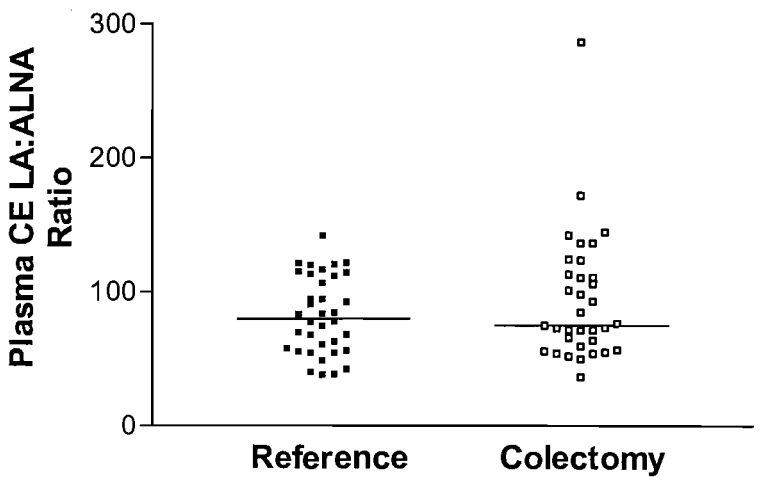


Figure 6.15 Ratio of LA:ALNA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

6.6.4 ESSENTIAL FATTY ACID METABOLITE COMPOSITION: EICOSAPENTAENOIC AND ARACHIDONIC ACIDS

EPA and ARA are examples of *n*-3 and *n*-6 fatty acid metabolites of the EFA precursors, respectively. The concentration and proportion of these fatty acids was measured in the plasma PC, TAG, NEFA and CE fractions. The results are summarised in Figure 6.16 to Figure 6.19 and Figure 6.20 to Figure 6.23, respectively.

Eicosapentaenoic Acid

It was observed that plasma PC EPA concentration was similar between the reference and colectomy groups (Figure 6.16). The relative proportion of EPA was approximately 13% lower in the colectomy group, but this was not significant.

In the TAG fraction, the concentration of EPA was roughly 40% higher in the colectomy patients (Figure 6.17), but this was not significant. Despite a tendency for a higher concentration of EPA in this group, the proportion of this fatty acid was around 25% lower, although this was not significant.

The concentration of EPA in plasma NEFA was similar between the two groups (Figure 6.18). Generally, the proportions of EPA in NEFA were similar between the groups, although the median value in the colectomy group was lower, but this was not a significant finding. It was noted that many of the patients in both groups did not exhibit any EPA in their plasma NEFA. It should be considered that concentration of EPA in this plasma fraction is typically particularly low and close to the minimum detection limits of the equipment used to measure it.

The proportion of EPA in the CE fraction was significantly lower in the colectomy group (Figure 6.19). The colectomy patients had 33% less EPA in their plasma CE than the reference patients ($p<0.001$).

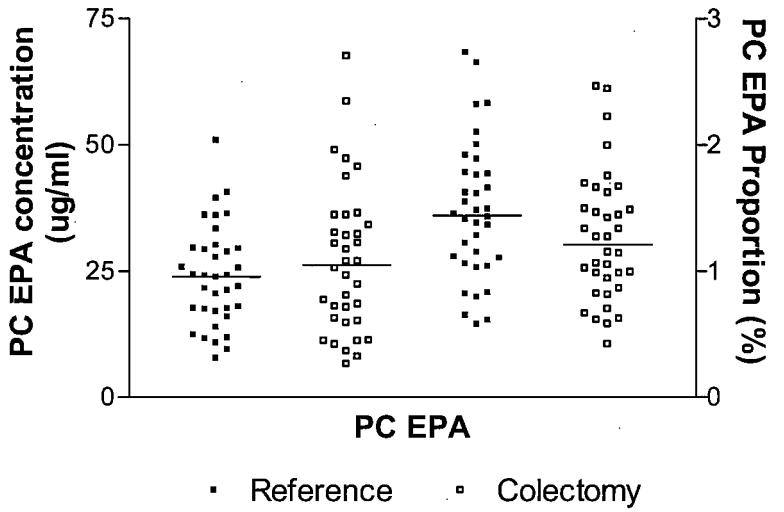


Figure 6.16 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of EPA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

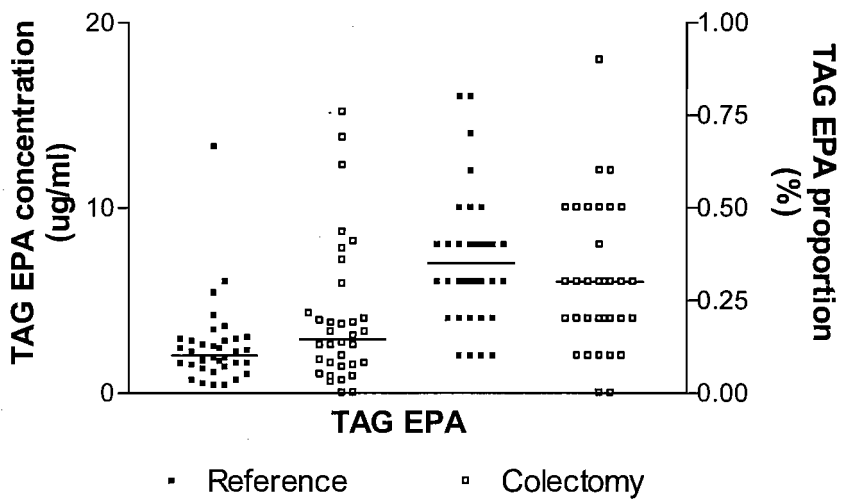


Figure 6.17 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of EPA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

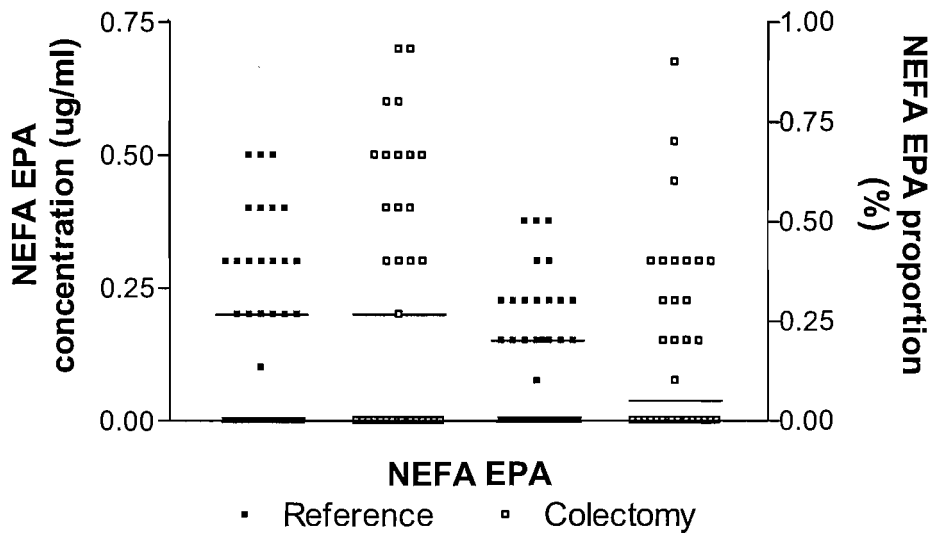


Figure 6.18 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of EPA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

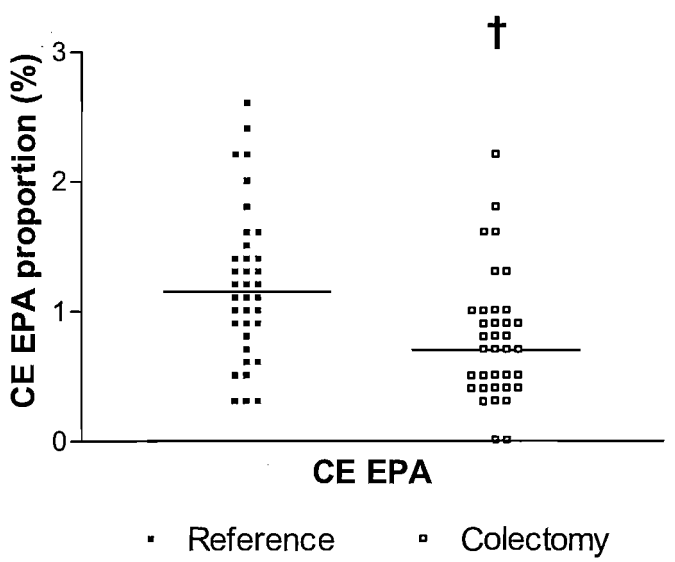


Figure 6.19 Relative proportion (%) of EPA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

Arachidonic Acid

In the plasma PC fraction there was a higher concentration of ARA in the colectomy group than the reference group (Figure 6.20). The colectomy patients had an approximately 25% higher concentration of ARA than the reference patients and this was significant ($p<0.01$). Despite this significantly higher concentration, the proportion of this fatty acid was not different between the two groups.

In plasma TAG, the concentration of ARA was also significantly higher in the colectomy group ($p<0.001$); these patients had roughly 120% more ARA in their plasma TAG than the reference patients (Figure 6.21). The proportion of ARA in this fraction was also significantly higher in the colectomy group ($p<0.05$). These patients had almost a 25% higher proportion of ARA than the patients in the reference group.

The concentration of ARA in plasma NEFA was slightly higher in the colectomy group than in the reference group but this was not significant (Figure 6.22). The proportion of ARA in plasma NEFA was similar between the two groups.

In plasma CE, the proportion of ARA was approximately 13% lower in the colectomy patients (Figure 6.23). This difference was significant ($p<0.05$).

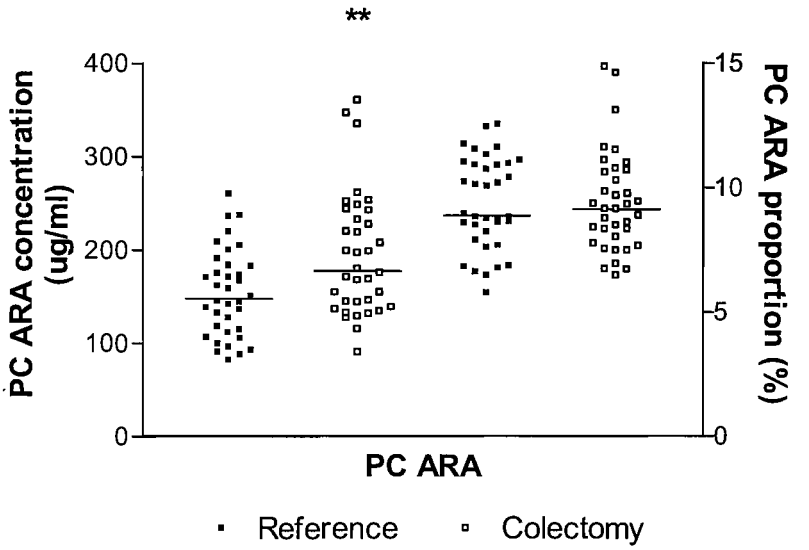


Figure 6.20 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of ARA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

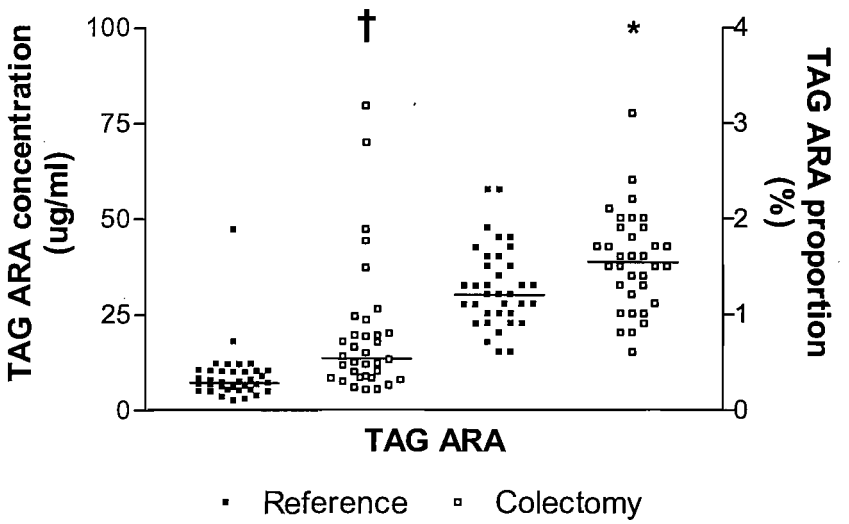


Figure 6.21 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of ARA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. * = p<0.05, † = p<0.001 (Mann-Whitney U).

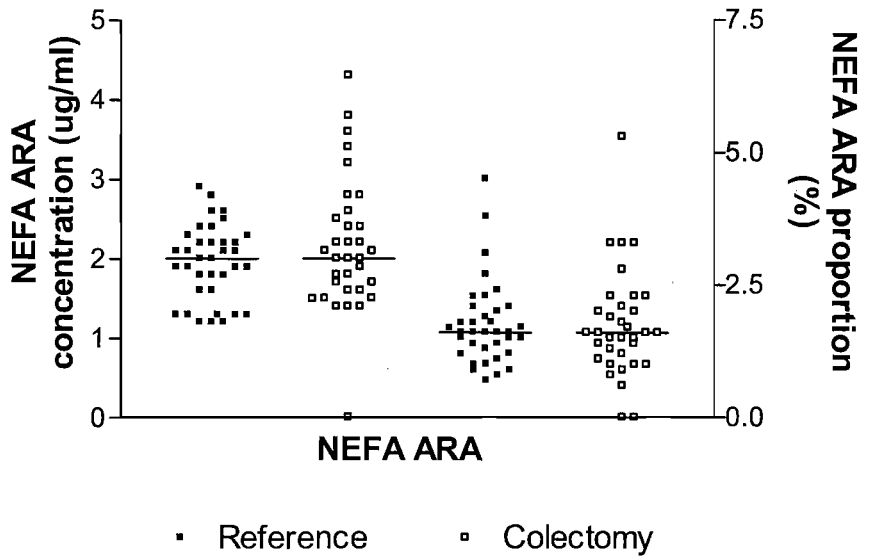


Figure 6.22 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of ARA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

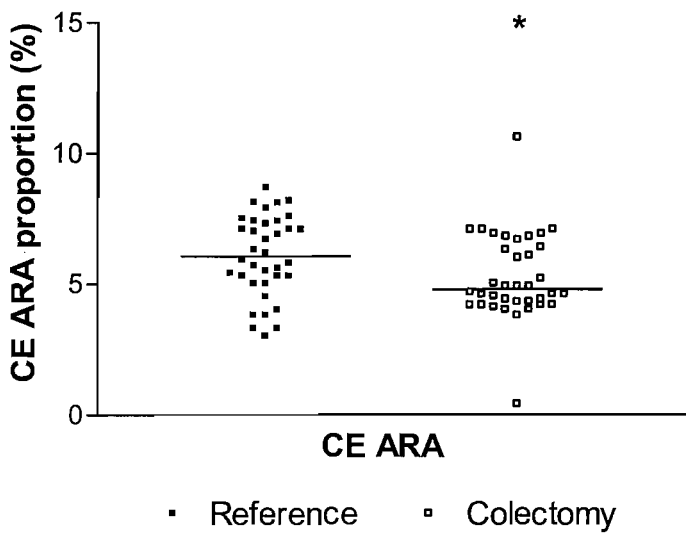


Figure 6.23 Relative proportion (%) of ARA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. * = $p<0.05$ (Mann-Whitney U).

6.6.5 EICOSAPENTAENOIC ACID AND ARACHIDONIC ACID RATIO

As described above, the concentrations and proportions of ARA and EPA were measured in four plasma lipid fractions of reference and colectomy patients. In this section, the ratio of one fatty acid to the other is considered. This may help to act as an indicator of the production of EFA metabolites in both the *n*-3 and *n*-6 families and may be a possible marker of *n*-6:*n*-3 PUFA imbalance. The results are shown in Figure 6.24 to Figure 6.27.

In the plasma PC fraction, the ratio of ARA:EPA was approximately 20% higher in the colectomy patients although this was not significant (Figure 6.24).

The ratio of ARA:EPA was almost 35% higher in the plasma TAG fraction of the colectomy patients compared to the reference patients (Figure 6.25). This result was significant ($p<0.01$).

The ratio of ARA:EPA was marginally lower in plasma NEFA of the colectomy patients but this was not significant (Figure 6.26).

In the plasma CE fraction, the ratio of ARA:EPA was roughly 35% higher in the colectomy group; this was a significant difference ($p<0.05$) (Figure 6.27).

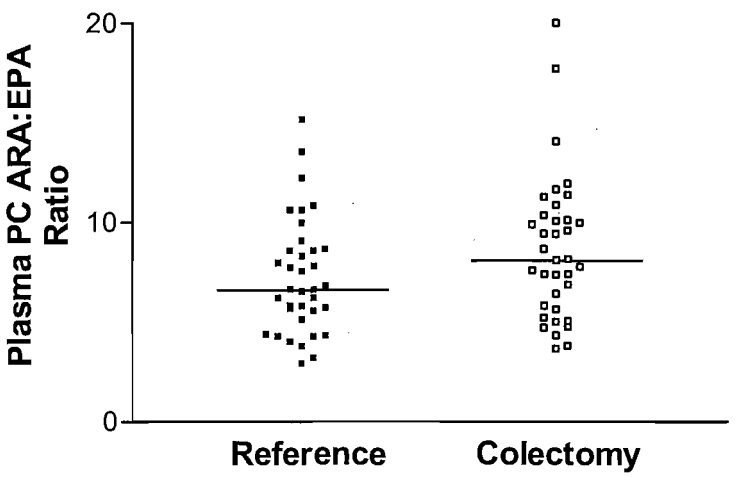


Figure 6.24 Ratio of ARA:EPA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

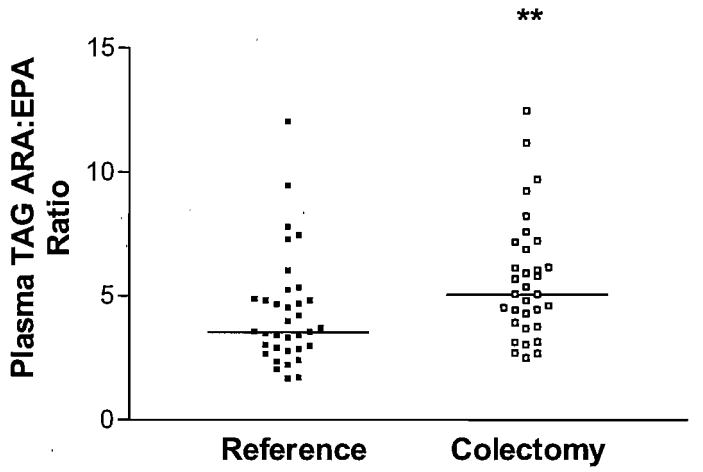


Figure 6.25 Ratio of ARA:EPA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

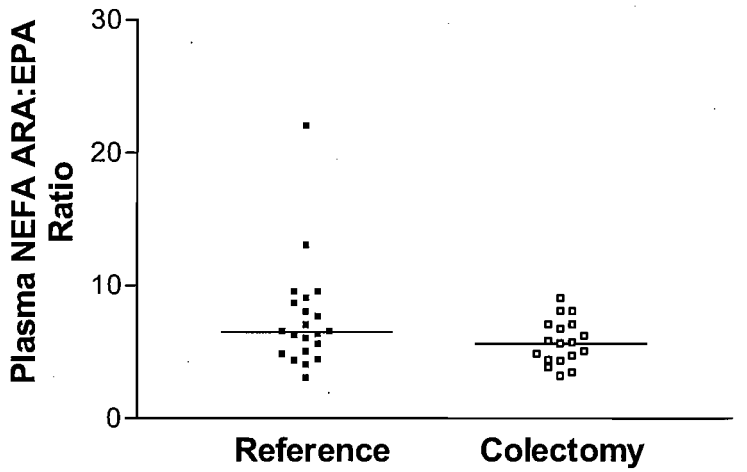


Figure 6.26 Ratio of ARA:EPA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

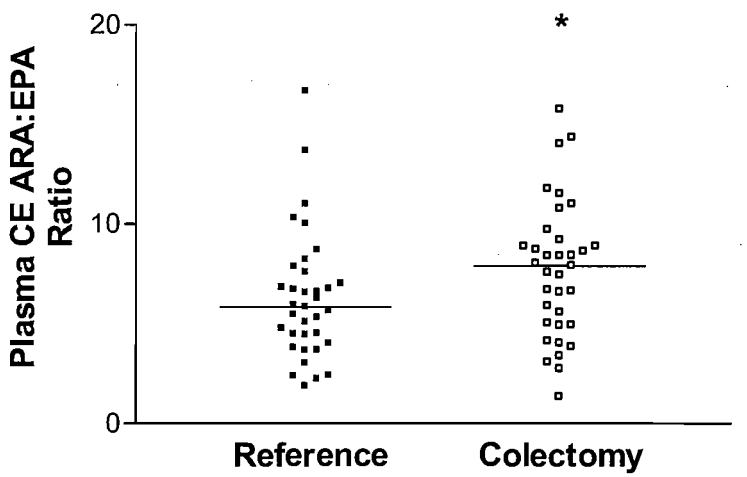


Figure 6.27 Ratio of ARA:EPA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. * = p<0.05 (Mann-Whitney U).

6.6.6 N-6 ESSENTIAL FATTY ACID METABOLITE COMPOSITION: γ-LINOLENIC AND DIHOMO-γ-LINOLENIC ACIDS

The metabolism of the *n*-6 EFA LA results in the production of GLA and DGLA, then ultimately ARA. DGLA is a twenty-carbon fatty acid and can compete with ARA (20:4*n*-6) and EPA (20:5*n*-3) for the actions of PLA_2 which yield eicosanoids. The concentration and proportion of GLA and DGLA was measured in the reference and colectomy groups. The results are presented in Figure 6.28 to Figure 6.30 and Figure 6.31 to Figure 6.34, respectively.

γ-Linolenic Acid

GLA was not measured in the PC fraction due to an analytical error.

In the plasma TAG fraction, the concentration of GLA was approximately 170% higher in the colectomy group (Figure 6.28). This finding was statistically significant ($p<0.001$). The GLA proportion was also higher, but less marked than the concentration. The colectomy patients had an approximately 50% higher proportion of GLA in their plasma TAG. This finding was also significant ($p<0.01$).

In the NEFA fraction, both the concentration and proportion of GLA was lower in the colectomy group (Figure 6.29). The concentration of GLA was nearly 60% lower in the colectomy patients ($p<0.001$). The proportion of GLA was also significantly lower as the colectomy patients presented with only 50% of the proportion of GLA of the reference group ($p<0.001$).

In the CE fraction, the proportion of GLA was significantly higher in the colectomy group ($p<0.01$). These patients had almost 40% more GLA in their plasma CE than their counterparts in the reference group (Figure 6.30).

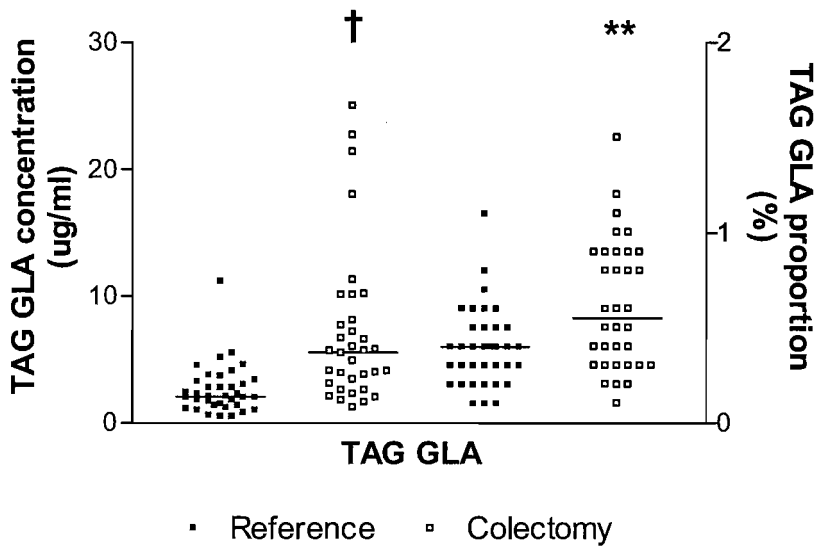


Figure 6.28 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of GLA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001; ** = p<0.01 (Mann-Whitney U).

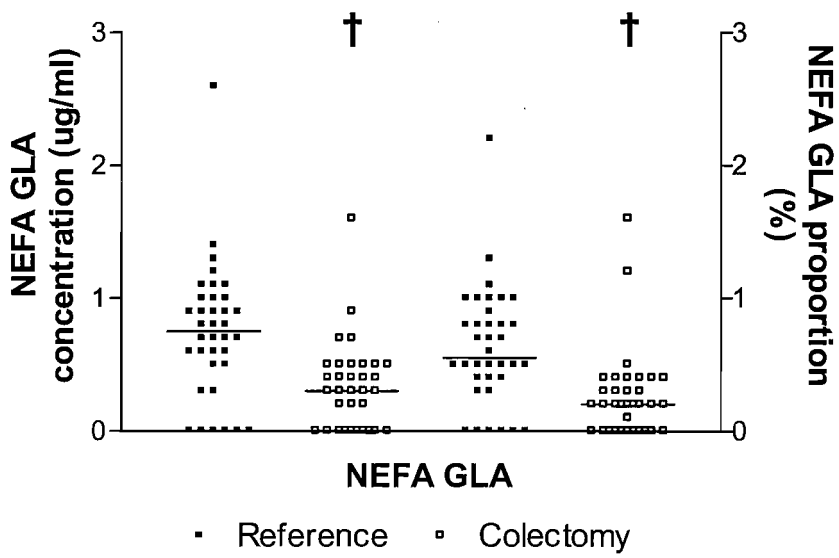


Figure 6.29 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of GLA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

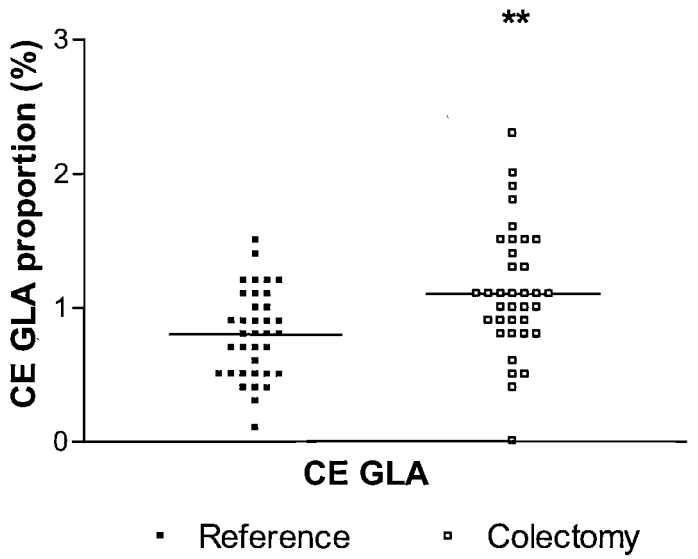


Figure 6.30 Relative proportion (%) of GLA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

Dihomo-γ-linolenic Acid

In the plasma PC fraction, the concentration of DGLA was almost 40% higher in the colectomy patients (Figure 6.31) and this difference was statistically significant ($p<0.001$). Despite the markedly higher concentration, the proportion of DGLA was similar between the two groups.

The concentration of DGLA was also significantly higher in plasma TAG of the colectomy patients (Figure 6.32). This group exhibited a concentration of DGLA approximately 60% higher than that of the reference group ($p<0.01$). However, the proportion of DGLA in plasma TAG did not differ between the groups.

In the NEFA fraction, both the concentration and proportion of DGLA was negligible in the colectomy patients so the differences in this fatty acid between the two groups was most marked in this fraction (Figure 6.33). The concentration of DGLA was 94% lower in the colectomy patients which was highly significant ($p<0.001$). The proportion of this fatty acid in NEFA was also remarkably lower in this group: the colectomy patients exhibited a 97% lower proportion of DGLA than the reference patients ($P<0.001$).

The proportion of DGLA in plasma CE was 20% lower in the colectomy patients (Figure 6.34) and this was significantly different ($p<0.001$).

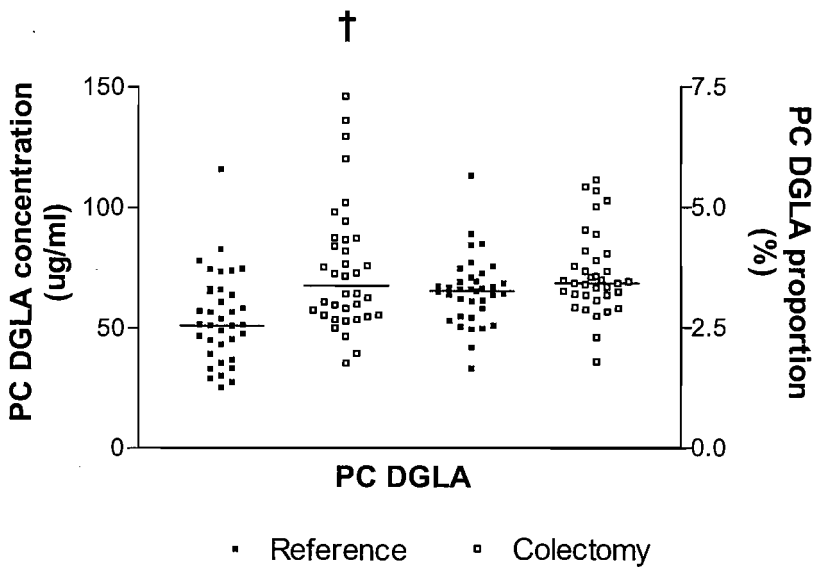


Figure 6.31 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DGLA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

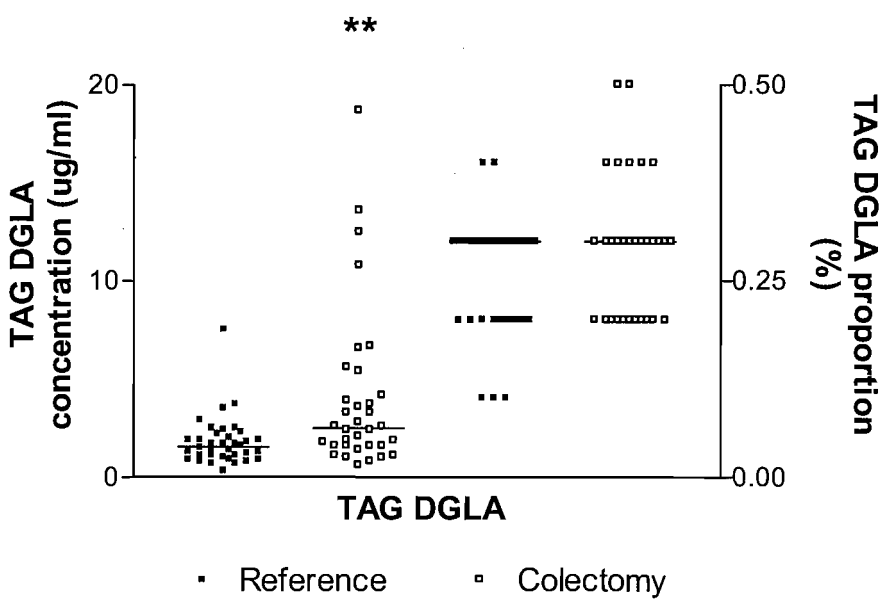


Figure 6.32 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DGLA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

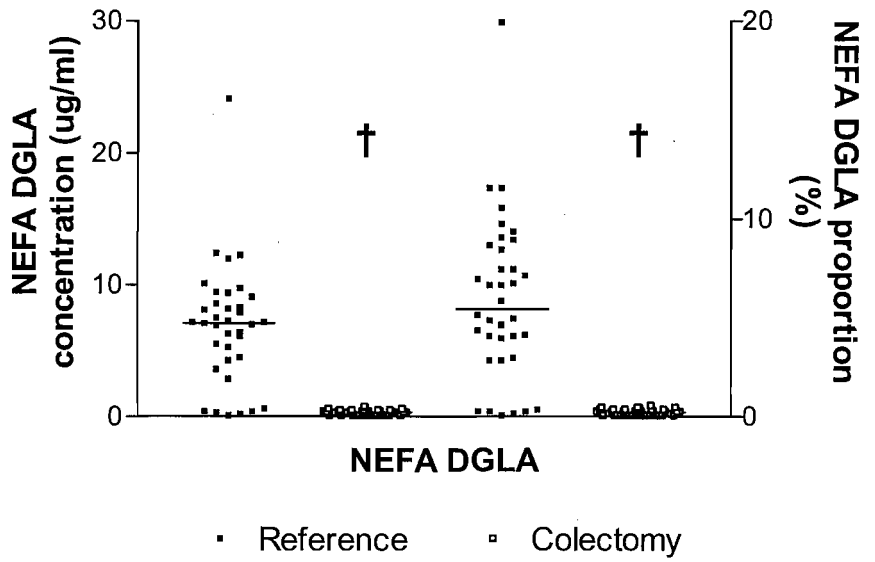


Figure 6.33 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DGLA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

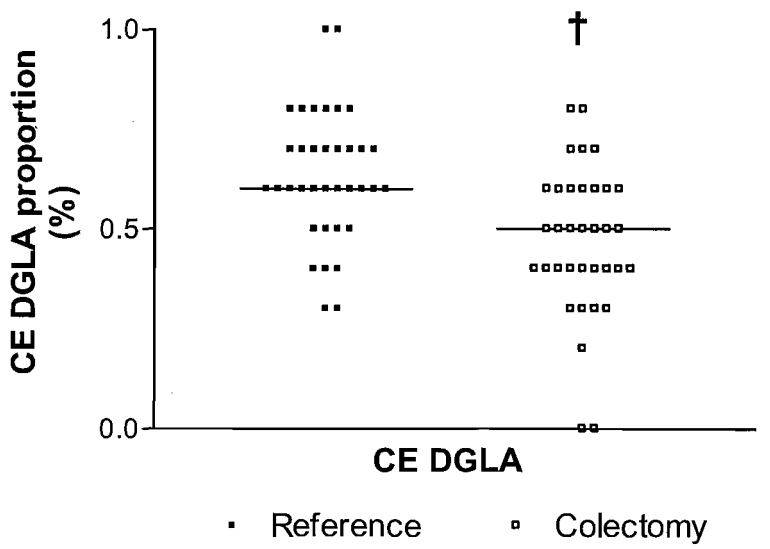


Figure 6.34 Relative proportion (%) of DGLA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

6.6.7 N-3 ESSENTIAL FATTY ACID METABOLITE COMPOSITION: DOCOSAPENTAENOIC AND DOCOSAHEXAENOIC ACIDS

The elongation and desaturation of the *n*-3 EFA (ALNA) produces EPA and then its longer-chain PUFA derivatives DPA and DHA. The concentration and proportion of DPA and DHA in plasma PC, TAG, NEFA and CE was measured in the reference and colectomy groups. The results are presented in Figure 6.35 to Figure 6.38 and Figure 6.39 to Figure 6.42, respectively.

Docosapentaenoic Acid

The concentration of DPA in plasma PC was approximately 20% higher in the colectomy patients than the reference patients (Figure 6.35) and this was significant ($p<0.01$). Despite this higher concentration, the proportion of fatty acid that was DPA was slightly lower in the colectomy group but this was not significant.

In the TAG fraction, the concentration of DPA was also higher in the colectomy patients (Figure 6.36). The concentration of DPA was roughly 90% higher in the colectomy group and this was highly significant ($p<0.001$). The proportion of this fatty acid in TAG was also higher in the colectomy group but to a lesser extent than the concentration; this was also significant ($p<0.05$).

A higher concentration and proportion of DPA was also seen in the plasma NEFA of the colectomy patients (Figure 6.37). The concentration of DPA was 110% higher in the colectomy patients and the proportion of DPA was over 20-fold higher. Both results were highly significant ($p<0.001$).

In general there was an almost negligible proportion of DPA in the CE fraction (Figure 6.38). In the reference patients it was not possible to detect DPA with confidence. This was also the case for the majority of the colectomy patients. However, a small proportion of patients did have detectable amounts of this fatty acid although the overall proportion of this fatty acid was very low (0.04%). This difference was significant ($p<0.01$).

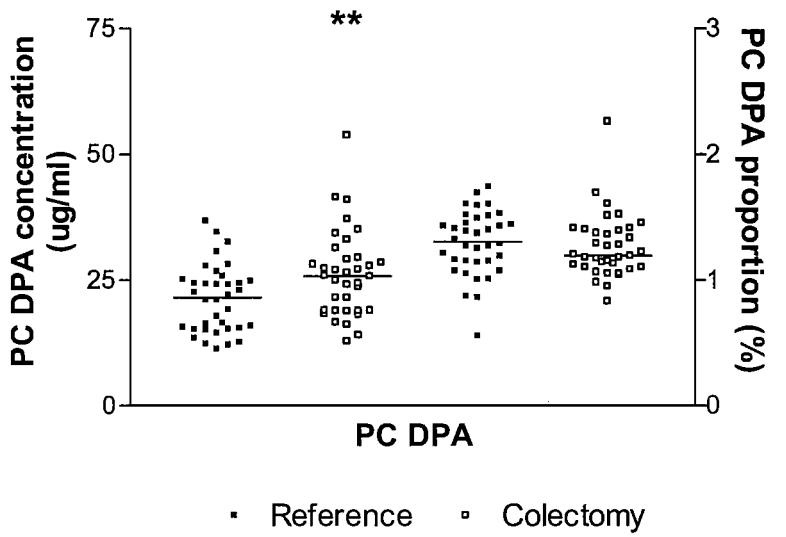


Figure 6.35 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DPA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

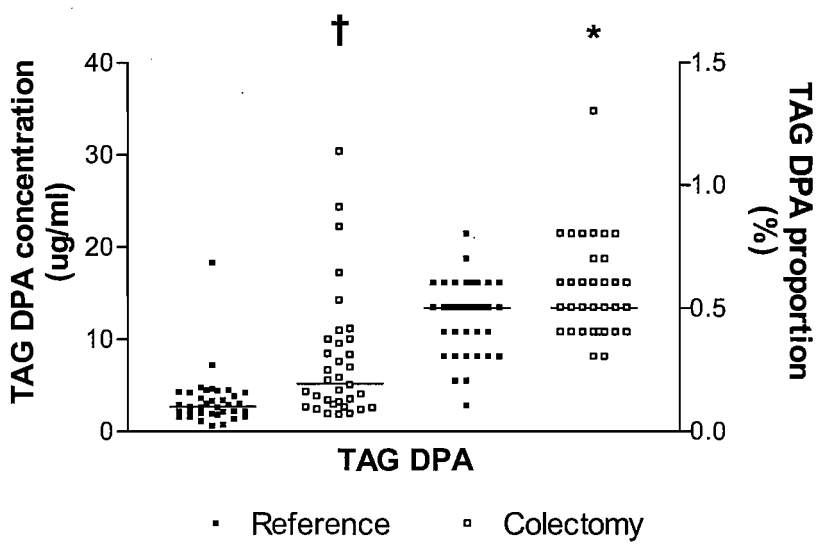


Figure 6.36 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DPA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001, * = p<0.01 (Mann-Whitney U).

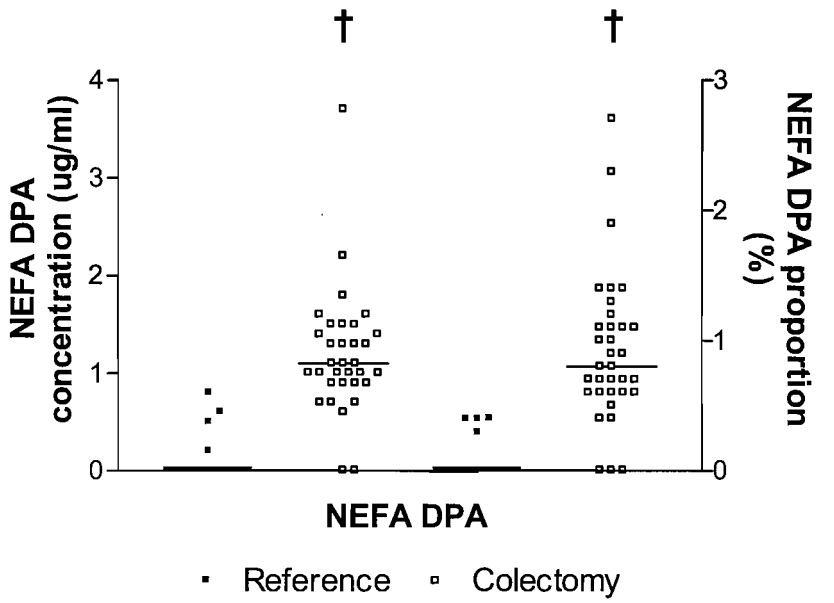


Figure 6.37 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DPA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

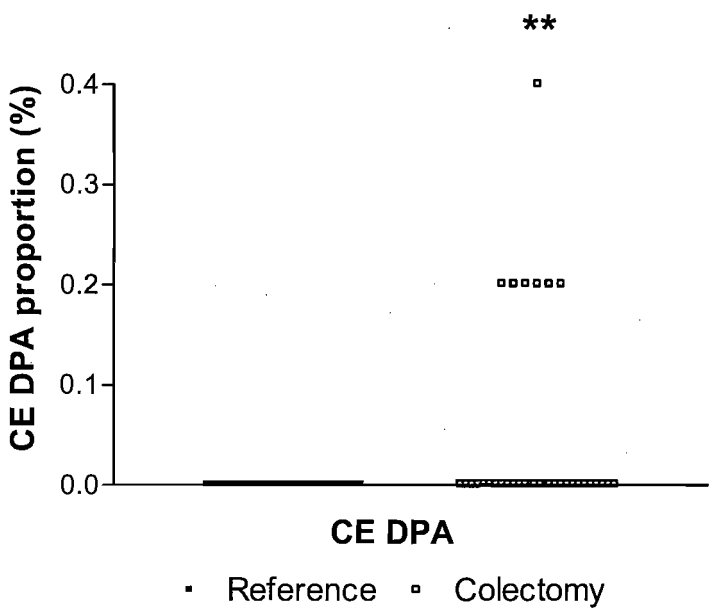


Figure 6.38 Relative proportion (%) of DPA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

Docosahexaenoic Acid

The concentration of DHA in plasma PC was marginally lower in the colectomy group but this was not significant (Figure 6.39). However, the proportion of fatty acid that was DHA was roughly 30% lower in the colectomy group. This difference was highly significant ($p<0.01$).

In the plasma TAG fraction, the concentration of DHA was slightly higher in the colectomy group, but this was not significant (Figure 6.40). However, the proportion of this fatty acid was 25% lower in the colectomy group when compared to the reference group and this finding was significant ($p<0.05$).

In plasma NEFA both the concentration and proportion of DHA were similar between the reference and colectomy groups (Figure 6.41).

In the CE fraction of plasma, the proportion of DHA was also similar in both groups (Figure 6.42).

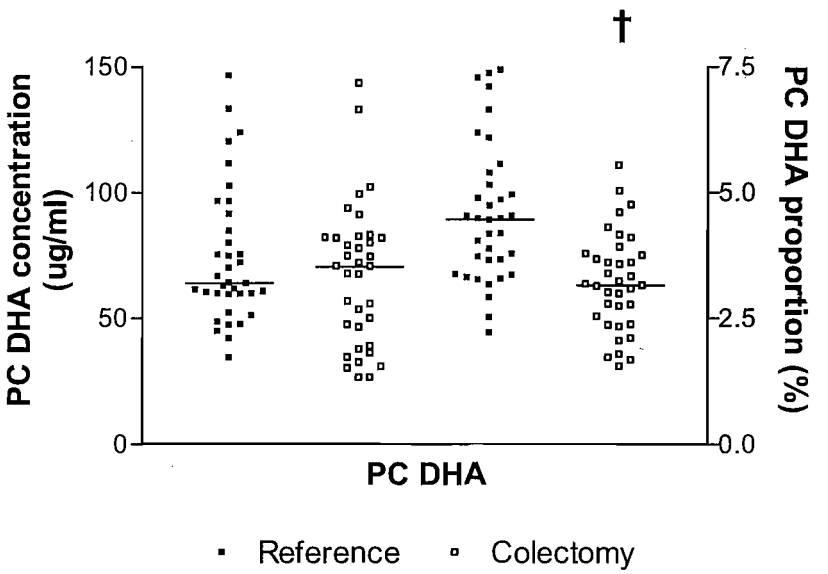


Figure 6.39 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DHA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = $p<0.001$ (Mann-Whitney U).

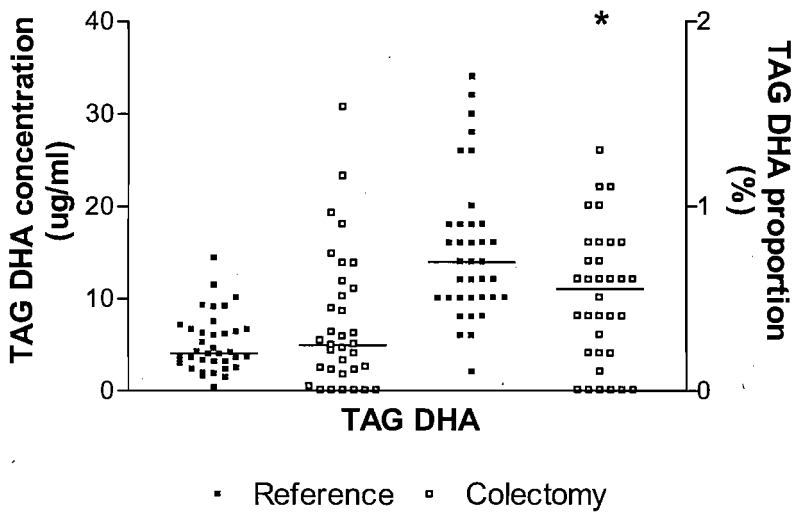


Figure 6.40 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DHA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. * = p<0.05 (Mann-Whitney U).

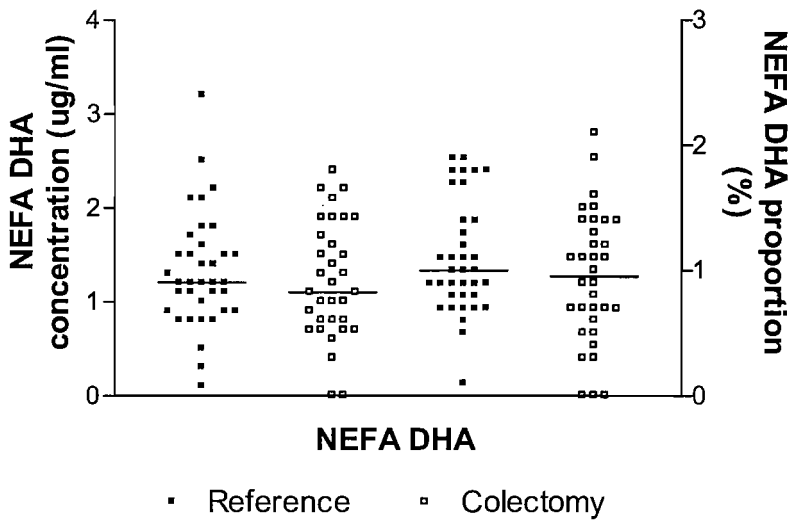


Figure 6.41 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of DHA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

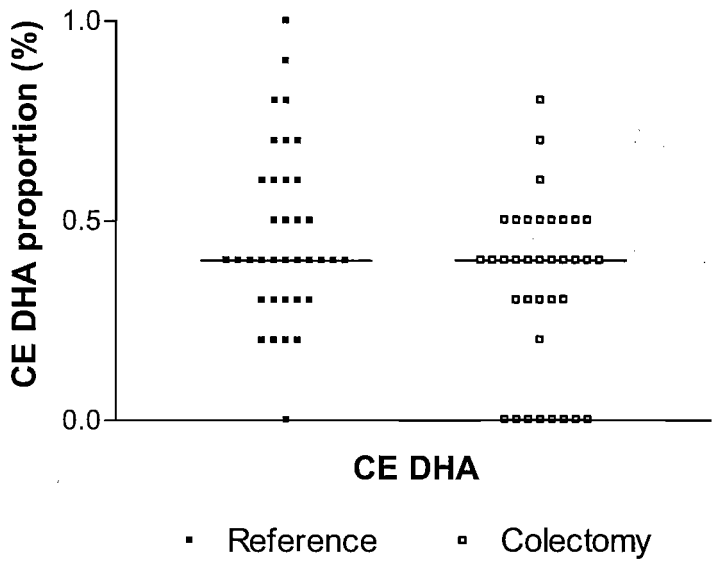


Figure 6.42 Relative proportion (%) of DHA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

6.6.8 TOTAL N-6 POLYUNSATURATED FATTY ACIDS

The sum of LA, GLA, DGLA and ARA was calculated to give a value for total *n*-6 PUFA concentration and the total proportion of these fatty acids in plasma lipids. The results are shown in Figure 6.43 to Figure 6.46.

In plasma PC, the concentration of *n*-6 PUFA was approximately 25% higher in the colectomy group (Figure 6.43). This finding was highly significant ($p<0.001$). Despite a considerably higher concentration of *n*-6 PUFA in plasma PC, overall the proportion of this group of fatty acids was not different between the two groups.

A similar pattern was seen in the TAG fraction (Figure 6.44). The concentration of *n*-6 PUFA was almost 55% higher in the colectomy group ($p<0.001$) but the proportion was not different between the groups.

The NEFA fraction exhibited a completely different pattern to the PC and TAG fractions (Figure 6.45). The concentration of *n*-6 PUFA in this fraction was 20%

lower in the colectomy patients than the reference patients ($p<0.01$). The proportion of these fatty acids in this fraction was also significantly lower; the colectomy patients had 30% less *n*-6 PUFA than the reference patients ($p<0.001$).

In plasma CE, the percentage of fatty acids that were *n*-6 PUFA was again lower in the colectomy group (Figure 6.46). In this fraction the colectomy patients had 15% less *n*-6 PUFA than the reference group and this was highly significant ($p<0.001$).

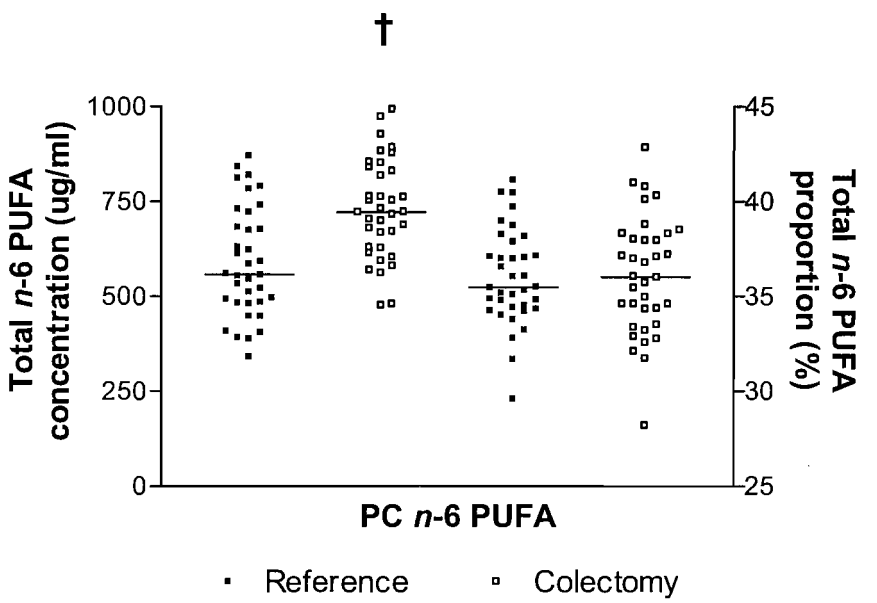


Figure 6.43 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of total *n*-6 PUFA in plasma PC (sum LA, GLA, DGLA and ARA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = $p<0.001$ (Mann-Whitney U).

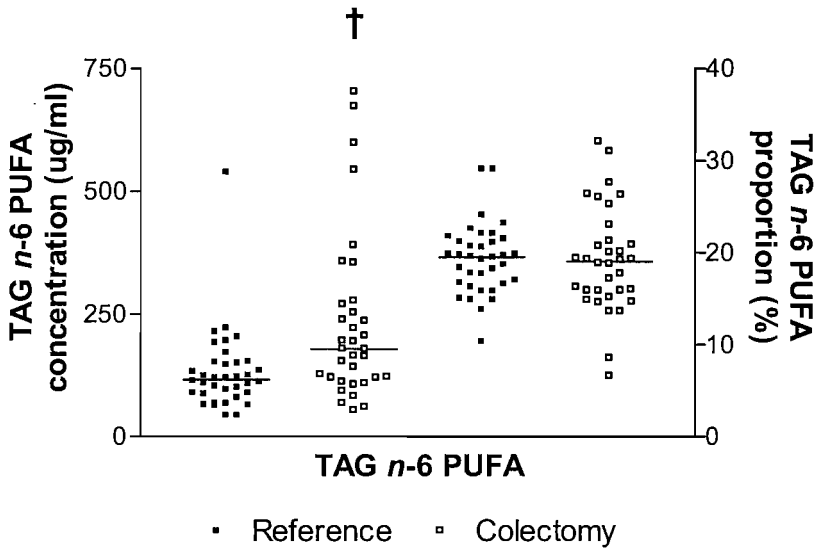


Figure 6.44 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of total *n*-6 PUFA in plasma TAG (sum LA, GLA, DGLA and ARA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

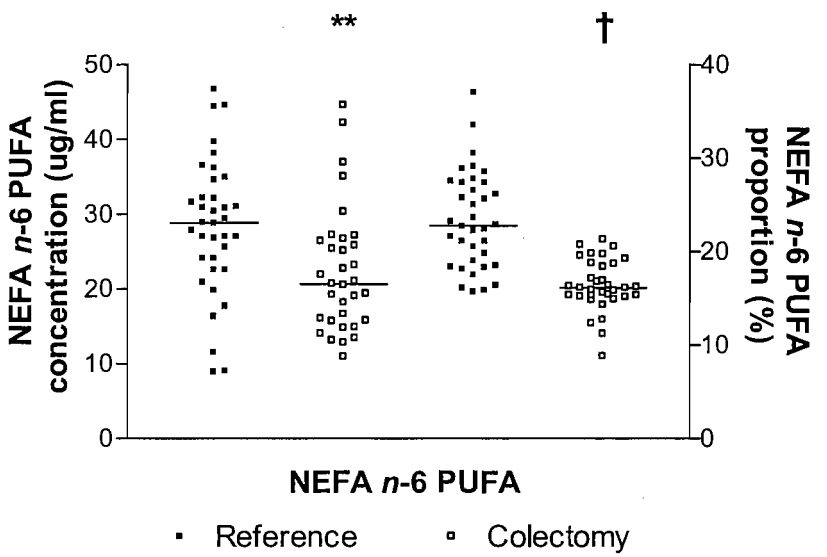


Figure 6.45 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of total *n*-6 PUFA in plasma NEFA (sum LA, GLA, DGLA and ARA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01; † = p<0.001 (Mann-Whitney U).

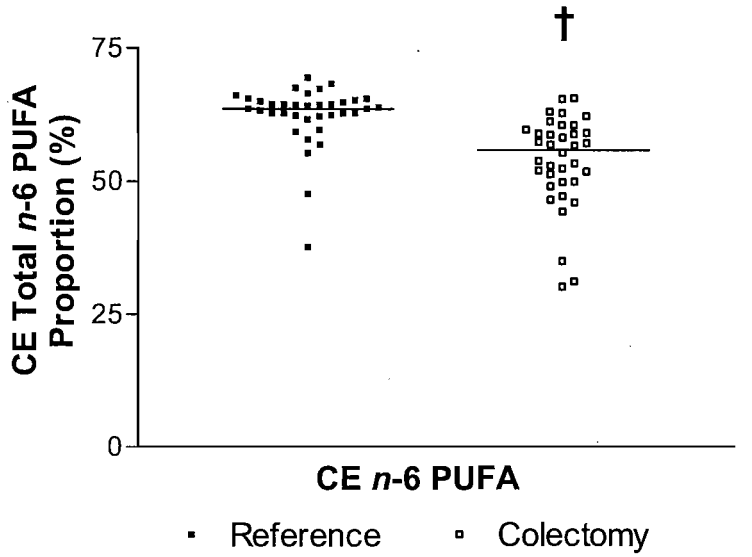


Figure 6.46 Relative proportion (%) of total *n*-6 PUFA in plasma CE (sum LA, GLA, DGLA and ARA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

6.6.9 TOTAL N-3 POLYUNSATURATED FATTY ACIDS

The sum of ALNA, EPA, DPA and DHA was calculated to give a value for total *n*-6 PUFA concentration and the total proportion of these fatty acids in plasma lipids. The results are shown in Figure 6.47 to Figure 6.50.

In plasma PC, the concentration of *n*-3 PUFA was similar between the two groups (Figure 6.47). However, the proportion of this group of fatty acids was roughly 20% lower in the colectomy patients and this was highly significant ($p<0.001$).

In the TAG fraction, the *n*-3 PUFA concentration was almost 40% higher in the colectomy group (Figure 6.48) but this was not significant. Despite this higher concentration, the proportion of *n*-3 PUFA was significantly lower; the colectomy patients had approximately 15% less TAG *n*-3 PUFA than the reference patients ($p<0.01$).

The pattern of *n*-3 PUFA in plasma NEFA was different to that seen in the PC and TAG fractions (Figure 6.49). The concentration of *n*-3 PUFA was almost 70% higher in the colectomy patients and this was highly significant ($p<0.001$).

The proportion of these fatty acids in this fraction was also significantly higher: the colectomy patients had 50% more *n*-3 PUFA in their plasma NEFA than the reference patients ($p<0.001$).

In the CE fraction, the proportion of *n*-3 PUFA was again found to be significantly lower in the colectomy group (Figure 6.50). These patients had approximately 30% less *n*-3 PUFA than the patients in the reference group and this was highly significant ($p<0.001$).

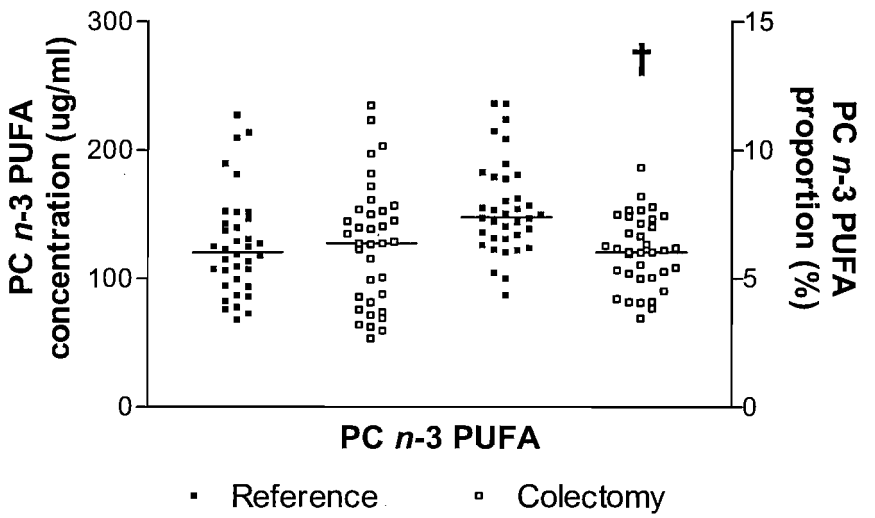


Figure 6.47 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of total *n*-3 PUFA in plasma PC (sum ALNA, EPA, DPA and DHA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = $p<0.001$ (Mann-Whitney U).

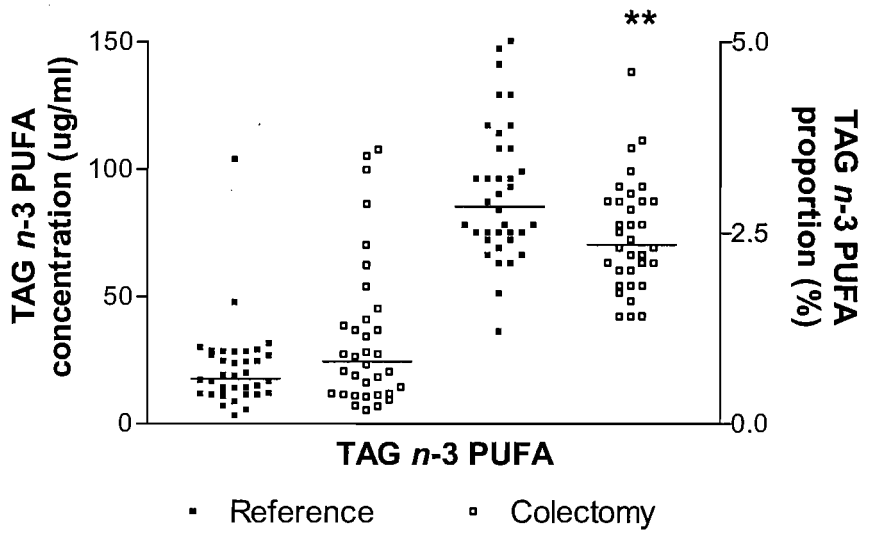


Figure 6.48 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of total *n*-3 PUFA in plasma TAG (sum ALNA, EPA, DPA and DHA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = p<0.01 (Mann-Whitney U).

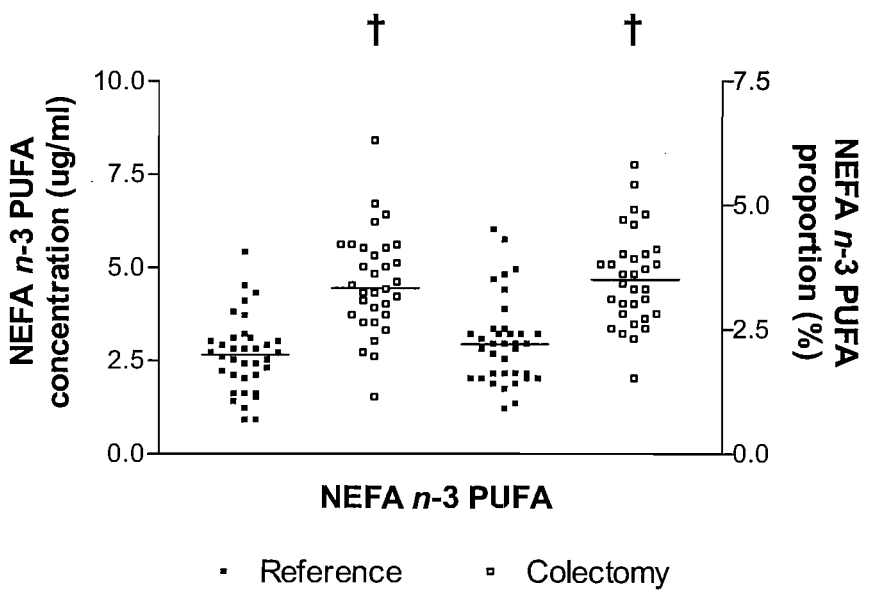


Figure 6.49 Concentration (µg/ml; left axis and data points) and relative proportion (%; right axis and data points) of total *n*-3 PUFA in plasma NEFA (sum ALNA, EPA, DPA and DHA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

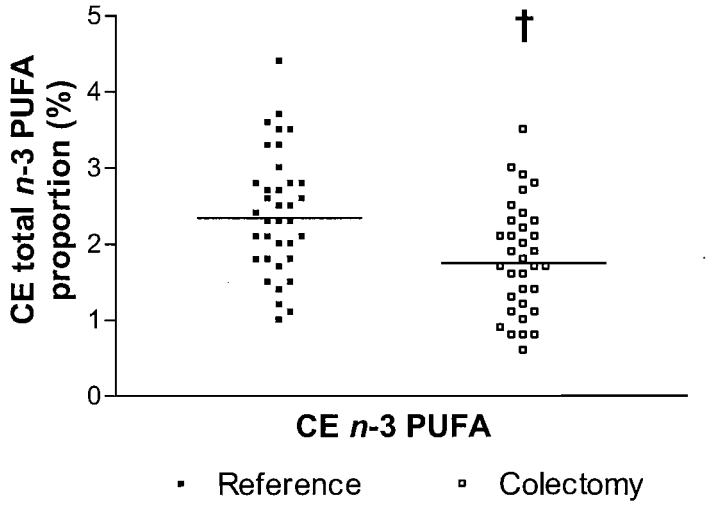


Figure 6.50 Relative proportion (%) of total *n*-3 PUFA in plasma CE (sum ALNA, EPA, DPA and DHA). Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

6.6.10 TOTAL N-6 TO N-3 PUFA RATIO

The concentration and proportion of *n*-3 and *n*-6 PUFA in plasma lipids of the reference and colectomy patients has been described in previous sections, where it was seen that the colectomy patients often had a different concentration and/or proportion of these fatty acids when compared to the reference group. In order to gain more information on the balance between these two groups of fatty acids, the ratio of *n*-6:*n*-3 PUFA was calculated by simple division of the total *n*-3 and *n*-6 PUFA in each of the plasma lipid fractions. The results are given in Figure 6.51 to Figure 6.54.

The ratio of *n*-6 to *n*-3 PUFA in plasma PC was roughly 30% higher in the colectomy group than in the reference group (Figure 6.51). This finding was significant ($p<0.01$). From the previous observations, it was noted that this increased ratio of *n*-6 to *n*-3 PUFA was primarily a feature of a lower proportion of *n*-3 PUFA in the colectomy group, rather than a higher proportion of *n*-6 PUFA.

The ratio of *n*-6 to *n*-3 PUFA was also higher in plasma TAG of the colectomy group but this was not significant (Figure 6.52). This altered ratio was also feature of a lower proportion of *n*-3 PUFA in this fraction.

In the plasma NEFA fraction, there was a lower ratio of *n*-6 to *n*-3 PUFA in the colectomy patients (Figure 6.53) and this was highly significant ($p<0.001$). As described in previous sections, the colectomy patients were found to have a higher concentration and proportion of *n*-3 PUFA accompanied by a lower concentration and proportion of *n*-6 PUFA, which explains the lower *n*-6 to *n*-3 PUFA ratio seen here.

In the CE fraction, the ratio of *n*-6 to *n*-3 PUFA was similar between the two groups (Figure 6.54). This was expected, as previous observations noted that decreases of approximately of equivalent magnitude were present in both the proportions of *n*-6 and *n*-3 PUFA.

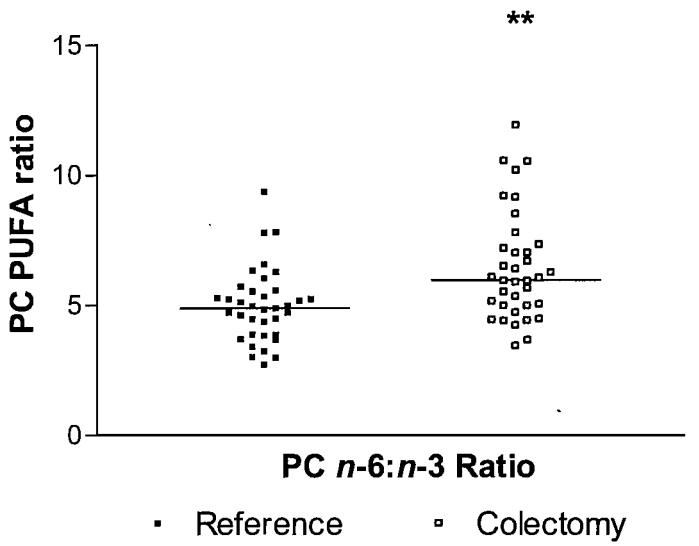


Figure 6.51 Ratio of *n*-6:*n*-3 PUFA in plasma PC. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. ** = $p<0.01$ (Mann-Whitney U).

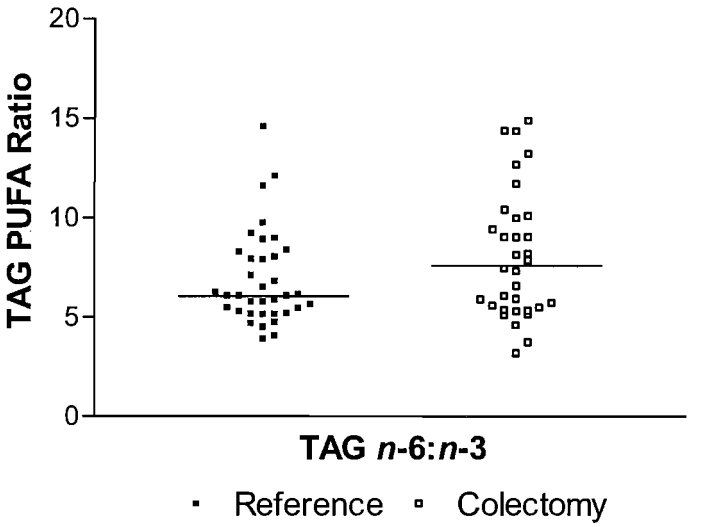


Figure 6.52 Ratio of *n*-6:*n*-3 PUFA in plasma TAG. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

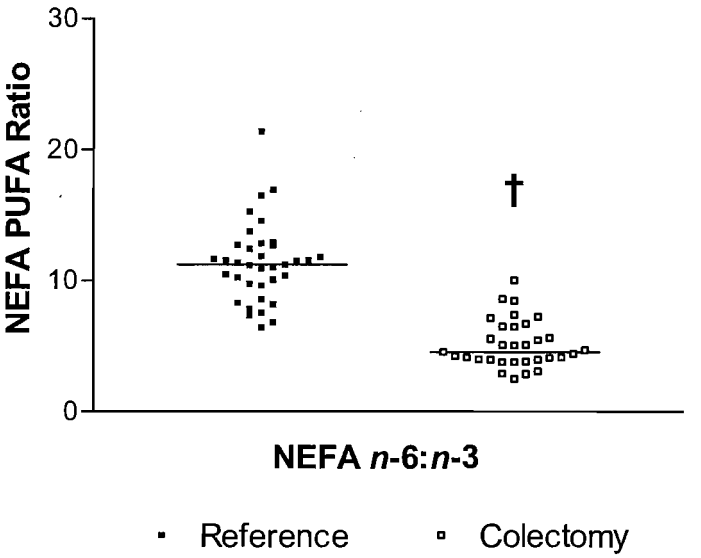


Figure 6.53 Ratio of *n*-6:*n*-3 PUFA in plasma NEFA. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. † = p<0.001 (Mann-Whitney U).

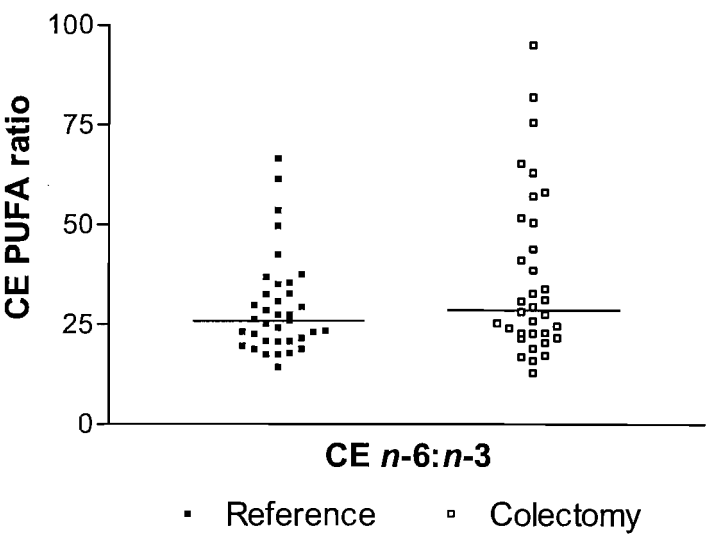


Figure 6.54 Ratio of *n*-6:*n*-3 PUFA in plasma CE. Horizontal lines indicate the median values. Filled squares are the reference group; hollow squares the colectomy group. Reference and colectomy groups were not significantly different (Mann-Whitney U).

6.7 SUMMARY OF RESULTS

In summary, the salient observations are given below (comparisons with respect to the colectomy patients). The results are also summarised in a tabular fashion in Table 6.1.

Total Fatty Acid Concentration

- Markedly higher in PC and TAG
- Similar to reference group in NEFA fraction

EFA

- ALNA concentration similar between groups in PC and TAG, but higher in NEFA.
- Proportion of ALNA lower in PC, TAG and CE but higher in NEFA.
- Concentration of LA higher in both PC and TAG, but proportions similar.
- Significantly lower proportion of LA in CE.
- NEFA LA composition similar between groups.
- Ratio of LA:ALNA concentration was higher in PC and TAG but lower in NEFA. CE LA:ALNA ratios similar.

EFA Metabolites: ARA & EPA

- EPA concentration higher in TAG but similar in the other fractions.
- Proportion of EPA lower in PC, TAG and CE.
- ARA concentration higher in PC, TAG and NEFA.
- ARA proportion also higher in TAG, but lower in CE.
- Ratio of ARA:EPA generally higher.

Longer Chain EFA Metabolites: GLA, DGLA, DPA & DHA

- GLA concentration higher in TAG but lower in NEFA.
- GLA proportion higher in TAG and CE but lower in NEFA.
- DGLA concentration higher in PC and TAG and proportion also higher in TAG, but both lower in NEFA.
- DPA concentration higher in PC, TAG and NEFA. Proportion also higher in TAG, NEFA and CE.

- Similar concentrations of DHA observed in all fractions. Proportion of DHA lower in all fractions.

Fatty acid concentrations			EFA			Eicosanoid Precursor FAs			LC-PUFA				Totals		
	Fraction	Total FA	LA	ALNA	LA:ALNA	ARA	EPA	ARA:EPA	GLA	DGLA	DPA	DHA	*n*-3	*n*-6	*n*-6:*n*-3
	PC	↑†	↑†	↓	↑**	↑**	↔	↑	NA	↑†	↑**	↑	↔	↑†	↑**
	TAG	↑**	↑**	↔	↑*	↑†	↑	↑**	↑†	↑**	↑†	↔	↑	↑†	↑
	NEFA	↔	↔	↑†	↓†	↔	↔	↔	↑†	↓†	↑†	↔	↑†	↓**	↓†
	CE	NA			↔	NA		↑*	NA						↔

Fatty acid proportions (%)		EFA		Eicosanoid Precursor FAs		LC-PUFA				Totals	
	Fraction	LA	ALNA	ARA	EPA	GLA	DGLA	DPA	DHA	*n*-3	*n*-6
	PC	↔	↓†	↔	↓	NA	↔	↓	↓†	↓†	↔
	TAG	↓	↓†	↑*	↓	↑**	↔	↑*	↓†	↓**	↔
	NEFA	↓	↑†	↔	↔	↑†	↓†	↑†	↔	↑†	↓†
	CE	↓†	↓†	↓*	↓†	↑**	↓†	↑**	↔	↓†	↓†

Table 6.1 A summary of the results seen when comparing the fatty acid concentrations and proportions of the reference and colectomy groups. Upwards arrows represent higher; and downwards arrows lower; values in the colectomy group in comparison to the reference group. Horizontal arrows represent no difference between the groups. Significant differences (as analysed by Mann-Whitney U) are indicated with *, ** or † and represent $p<0.05$, <0.01 and 0.001, respectively. NA – not applicable or no results available.

6.8 DISCUSSION

The results presented above describe the fatty acid composition of plasma PC, TAG, NEFA and CE in reference and colectomy patients. The results supported the initial hypotheses that predicted the colectomy patients would exhibit alterations in fatty acid composition despite apparent well being and that these differences would vary between lipid fractions.

6.8.1 TOTAL FATTY ACID CONCENTRATION

Overall, the total fatty acid concentration in PC and TAG was significantly higher in the colectomy patients. The concentration of TAG was almost two-fold higher in the colectomy group. As all TAG molecules are surrounded with a PL coat, it would be expected that a higher TAG concentration would also be accompanied by a higher PC fatty acid concentration. This was certainly the case as the PC fatty acid concentration was 25% higher in the colectomy patients. However, in contrast to the higher concentration of PC and TAG, the NEFA concentration was similar between the groups.

The reasons for this higher TAG concentration were not clear. If indeed these patients are lacking in some fatty acids it could be that the higher TAG (and therefore PC) concentration is a compensatory mechanism to increase the availability of fatty acids to target cells and tissues.

Higher TAG concentrations in the colectomy patients could also be a result of insulin resistance; a condition which is a common feature in obese individuals, especially around the age of 40 years (Frayn, 1996e). However, it is interesting to note that the NEFA concentration, which is a proxy measure for release of fatty acids from adipose tissue, was not higher in the colectomy patients. This may be because NEFA utilisation by cells is dependent on plasma concentration, therefore an increase in NEFA concentration in the plasma will result in their increased uptake and utilisation (Frayn, 1996f).

The total fatty acid concentration was different between the two groups as the colectomy patients had a considerably higher concentration of fatty acids in the

form of PC and TAG. Thus, the fatty acid transport pool was much larger in this group.

6.8.2 ESSENTIAL FATTY ACID STATUS

Despite a higher concentration of total fatty acids in PC and TAG, an equivalent increase in ALNA concentration was not observed in these fractions. Indeed, the proportion of this fatty acid was significantly lower in PC and TAG, and also in CE. Recall that total fatty acid concentration in NEFA was similar between the two groups. However, ALNA concentration and proportion was higher in this fraction.

These results suggest that ALNA supply to target cells and tissues from the liver in the form of LPs may be limited. This could be a result of inadequate dietary supply, insufficient digestion and/or absorption, or demands for synthesis of longer chain *n*-3 PUFAs which cannot be met. Increased demands or utilisation of LC-PUFA (either *n*-3 or *n*-6) would result in up-regulated conversion of ALNA to EPA and DHA via the Δ^5 and Δ^6 desaturase enzymes. It could also be a result of selective partitioning of this fatty acid at the level of the liver which targets the storage of this fatty acid in adipose tissue. This could explain why there was a higher availability of ALNA from NEFA in these patients in comparison to the colectomy group.

The higher concentration of total fatty acid in TAG and PC was accompanied by an almost equivalent increase in the concentration of LA in these two fractions. As a result of this, the proportion of this fatty acid in these fractions was similar between the study groups. However, the proportion of LA in the CE fraction was significantly lower, although this was less marked than the lower proportion of ALNA observed in the same fraction.

These results do not suggest that LA export from the liver is lower in the colectomy patients, although this may be a marginal status as the proportion of LA in CE is lower. LA supply from the diet is unlikely to be insufficient as the average Western diet contains ample LA and other *n*-6 PUFA. However, it is possible that the digestion and absorption could be limited in these patients. If

the previous suggestion of increased EFA conversion to LC-PUFA is true, the supply of LA may be only marginally sufficient to satisfy demands for this.

The ratio of LA:ALNA was also presented for each of the lipid fractions and the findings served to re-iterate what was discussed above. The ratio of LA:ALNA was significantly higher in the PC and TAG fractions. This supports the suggestion that ALNA may be limited in these fractions but LA is not. The ratio of LA:ALNA was lower in the NEFA fraction of the colectomy group and this was a result of a higher proportion of ALNA in this fraction, accompanied by a lower proportion of LA. Interestingly, despite a lower proportion of both ALNA and LA in the CE fraction, the ratio in the colectomy group was not different to the reference group. This could suggest that the availability of both EFAs is slightly limited in this fraction, but to a similar extent for each fatty acid.

In summary, it appears that the colectomy patients may be lacking ALNA, but are probably not deficient in LA. It appears there may be a drain on EFAs in the colectomy patients, possibly due to up-regulation of LC-PUFA synthetic pathways, which in turn may be driven by increased demands for eicosanoid precursors or other LC-PUFA. If this were the case, it would appear that LA supply is adequate to sustain the requirements of this possible up-regulation, whereas ALNA supply is not.

6.8.3 ESSENTIAL FATTY ACID METABOLITES: ARACHIDONIC AND EICOSAPENTAENOIC ACIDS (EICOSANOID PRECURSORS)

The concentration of EPA in plasma PC did not reflect the higher concentration of total fatty acids in this fraction. As a consequence, the proportion of this fatty acid was lower in the colectomy patients. EPA proportion was also lower in the TAG fraction, despite a higher concentration. This was because the difference between the two groups in EPA concentration was less than that of total fatty acid concentration. CE EPA proportion was also lower in the colectomy group. NEFA EPA composition was similar between the two groups.

These results suggest that EPA availability, perhaps for supply to target tissues, may be limited in the colectomy patients. As suggested previously, this could be

a result of increased demands for EPA either as an eicosanoid or for DPA/DHA synthesis. The supply of EPA from the diet is likely to be low in these groups as a typical Western diet contains little *n*-3 PUFA, especially LC-PUFA. Thus, the main source of EPA will be from ALNA conversion. As ALNA also appeared to be limited, and desaturase activity may be up-regulated for formation of DPA and DHA, these factors could further exacerbate a marginal EPA status in these patients.

The higher concentration of ARA observed in the PC fraction of the colectomy patients was of an equivalent magnitude to the higher concentration of total fatty acids seen in this fraction. Consequently, the proportion of ARA was not different between the two groups. ARA concentration in TAG was also higher, but in this case, of a greater magnitude than seen in total fatty acid concentration in TAG. Accordingly, the proportion of ARA was also higher in the colectomy group. This pattern was not seen in the NEFA fraction as ARA composition was similar between the groups. In contrast to the other fractions, ARA proportion in CE was lower in the colectomy group.

These observations suggest that the availability of ARA to target cells is probably not limited as both the precursor and ARA itself appear to be in adequate supply. The demands for this fatty acid as an eicosanoid precursor may well be increased if there is inflammation present, but the supply of LA may appears to be sufficient to supply this increased conversion to ARA. The conversion of LA to ARA could also be up-regulated in conjunction with increased demands for longer chain *n*-3 PUFA as the enzymes are common to both pathways.

The ratio of ARA:EPA was also expressed graphically in the results section. An increased ratio of ARA:EPA was seen in the PC, TAG and CE fractions. However, the reasons for this differ between the fractions. The increased ratio in the PC fraction appeared to be due to decreased EPA availability. In the TAG fraction it appeared to be a consequence of both increased ARA and decreased EPA availability. In the CE fraction, EPA and ARA proportions were lower, but to a greater extent for EPA.

To summarise, the results suggest that the EPA content of the plasma transport pool is limited. In contrast, the evidence does not suggest that ARA availability is limited. The imbalance of these fatty acids may be indicative of increased demands for ARA (perhaps for eicosanoid synthesis) or for DHA. In the case of the *n*-3 PUFA, it would seem that the supply of the precursor EFA is not sufficient to meets the requirements of the conversion pathway.

6.8.4 LONGER CHAIN ESSENTIAL FATTY ACID METABOLITES: γ-LINOLENIC, DIHOMO-γ-LINOLENIC, DOCOSAPENTAENOIC AND DOCOSAHEXAENOIC ACIDS

The total fatty acid concentration in plasma TAG was approximately 93% higher in the colectomy group. However, it was observed that the GLA concentration in these patients was a staggering 170% higher and DGLA concentration was 120%. The proportions of GLA and DGLA were also higher; 50% and 16%, respectively. PC DGLA concentration was also higher. These findings, together with the observations for ARA, could support the suggestion that the EFA conversion pathways are up-regulated, stimulating the synthesis of longer chain *n*-6 PUFA.

Findings related to the CE fraction were slightly different to above. Although the proportion of ARA in this fraction was lower in the colectomy patients, the proportion of GLA was higher and DGLA was not different.

In the NEFA fraction, the concentration and proportion of GLA and DGLA were both markedly lower in the colectomy group, although ARA comnposition was similar. The results overall do not suggest that the longer chain *n*-6 PUFAs are lacking in the colectomy patients.

With regard to *n*-3 PUFA, the concentration of DPA in PC, TAG and NEFA was significantly higher. The proportion of this fatty acid was also higher in TAG, NEFA and CE. However, both the concentration and proportion of DHA was lower in the colectomy patients in all fractions. This apparent good availability, coupled with an apparent lack of DHA could suggest that the conversion of DPA

to DHA is insufficient to meet the demands of DHA. If indeed the EFA conversion pathways are up-regulated as previously suggested, this would not be limited to purely the *n*-3 pathway as the enzymes are common to both the *n*-3 and *n*-6 pathways.

In summary, the results suggest that the availability of the *n*-6 LC-PUFA are not limited as concentration and proportion of these fatty acids are generally higher in the colectomy group. This could be indicative of up-regulation of the EFA conversion pathways. However, the results for the *n*-3 PUFA appear to show an apparent abundance of DPA. This was accompanied by a shortage of DHA which was consistent across all lipid fractions. DHA was the only fatty acid measured whose concentration and proportion was lower in the colectomy patients in every fraction. These results again seem to point to up-regulated conversion of EFA to LC-PUFA which could be to satisfy demands for ARA and EPA or for DHA. The lack of DHA coupled with high amounts of DPA suggests that the synthesis of DHA is not sufficient to meet demands.

6.8.5 TOTAL N-3 AND N-6 PUFA COMPOSITION

The overall balance of *n*-3 and *n*-6 PUFA in the four fractions did not give a particularly good representation of what was observed for each of the fatty acids in isolation. This illustrated the importance of studying each of the fatty acids individually.

N-6 PUFA concentration in both PC and TAG was significantly higher in the colectomy patients, although the proportion was similar. The *n*-6 PUFA concentration in these fractions had risen in line with the total fatty acid concentration, hence why their proportion had remained the same. The *n*-3 PUFA concentration however, had not risen in line with the total fatty acid concentration, thus the *n*-3 PUFA proportion overall was lower in the colectomy group.

In the NEFA fraction, both the concentration and proportion of *n*-6 PUFA was lower, but *n*-3 PUFA concentration and proportion was higher in the colectomy group. The proportion of both *n*-3 and *n*-6 PUFA was lower in the CE fraction,

which means that the SFA or MUFA proportion must be contributing a higher proportion instead.

6.8.6 RATIONALE FOR SEPARATING PLASMA LIPID FRACTIONS

In this study, the fatty acid composition of individual plasma lipid fractions was determined (PC, TAG, NEFA and CE) rather than the more general measurement of total plasma lipids often employed in other studies, presumably because it is less laborious and costly. The reasons for separating the plasma into its individual component fractions was briefly eluded to in Chapter 2 and will be more fully discussed here.

When reviewing the overall results, generally where the relative compositions of the PC, TAG and CE fractions often showed similarities to each other, the pattern of fatty acids making up the NEFA fraction often showed the opposite. This was seen particularly for *n*-3 PUFA proportions. NEFA composition represents that of adipose tissue; formed from fatty acids which are partitioned for storage in the fed state. Perhaps the NEFA may also be more long term representation of historic dietary intake, as fatty acids consumed in the diet and appearing in the circulation are then stored as adipose tissue in the fed state. Then in the fasted state (as in this study), NEFA release from adipose tissue is stimulated and the composition reflects principally the fatty acids mobilised from adipose tissue (storage pool).

In the fasting state, the fatty acid compositions of plasma PC, TAG and CE are the net product of: a) hepatic lipid synthesis b) VLDL export and c) turnover within the plasma transport pool. The composition these pools particularly is also influenced by partitioning of certain fatty acids towards oxidation and storage. This may be particularly important for the EFAs (ALNA and LA) which are precursors for the LC-PUFAs so may be metabolised for synthesis of such fatty acids. In addition, fatty acids may be selectively partitioned towards lipids secreted by the liver into the transport pool. In consideration of these influences on the composition of hepatically-derived lipids (i.e. PC, TAG and CE), perhaps the NEFA could be considered as a representation of the fatty acid composition

of these lipids, subsequent to any partitioning and utilisation for cellular demands. This is represented pictorially in Figure 6.55.

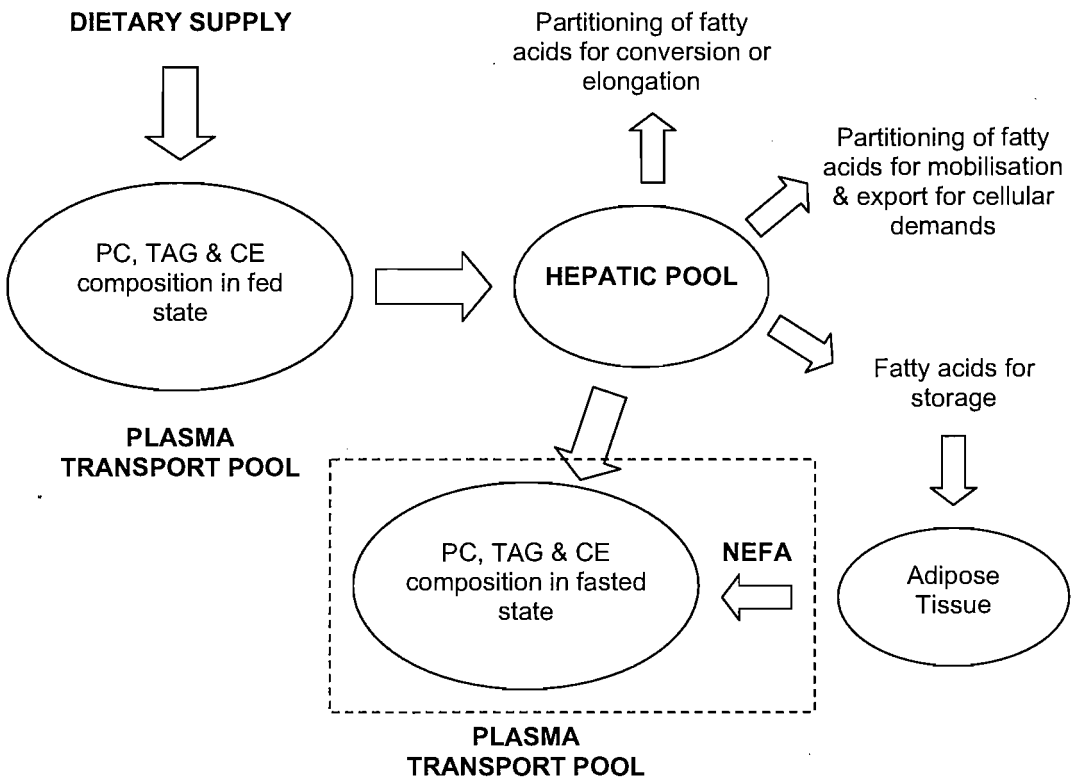


Figure 6.55 Diagram to illustrate the transport pathways for lipids. The dashed line indicates the compartments which were measured and analysed for this study.

In view of the discussions above, it was vitally important to measure not just the total fatty acid composition of the plasma, but to sub-divide this into its constituent fractions. The diagram in Figure 6.55 quite clearly indicates that NEFA and PC fatty acids originate from fundamentally different compartments in the body.

In addition to the importance of analysing the composition of plasma NEFA, it was also imperative to analyse the PC, TAG and CE fractions individually. Although the definitive biological role and importance of these fractions is not fully understood, it is clear from the results presented in this chapter that each fraction has its own characteristic composition and that although general agreement exists at least between the hepatic fractions, they are not the same.

it is generally considered that PC is the most functionally relevant fraction as it is from this pool of fatty acids that exchange with cells and tissues takes place.

With respect to these concepts, it is possible that the pattern of fatty acids seen between the four lipid fractions could suggest a shortage of *n*-3 PUFA in the plasma pool of the colectomy patients. Recall the overall pattern of results presented in Table 6.1. Total fatty acid and *n*-6 PUFA concentrations were markedly higher in the colectomy group. However, the pattern was not generally observed with the *n*-3 PUFA. As a result, proportions of the *n*-3 PUFA were generally much lower in the colectomy patients. However, this was only generally true of the hepatically-derived fractions – not NEFA. Where lower proportions of ALNA, EPA and DHA were observed in the hepatically-derived fractions, NEFA proportions of these fatty acids were either higher or not different in the colectomy patients. This indicates that the *n*-3 PUFA are not being exported from the liver into the plasma, therefore they are being partitioned for oxidation, elongation or conversion, or storage. Presumably, fatty acid requirements for storage are less critical, and perhaps hepatic mechanisms are more likely to selectively partition fatty acids for processes other than lipogenesis. This could suggest up-regulated ALNA conversion pathways and/or demands for these fatty acids which are draining the hepatic pool such that exported VLDL have a much lower proportion of these fatty acids.

6.8.7 RATIONALE & JUSTIFICATION FOR MEASURING CONCENTRATION AND PROPORTION OF FATTY ACIDS IN PLASMA

In Chapter 2, a brief explanation into the reasons for expressing the results of this thesis as both concentrations and proportions were given. This topic will be more fully discussed here.

Calculation of absolute concentrations of fatty acids in plasma lipids are often overlooked in composition studies as it is the proportionate values which are considered to be the most relevant; providing a better indication of fatty acid availability to the tissues. Historically, neglecting to assess the concentration of fatty acids in a lipid sample by the addition of lipid standards and calculating recovery may have been more a function of inadequate methods and

procedures rather than simple oversight. However, there is no doubt that today there are several excellent methods for the calculation of fatty acid concentration down to even the smallest amounts and that lipid recoveries of >90% from plasma samples are achievable with careful processing techniques. Although there is an obvious cost implication to performing these extra techniques, the added value of the data which can be obtained from this should be carefully considered.

The main limitation of assessing purely the relative proportion of fatty acids in a sample is that it is very difficult to compare values across studies as the choice of fatty acids reported between studies are rarely the same. The omission or addition of fatty acids to the analysis directly affects the percentage contribution made by the species which are reported; particularly those contributing a small percentage (which are often of highest interest). This results in data which are incredibly difficult to compare with other study studies as the fatty acids reported are rarely the same between groups.

If it were to become a standard within this field of research to report the total concentration of fatty acids alongside values for the fatty acids of interest to that group, then it would provide much greater scope for direct comparisons of data between groups. In the reporting of proportionate data in isolation, this comparative work is limited and difficult at best.

In this study, the results clearly illustrate the significant benefits of assessing both concentration and proportion of fatty acids in a sample. This is well illustrated in the PC fraction. The consideration of the proportionate data alone may have led the investigator to the conclusion that the colectomy patients are simply lacking in *n*-3 PUFA as the proportions of ALNA, EPA, DPA and DHA were all lower in this group. Although this is partly true, the proportionate data do not reveal the marked increase of PC fatty acid concentration in these patients. Although the biological reasons and implications of this increase are not understood in these patients, it is clearly an important observation. In addition, when reviewing the concentration data, DPA and DHA concentrations were seen to be higher in the colectomy patients in comparison to the reference

group. However, when reviewing the proportionate data, it was clear that despite this increase in concentration, the overall contribution of both these fatty acids was not of the same magnitude as the increase in total fatty acid concentration. In other words, whatever the reason for a higher concentration of fatty acids in the colectomy patients, there appeared to be an ability of these subjects to upregulate *n*-6 PUFA concentration to match, but not *n*-3 PUFA. Thus, if concentrations (or the *size* of the fatty acid pool) vary, the qualitative composition is not necessarily affected. Therefore expressing fatty acid data as either concentration or proportion limits the understanding which can be gained by assessing the composition of the plasma.

6.9 CONCLUSIONS

- Fatty acid concentrations, therefore the size of the fatty acid transport pool, in the colectomy patients were higher. This may be explained partly by possible insulin resistance in these patients but may also be an adaptive response to increase the availability of certain fatty acids to the target cells and tissues. This may be due to increased demands or decreased availability of particular fatty acids.
- The colectomy patients did not appear to lack LA. However, the results did suggest limited ALNA availability. Increased conversion of the EFAs into LC-PUFA could explain the marginal ALNA status as this fatty acid is less abundant in the diet than its *n*-6 counterpart.
- The EPA content of the transport pool also appeared to be limited. In contrast, the results did not suggest low ARA availability. Again this could suggest a low *n*-3 PUFA intake, limited conversion of ALNA to LC-PUFA or demands for *n*-3 PUFA which cannot be adequately met.
- LC-PUFA of the *n*-6 family did not seem to have limited availability in the transport pool. However, the results did strongly suggest that DHA availability is low, despite high levels of DPA in all fractions. This suggests that the conversion of DPA to DHA is insufficient in these patients, perhaps due to demands for DHA that cannot be satisfied or a marginal capacity for DHA biosynthesis.

6.10 COMPARISON WITH OTHER STUDIES

The findings of this study can only be put into context if they are related in a comparable way to the findings of other similar studies. As discussed at the beginning of this chapter, this is often very difficult due to much disparity between studies in terms of fatty acids analysed, lipid fractions separated and the form in which results are reported (i.e. concentration versus proportion of fatty acid).

This section aims to compare the results of this study with those of other groups who have studied fatty acids in a similar context. For simplicity, just the ratio of each fatty acid in the patient group compared to the control or reference group has been calculated, and the results presented graphically below. These graphs allow the overall fatty acid profile to be viewed in a single graph to help establish if particular patterns are characteristic of certain conditions.

6.10.1 RESULTS

The graphs demonstrating the results of this current study are show in Figure 6.56. Adapted results from a study of patients with inactive IBD and recent colectomy by Esteve-Comas *et al.* are presented in Figure 6.57, and of patients with active IBD in Figure 6.58. Adapted results from a cohort of established colectomy patients by the same author are given in Figure 6.59. Results from an unpublished study of CF patients are given in Figure 6.60 and Figure 6.61 (Wootton, pers. comm., 2003). Finally, adapted results from a study by Geerling *et al.* of newly diagnosed and long-term CD patients are presented in Figure 6.62.

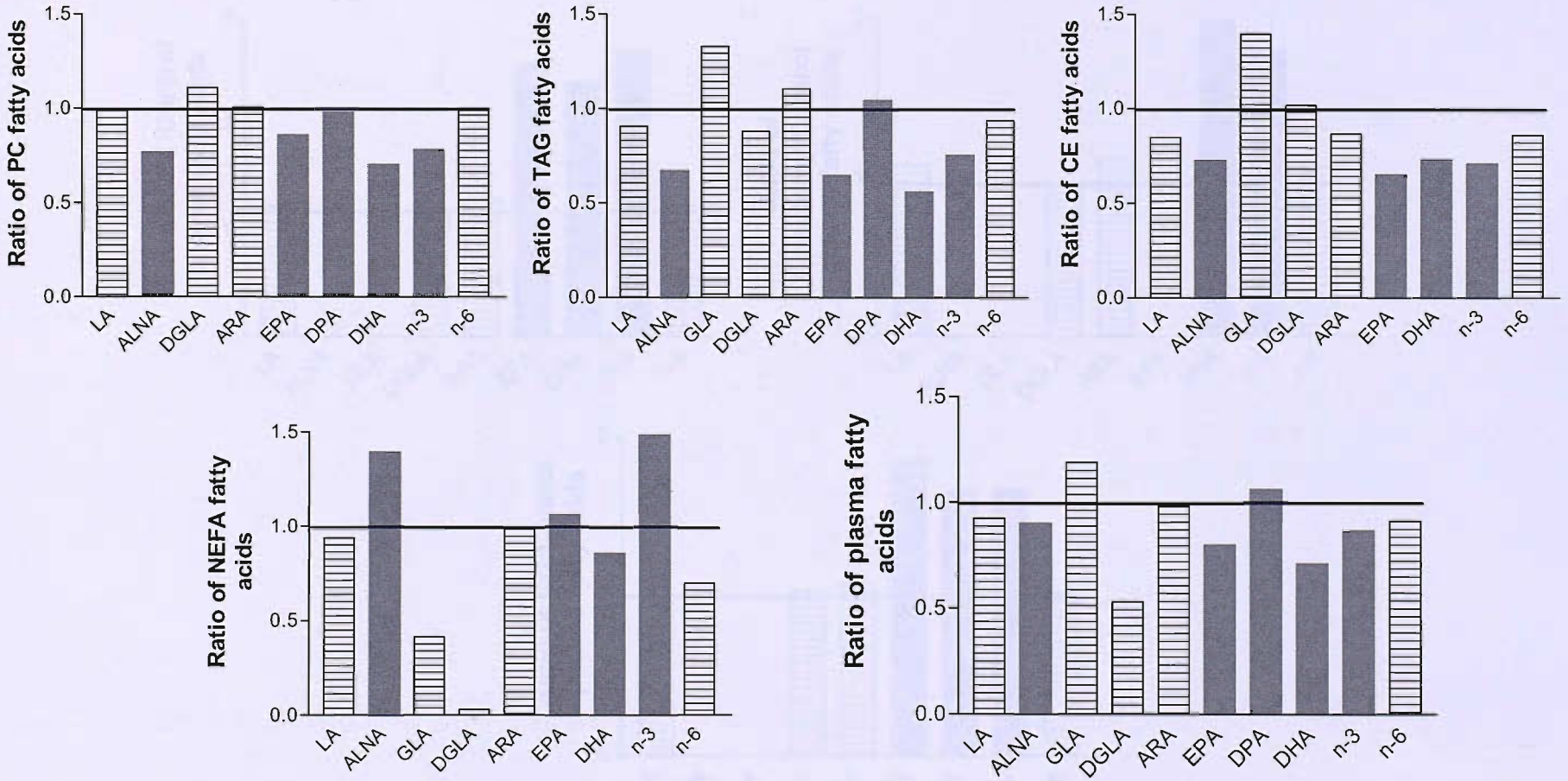


Figure 6.56 The ratio of fatty acids (Colectomy:Reference) in PC, TAG, NEFA, CE and Total Plasma (sum PC, TAG, NEFA & CE). Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA.

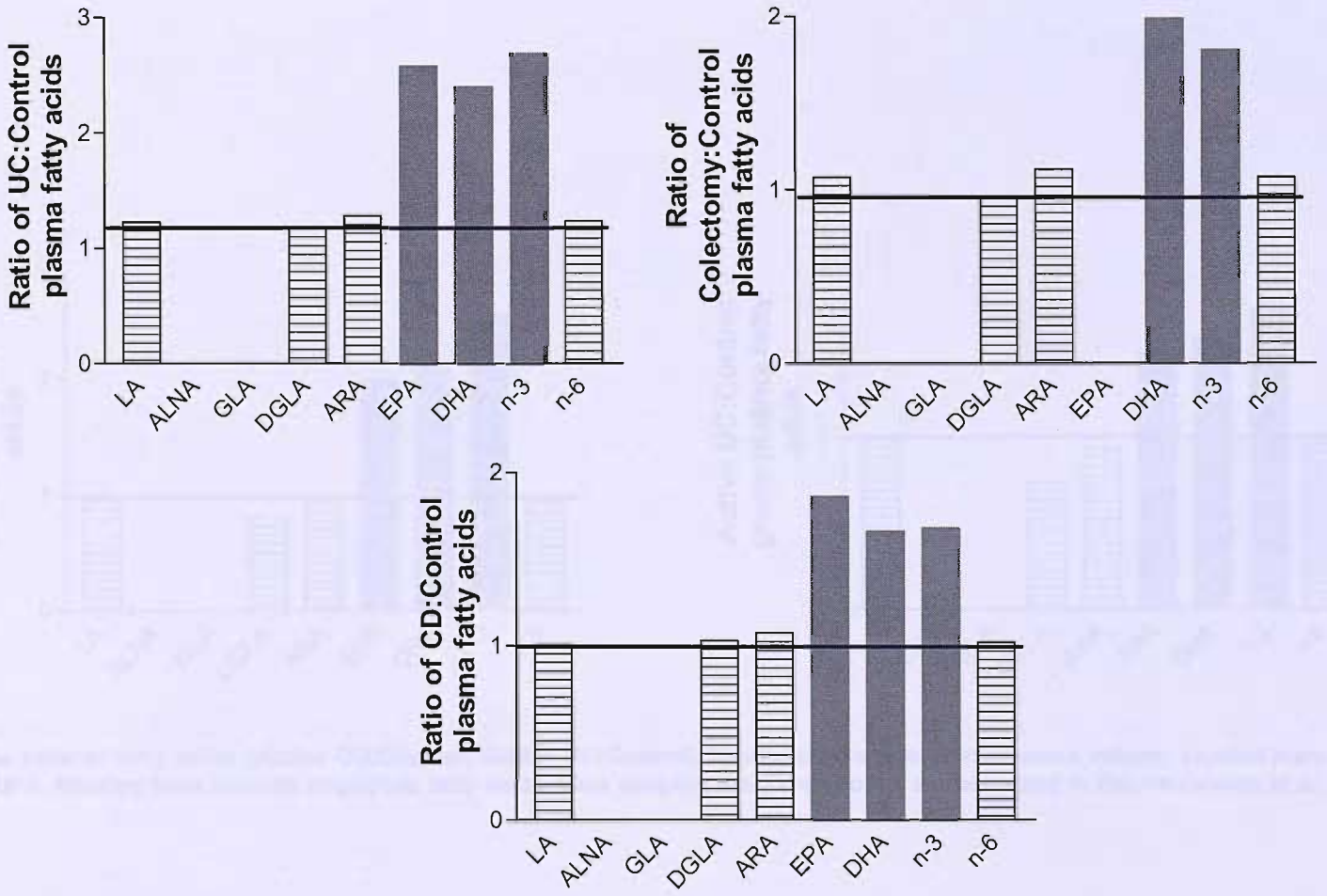


Figure 6.57 The ratio of fatty acids (Inactive UC:Control; Short-Term Colectomy:Control; Inactive CD:Control) in plasma. Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA. Missing bars indicate negligible fatty acids. Data adapted from and groups as described in Esteve-Comas *et al.*, 1993.

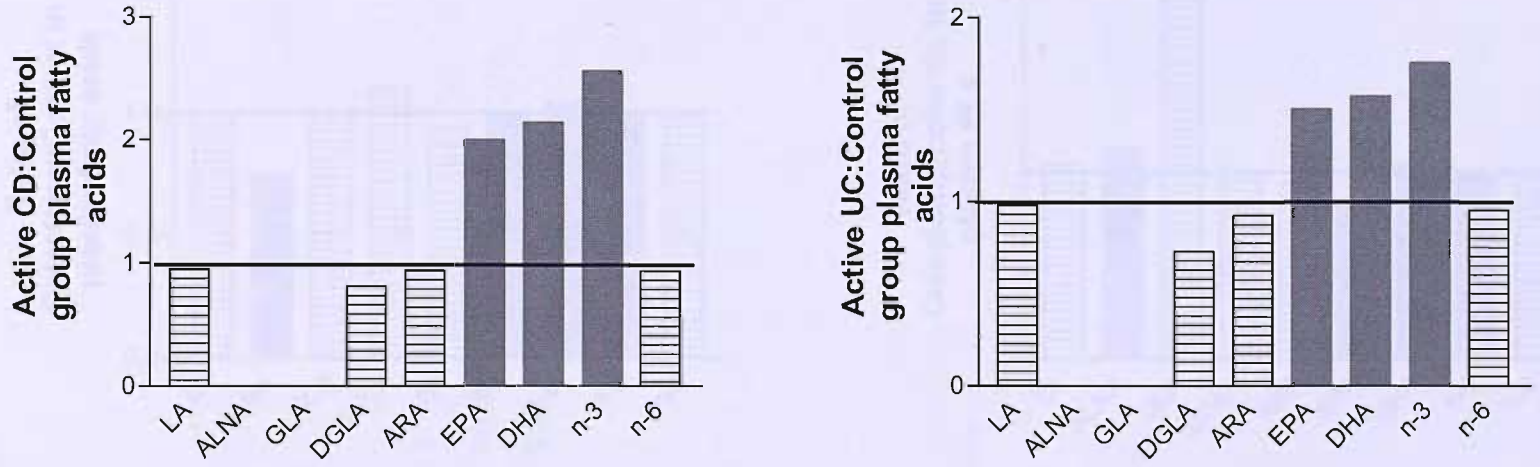


Figure 6.58 The ratio of fatty acids (Active CD:Control; Active UC:Control) in plasma. Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA. Missing bars indicate negligible fatty acids. Data adapted from and groups as described in Esteve-Comas *et al.*, 1992.

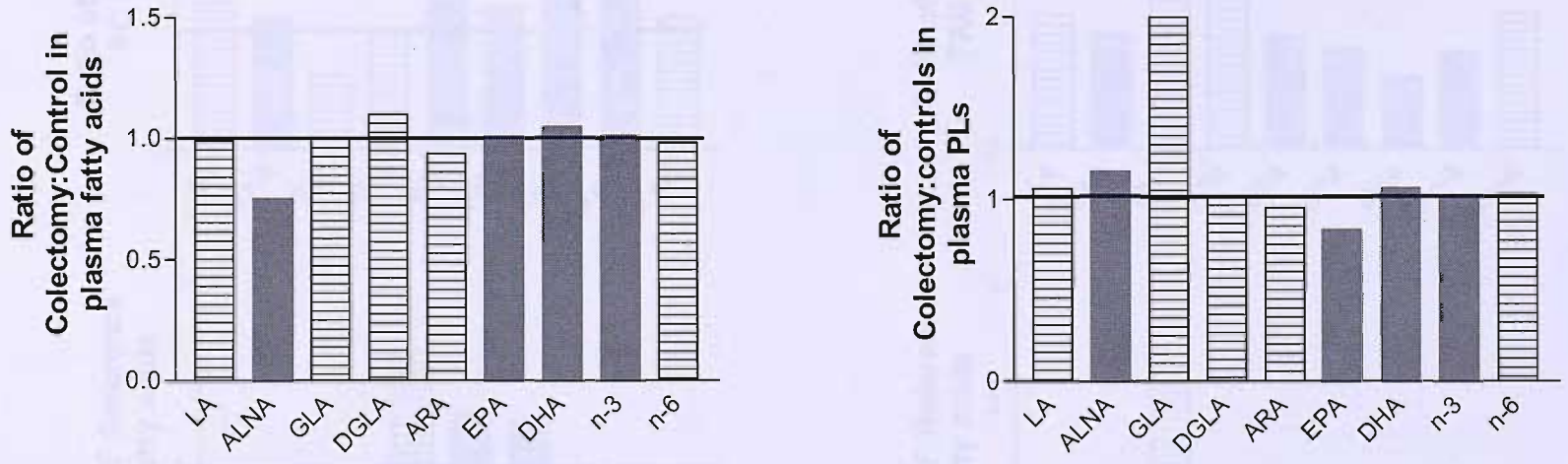


Figure 6.59 The ratio of fatty acids (Established Colectomy:Control Plasma Fatty Acids; Established Colectomy:Control Plasma PL Fatty Acids). Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA. Data adapted from and groups as described in Esteve *et al.*, 1998.

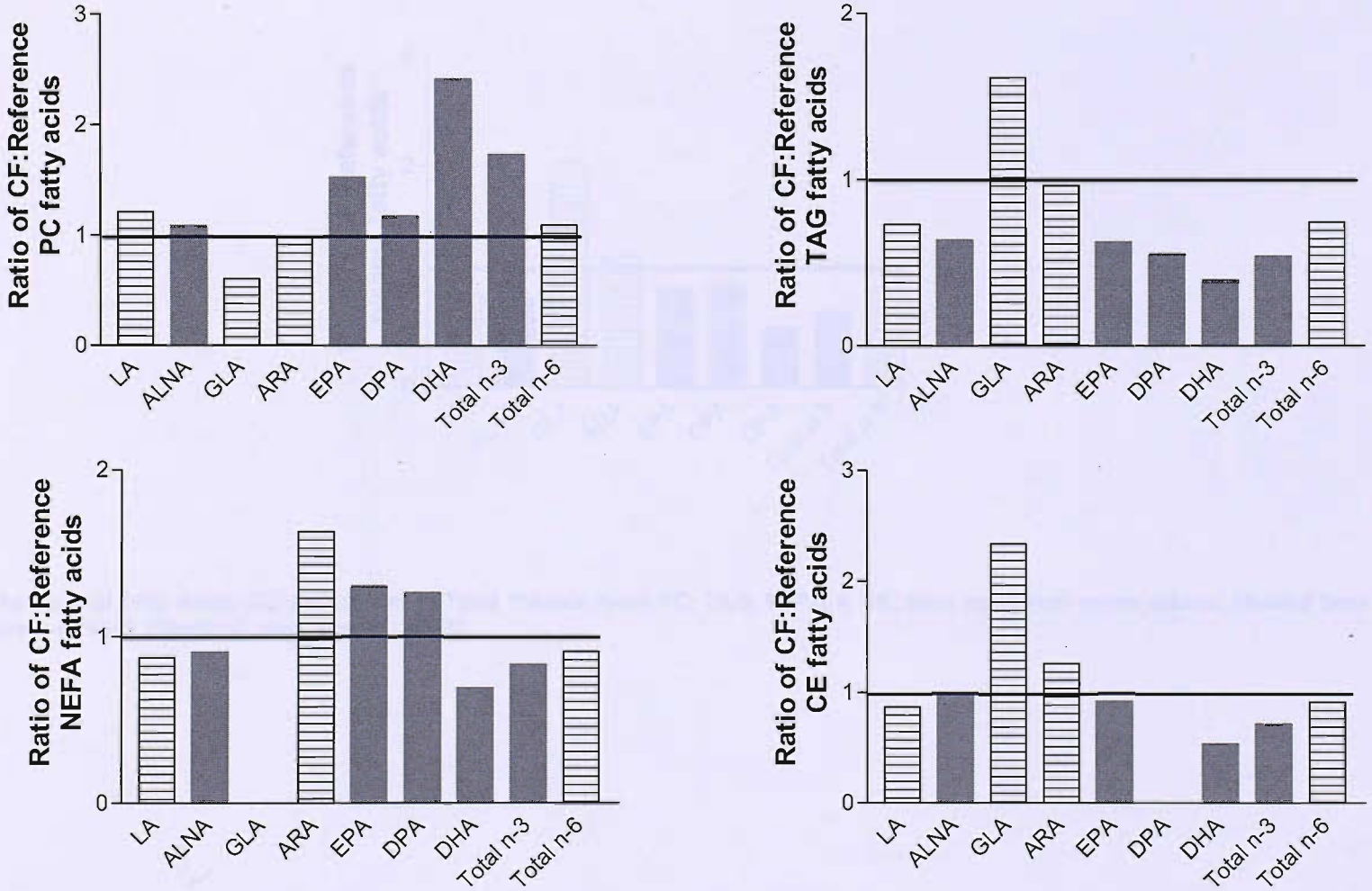


Figure 6.60 The ratio of fatty acids (CF:Reference) in PC, TAG, NEFA & CE. Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA (Wootton, pers. comm., 2003).

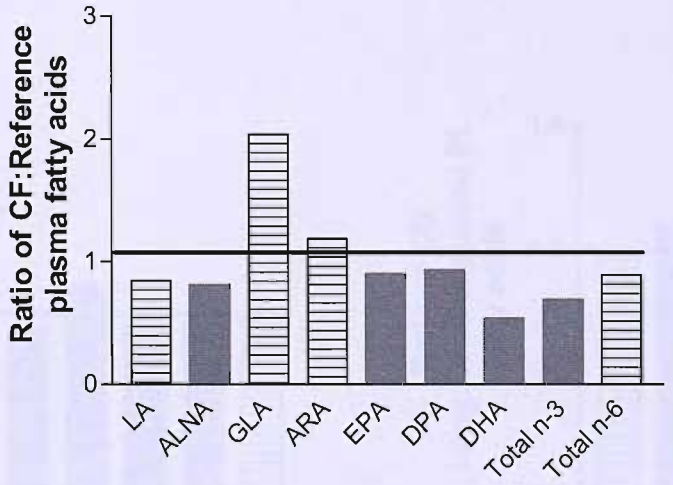


Figure 6.61 The ratio of fatty acids (CF:Reference) in Total Plasma (sum PC, TAG, NEFA & CE. Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA (Wootton, pers. comm., 2003).

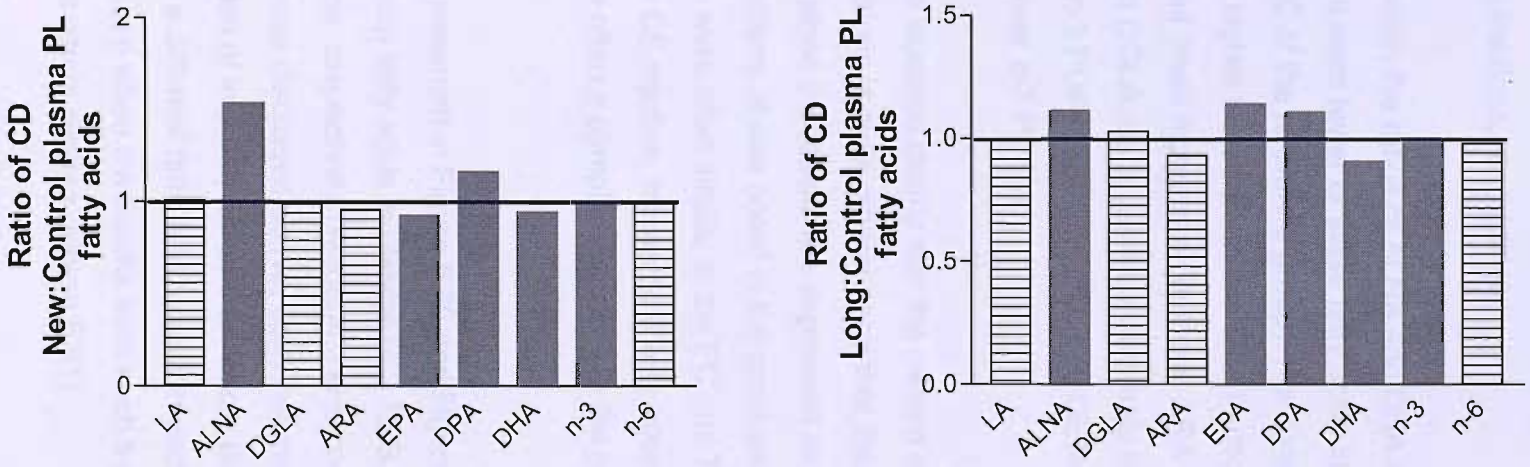


Figure 6.62 The ratio of fatty acids (Newly Diagnosed CD:Control; Long-Term CD:Control). Bars represent mean values; shaded bars are *n*-3 PUFA; striped bars are *n*-6 PUFA. Data adapted from and groups as described in Geerling e*t al.*, 1999.

6.10.2 DISCUSSION

The graphs presented in Figure 6.56 show the ratio of colectomy group to reference group fatty acids in the PC, TAG, NEFA and CE fractions, and the sum of these fractions, respectively.

In the PC fraction the ratios of ALNA and DHA were less than one (i.e. on average there were fewer of these fatty acids in PC of the colectomy patients than in the PC of the reference group). However, the ratios of DGLA and ARA were slightly higher than one. In the TAG fraction, the ratio of GLA was almost one-and-a-half times higher than one and ARA was also higher. In contrast to this, GLA and DGLA ratios were considerably less than one in the NEFA fraction and *n*-3 PUFAs were higher. The CE fraction showed higher GLA and DGLA and lower *n*-3 PUFAs.

These results illustrate clearly that the pattern of fatty acid in each of the separate fractions is quite different and that these patterns are not necessarily represented when the results are expressed as whole plasma rather than individual fractions. It was noted in the previous results section that the fatty acid patterns were often similar in the PC and TAG fractions and to a certain extent, in the CE fraction. However, it was noted that the pattern seen in these fractions was often a complete contrast to the pattern seen in the NEFA fraction.

The graphs presented in Figure 6.60 and Figure 6.61 show the ratio of CF to reference group fatty acids in plasma PC, TAG, NEFA, CE and in the sum of these fractions, respectively (Wootton, pers. comm., 2003). These results, in addition to those discussed above, demonstrate the importance of analysing the plasma fraction of interest, rather than whole plasma alone. These figures also clearly show a different fatty acid pattern in each fraction, which is not necessarily seen when the results from each fraction are summed and expressed as whole plasma (Figure 6.61).

On an individual fraction basis, the findings from the CF patients show few similarities to the results presented in this thesis for the colectomy patients.

However, a high GLA ratio in TAG and CE can be seen in both groups. When the results for whole plasma are compared, several similarities can be seen: lower LA & ALNA ratios, considerably higher GLA ratio and lower DHA, total *n*-3 and *n*-6 ratios. This could suggest some similarities in lipid metabolism between the colectomy patients and those with CF. Indeed, these patient groups may both have GI difficulties, resulting in less than optimal digestive and absorptive abilities. However, similarities between the groups in whole plasma alone, but not between the individual fractions could illustrate the importance of analysing each lipid fraction separately. Here we have two completely different patient groups, each likely to have its own unique pathology resulting in altered lipid metabolism, yet comparison of the total plasma lipids would potentially suggest common pathways. However, analysis of the individual lipid fractions suggests a rather different conclusion.

The graphs in Figure 6.57 are results adapted from Esteve-Comas *et al.,* 1993, who performed a study on patients diagnosed with UC or CD who had been in remission for three months (previously discussed in Chapter 2). These results show a higher ratio of *n*-3 PUFA in the patient cohort which was suggested by the researchers to be due to up-regulated PUFA biosynthesis. This observation of higher *n*-3 PUFA ratios in the UC group was not corrected three to six months post-colectomy surgery. This pattern of higher *n*-3 PUFA ratios was also observed in Esteve-Comas' group of UC and CD patients with active inflammatory disease (Figure 6.58). However, in the active patients, these observations were coupled with lower ratios of the *n*-6 PUFA series. The results of this current study are not at all in agreement with those of Esteve-Comas, the results are almost completely the opposite of one another. Esteve-Comas' results quite clearly show that *n*-3 PUFAs are higher in her patient groups. On the contrary, the data from this study show a fairly consistent trend for lower availability of *n*-3 PUFA.

The graphs presented in Figure 6.62 shows results adapted from Geerling *et al.,* 1999. The newly diagnosed CD group could be considered a representation of active IBD. The ratio of ALNA is considerably higher, along with a marginally higher ratio of DPA. Adapted from the same paper, results are also shown for long term CD patients. This group could be considered as a representation of

more inactive disease. These results show a higher ratio of ALNA, EPA and DPA, but a lower ratio of DHA. These findings do not agree with those of Esteve-Comas as longer chain *n*-3 PUFAs are not elevated, with the exception of DPA, which was not reported by Esteve-Comas' group. However, the results from Geerling's 'inactive' patients show some similarity to the pattern seen in whole plasma of the colectomy patients in the study presented in this thesis. This could suggest that a particular pattern of fatty acids is characteristic of a post-inflammatory state.

The graphs presented in Figure 6.59 show results adapted from Esteve *et al.,* 1998. This was a study of patients with established colectomy (minlmum of two years post-surgery). These results were presented as fatty acids in whole plasma and in plasma PL. The patterns observed for Esteve's colectomy patients did not necessarily agree with what was seen in the colectomy patients of this study. However, it is interesting to note that Esteve's group did report a significantly high GLA ratio in plasma PL, although this was not seen in whole plasma. This high GLA ratio was also observed in the colectomy patients of this study, in several individual fractions and in whole plasma.

The results from the current study seem to have little in common with the findings of Esteve's colectomy patients, but are in some agreement with the findings of Geerling's inactive patient group and the CF patient group. This disagreement seen between the different sets cannot be fully explained. However, some of the issues raised in Chapter 2 (Section 2.10) may have some relevance. The disagreement between the results of this study in comparison to the findings of others seems most apparent when comparing with the studies of Esteve. Indeed, the methodological disparities are greatest between this study and Esteve's than between the other groups.

The comparison of the results from this study to those of other groups could possibly suggest that the fatty acid profiles of the colectomy patients in this study exhibit characteristics of previous inflammatory process or alterations due to altered GI lipid handling. However, significant further analyses of the data and consideration and testing of the other stated hypotheses are needed before any firm interpretations or conclusions can be made. It remains to be

investigated in subsequent chapters the processes of GI digestion or absorption processes and hepatic handling of fatty acids and whether these processes may have a significant influence on the plasma fatty acid composition in colectomy patients.

6.10.3 CONCLUSIONS

The findings from the current study in isolation and in comparison to other studies showed:

- That the colectomy patients had a significantly altered fatty acid profile in plasma lipids in comparison to the reference group

- That the pattern of fatty acids was different in each of the lipid fractions and that the pattern of fatty acids seen in whole plasma did not generally represent what was seen in the individual fractions.

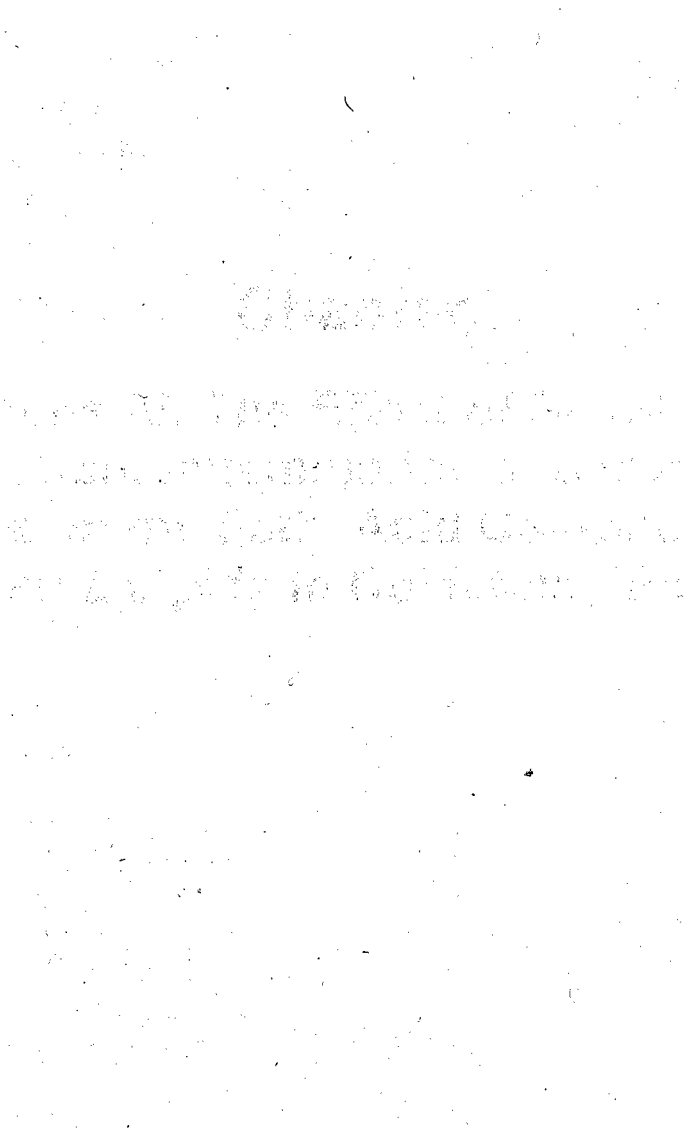
- That some *n*-6 PUFA appeared more in abundance, suggesting LA supply (and conversion to LC-PUFA) is not limited and/or elongation and desaturation pathways are up-regulated.

- That the composition patterns of fatty acids in the colectomy patients showed some similarities to patients with CF. This could suggest a possible GI involvement as lipid maldigestion and malabsorption are characteristic of CF.

- That *n*-3 PUFA appeared to be limited in the colectomy patients, especially ALNA and DHA. This could suggest the supply of these fatty acids is limited, or that demands are higher and cannot be met.

These results show that despite apparent good health, these patients have significant disturbances to their fatty acid metabolism. The cause(s) of these alterations is not known. In subsequent chapters, the remaining hypotheses of this thesis will be tested to establish the potential involvement of

digestion/absorption problems and inflammatory process in the pathogenesis of these disturbed lipid profiles.



Chapter 7

Results IV: The Effect of Small Bowel Resection, Inflammation and Body Mass Index on the Fatty Acid Composition of Plasma Lipids in Colectomy Patients

7.1 INTRODUCTION

From the previous chapter, it was seen that the fatty acid composition of plasma lipids was altered in the colectomy patient group when compared to the healthy reference group. Alterations were evident in all four of the lipid fractions studied, although the extent and nature of these changes varied between the fractions. It was not possible to establish whether the supply or demands for fatty acid were the reason for the altered composition of the fatty acid transport pool, or if indeed both were involved. In this chapter, the possible influences of inflammation and disturbed fatty acid supply are explored by further investigation of the patients in the colectomy group. This may help to explain what aspect of the colectomy patients' physiology could be responsible for the disturbances to the fatty acid composition in this group. In this way, the ability to separate out the subsets of the colectomy group means that the patients can be used as a tool to try to establish the influences responsible for fatty acid composition change

As presented in (Chapter 4), a subset of the colectomy patients had undergone a degree of small bowel resection in addition to total colectomy. It was suggested that fatty acid supply (in terms of digestion and absorption as intake has already been shown to be normal) could be an influence on the disturbed fatty acid profiles in the colectomy patients. Therefore, studying patients with additional small bowel resection may help to elucidate some of the effects of altered supply on plasma lipid fatty acid composition as these patients are more likely to have a limited ability to digest and absorb lipids efficiently through the GI tract.

It is also possible that some or all of the patients in the colectomy group were in a current raised inflammatory state which would be highlighted by raised inflammatory markers. As inflammation is known to influence fatty acid metabolism, this chapter also aimed to establish the inflammatory status of the colectomy patients and to relate this to fatty acid composition of the plasma.

7.2 AIMS

- To further explore the fatty acid data by dividing the colectomy group into two subsets; patients with colectomy only and with colectomy and small bowel resection.
- To characterise the plasma fatty acid composition of the two colectomy group patient subsets.
- To perform three-way comparisons of the fatty acid data using these subject groups.
- To explore whether limited fatty acid supply can explain differences in plasma fatty acid composition in colectomy patients.
- To establish any evidence for ongoing inflammation in the colectomy patients and if appropriate, relate this to plasma fatty acid composition.

Questions to be addressed include:

- Do patients with small bowel resection exhibit a different fatty acid composition pattern to patients with no small bowel resection?
- Can small bowel resection account for disturbances in fatty acid profiles seen in the colectomy group; does this implicate impaired digestion and absorption?
- Do patients in the SB group have higher inflammatory markers than those in the CO group? If so, can this be related to differences in fatty acid profiles?

7.3 HYPOTHESES

III. Colectomy patients have disturbed fatty acid profiles due to maldigestion or malabsorption in the intestine as a result of colon removal.

IV. Colectomy patients have disturbed fatty acid profiles due to the altered metabolic handling of fatty acids, either due to altered hepatic metabolism or abnormal demands due to active inflammation.

7.4 METHODS

The 36 patients in the colectomy group were sub-divided into two groups according to whether they had undergone significant small bowel resection in addition to total colectomy. Within the colectomy group, 13 patients (36%) had undergone more than 20 cm of small bowel resection in addition to total colectomy. These patients formed the small bowel resection group (SB) and the remaining 23 patients (64%) formed the colectomy-only (CO) group. The characteristics of the populations are shown below in Table 7.1. Differences in age and BMI were tested between the groups using ANOVA with Bonferroni *post hoc* analysis. The three groups were statistically comparable, apart from the CO group which was significantly older than the reference group ($p<0.001$).

Measurement	Study Group		
	Reference	CO	SB
Number of subjects	36	23	13
Males/Females	19/17	13/10	6/7
Age range (years)	27-64	27-74	26-66
Mean (±SD) age	42.7±11.3[a]	54.2±14.7[a]	48.8±12.1
BMI range (kg/m^2)	19.6-36.6	14.3-43.0	17.4-39.9
Mean (±SD) BMI	25.4±4.0	26.3±6.7	26.4±5.9

Table 7.1 Age and BMI characteristics of the Reference group and CO and SB subsets of the colectomy group. [a] CO patients were significantly older than the reference group as analysed by ANOVA, $p<0.001$.

In Chapter 3 it was established that the majority of the data analysed were not normally distributed as they failed the normality tests. It is generally accepted as more appropriate to use non-parametric statistics when analysing data which do not display a normal distribution. However, for the comparison of these three groups the ANOVA was used. It has been suggested that as the ANOVA is very robust, it may be more valid than many tests assuming homogeneity of variance and that it can still be appropriately performed provided there are sufficient data with a balanced design (Box, 1953).

The three subject groups were compared using ANOVA with Bonferroni *post-hoc* analysis. The results of these comparisons are summarised in Figure 7.1 to Figure 7.15.

7.5 RESULTS

7.5.1 PLASMA PHOSPHATIDYLCHOLINE

It was previously described that the total concentration of fatty acids in plasma PC was significantly higher in the colectomy group than in the reference group (Chapter 6). From the current analyses, it was noted that this higher concentration was seen to a greater extent in the SB group ($p<0.001$), although the higher concentration in the CO was also significant ($p<0.05$) (Figure 7.2).

Previously, a significantly higher concentration of LA was described in the colectomy group when compared to the reference group. The current analyses are in agreement with these results as LA concentration was higher in both the CO and SB subsets when compared to the reference group ($p<0.01$ and 0.05, respectively). The mean concentration was roughly similar between the subsets (Figure 7.1). Results for LA proportion were in agreement to previous analyses as no differences were observed between the reference and colectomy subsets (Figure 7.3). However, the mean concentration tended to be slightly lower in the SB subset, but this was not significant.

ALNA concentration had not been previously observed to be different between the reference and colectomy groups. The results from the current analyses were consistent with these previous findings as ALNA concentrations were similar between the reference, CO and SB groups. Similar again to previous findings, ALNA proportion was significantly lower in the colectomy patients ($p<0.05$), but there was no difference between CO and SB subsets.

The concentration of DGLA was previously found to be significantly higher in the colectomy group than in the reference group. In the current analyses, it was noted that DGLA concentration was higher in both subsets, but this was only significant for the SB subset ($p<0.001$). However, this did appear to be

influenced by a small minority of patients within this group. Consistent with previous results, DGLA proportions were not different between the groups.

Similar to the findings for DGLA, ARA concentration was previously found to be significantly higher in the colectomy group and this was also seen in the current analyses. However, the higher ARA concentration was only significant in the SB subset ($p<0.001$) but this again appeared to be influenced only by a small minority of patients. Consistent again with previous findings, ARA proportions were not different between the groups.

The concentration of EPA had not previously been found to be significantly different between the reference and colectomy group and this was consistent with the current findings. EPA proportion was previously described as similar between the reference and colectomy groups. In the current analyses, the proportion of EPA was lower in both the CO and SB subsets, and this effect was greater in the CO patients. However, neither colectomy group subset was significantly different to the reference group.

DPA concentration was previously found to be significantly higher in the colectomy group than in the reference group. Consistent with this, the concentration of DPA was higher in both the CO and SB subsets but this was only significant in the latter ($p<0.01$). The proportion of DPA was not previously found to be different and this was consistent with the current analyses. However, the proportion had a tendency to be lower in the CO, but higher in the SB subset.

In previous comparisons, PC DHA concentration was not different between the reference and colectomy groups. This was consistent to current findings and concentration was roughly similar between the CO and SB subsets. The proportion of this fatty acid was previously found to be lower in the colectomy group. The current analyses showed that the proportion of this fatty acid was lower in the CO subset than in the reference group ($P<0.01$). This was seen to an even greater extent in the SB subset ($p<0.001$).

Total *n*-3 PUFA concentration was not previously found to be different between the reference and colectomy groups and this finding was consistent with the current analyses (Figure 7.2). The proportion of *n*-3 PUFA however, was significantly lower in the colectomy group, and the current findings showed that the mean proportion of *n*-3 PUFA was similar between the CO and SB subsets, both of which were also significantly lower than the reference group ($p<0.05$) (Figure 7.4).

Total *n*-6 PUFA was significantly higher in the CO subset, and higher still in the SB subset when compared to the reference group ($p<0.01$ and <0.001, respectively). These findings were consistent with previous results presented in Chapter 6. However, previous analyses did not show a difference between the reference and colectomy groups in terms of the proportion of *n*-6 PUFA in this fraction. This was also in keeping with the findings of the current analyses.

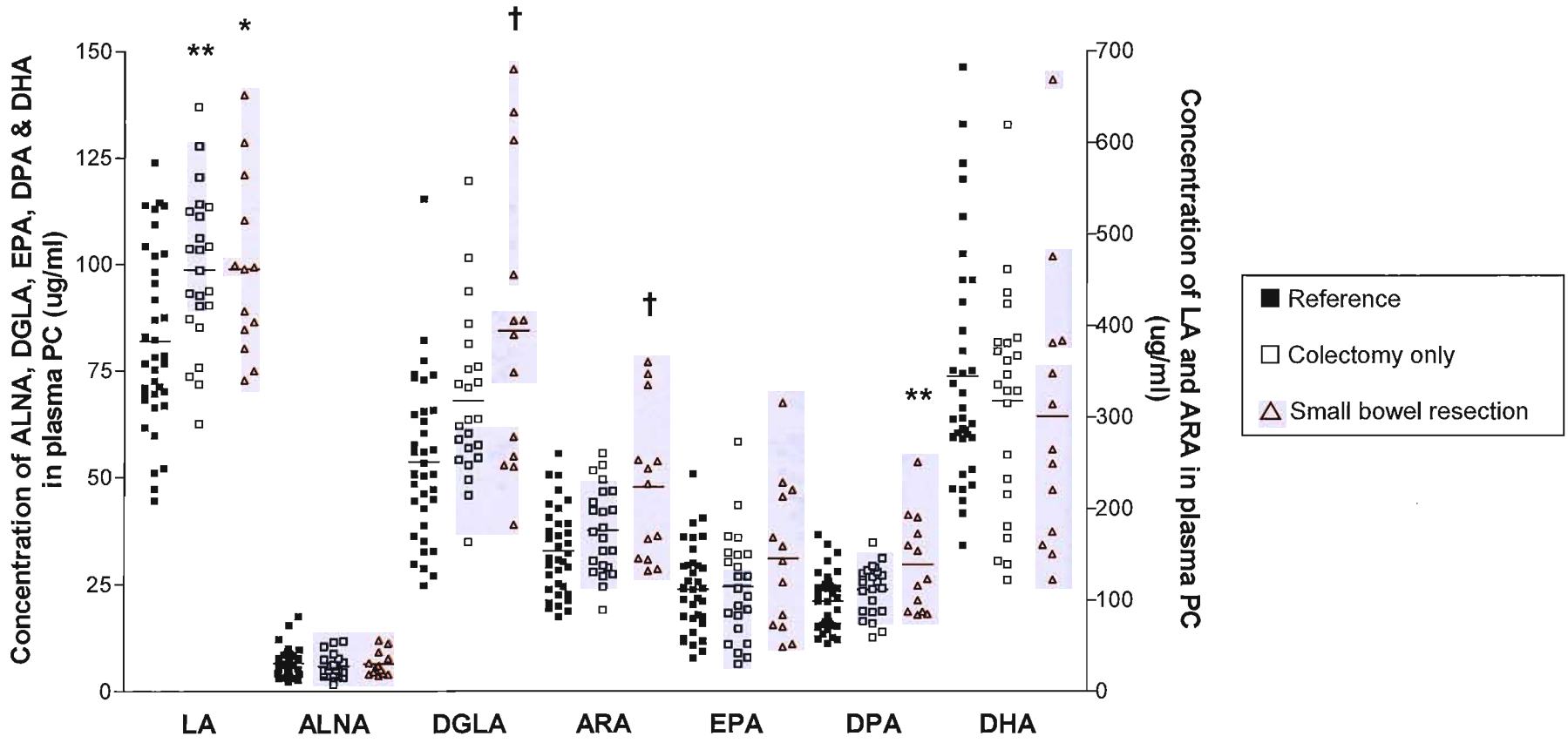


Figure 7.1 Graph showing the concentration of fatty acids in plasma PC of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Fatty acid concentrations (mg/ml) plotted on left Y axis except LA and ARA plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

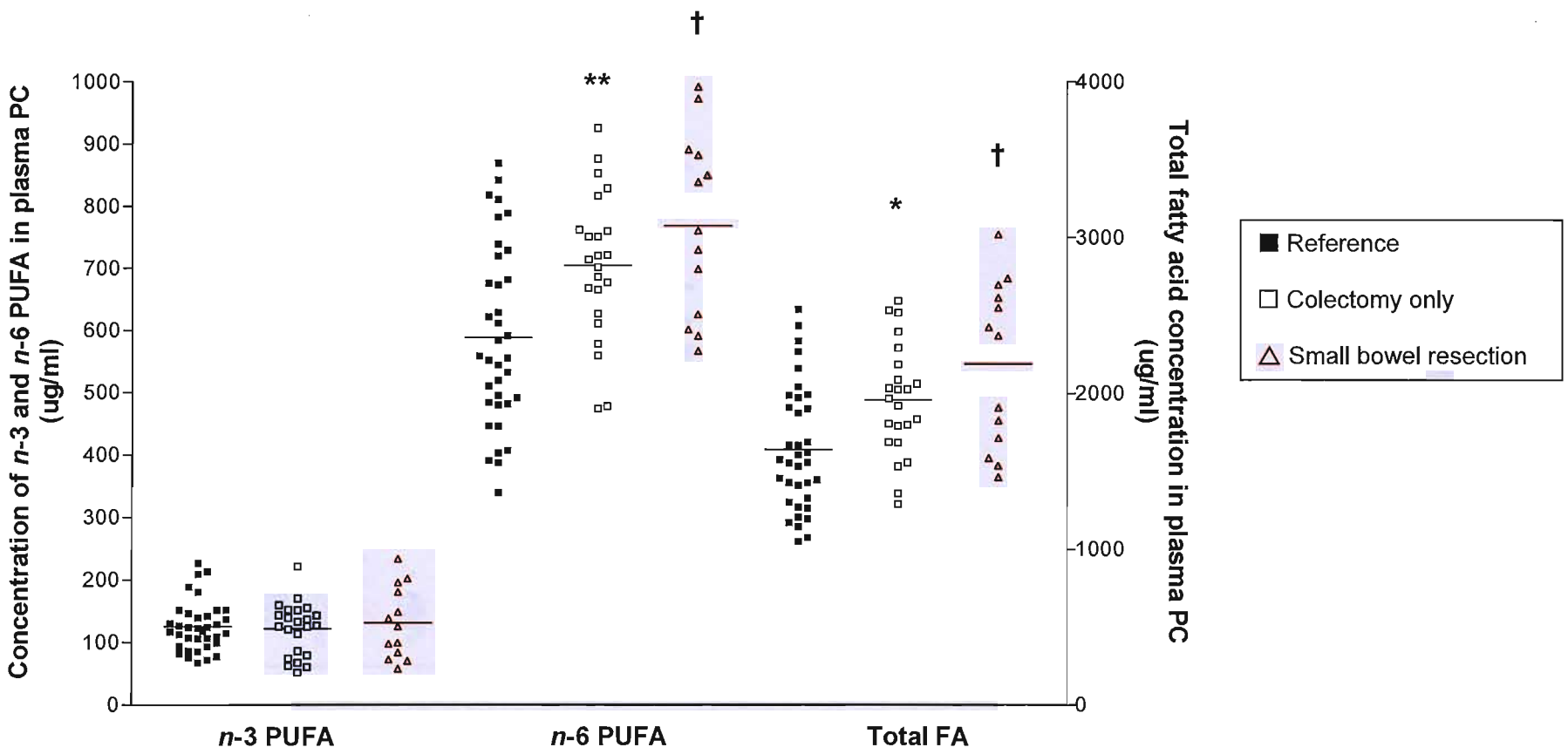


Figure 7.2 Graph showing the concentration of fatty acids in plasma PC of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. *N*-3 & *n*-6 PUFA concentrations (mg/ml) plotted on left Y axis; Total Fatty Acid concentration plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = p<0.05, ** p<0.01 and † p<0.001.

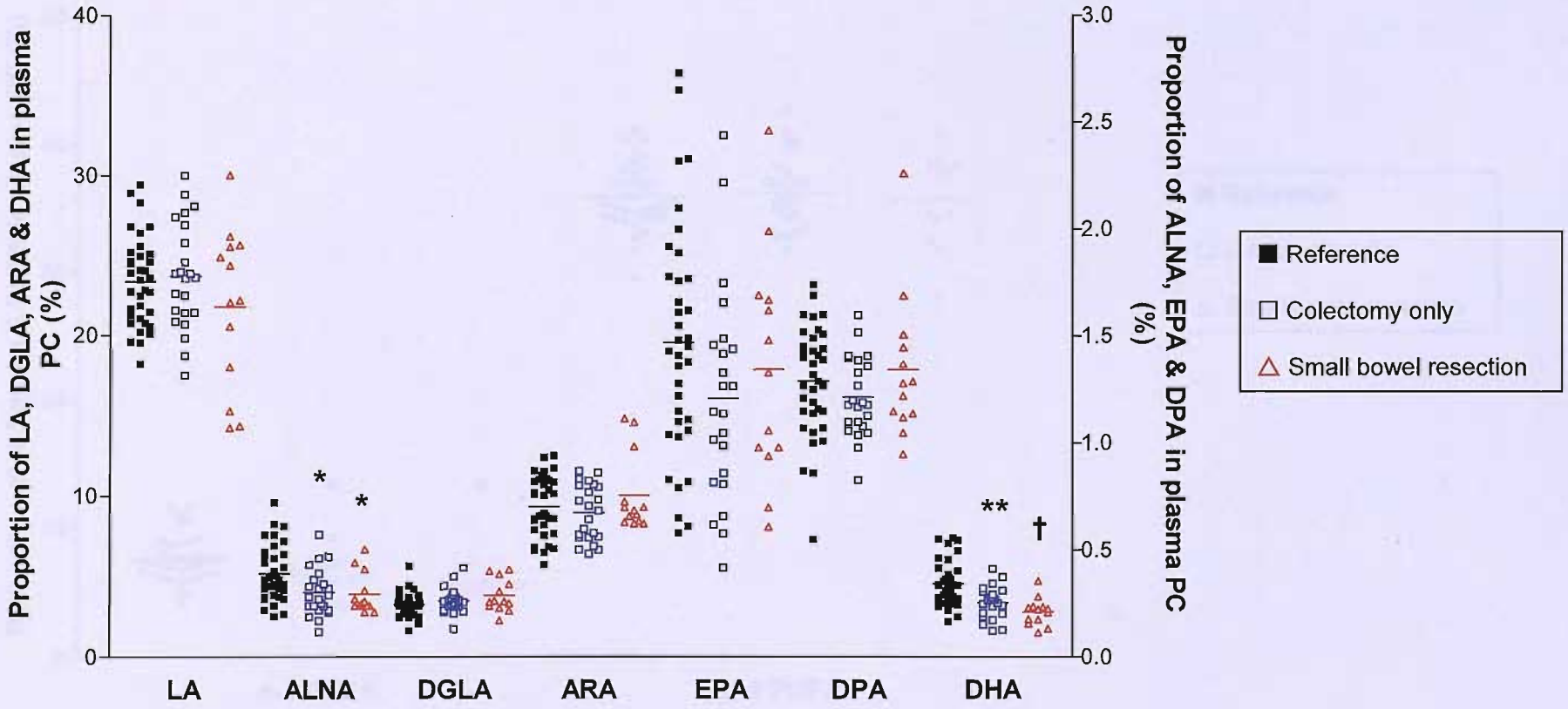


Figure 7.3 Graph showing the proportions of fatty acids in plasma PC of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Fatty acid proportions (%) plotted on left Y axis except ALNA, EPA and DPA plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

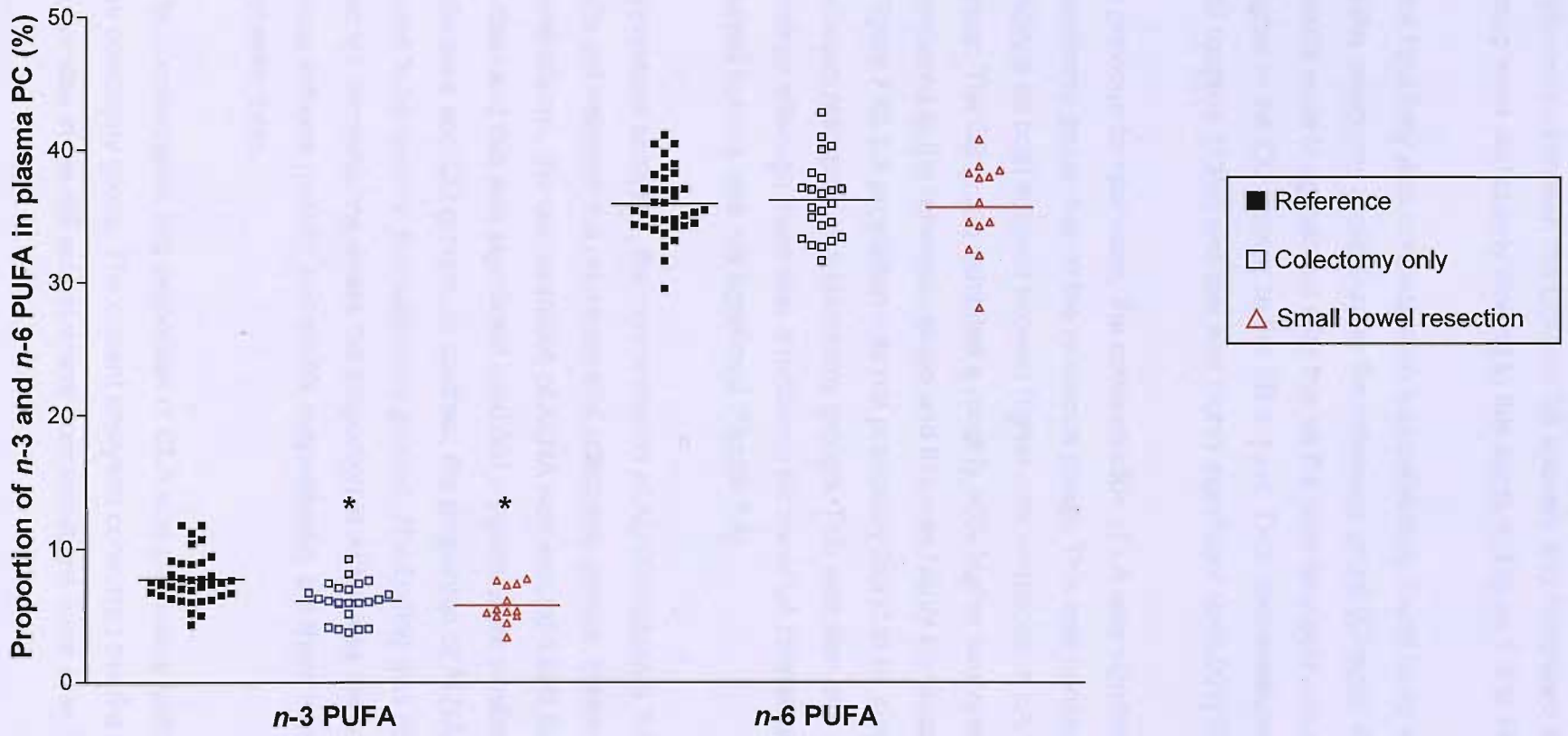


Figure 7.4 Graph showing the proportion of fatty acids in plasma PC of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = p<0.05, ** p<0.01 and † p<0.001.

7.5.2 PLASMA TRIACYLGLYCEROL

Differences between the CO and SB subsets and compared to the reference group were particularly striking in this fraction (Figure 7.6 to Figure 7.9).

The total fatty acid concentration was previously found to be significantly higher in the colectomy group than in the reference group (Chapter 6). The current results were in agreement with this as the total fatty acid concentration was higher in the CO subset. In the SB subset, TAG concentration was yet higher still (approx 113%) and this was highly significant ($p<0.001$) (Figure 7.7).

In previous comparisons, the concentration of LA was significantly higher in the colectomy group than in the reference group. This was consistent with current findings as both subsets showed higher concentrations of LA than the reference group. The SB group exhibited a roughly 80% higher concentration of LA compared to the reference group and this was highly significant ($p<0.001$) (Figure 7.6). LA proportion was not previously found to be significantly different between reference and colectomy groups. This was also true of the current findings although there was a tendency for lower LA proportions in the SB subset but this was not significant (Figure 7.8).

In previous analyses, the concentration of ALNA in plasma TAG was not different between the reference and colectomy groups. However, in the current comparisons, the concentration of ALNA was around 130% higher in the SB subset and this was significant ($p<0.001$). Values were similar between the reference and CO groups. In contrast, the proportion of ALNA was previously found to be lower in the colectomy ground. The finding was also true for the current comparisons where the proportion of ALNA was lower in both colectomy group subsets ($p<0.01$ and <0.05, respectively), but there was no difference between them.

The concentration and proportion of GLA was previously found to be higher in the colectomy group. The current analyses concurred but the effect was more noticeable in the SB subset where concentrations were over 140% higher than

in the CO group. Concentrations in the SB subset were significantly higher than in the reference group ($p<0.001$).

Similar findings were seen for DGLA: the concentration of this fatty acid had previously been found to be higher in the colectomy group and this was also seen in the current comparisons. This was largely restricted to concentrations in the SB group which were almost 140% higher than in the CO subset. These values were significantly higher than those found in the reference group ($p<0.001$). However, where previous analyses had found the proportion of DGLA to be higher in the colectomy group, this did not concur with the current findings as values were similar between all three groups.

The concentration and proportion of ARA had been found previously to be higher in the colectomy patients. A higher ARA concentration was also seen in the current comparisons although this was only significant in the SB subset ($p<0.001$) where the concentration of ARA was over 120% higher than in the CO subset. The proportions of this fatty acid also tended to be higher in the CO and SB subsets, but these were not significant.

EPA concentration was previously found to be higher in the colectomy group. This was also seen in the current comparisons, although this was only significant for the SB subset ($p<0.001$) where the concentration of EPA was roughly 125% higher than in the CO group. The proportion of EPA was not different between the groups in either the previous or current comparisons.

Previous analyses found that DPA concentration was higher in the colectomy group. This was also reflected in the current comparisons, although data were only significant in the SB subset ($p<0.001$) where the concentration was over 140% higher than in the CO group. Previous comparisons had also described a higher proportion of DPA in this fraction, which was also true of the current findings, but differences between the reference population and colectomy group subsets were not significant.

In previous analyses, the concentration of DHA in plasma TAG was not different between the reference and colectomy groups. In the current findings, DHA

concentration tended to be higher in the SB subset, but this was not significant. The proportion of DHA was previously described as lower in the colectomy group and this was also seen in the current comparisons however, differences were more marked and only significant in the SB subset ($p<0.001$).

Total *n*-3 PUFA concentration had previously been found to be higher in the colectomy group. This finding was consistent with the current analyses, but was only significant in the SB subset (Figure 7.7, $p<0.001$). Conversely, the proportion of total *n*-3 PUFA was found to be lower in the colectomy group and this again was seen in the current comparisons (Figure 7.9). However, this was only significant in the SB subset ($p<0.05$).

Higher *n*-6 PUFA concentrations in the colectomy group were previously described. These findings were also true of the current analyses, but the higher concentration was more marked and significant only in the SB subset ($p<0.001$). Conversely, *n*-6 PUFA proportion was not different between the reference and colectomy groups and this was also true of the current findings, although a tendency for lower proportions were seen in the SB subset.

It was noted that in the SB subset, the measurements for all fatty acid concentrations covered a very large range, much wider than that for either of the other two groups for any fatty acid. In some cases, the highest value within the group was over eight-fold higher than the lowest value.

Total fatty acid concentration in TAG was over 110% higher in the SB subset when compared to the CO subset. The graph in Figure 7.5 shows the contribution (%) to the total made by each individual fatty acid concentration. It shows that although all fatty acid concentrations were higher in the SB group, they were not all increased by the same extent; some show a relatively higher raise than others. Despite more than 110% higher total fatty acid concentration in the SB group, the concentration of LA was only 80% higher. In contrast, the other *n*-6 PUFA GLA, DGLA and ARA were all over 125% higher. The *n*-3 PUFA also showed higher concentrations in the SB patients. ALNA and EPA showed increases of approximately 130% in this group, and DPA was nearly 145% higher. This contrasted starkly with the concentration of DHA which was

merely 23% higher in the SB group. Overall, *n*-6 concentration was 89% and *n*-3 concentration was 102% higher in the SB group.

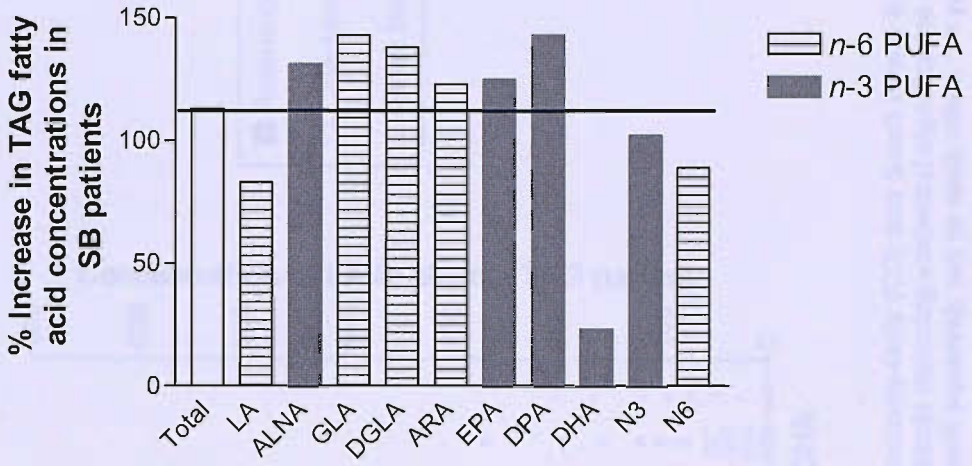


Figure 7.5 Higher TAG fatty acid concentration in SB colectomy patients in comparison to the CO subset and the relative increases contributed by each fatty acid. Bars represent average values; grey bars are *n*-3 PUFA and striped bars are *n*-6 PUFA. The horizontal line indicates the higher total fatty acid concentration in the SB subset.

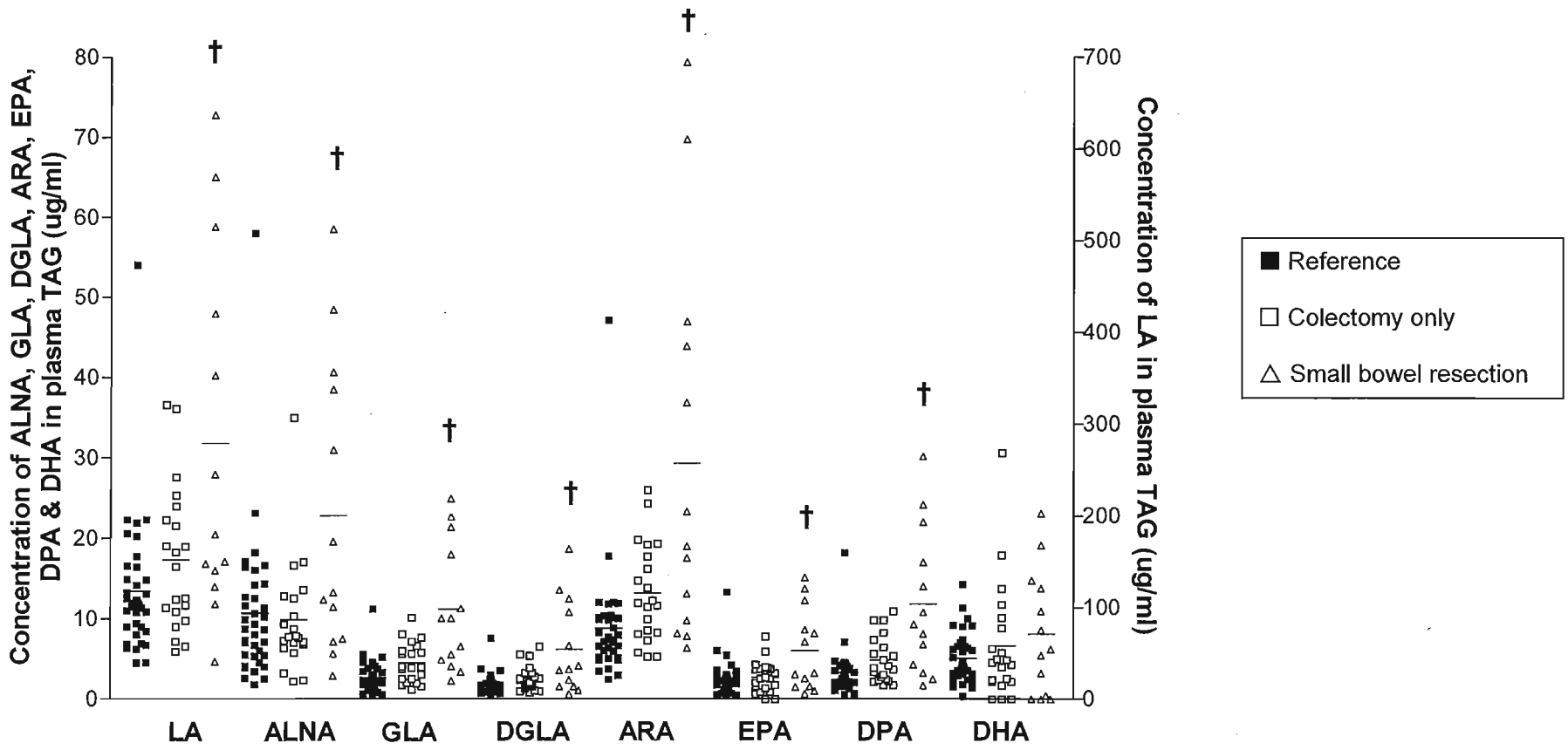


Figure 7.6 Graph showing the concentration of fatty acids in plasma TAG of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Fatty acid concentrations (mg/ml) plotted on left Y axis except LA plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

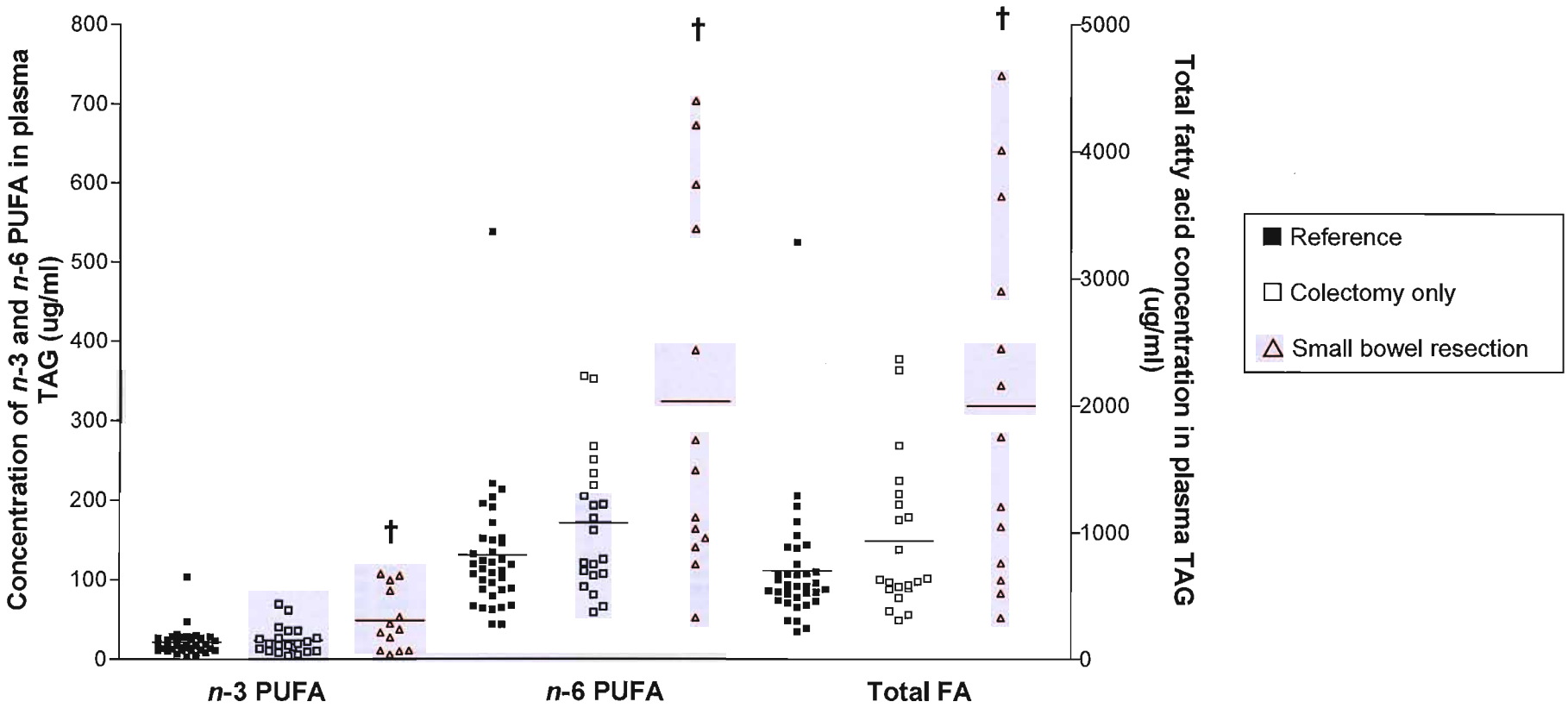


Figure 7.7 Graph showing the concentration of fatty acids in plasma TAG of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. *N*-3 and *n*-6 PUFA concentrations (mg/ml) plotted on left Y axis; Total Fatty Acid concentration on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

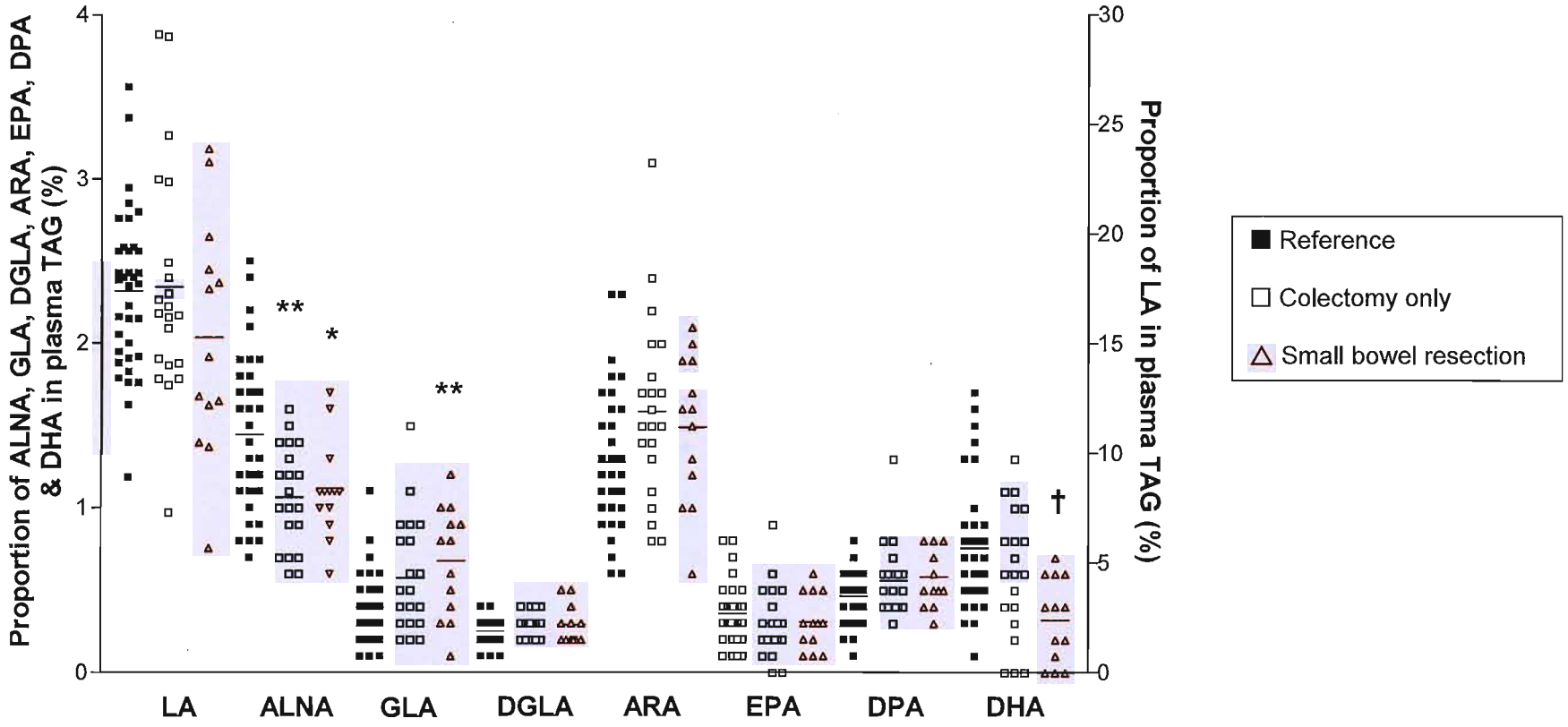


Figure 7.8 Graph showing the proportion of fatty acids in plasma TAG of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Proportions (%) plotted on left Y axis except LA proportion on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = p<0.05, ** p<0.01 and † p<0.001.

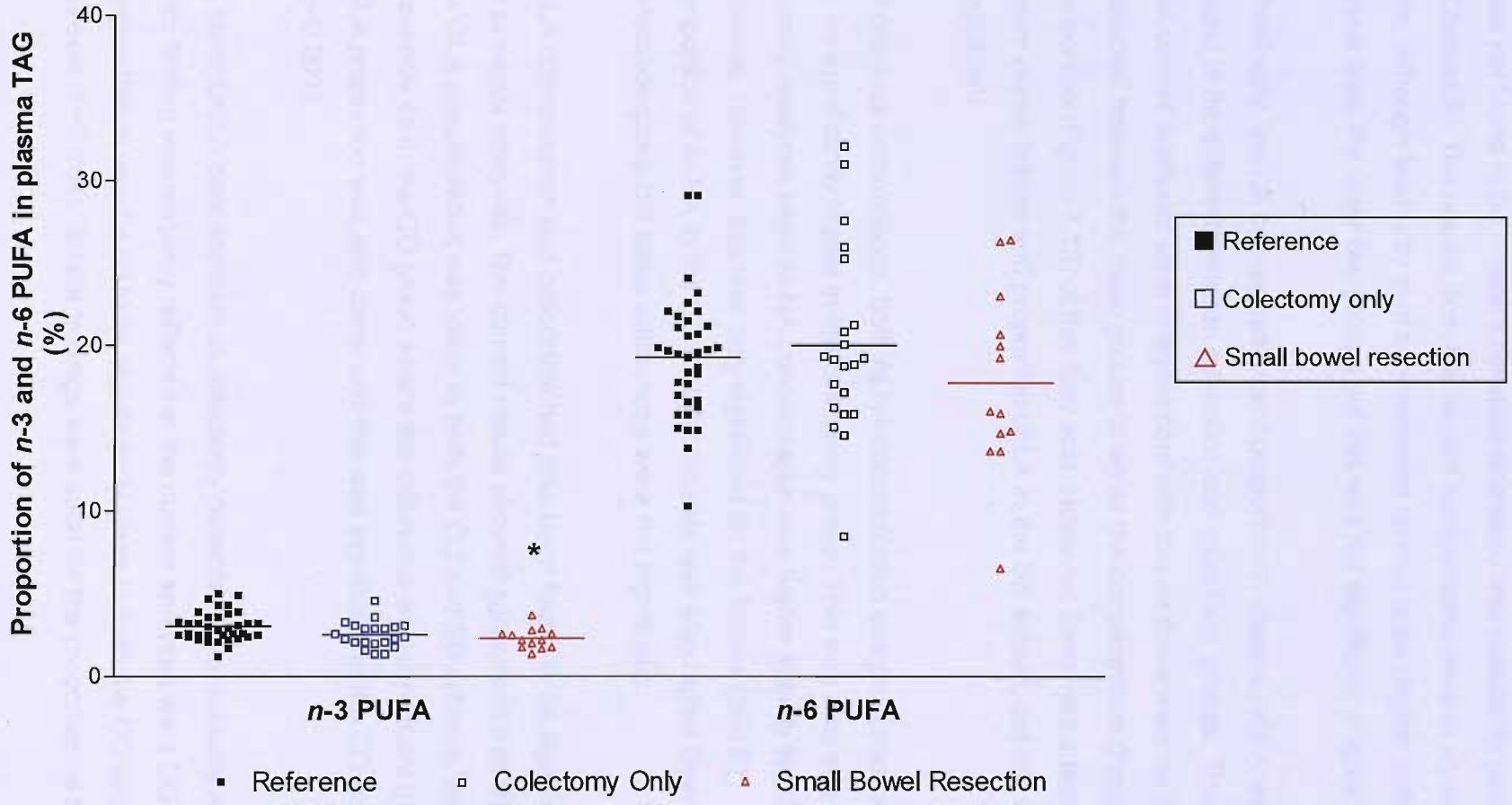


Figure 7.9 Graph showing the proportion (%) of fatty acids in plasma TAG of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = p<0.05, ** p<0.01 and † p<0.001.

7.5.3 PLASMA NON-ESTERIFIED FATTY ACID

In previous results sections, the total fatty acid concentration in plasma NEFA was not found to be different between reference and colectomy patients (Chapter 6). The results from the current comparisons were in agreement with this, although total fatty acid concentration tended to be slightly higher in the CO group than the other two groups but this was not significant (Figure 7.10).

Previously, the LA concentration and proportion in plasma NEFA were not found to be different between reference and colectomy groups. The results from the current analyses were in agreement with this as there were no differences detected between the three groups in either the concentration (Figure 7.10) or proportion (Figure 7.12) of this fatty acid. However, there was a tendency for a lower concentration and proportion of LA in the SB subset, but this was not significant.

In previous comparisons, both ALNA concentration and proportion were found to be significantly higher in the colectomy group. This was also true of the current analyses were ALNA concentration was higher in both the CO and SB subsets. However, this was only significant for the former ($p<0.01$). The proportion of ALNA in the CO and SB subsets was also higher than in the reference group but these differences were not significant.

GLA concentration and proportion had also been found to be significantly lower in previous analyses. The current results showed agreement with these findings as GLA concentration was lower in both the CO and SB subsets, but more markedly so in the CO group where the difference was significant ($p<0.01$). GLA proportion was also lower and this was significant in the CO group ($p<0.001$).

A lower DGLA concentration in colectomy patients was previously reported. This finding was certainly reflected in the current analyses were DGLA concentration was found to be significantly lower in both the CO and SB subsets ($P<0.001$). Similar findings were seen for the proportion of this fatty

acid where this was also significantly lower in the CO and SB subsets ($p<0.001$).

No differences in ARA concentration or proportion between the reference and colectomy groups were previously reported. However, in the current analyses, there was a tendency for a higher concentration of ARA in both the CO and SB groups which was more marked in the latter. However, neither of the findings was significant. The proportion of ARA was not significantly different between the reference, CO and SB groups. However, the tendency was for a lower proportion in the CO group and a higher proportion in the SB group.

In previous results, the concentration and proportion of EPA in NEFA was found to be similar between the study groups. This was consistent with current findings as both measurements were similar between the three groups.

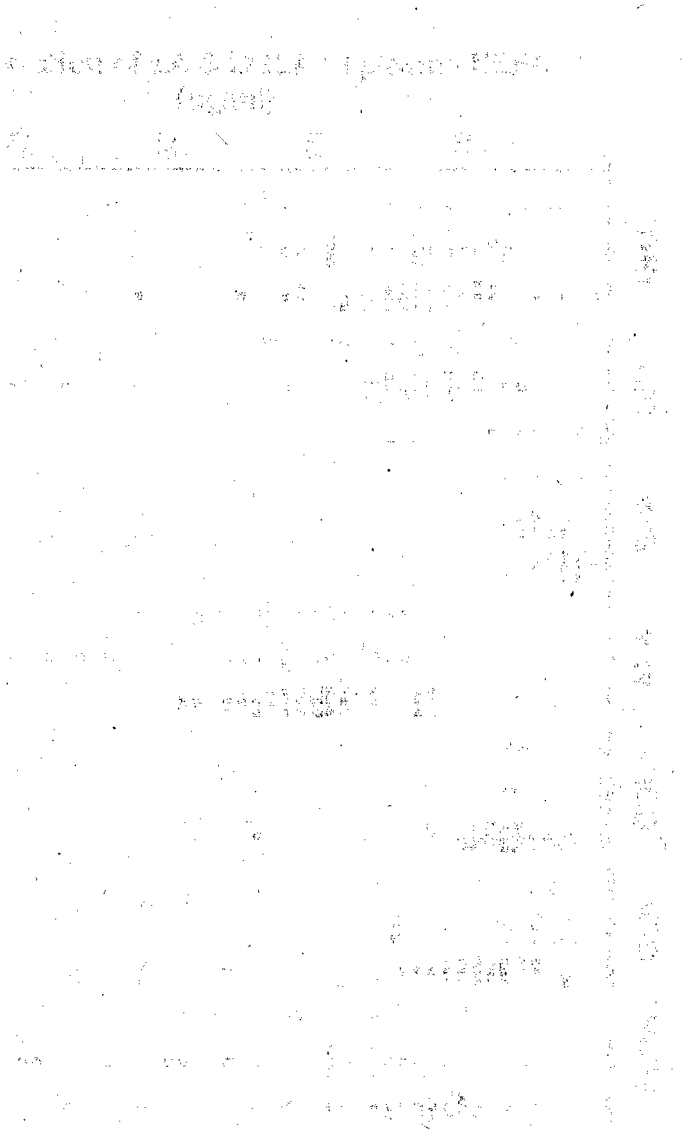
Previous analyses described a significantly higher concentration and proportion of DPA in colectomy patients. These findings were consistent with those presented in the current analyses; both the concentration and proportion of DPA was significantly higher in the CO and SB subsets ($p<0.001$).

DHA concentration and proportion was not previously found to be different in the colectomy group when compared to the reference group. This was again consistent with current findings, although both measurements of DHA did tend to be lower in the SB group but this was not significant.

The concentration and proportion of total *n*-3 PUFA were previously found to be higher in the colectomy group. These findings were consistent with the current results. The concentration of *n*-3 PUFA was higher in both the CO and SB subsets ($p<0.001$ and <0.05, respectively) but this was more marked in the SB patients (Figure 7.13). The findings for *n*-3 PUFA proportion showed a similar pattern as this was higher in the CO and SB subsets ($p<0.001$ and <0.01, respectively).

On the contrary, *n*-6 PUFA concentration and proportion was previously found to be significantly lower in the colectomy group. This was consistent with current

findings as both the CO and SB subsets showed a lower concentration of *n*-6 PUFA, although this was only significant for the SB subset ($p<0.01$). The proportion of *n*-6 PUFA however, was significantly lower in both the CO and SB subsets ($p<0.001$).



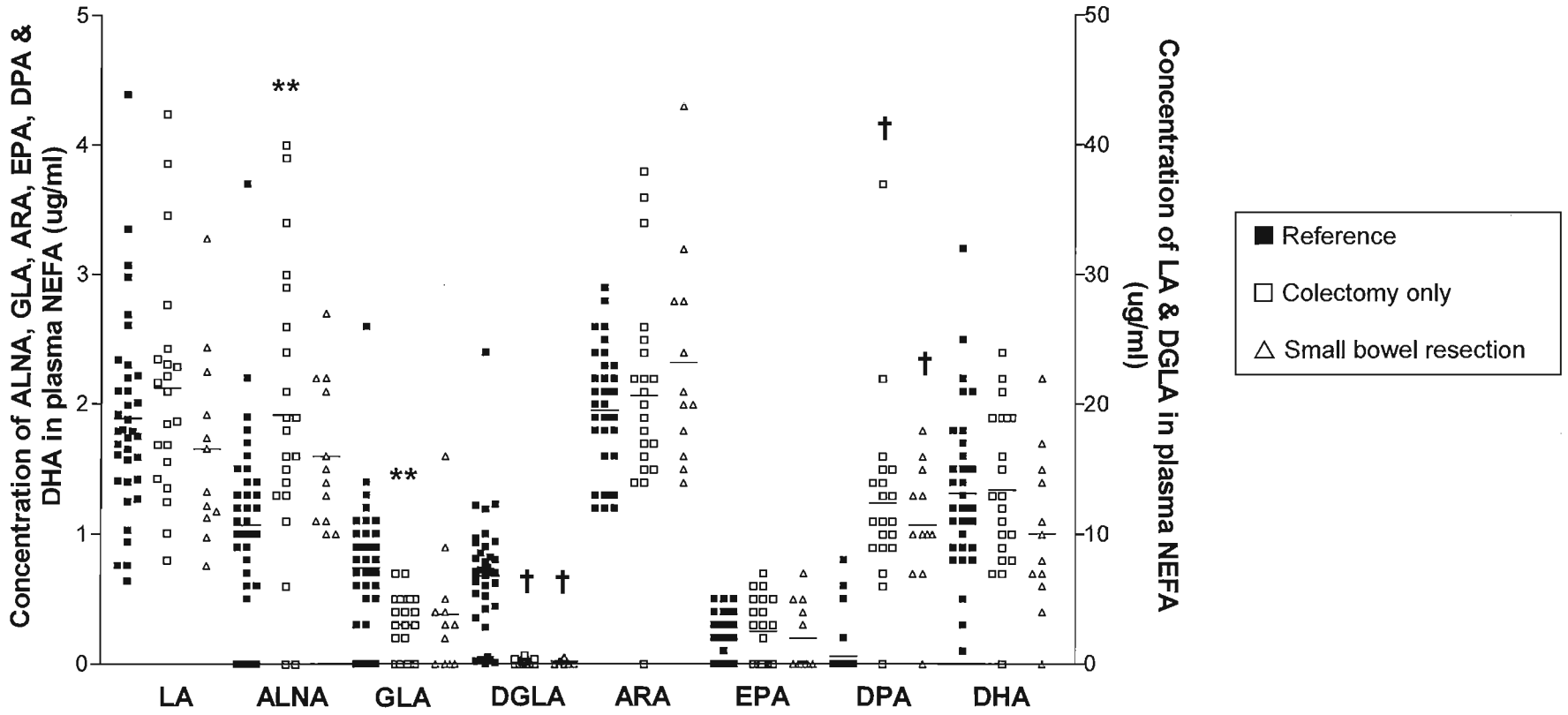


Figure 7.10 Graph showing the concentration of fatty acids in plasma NEFA of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Fatty acid concentrations (mg/ml) plotted on left Y axis except LA and DGLA plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

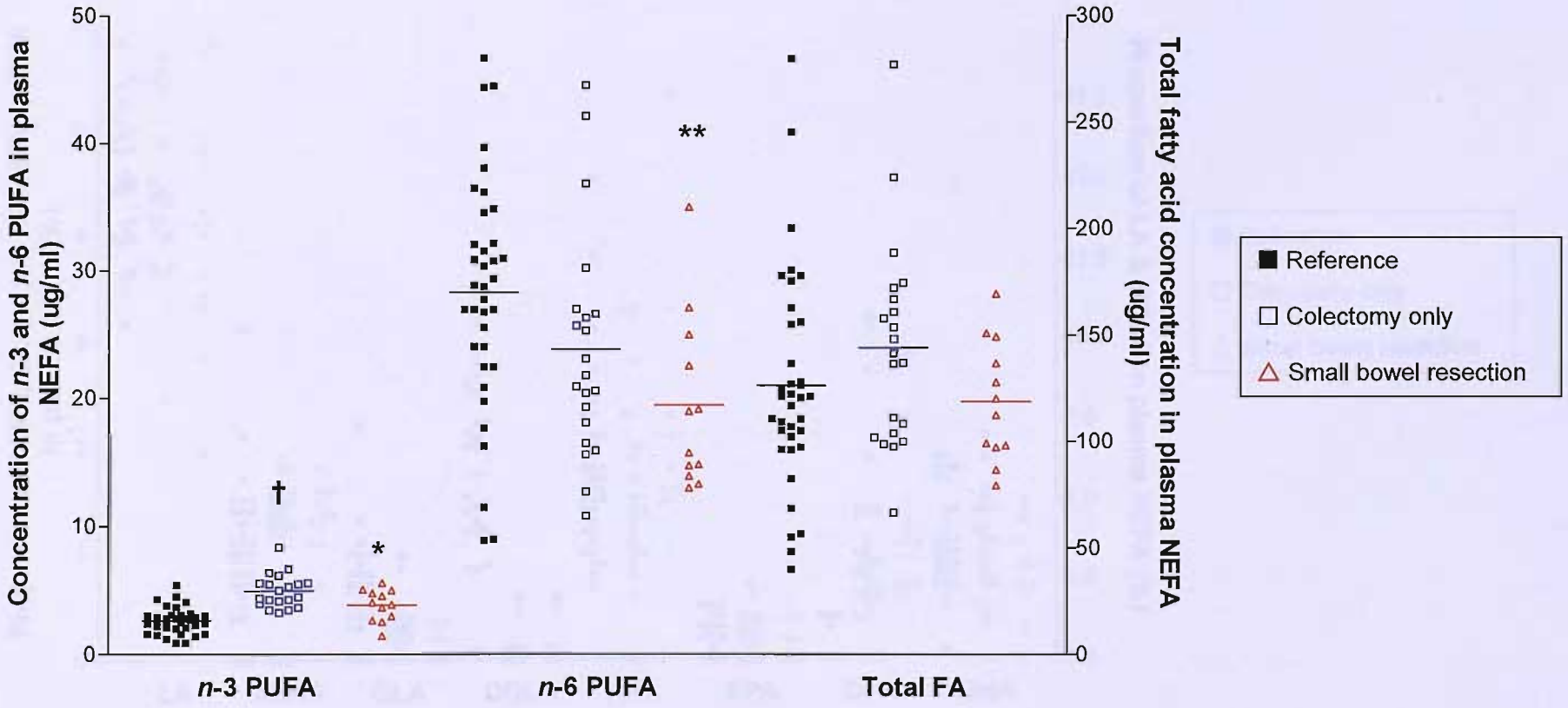


Figure 7.11 Graph showing the concentration of fatty acids in plasma NEFA of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. *N*-3 and *n*-6 PUFA concentrations (mg/ml) plotted on left Y axis; Total Fatty Acid concentration plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = p<0.05, ** p<0.01 and † p<0.001.

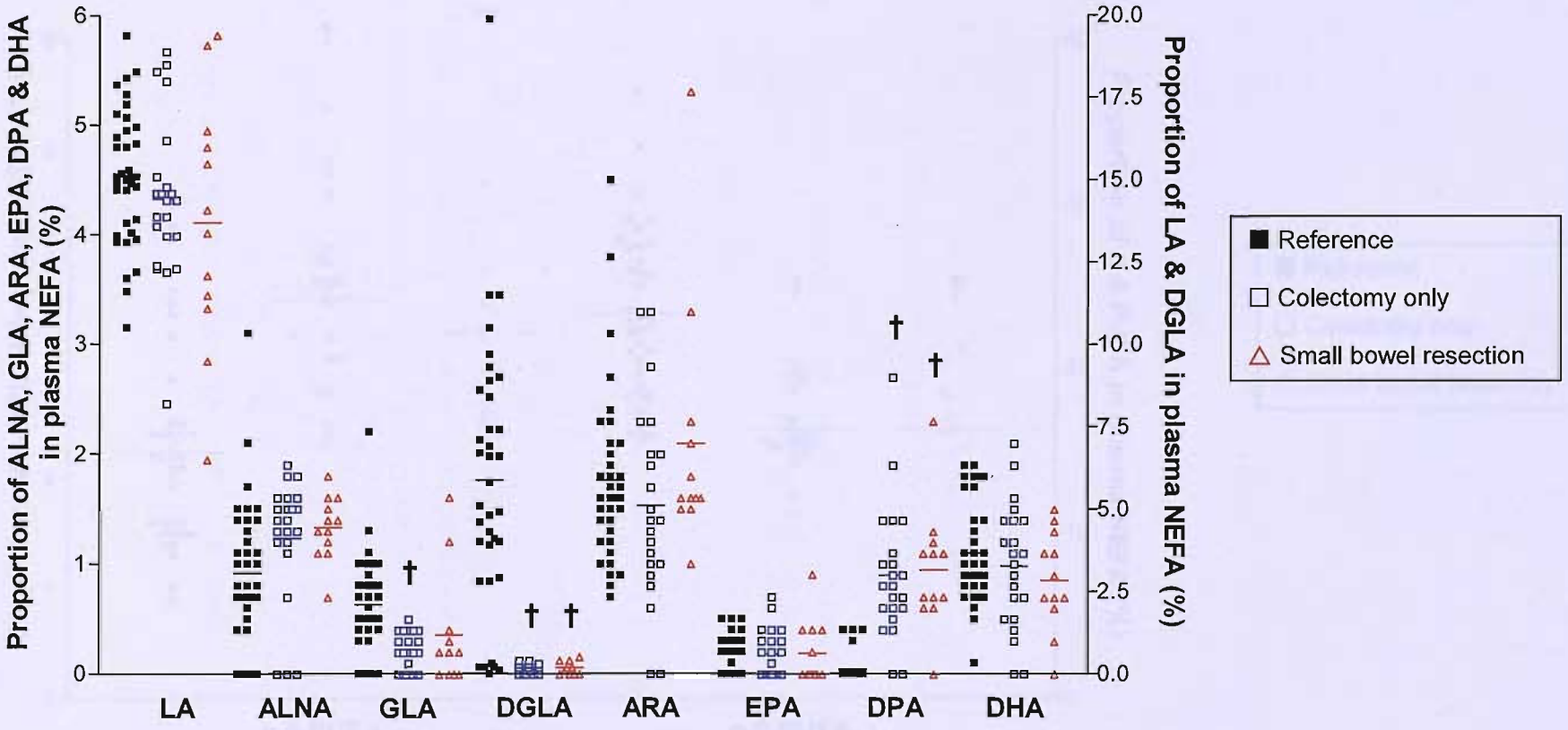


Figure 7.12 Graph showing the proportion of fatty acids in plasma NEFA of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Proportions in plasma (%) plotted on left Y axis except LA and DGLA plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

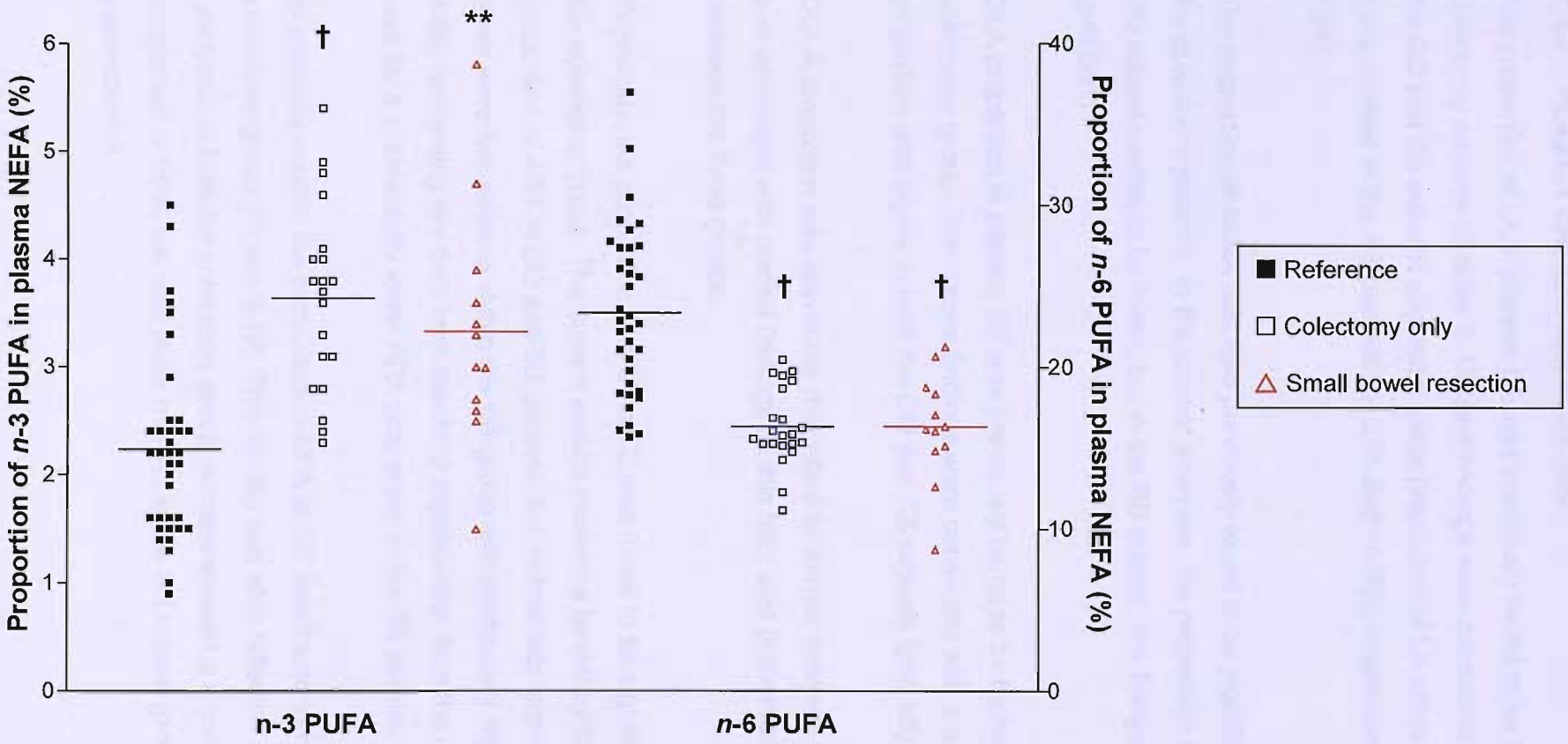


Figure 7.13 Graph showing the proportion of fatty acids in plasma NEFA of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. *N*-3 PUFA proportion (%) plotted on left Y axis; *n*-6 PUFA proportion on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = p<0.05, ** p<0.01 and † p<0.001.

7.5.4 PLASMA CHOLESTERYL ESTER

The proportion of LA in plasma CE was previously found to be lower in the colectomy patients Chapter 6. Current findings were consistent with this as both the CO and SB subsets showed a lower proportion of LA although this was more marked in the SB patients ($p<0.05$ and <0.001, respectively) (Figure 7.14).

The proportion of ALNA was also previously found to be significantly lower in the colectomy patients. In the current analyses, the proportion of ALNA in the CO subset tended to be lower, but in the SB subset, this finding was significant ($p<0.001$).

GLA proportion in plasma CE was previously found to be higher in the colectomy group. The current findings were consistent with this as the GLA proportion was higher in both the CO and SB subsets ($p<0.05$).

DGLA proportion was previously described as similar between the groups. This was consistent with current findings as this fatty acid proportion was similar between the three groups.

Previously, the proportion of ARA in CE was found to be significantly lower in the colectomy group. The current results showed a tendency for a lower proportion of ARA in CO and SB subsets, but neither was significant. However, there were two patients within the SB group with particularly high proportions of ARA, preventing the data from reaching significance, thus the overall pattern was for a substantially lower ARA proportion in the SB subset.

In previous results, the proportion of EPA in CE was found to be lower in the colectomy group (Figure 6.19). This finding was also reflected in the current analyses as both the colectomy group subsets showed a significantly lower proportion of EPA, this was more marked in the SB subset ($p<0.05$ and <0.01, respectively).

Previous results had shown that DPA proportion was significantly higher in colectomy patients. This was also seen in the current analyses, although the higher proportion of DPA was only significant in the SB subset ($p<0.05$).

DHA proportion had previously been found as significantly lower in colectomy patients. This finding was also true of the current analyses, although this was only significant again in the SB subset ($p<0.01$).

Total *n*-3 PUFA proportion was previously described as lower in the colectomy patients (Figure 6.50). This was also seen in the current analyses, although the effect was more marked in the SB subset ($p<0.001$) than in the CO subset ($p<0.05$).

Total *n*-6 PUFA had also been previously found as lower in the colectomy patient group (Figure 6.46). Again, this was also true of the current findings and was consistently more marked in the SB subset ($p<0.001$) than in the CO subset ($p<0.01$).

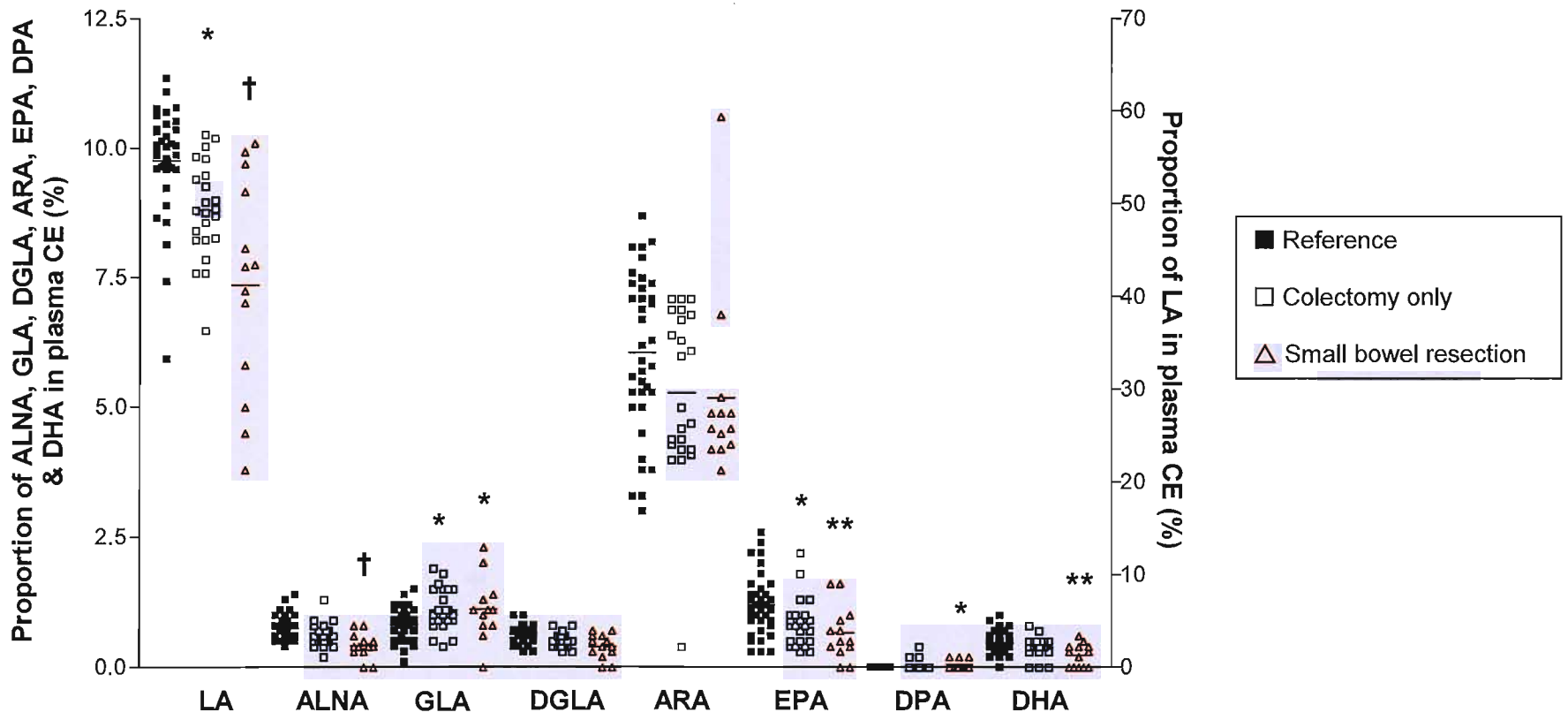


Figure 7.14 Graph showing the proportion of fatty acids in plasma CE of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. Fatty acid proportions in plasma (%) plotted on left Y axis except LA plotted on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

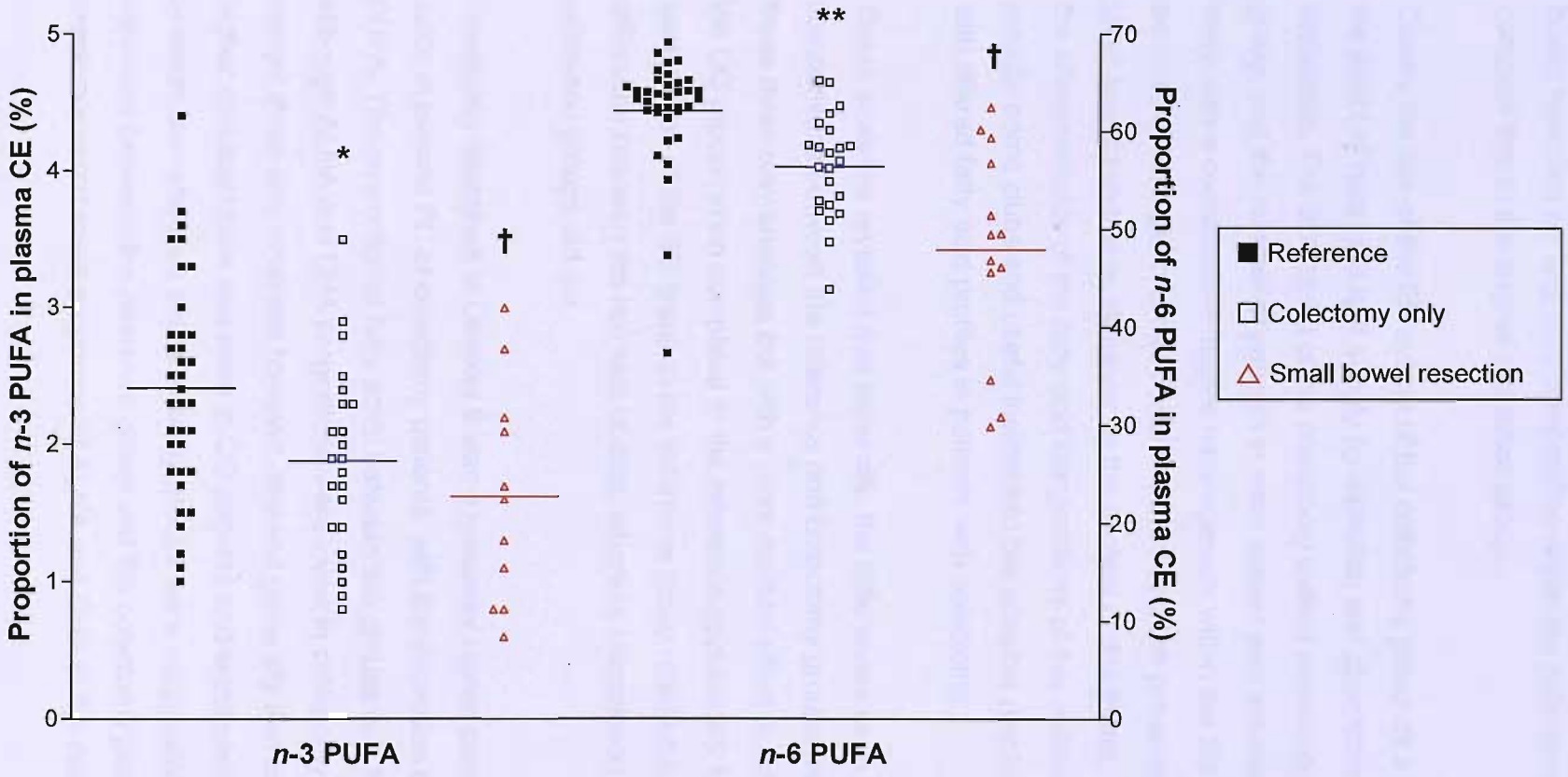


Figure 7.15 Graph showing the proportion of fatty acids in plasma CE of reference, Colectomy-Only (CO) and Small Bowel Resection (SB) groups. *N*-3 PUFA proportions in plasma (%) plotted on left Y axis; *n*-6 PUFA proportions on right Y axis. Symbols denoting statistical significance are comparison of CO (hollow blue squares) and SB (hollow red triangles) groups to the reference group (solid black squares); not to each other. * = $p<0.05$, ** $p<0.01$ and † $p<0.001$.

7.6 DISCUSSION

The results presented above served to investigate the possible effect of small bowel resection on fatty acid composition within the colectomy group and to compare this to the original reference group.

Clearly the use of the SB subset of the colectomy group as a tool for exploring the effect of restricted lipid supply by digestion and absorption is not without its limitations. The SB subset of the colectomy patient population was a minority group, and the number of patients in each subset was imbalanced. In addition, there was a considerable degree heterogeneity within the SB subset, in particular the length of small bowel removed and the prevalence of symptoms of short bowel syndrome. However, in the context of this thesis, the exploration of the characteristics of the fatty acid compositions of this subset group may provide some clues and useful insight into the possible mechanisms involved with altered fatty acid profiles in patients with colectomy.

These analyses revealed that generally, the differences seen in previous comparisons between the reference and colectomy groups were also seen in these three-way analyses but with a more marked effect in the SB group than the CO group (when compared to the reference population). In some cases, comparison of the SB group to the reference group resulted in a significant difference between the two sets of data, whereas comparison of the CO and reference groups did not.

Previously described in Chapter 6 was a generally higher concentration of fatty acids in plasma PC of colectomy patients, with the exception of some *n*-3 PUFA. The proportion of fatty acids between the groups was largely similar, although ALNA and DHA proportions were lower in colectomy patients. The current three-way analyses however, showed generally that only a tendency for higher concentrations was seen in CO patients and significant differences between the reference and colectomy groups were attributable principally to variations between the reference group and SB colectomy patients. The previously noted lower proportion of ALNA and DHA in the colectomy patients

was still seen in the CO, but was highlighted by the SB patients in whom these disturbances were more marked.

A very similar pattern to that described above was seen also in the plasma TAG fraction. However, the results were more marked in this fraction. Previously in Chapter 6, higher TAG fatty acid concentrations were identified in the colectomy group. However, the current comparisons clearly showed that the higher concentrations were attributable only to the SB subset of the colectomy group. The TAG fatty acid concentrations in the CO subset showed a slight tendency to be higher than in the reference group, but this was not significant in any instances. The results also quite clearly demonstrated the huge variation of concentrations within the SB group; in some cases the highest individual fatty acid concentration was eight-fold greater than the lowest (e.g. ARA).

Findings from plasma NEFA demonstrated a different pattern of results to the other two fractions. Previously in PC and TAG, differences between the reference and colectomy groups were largely attributable to changes within the SB group. However, in this fraction, the opposite was true in many cases as it was the CO subset exhibiting the largest changes (e.g. ALNA, GLA and DPA concentration). The results from this fraction contrasted with those of PC and TAG in that both the total *n*-3 PUFA concentration and proportion was higher in the colectomy groups than the reference group, and to a greater extent in the CO subset. This was accompanied by a lower concentration and proportion of *n*-6 PUFA in this fraction.

The findings from the CE fraction were largely similar to those found in the PC and TAG fractions; differences between the reference and colectomy groups were more marked in the SB subset. However, in this fraction, there also appeared to be a greater degree of influence on the CO subset than seen in the other fractions as several significant differences were observed between the reference group and the CO subset - in other fractions often there was only a tendency within these subjects and more marked changes were noted in the SB group.

These results clearly illustrate an important effect of small bowel resection on fatty acid composition of plasma lipids. In general, more marked differences in fatty acid composition were seen between the reference and SB groups than between the reference and colectomy groups. Indeed, often there were no differences between the reference and CO groups. This could suggest that the processes influencing fatty acid profile changes in colectomy patients are digestion or absorption related as such processes would be strongly affected by small bowel resection. It is possible that decreased fatty acid digestion and/or absorption could lead to altered hepatic fatty acid metabolism as insufficient fatty acid availability could possibly stimulate hepatic lipid export in an effort to increase the availability of certain fatty acids. The results from this study certainly indicate limited *n*-3 PUFA availability in colectomy patients, in particular the SB subset, as the relative proportion of ALNA and DHA were consistently lower in all lipid fractions.

It has already been shown that the dietary intake of the colectomy patients is probably not different to that of the reference group and it is unlikely that variations in dietary intake of *n*-3 PUFA can account, even in part, for the marked changes in fatty acid composition in the colectomy patients. This is also supported by an intervention study published by Burdge *et al.*, who demonstrated that even with an almost 6-fold increase in ALNA intake, ALNA and DHA concentrations were not significantly changed in plasma PC, TAG and NEFA in the intervention group compared to controls (Burdge et al., 2003).

7.6.1 THE EFFECT OF INFLAMMATION

One of the original hypotheses of this thesis predicted that any alterations to plasma fatty acid composition in colectomy patients could be a result of altered metabolic handling of fatty acids, either due to altered hepatic metabolism or abnormal demands due to active inflammation. This is because inflammatory processes are known to affect lipid metabolism, such as PGE_2 production from membrane ARA, and less often EPA. Although an alteration in the hepatic handling of fatty acids in the colectomy patients has already been identified as a possible influence on plasma composition alterations in this group, the confounding effect of inflammation also needs to be considered.

Inflammatory markers were measured using blood samples taken from the patients in the colectomy group as described previously. The markers assessed were CRP and ESR. Only though these are both very non-specific markers of inflammation, they may be a useful indicator of whether an individual is in a raised inflammatory state.

Unfortunately, due to financial constraints, it was not possible to collect samples for measurement of CRP and ESR from the reference patients. Instead, the CRP values for the colectomy patients were compared to results published in the HSFE (see Chapter 4 for details). This was a large data set collected as part of a UK national survey into risk factors for cardiovascular disease. Unfortunately, raw data were not available from the HSFE, so the published means and standard deviations were used to make an arbitrary comparison between this population of 'normal' adults and the colectomy patients. These comparisons showed that the mean CRP concentrations in the colectomy patients were lower than those reported for the general population. Unfortunately, it was not possible to identify any reference data for the ESR measurements in the colectomy patients so they were instead assessed against the hospital laboratory normal range. As presented, a small number of the colectomy patients had ESR measurements outside of the normal range, but values were not particularly high. Overall, there were no CRP or ESR values found to be extremely high as would be seen in cases of active inflammatory disease. Therefore, this did not generally lend support to the hypothesis that altered fatty acid profiles were related to a raised inflammatory status in the colectomy patients. For this reason, the hypothesis that a raised inflammatory state could part-explain altered plasma fatty acid profiles in the colectomy patients is unlikely to be supported by these data.

The limitations of the use of these markers as a true representation of inflammatory status must be considered as there may be other indicators of inflammation which were not measured and have not been considered in this study. However, the results from the measurement of CRP and ESR in this study were considered to provide good general evidence that the colectomy patients were not in a significantly active inflammatory state.

7.6.2 THE EFFECT OF BODY MASS INDEX

It is well documented that a positive relationship exists between BMI and plasma TAG concentrations (Gensini et al., 1998; Simons et al., 1984). Indeed, within the data presented in this thesis, a significant positive association between BMI and plasma total TAG fatty acid concentration ($r = 0.371$, $p<0.01$) was found, although a similar relationship was not seen between BMI and total fatty acid concentration in either NEFA or PC (Table 7.2).

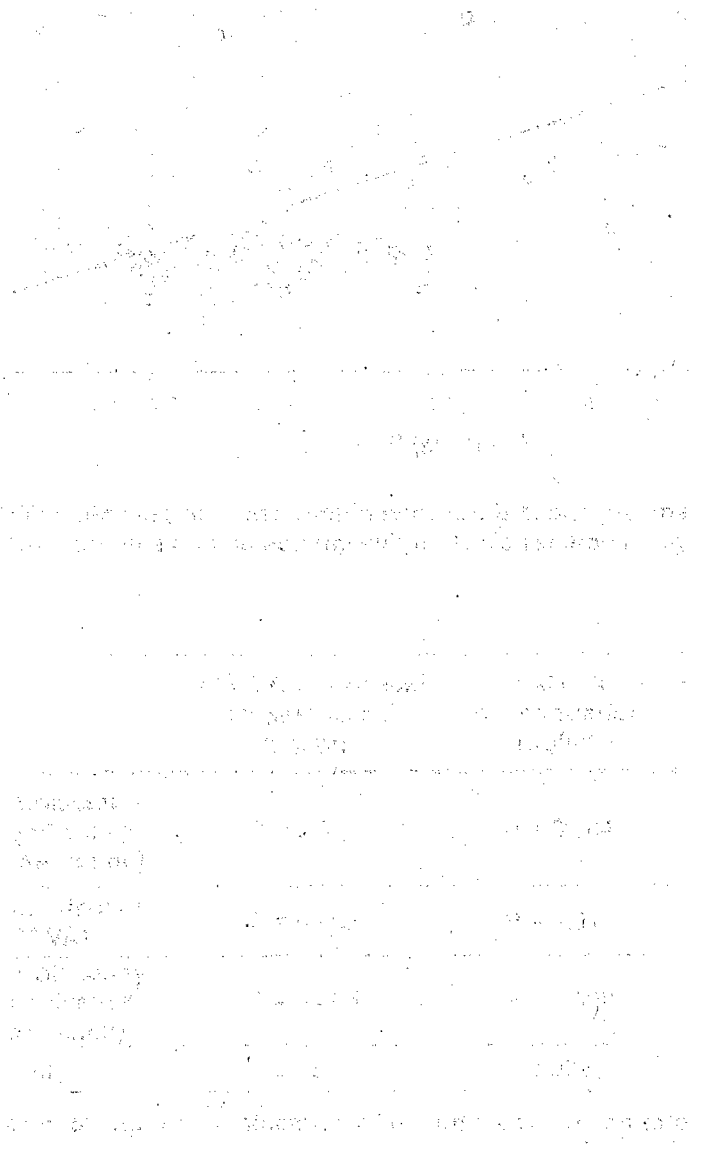
	Total fatty acid concentration (µg/ml)		
	TAG	PC	NEFA
Pearson correlation with BMI (kg/m^2)	0.371**	0.167	-0.172

Table 7.2 Pearson correlations of BMI (kg/m^2) with the total fatty acid concentration in plasma TAG, PC and NEFA. ** = $p<0.01$.

The relationship between plasma total fatty acid concentration in TAG and BMI is shown graphically in Figure 7.16 Graph showing the relationship between BMI and plasma TAG total fatty acid concentration in reference and colectomy subjects. Correlation analysis performed using Pearson.Figure 7.16. This shows that BMI accounts for 14% of the variance in plasma total TAG concentrations. Therefore, 86% of the variance in TAG concentrations remain unexplained by BMI.

It was previously shown in Chapter 4, and earlier in 7.4, that the Reference, CO and SB subject groups were not significantly different in terms of BMI measurement, although a wider range of BMI values was exhibited in the colectomy subsets. However, due to the significant relationship between BMI and plasma TAG concentrations, the data were explored further to establish if differences in TAG fatty acid composition in the study groups could be attributable to differences in BMI. To perform this test, an analysis of covariance (ANCOVA) was performed to test for the effects of BMI (covariate) on the total fatty acid concentration in TAG in the study subjects. As an additional example, as a significant difference in LA concentration was seen between the reference and colectomy groups (as described in previous chapters), the effect of BMI on this variable was also tested. Although there was no significant difference between the populations in TAG DHA concentration, this variable was also

analysed to establish if differences between the reference and colectomy groups may become significant when correcting for BMI.



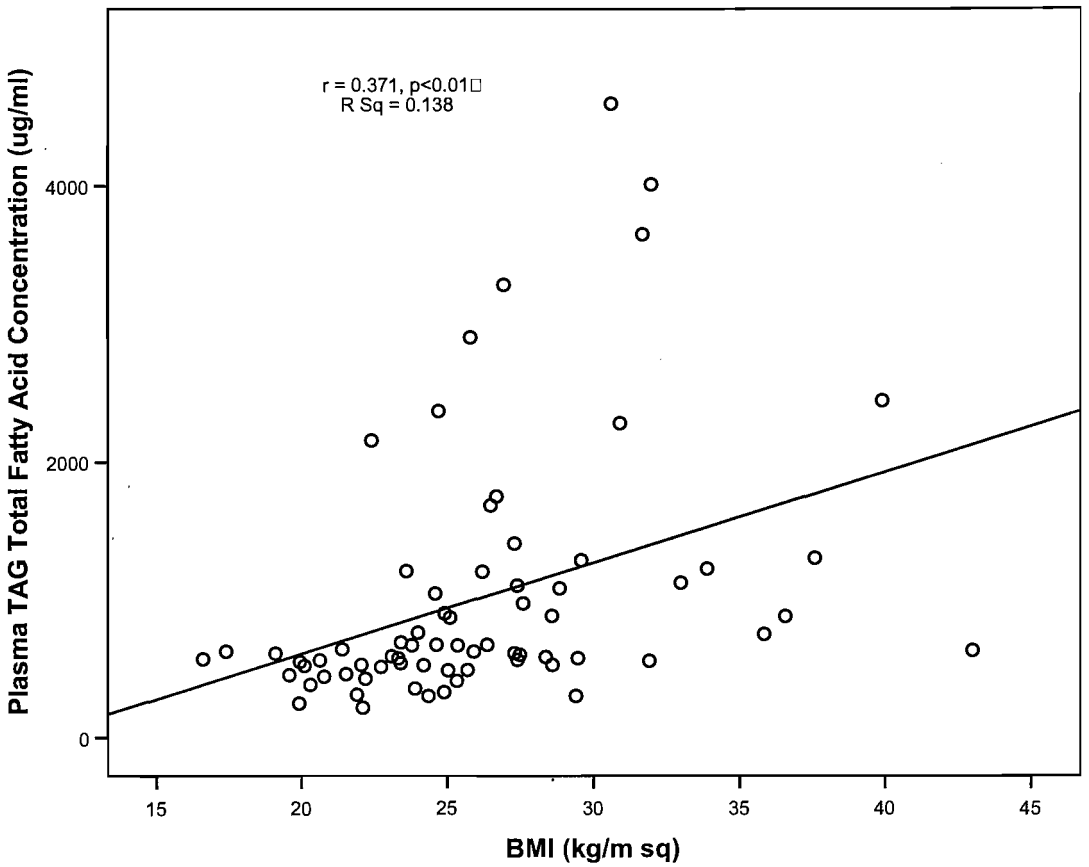


Figure 7.16 Graph showing the relationship between BMI and plasma TAG total fatty acid concentration in reference and colectomy subjects. Correlation analysis performed using Pearson.

	Total TAG fatty acid concentration (µg/ml)	TAG LA concentration (µg/ml)	TAG DHA concentration (µg/ml)
Reference vs Colectomy statistical significance (without BMI correction)	P = 0.002	P = 0.004	NS
Significance of effect of BMI (ANCOVA)	P = 0.003	P = 0.011	P = 0.026
Reference vs Colectomy statistical significance (with BMI correction)	P = 0.005	P = 0.008	NS
R^2 for BMI	0.138	0.082	0.083

Table 7.3 Table showing the significance of the effect of BMI on total TAG fatty acid and selected TAG fatty acid concentrations. The first row gives the ANOVA statistic of the reference versus colectomy comparison (i.e. with no correction for BMI). The second row gives the ANCOVA statistic for the significance of the effect of BMI on the total TAG or TAG fatty acid concentration. The third row states the R^2 value for the inclusion of BMI into a multiple regression analysis.

ANCOVA showed that there was a significant effect of BMI on total TAG fatty acid, LA and DHA concentrations ($p = 0.003$, 0.011 and 0.026, respectively) (Table 7.3). However, if the effect of BMI was removed from the model, the differences which previously existed between the reference and colectomy groups (for total TAG fatty acid and LA concentration) were still highly significant. In addition, for TAG DHA concentration, where originally no significant difference was detected between the reference and colectomy groups, when the effect of BMI from the model was removed, differences between thee reference and colectomy groups again failed to reach significance. Furthermore, the regression analyses showed that only a small amount of the variance in total fatty acid and LA and DHA concentration in TAG could be accounted for by BMI (<14%). Thus over 86% of the variance in the various TAG fatty acid concentrations could not be accounted for by BMI.

In summary, although there was a significant association between BMI and TAG concentration and adjusting for the effects of BMI may explain a small amount of the variance in TAG fatty acid concentrations, it cannot adequately account for the differences seen between the reference and colectomy groups. This finding is perhaps not surprising given that the mean BMI of the study groups was not different.

In conclusion, the results presented here show a clear effect in the SB patients as this group exhibited the greatest perturbations to fatty acid compositions. Some effects were also seen in the CO group and these patients generally showed the same pattern of findings, but the changes were generally more marked than those seen in the SB subset.

It was not clear exactly as to the nature of the effects on fatty acid composition in the SB subset, but the evidence could suggest a possible influence of lipid maldigestive or malabsorptive processes in these patients. Clearly such patients are likely to have significant alterations to GI function and potentially the ability to digest and absorb dietary lipids. However, it is important to consider that the SB patients represented a small number of subjects whose physiology was very heterogeneous. As previously documented, in the majority of cases it was impossible to establish precisely the extent of ileal resection and

this often only an estimate was possible. Added to this the fact that varying degrees of adaptation are seen between individuals, it is very difficult to characterise these patients and much larger numbers would be required to establish more robust data.

Significantly higher concentrations of PC and TAG fatty acids were observed in the colectomy patients and again, these effects were considerably more obvious in the SB subset of this group. This could possibly suggest the manifestation of a compensatory increase in the size of the hepatically-derived fatty acid pool; potentially the response to a decrease in the exogenous supply of lipids to the body due to impaired abilities to digest or absorb dietary fat. However, as was observed when considering the relative proportions of the fatty acids compromising the TAG and PC fractions, this increase in total concentration was not reflected equally in all fatty acid species within the fractions. Indeed, the proportion of *n*-3 PUFA was considerably lower in most cases, and this was consistent across the lipid fractions. The apparent lack of *n*-3 PUFA across the fractions could suggest cellular demands for *n*-3 PUFA which cannot be met. Alternatively, it could suggest that despite the significant expansion of the size of the plasma lipid pool, availability from exogenous sources and from biosynthetic pathways simply cannot meet the requirements for *n*-3 PUFA to maintain a desirable fatty acid composition. It has been shown in several studies that the extent of ALNA conversion to EPA is limited in humans (<8%) and DHA synthesis is only marginal (<0.02-4%) and it is been suggested that this may represent tight regulation of the synthesis of DHA, perhaps to ensure adequate availability within the body independently of variations in dietary intake (Burdge et al., 2003) which may be pertinent.

The changes to fatty acid composition of the plasma pool are unlikely to be a result of an abnormal inflammatory state in the colectomy patients. Although the evidence is limited, the data available do generally indicate that the colectomy patients did not exhibit any obvious signs of significant inflammatory burden which could significantly affect PUFA metabolism, in particular the *n*-6 family.

7.7 CONCLUSIONS

From the results presented and discussed above, the following conclusions were drawn:

- Small bowel resection was associated with changes in fatty acid composition of plasma lipids; however, the extent and full nature of these changes could not be fully elucidated due to the limited number of patients studied.
- Changes in fatty acid composition were also evident in patients with no small bowel resection, but these were generally less marked than those exhibited by the SB subjects.
- The greater magnitude of fatty acid perturbations within the SB group could possibly indicate a component of lipid maldigestion and/or malabsorption in the pathogenesis of these disturbances. However, additional research is needed to investigate this further.
- Similar, but generally less marked changes in the CO group could possibly suggest that even patients with no small bowel involvement could be at risk of more significant disturbances to fatty acid profiles.
- It is possible that colectomy patients, in particular those with small bowel resection, could have a decreased exogenous supply due to an impaired digestive and absorptive capacity. These in turn could possibly explain the significantly higher TAG and PC concentrations in colectomy patients as this could be a compensatory increase in the size of the fatty acid pool in the face of decreased availability of certain fatty acids, perhaps in particular the *n*-3 PUFA.
- The changes observed in the colectomy patients were unlikely to be explained by the influence of a raised inflammatory status as the colectomy patients did not exhibit any obvious signs of ongoing inflammation. Inflammatory pathways and their influences on fatty acid metabolism are unlikely to be able to explain the differences between the study groups.
- A significant relationship between plasma TAG concentrations and BMI was noted and this would be expected. However, mean BMI measurements were not different between the study populations so it

was unlikely that differences in fatty acid composition could be attributed to differences in BMI. A brief covariance analysis on the effect of BMI on fatty acid proportions did not reveal any significant interaction. Therefore it is unlikely that BMI had a significant bearing on the results.

Hypothesis III could neither be accepted nor refuted, as the results presented were not fully conclusive. There were clear patterns emerging from the results which appeared to indicate that whatever the influences affecting the fatty acid profiles, these were more profound in patients with small bowel resection. However, it is not known if small bowel resection is the causal link to the changes in fatty acid status. Neither is it known if the changes in fatty acid profiles in colectomy patients in general are a result of maldigestion and/or malabsorption processes.

Hypothesis IV could also neither be fully accepted nor rejected. The results were not generally indicative of any inflammatory influence on fatty acid metabolism in the colectomy patients, but the assessments of inflammatory status were limited. However, it is possible that the colectomy patients may have a digestive and/or absorptive impairment, potentially as a result of colectomy surgery. It is also possible that this could confer a limited exogenous lipid supply in these patients and a considerably higher concentration of fatty acids in the plasma of these patients could be indicative of adaptive mechanisms as a result of this limited availability from the diet.

However, the data available from this study are limited and the patient numbers small. Further and more extensive research would be required to fully investigate these phenomena and to possibly support or refute the mechanisms that have been proposed.

Chapter 8

General Discussion

8.1 INTRODUCTION

There is little clinical interest in patients with colectomy and they are only regarded as being at nutritional risk if they present with obvious clinical problems or symptoms. However, patients with colectomy often present with steatorrhoea and other absorption problems. In addition, some patients with short bowel syndrome have significant electrolyte balance problems. There is good reason to believe that following colon resection surgery, patients may have fatty acid handling problems, but this issue has not been well researched.

Although there are many studies in IBD and fatty acids, the focus is generally more in the role of fatty acids in the inflammatory process and how the *n*-3 and *n*-6 PUFA balance may have a role in the pathogenesis of active disease or in maintaining remission. There is significantly less interest into 'cured' patients with no recent history of active inflammation. In those limited studies which have investigated such subjects, there is considerable disparity between the studies in the type of patients enrolled, analytical methods for isolating and measuring fatty acids and the reporting of results. Direct comparison of data between studies is rarely possible therefore limiting the information and conclusions which can be extracted. In addition, the concept of different fatty acid compartments is not considered; usually plasma or serum fatty acids are measured only. However, plasma is constituted of several discreet compartments of which the fatty acids are from varying sources and serving different purposes. Knowledge of the individual compositions may contribute to our understanding and help to unravel the complex enigma which is fatty acid metabolism in these patients. This is not the only barrier to our understanding; current knowledge is also hindered partly by how data are expressed, either as absolute or relative proportions of fatty acids. This is believed to be the first study to investigate the fatty acid composition individually for all plasma lipid fractions in colectomy patients, in addition to reporting both the absolute concentration and relative proportion of each fatty acid.

This study set out to describe in detail the plasma fatty acid composition in colectomy patients and to consider the possibilities as to why these patients may have altered lipid metabolism. This chapter summarises the findings of the

study in relation to the original hypotheses. Then a discussion on how the results compare to what is already known and how the work contributes to our understanding will be presented. The next section considers the implications of these findings on individuals such as those studied in this project, including some general nutritional recommendations and considerations. Finally, some ideas for future studies and research are proposed.

8.2 HYPOTHESES

This study has characterised the fatty acid composition of plasma lipids of thirty-six colectomy patients and a reference group and examined some of the possible influences on altered fatty acid balance in the former.

8.2.1 HYPOTHESIS I

Colectomy patients will have disturbed fatty acid compositions when compared to a reference population.

8.2.2 HYPOTHESIS II

Colectomy patients have disturbed fatty acid profiles due to an altered exogenous (dietary) supply of fatty acids to the body.

8.2.3 HYPOTHESIS III

Colectomy patients have disturbed fatty acid profiles due to maldigestion or malabsorption in the intestine as a result of colon removal.

8.2.4 HYPOTHESIS IV

Colectomy patients have disturbed fatty acid profiles due to the altered metabolic handling of fatty acids, either due to altered hepatic metabolism or abnormal demands due to active inflammation.

8.3 SUMMARY OF FINDINGS

8.3.1 HYPOTHESIS I

Extensive study of the fatty acid composition of plasma PC, TAG NEFA and CE in reference and colectomy patients revealed significant alterations to the fatty acid composition profiles in the colectomy patients. In particular, fatty acid concentrations were significantly higher in the TAG and PC fractions, but not in NEFA, in the colectomy group. These changes were not reflected in the relative proportions of fatty acids in these fractions. *N*-3 PUFA proportions were lower in PC, TAG and CE fractions but higher in NEFA. EFA proportions were lower in CE. These data supported the suggested hypothesis and as such, it was accepted.

8.3.2 HYPOTHESIS II

To test the hypothesis of a possible influence of exogenous fatty acid supply on fatty acid compositions, data collected from the administration of a FFQ were analysed. Although the data were limited, they were generally not suggestive of any considerable differences between the two groups in terms of intakes of dietary fat, including PUFA consumption. In addition, the degree of alteration in PUFA intake in the diet required to elicit significant changes to plasma fatty acid composition is generally large, thus it was unlikely that modest differences between the groups, which may have been undetectable using the assessment tools employed in this study, could account for the significant changes seen between the study populations.

The results suggested that differences in dietary intake were unlikely to explain plasma fatty acid composition differences between the groups and as such, the hypothesis was unlikely to be true (Figure 8.1).

It is worth mentioning that many similar studies assessed or took into account possible differences in dietary intake between their study groups. This of course could have a significant influence on results. The dietary assessment methods employed in this study were deemed to be appropriate to establish that the long term habitual intakes of the groups were similar and that a potential

confounding influence of diet on fatty acid composition of plasma lipids could be reasonably ruled out.

8.3.3 HYPOTHESIS III

The principal approach employed to test this hypothesis was to sub-divide the colectomy group into patients with and without small bowel resection. Although patients without small bowel resection may still exhibit disturbances to fatty acid metabolism due to colonic bacterial relocation to the terminal ileum, it is more likely that those patients with ileal involvement requiring additional resection will show more marked changes to fatty acid metabolism. Indeed, such patients are more likely to suffer complications of colectomy surgery such as electrolyte imbalance and steatorrhoea.

The data from patients in the CO group were separated from and compared to those from individuals with >20 cm of small bowel resection in addition to colectomy (SB group). The data from the two colectomy sub-groups were compared against the reference group using ANOVA. Many of the fatty acid composition changes seen in the original analyses for Hypothesis I were now only significant when comparing the reference and SB groups; CO versus reference group comparisons were largely insignificant. However, it was clear from the graphical data that clear tendencies existed within the CO group which reflected the significant marked differences between the reference and SB groups.

These results were suggestive of a common process, influencing both the CO and SB subsets of the colectomy group. From these observations, it was suggested that the colectomy patients could have a degree of lipid maldigestion or malabsorption, probably as a result of the loss of the colon. This could potentially influence the availability of *n*-3 PUFA within the body, particularly as the average Western diet contains little *n*-3 PUFA. Marginal exogenous supply coupled with limited digestive and absorptive capacity could result in a limited availability of these fatty acids to target cells and tissues and perhaps the alterations to the *n*-3 PUFA composition of the hepatically-derived lipids were indicative of this. This in turn could result in increased lipid export from the liver

in an attempt to boost *n*-3 availability. This could explain the remarkable increases in concentrations of fatty acids in the plasma; perhaps an adaptive response to increase the size of the plasma fatty acid pool and thus availability to cells. *N*-3 PUFA, in particular ALNA and DHA, were consistently in a lower proportion in all fractions apart from NEFA. This could suggest that despite a potential increase in hepatic export to possibly increase fatty acid availability, supplies of ALNA and DHA were insufficient to maintain a 'desirable' level of these fatty acids in colectomy patients.

8.3.4 HYPOTHESIS IV

There were two facets to this hypothesis; one being an influence of inflammation which would increase fatty acid demands, the other being altered hepatic fatty acid metabolism.

The potential influence of inflammation on fatty acid metabolism was first tested by establishing the acute phase protein status of the colectomy group. Unfortunately, due to limitations of finance and design, inflammatory markers could not be measured in the reference group, so instead published results from a large population study in the UK were used for CRP reference values. These indicated that the CRP concentrations in the colectomy group were probably not raised, but conclusions were restricted by the absence of raw data for the reference values. The results were not indicative of any significant inflammatory processes in the colectomy patients so it was deemed unlikely that fatty acid metabolism would be affected by this.

From the results described previously, it was considered that altered hepatic fatty acid metabolism may be secondary to disturbances in fatty acid metabolism in colectomy patients, particularly those with additional small bowel resection. It was postulated that reduced lipid absorption/digestion in the GI tract could result in increased lipid export from the liver to improve the availability of *n*-3 PUFA (namely ALNA and DHA) to target cells and tissues.

In summary, the interpretations of the results presented in this thesis suggest that:

- Colectomy patients have altered plasma lipid fatty acid composition when compared to a reference group.
- Altered fatty acid compositions are associated to a greater degree with small bowel resection than to colectomy alone.
- Altered fatty acid profiles are unlikely to be the result of dietary disparities between the reference and colectomy groups.
- The colectomy patients are unlikely to be in an active inflammatory state.
- Altered fatty acid profiles could potentially be a result of maldigestion and/or malabsorption processes in the GI tract, particularly in SB patients.
- Altered hepatic metabolism could potentially be an adaptive response to impaired digestive and absorptive abilities as the body attempts to increase *n*-3 PUFA availability to the cells and tissues.

In conclusion, although gross nutritional intakes in these patients may not be considered a problem, there could be a rationale for PUFA supplementation in these patients, especially *n*-3 PUFA.

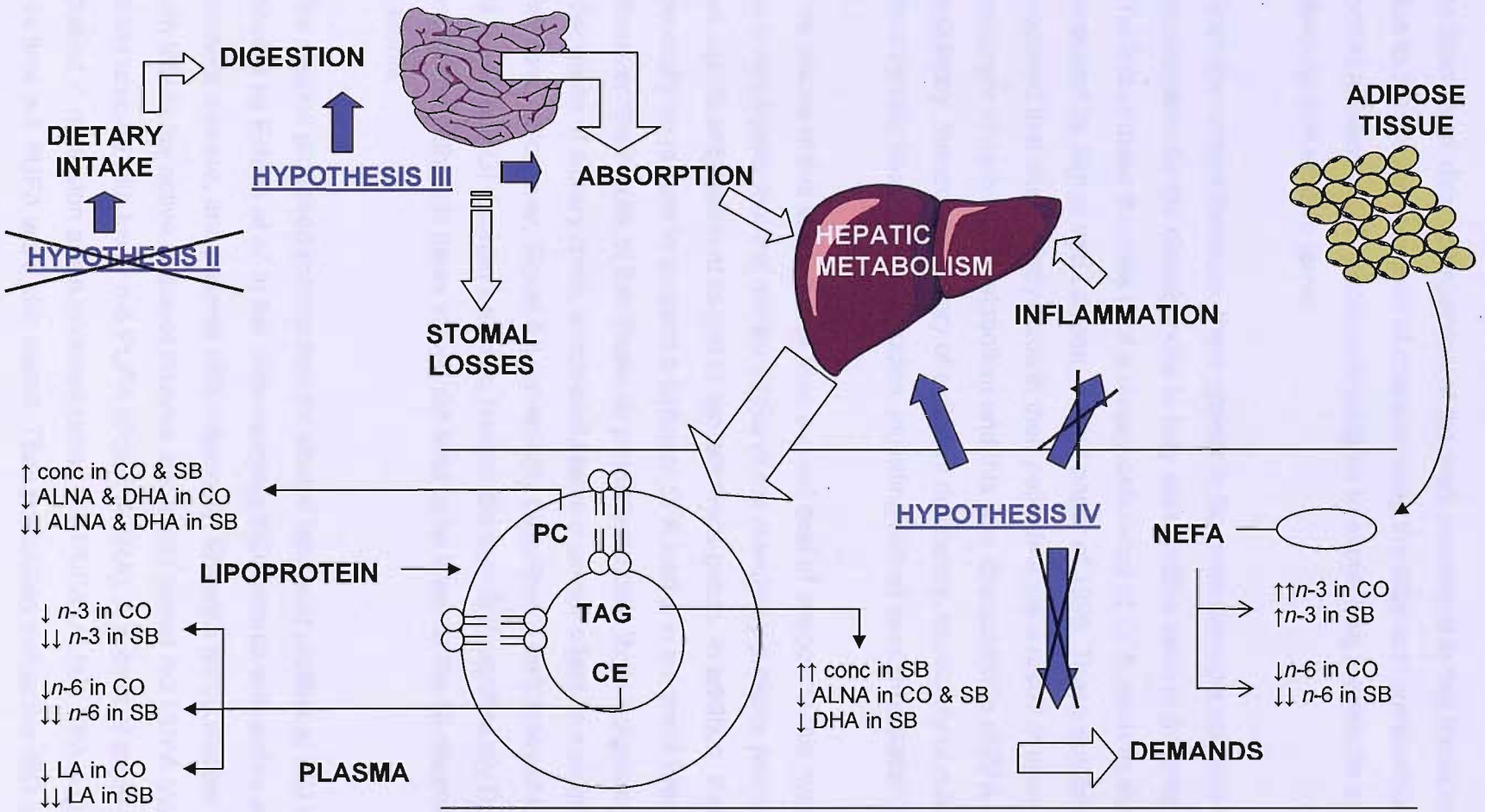


Figure 8.1 A pictorial summary of the findings of this thesis and the principal hypotheses.

8.4 CONTRIBUTION TO PRESENT UNDERSTANDING

As discussed, direct comparison of the work presented in this thesis is difficult due to the unique approach of characterising the fatty acid composition of individual plasma lipid fractions in addition to expressing the results in both absolute and relative terms.

From the current literature, there appear to be three principal suggested mechanisms for the disturbances to fatty acid profiles seen in patients with IBD. The first of these theories is of a dietary deficiency of EFA, such as that proposed by Siguel and Lerman, in their paper of 1996. These authors proposed that altered fatty acids in their patients were a result of up-regulated precursor to derivative metabolism and this was characteristic of EFA deficiency. Indeed, the theory of a dietary deficiency, especially of *n*-3 PUFA, is the scientific basis of many studies, including fish oil supplementation trials.

The results of this study do not lend a great deal of support to this hypothesis, as it was shown that the dietary intake of the colectomy patients probably were not significantly different to that of the reference group. In addition, there is generally no reason to suspect a deficient EFA intake in a normal Western diet. However, the results of this thesis do possibly indicate limited digestion and/or absorption of dietary lipids, which could have a similar effect to a simple dietary deficiency. However, Siguel & Lerman only described a deficiency of LA, not ALNA in their GI disorder patients. Neither did they find significantly DHA proportion, although these values did tend to be lower in the GI disorder patients.

The second proposed mechanism for altered fatty acid profiles in IBD was put forward by Esteve *et al.* in her trials studying IBD patients with active and dormant disease, and patients with colectomy. Esteve's group studies patients with IBD in the active phase of disease and found raised *n*-3 PUFA (ALNA & DHA) coupled with lower *n*-6 PUFA (DGLA & ARA). A subset of these patients studied in remission again revealed raised *n*-3 PUFA (ALNA, EPA & DHA) but this time *n*-6 PUFA were also raised. They postulated that active IBD increased fatty acid utilisation (possibly for ARA-derived eicosanoids) and therefore fatty

acid biosynthesis was up-regulated to meet these demands. They suggested this metabolic change was also present in patients with inactive disease, but in the absence of increased fatty acid demands, *n*-6 PUFA synthesis (with respect to utilisation) was also raised when patients were in remission. In both papers they strongly opposed recommendations for fish oil supplementation in such patients. In a later paper, they suggested that the metabolic change was directly due to the presence of an inflamed intestine as fatty acid profiles normalised in patients with established colectomy. The results presented in this thesis do not agree with the findings of Esteve, in fact they are quite the opposite.

An additional mechanism was proposed by Geerling *et al* in 1999. This group studied two groups of CD patients; one a set of newly diagnosed patients and the other a long-term disease group. These groups were probably more representative of patients in the active and inactive disease states, respectively. Geerling described lower ARA proportions in the CD groups along with marginally higher ALNA, higher DPA and lower DHA proportions in plasma PL. Although Geerling heavily criticised Esteve's studies, she also proposed a mechanism of altered fatty acid metabolism, but suggested that this could be due to steroid-induced alterations to Δ^6 desaturase activity.

The results of this study are generally not in agreement with the findings of any of the other groups as discussed in this thesis. In particular, the results contrast dramatically with those of Esteve *at al.* who reported higher *n*-3 PUFAs in her colectomy groups which later normalised in patients with long term colectomy. Esteve consistently suggests that *n*-3 PUFA supplementation in active or dormant IBD or in colectomy should not be given.

These data are certainly in agreement with the findings of (Johansson *et al.*, 1987), (Jeppesen *et al.*, 2000) and (Färkkilä *et al.*, 1987) who on the whole also described lower *n*-3 PUFA in plasma lipids. However, all of these studies have only considered patients with ileal resection; they do not consider that this problem may also extend to colectomy patients without ileal resection. The results of this study are generally suggestive of a component of ileal resection involvement; however certain observations of fatty acid imbalance are also seen in the colectomy-only group but to a lesser extent.

The fatty acid concentration data of this thesis, even in isolation, would still have shown that fatty acid concentrations were higher in colectomy. However, they would not have shown that *n*-3 PUFA proportions were still lower, despite this increase in concentration. Conversely, the proportion data in isolation would not have highlighted the considerably increased concentration of fatty acids in colectomy patients which has been postulated to be a result of decreased assimilation of dietary lipids. If the proportionate data had been considered in isolation, this could potentially have led to a conclusion of a relatively straightforward lack of *n*-3 PUFA as the proportions of these fatty acids were consistently lower in most fractions. Although this is one of the key observations of the study, the discovery of a considerable increase in fatty acid concentrations in the colectomy patients is also an important finding and simply would have gone undetected without the measurement of both concentration and proportionate data. The results and information available from them would be considerably less revealing and informative if either had been considered in isolation.

8.5 IMPLICATIONS OF FINDINGS

It is difficult, and probably inappropriate to make strong recommendations regarding the treatment of colectomy patients based on such a limited number of subjects. However, some tentative suggestions may help contribute to a better management and treatment of patients like those included in this study.

The results of this study suggested a possible limited availability of ALNA and DHA in colectomy patients, particularly in subjects with small bowel resection. Although results from the dietary analyses did not generally indicate significant dietary differences between the study groups, there was evidence to suggest lower PUFA availability, due to compromised digestion and absorption processes. It may be beneficial to these patients to increase *n*-3 fatty acid intake, possibly with a concurrent decrease in *n*-6 PUFA intake to decrease the *n*-6:*n*-3 PUFA intake ratio of the diet. This may help to supply the body with

more long chain PUFA in the face of limited digestive and/or absorptive capabilities.

The findings of this study may also have implications for women with colectomy who are of child-bearing potential and who wish to become pregnant. Adequate LC-PUFA availability is important for optimal brain and neuronal development and function. During pregnancy, the developing foetus depends completely on maternal sources of DHA from lipid stores & dietary supply (including supplements) (Uauy *et al.*, 2000). The apparent lack of ALNA and in particular, DHA, could have significant implications on the developing foetus. It may therefore be especially beneficial for such people to increase their intake of *n*-3 PUFA, or possibly to follow a supplementation regimen.

Many patients in the colectomy group had a BMI which classified them either as overweight or obese and physical examinations confirmed that these high BMI measurements were indeed a result of increased fat mass in these patients. It was also noted that many patients had high TAG concentrations in comparison to a reference population of the same age. This has been suggested as a possible adaptive response in the face of an impaired ability to digest and absorb dietary lipids. This may have particular implications to the coronary health of these patients, especially if coupled with a high alcohol intake, smoking, lack of exercise or an unfavourable genotype. It may therefore be pertinent to suggest that these patients pay particular attention to their cardiovascular risk factors and take steps to reduce them and this may also include increasing *n*-3 PUFA intake.

Overall, it would appear that the colectomy patients studied in this thesis may be in a poorer clinical and nutritional state than would generally be expected. Although superficially these patients generally showed few or no signs of major health concerns, it is clear from the investigations of this thesis that these patients may not be as healthy as they appeared.

8.6 LIMITATIONS OF THE STUDY

As with every scientific study, there are of course limitations of design, collection and analysis of results which must be considered when interpreting the results. The limitations of this study are discussed below:

- **Patient Availability**

There were a limited number of suitable patients available for inclusion into the study and naturally, not all invited patients were willing to participate. Although we were able to recruit all available and willing patients onto this study, unfortunately due to time and financial constraints, it was not possible to analyse every sample collected, hence a subset of patients were chosen at random. A larger sample population would have added statistical strength to the analysis of the results, and perhaps in cases of marginal statistical significance, a larger group number would have resulted in a significant difference. However, the number of patients in the group was of a reasonable size and many of the statistical differences were highly significant beyond the <0.05 level.

The patients had either a history of CD or UC resulting in their colectomy, but the results from both sub-groups were combined and termed the colectomy group. Some experts would argue that the two disease pathologies are too heterogeneous (especially in immunological terms) and data pooling is inappropriate. However, the two disease groups were compared to each other in the first instance and no significant differences existed between the groups. Thus, in the context of this study where plasma fatty acid profiles were the outcome under investigation, it was appropriate for these two groups to be combined.

- **Within- and Between-Group Heterogeneity**

There were further differences between the patients within the colectomy group also limiting the conclusions drawn from the results. There were very few patients in the group who had undergone small bowel resection which limited the ability to detect statistical differences between them and the patients with colectomy only. There was also a degree of heterogeneity in the length of small bowel removed in the SB patients, if indeed this could be assessed at all.

Review of the surgical notes for these patients commonly revealed limited detail in the documentation recording the surgical removal of the small bowel. Subsequently, it was often difficult to assess exactly how much ileum had been removed. Therefore the SB patients were classified as those with >20 cm of ileum removed as this was the best classification possible with the information available. This means that the grouping of the patients into SB and CO was not entirely definitive, and there is a possibility that a minority of patients could have been misclassified. However, despite this, the results presented in the thesis showed clear differences between the SB and CO groups. In addition, it was felt that the classification of subjects into the subgroups was as appropriate and accurate as the medical history would allow.

- **Dietary Assessment**

The dietary intake of both groups was assessed using a FFQ. This questionnaire was not specifically designed to assess *n*-3 fatty acid intake. However, the questionnaire did contain specific questions regarding fish consumption as these items are the principal source of long-chain *n*-3 PUFA in a typical Western diet.

It should be considered that dietary intake assessment in general is a very difficult task and no one tool in isolation can provide a truly accurate measure of dietary intake in a free-living population. Within the confines of this study where it was not possible to utilise several tools, the FFQ was taken to be the most appropriate method available to assess the habitual and longer-term dietary intake of the study groups.

8.7 FURTHER RESEARCH

Several hypotheses have been proposed for the mechanisms behind altered fatty acid composition in the colectomy patients. The work presented in this thesis offers a small contribution to our understanding, but our knowledge is far from complete. There are a number of ways to extend the research presented in this thesis and below are just a few examples.

As has already been discussed previously in this thesis, there were limitations to the dietary assessment data used to establish any obvious differences in intake between the reference and colectomy groups. Should the study be repeated or extended, it would be incredibly valuable to perform more thorough dietary assessments in order to be able to make a more definitive statement regarding the presence or absence of significant differences in dietary intake between the groups. For instance, it would be advantageous to extend the FFQ used in this study to capture potential significant sources of *n*-3 and *n*-6 PUFA in the diet. In addition, direct comparison of the intakes of the major sources of *n*-3 PUFA (such as oily fish) would provide more detailed and valuable insight into the *n*-3 content of the diet. Improvements to the dietary assessment component of this study could provide stronger evidence to either support or reject the hypothesis that dietary alterations could account for fatty acid differences between the groups.

One of the main interpretations of the results of this study was that colectomy patients, in particular those with small bowel resection, have altered fatty acid metabolism as a result of decreased availability of ingested lipids. To investigate this hypothesis further, an investigation of lipid metabolism of colectomy patients (with and without small bowel resection) could be performed which could involve:

- Administration of isotopically labelled test meal of known lipid composition, then
- Measurement and analyses of stomal output to establish the actual absorption of lipid from the meal.
- Measurement of blood lipids to trace incorporation of isotope into plasma lipids.

- *n*-3 PUFA supplementation (possibly parenterally administered to bypass absorption difficulties), then
- Measurement of blood lipids to establish composition change over time
- Establish if plasma fatty acid concentrations fall with increasing *n*-3 PUFA availability.

Such studies could provide more substantial evidence to support the hypothesis that the plasma fatty acid disturbances presented in this thesis are due to decreased lipid absorption through the GI tract. Such studies could also establish whether *n*-3 PUFA supplementation in these patients would offer a significant clinical benefit in terms of lowering plasma lipid concentrations.

8.7.1 POWER ANALYSES

As discussed in Chapter 3, this study was not designed according to statistical power because it was intended as a pilot study for several reasons. Firstly, because the number of potentially suitable patients was not expected to be large and indeed this was the case when recruitment started. In addition, there were significant restrictions of time and budget which would not have permitted the complete lipid analysis of any more samples than were actually processed for this study. Lastly, to the author's knowledge there were no other published studies providing sufficient comparable data which could have been utilised to perform power calculations for the current study.

Due to a potentially important biological significance of the results from this study, it is hoped that this work may be extended in the future. In this instance and subject to the availability of suitable study patients, it would be necessary for other researchers to perform a power analysis to ensure sufficient subject numbers. The data from this study could be used to provide the grounding for such analyses.

The results for the colectomy group from the current study were used as a basis for calculating the power for future research (DSS Research). To have an 80% power to detect a 20% difference between groups in TAG DHA proportion (given a mean of 0.51, SD of 0.36 and 5% confidence interval), approximately 100 subjects in each group would be required. To detect a difference in EPA and DHA in the NEFA fraction would require far greater numbers of subjects (low power), than to detect differences in other variables, such as PC ALNA (high power). Given these numbers of subjects in the study groups, this would provide much greater confidence in the observations on the latter than the former.

8.8 FINAL CONCLUSION

There is good reason to suspect that patients with colectomy have problems with digestion and absorption of dietary lipids, including those patients who have not undergone resection of the ileum in addition to colectomy surgery. However, such patients are generally considered to be cured and are discharged from specialist care. Certainly, the majority of patients entering this study did not present to the investigators as particularly unwell or malnourished.

The evidence presented in this thesis indicates that colectomy patients do appear to have underlying problems in terms of their lipid metabolism, possibly as a result of maldigestion and malabsorption of dietary lipids. Such disturbances to lipid metabolism were particularly evident in those patients who had undergone resection of the ileum in addition to the colon. Analysis of the fatty acid composition of plasma lipids in the patient group showed very high concentrations of certain lipids in the plasma and lower proportions of ALNA and DHA. These results showed some similarity to studies of fatty acids in patients with intestinal failure. This may place these patients at risk of EFA deficiency and problems associated with a lack of *n*-3 PUFA, in addition to compromising their cardiovascular health due to increased plasma lipid concentrations.

Further research is needed to further investigate the possibility of intestinal failure both in patients with colectomy and those with additional small bowel resection. Such investigations could include isotope label studies to quantify the degree of lipid malabsorption, in addition to PUFA supplementation trials. A more comprehensive understanding of lipid metabolism in this patient group could help to establish the effects of PUFA supplementation in these patients and whether this could provide any measurable clinical benefit.

Chapter 9

Appendices

9.1 METHOD VALIDATION

9.1.1 LABORATORY ANALYSIS

Plasma TAG

	PEAK AREA COUNTS										
Sample	PA	*C17:0	SA	OA	LA	GLA	ALNA	DGLA	ARA	EPA	*C23:0
1	183799.0	43244.7	24036.1	275351.0	155703.0	5474.0	8074.8	3626.8	9371.2	1419.8	63289.4
2	204988.0	49050.7	26442.7	306506.0	173778.0	6036.3	9086.1	4040.5	10353.6	1623.3	71497.6
3	183569.0	43376.7	23807.3	274977.0	155849.0	5528.9	8100.4	3493.5	9712.9	1472.8	63906.5
4	182084.0	41972.7	23833.7	273093.0	154028.0	5320.5	7884.1	3468.6	9357.2	1418.2	66101.8
5	328944.0	74056.3	43225.2	482265.0	274565.0	9966.3	14531.7	6461.9	17099.1	2599.2	127688.0
6	180833.0	43263.7	23807.5	272376.0	154309.0	5397.9	8009.6	3639.4	9245.2	1455.6	63439.2
7	179198.0	41770.0	23392.4	267562.0	151135.0	5277.6	7802.9	3543.2	9123.7	1463.3	63248.2
8	174160.0	41518.6	22620.8	258879.0	146504.0	5129.4	7519.9	3354.6	8713.6	1364.1	62631.7
9	178966.0	40278.9	23281.0	267403.0	151440.0	5169.6	7799.4	3507.6	9130.1	1390.6	65154.0

Table 9.1 Plasma TAG validation sample chromatogram fatty acid and standard peak area counts. **17:0 = added internal standard; *23:0 = added recovery standard*

PEAK AREA COUNTS		
DPA	DHA	TOTAL (EXC *STD)
3261.8	3441.0	673558.5
3820.3	3849.0	750523.8
3377.3	3470.5	673358.6
3368.3	3396.4	667252.0
5574.3	6290.2	1191521.9
3816.2	3447.2	666336.6
3327.3	3294.4	655119.8
3032.3	3145.3	634423.0
3245.3	3332.0	654664.6

Sample	FATTY ACID RELATIVE PROPORTION (%)											
	PA	SA	OA	LA	GLA	ALNA	DGLA	ARA	EPA	DPA	DHA	STANDARD RECOVERY (%)
1	27.3	3.6	40.9	23.1	0.8	1.2	0.5	1.4	0.2	0.5	0.5	68.3
2	27.3	3.5	40.8	23.2	0.8	1.2	0.5	1.4	0.2	0.5	0.5	68.6
3	27.3	3.5	40.8	23.1	0.8	1.2	0.5	1.4	0.2	0.5	0.5	67.9
4	27.3	3.6	40.9	23.1	0.8	1.2	0.5	1.4	0.2	0.5	0.5	63.5
5	27.6	3.6	40.5	23.0	0.8	1.2	0.5	1.4	0.2	0.5	0.5	58.0
6	27.1	3.6	40.9	23.2	0.8	1.2	0.5	1.4	0.2	0.6	0.5	68.2
7	27.4	3.6	40.8	23.1	0.8	1.2	0.5	1.4	0.2	0.5	0.5	66.0
8	27.5	3.6	40.8	23.1	0.8	1.2	0.5	1.4	0.2	0.5	0.5	66.3
9	27.3	3.6	40.8	23.1	0.8	1.2	0.5	1.4	0.2	0.5	0.5	61.8
Mean	**27.3**	**3.6**	**40.8**	**23.1**	**0.8**	**1.2**	**0.5**	**1.4**	**0.2**	**0.5**	**0.5**	**65.4**
SD	**0.1**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**3.6**
CV (%)	**0.48**	**0.82**	**0.32**	**0.17**	**1.67**	**1.02**	**1.83**	**1.69**	**1.83**	**5.97**	**1.77**	**5.54**

Table 9.2 Relative proportion (%) of TAG component fatty acids in the validation samples.

Sample	FATTY ACID CONCENTRATION (µg/ml)											
	PA	SA	OA	LA	GLA	ALNA	DGLA	ARA	EPA	DPA	DHA	TOTAL (EXC *STD)
1	425.0	55.6	636.7	360.1	12.7	18.7	8.4	21.7	3.3	7.5	8.0	1557.6
2	417.9	53.9	624.9	354.3	12.3	18.5	8.2	21.1	3.3	7.8	7.8	1530.1
3	423.2	54.9	633.9	359.3	12.7	18.7	8.1	22.4	3.4	7.8	8.0	1552.4
4	433.8	56.8	650.6	367.0	12.7	18.8	8.3	22.3	3.4	8.0	8.1	1589.7
5	444.2	58.4	651.2	370.8	13.5	19.6	8.7	23.1	3.5	7.5	8.5	1608.9
6	418.0	55.0	629.6	356.7	12.5	18.5	8.4	21.4	3.4	8.8	8.0	1540.2
7	429.0	56.0	640.6	361.8	12.6	18.7	8.5	21.8	3.5	8.0	7.9	1568.4
8	419.5	54.5	623.5	352.9	12.4	18.1	8.1	21.0	3.3	7.3	7.6	1528.0
9	444.3	57.8	663.9	376.0	12.8	19.4	8.7	22.7	3.5	8.1	8.3	1625.3
Mean	**428.3**	**55.9**	**639.4**	**362.1**	**12.7**	**18.8**	**8.4**	**21.9**	**3.4**	**7.9**	**8.0**	**1566.7**
SD	**10.4**	**1.5**	**13.5**	**7.7**	**0.3**	**0.5**	**0.2**	**0.7**	**0.1**	**0.4**	**0.3**	**34.6**
CV (%)	**2.4**	**2.7**	**2.1**	**2.1**	**2.7**	**2.4**	**2.9**	**3.3**	**2.6**	**5.6**	**3.3**	**2.2**

Table 9.3 Absolute concentration (µg/ml) of TAG component fatty acids in the validation samples.

Plasma PC

Sample	PEAK AREA COUNTS												
	*C15:0	PA	SA	OA	LA	ALNA	DGLA	ARA	EPA	*C23:0	DPA	DHA	TOTAL (EXC *STD)
1	99745.9	784884.0	372658.0	258769.0	619968.0	7057.9	62997.2	274687.0	76369.3	172994.7	32665.3	169737.0	2659792.7
2	97485.3	739633.0	348224.0	244267.0	583795.0	6724.4	59106.2	258083.0	71540.8	159849.0	30775.3	159738.0	2501886.7
3	85734.4	647073.0	303662.0	209040.0	505989.0	5704.9	52140.0	223344.0	61920.6	145659.0	26075.8	136161.0	2171110.3
4	96964.4	729267.0	341693.0	237994.0	570783.0	6510.3	57440.4	252153.0	69837.3	156389.0	29182.2	154471.0	2449331.2
5	82497.8	614725.0	283415.0	194796.0	475413.0	5333.5	47412.5	209167.0	58122.2	140531.8	24108.7	126305.0	2038797.9
6	88451.2	664525.0	310032.0	215144.0	518156.0	5912.2	52040.4	228064.0	63216.8	146320.5	26642.2	139074.0	2222806.6
7	88300.0	654132.0	301746.0	207038.0	502536.0	5576.3	50278.1	220971.0	61075.7	148803.5	25873.1	133444.0	2162670.2
8	88263.6	648944.0	298631.0	204651.0	497120.0	5560.1	49683.1	217266.0	60014.5	150115.5	25061.5	130239.0	2137170.2

Table 9.4 Plasma PC validation sample chromatogram fatty acid and standard peak area counts. **15:0 = added internal standard; *23:0 = added recovery standard*

Sample	FATTY ACID RELATIVE PROPORTION (%)										
	PA	SA	OA	LA	ALNA	DGLA	ARA6	EPA	DPA	DHA	STANDARD RECOVERY (%)
1	29.5	14.0	9.7	23.3	0.3	2.4	10.3	2.9	1.2	6.4	79.0
2	29.6	13.9	9.8	23.3	0.3	2.4	10.3	2.9	1.2	6.4	83.5
3	29.8	14.0	9.6	23.3	0.3	2.4	10.3	2.9	1.2	6.3	80.6
4	29.8	14.0	9.7	23.3	0.3	2.3	10.3	2.9	1.2	6.3	84.9
5	30.2	13.9	9.6	23.3	0.3	2.3	10.3	2.9	1.2	6.2	80.4
6	29.9	13.9	9.7	23.3	0.3	2.3	10.3	2.8	1.2	6.3	82.8
7	30.2	14.0	9.6	23.2	0.3	2.3	10.2	2.8	1.2	6.2	81.3
8	30.4	14.0	9.6	23.3	0.3	2.3	10.2	2.8	1.2	6.1	80.5
Mean	**29.9**	**14.0**	**9.7**	**23.3**	**0.3**	**2.3**	**10.3**	**2.8**	**1.2**	**6.3**	**81.6**
SD	**0.3**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.0**	**0.1**	**2.0**
CV (%)	**1.0**	**0.3**	**0.8**	**0.1**	**1.4**	**1.2**	**0.5**	**0.7**	**1.7**	**1.6**	**2.4**

Table 9.5 Relative proportion (%) of PC component fatty acids in the validation samples.

	FATTY ACID CONCENTRATION (µg/ml)										
Sample	**PA**	**SA**	**OA**	**LA**	**ALNA**	**DGLA**	**ARA**	**EPA**	**DPA**	**DHA**	**TOTAL (EXC *STD)**
1	786.9	373.6	259.4	621.5	7.1	63.2	275.4	76.6	32.7	170.2	2666.6
2	758.7	357.2	250.6	598.9	6.9	60.6	264.7	73.4	31.6	163.9	2566.4
3	754.7	354.2	243.8	590.2	6.7	60.8	260.5	72.2	30.4	158.8	2532.4
4	752.1	352.4	245.4	588.7	6.7	59.2	260.0	72.0	30.1	159.3	2526.0
5	745.1	343.5	236.1	576.3	6.5	57.5	253.5	70.5	29.2	153.1	2471.3
6	751.3	350.5	243.2	585.8	6.7	58.8	257.8	71.5	30.1	157.2	2513.0
7	740.8	341.7	234.5	569.1	6.3	56.9	250.3	69.2	29.3	151.1	2449.2
8	735.2	338.3	231.9	563.2	6.3	56.3	246.2	68.0	28.4	147.6	2421.3
Mean	**753.1**	**351.4**	**243.1**	**586.7**	**6.6**	**59.2**	**258.6**	**71.7**	**30.2**	**157.6**	**2518.3**
SD	**15.6**	**11.1**	**9.1**	**18.3**	**0.3**	**2.3**	**9.1**	**2.6**	**1.4**	**7.2**	**76.5**
CV (%)	**2.1**	**3.2**	**3.7**	**3.1**	**4.1**	**3.9**	**3.5**	**3.7**	**4.6**	**4.6**	**3.0**

Table 9.6 Absolute concentration (µg/ml) of PC component fatty acids in the validation samples.

Plasma NEFA

	PEAK AREA COUNTS								
Sample	**PA**	**SA**	**OA**	**LA**	**ALNA**	***C21:0**	***C23:0**	**DHA**	**TOTAL (EXC *STD)**
1	39510.7	18617.9	72969.6	32685.8	6807.1	72616.5	109034.4	6340.1	176931.2
2	44183.5	20304.7	82272.1	37363.2	6947.5	84804.9	105341.8	6622.4	197693.4
3	43711.9	20346.8	79119.7	35975.4	7243.4	82157.2	107532.4	6509.7	192906.9
4	47967.1	21845.8	86424.9	37547.6	7898.9	87654.6	107893.2	6483.4	208167.7
5	39169.1	18166.6	77937.5	34670.5	6963.0	84999.6	97519.2	6056.3	182963.0
6	47266.5	22260.9	90688.6	39738.1	8311.7	100086.0	108590.5	7755.4	216021.2
7	46394.3	22273.2	87663.8	38202.4	7933.8	97545.6	107190.4	7850.1	210317.6

Table 9.7 Plasma NEFA validation sample chromatogram fatty acid and standard peak area counts. **21:0 = added internal standard; *23:0 = added recovery standard*

FATTY ACID RELATIVE PROPORTION (%)							
Sample	PA	SA	OA	LA	ALNA	DHA	STANDARD RECOVERY (%)
1	22.3	10.5	41.2	18.5	3.8	3.6	66.6
2	22.3	10.3	41.6	18.9	3.5	3.3	80.5
3	22.7	10.5	41.0	18.6	3.8	3.4	76.4
4	23.0	10.5	41.5	18.0	3.8	3.1	81.2
5	21.4	9.9	42.6	18.9	3.8	3.3	87.2
6	21.9	10.3	42.0	18.4	3.8	3.6	92.2
7	22.1	10.6	41.7	18.2	3.8	3.7	91.0
Mean	**22.2**	**10.4**	**41.7**	**18.5**	**3.8**	**3.4**	**82.2**
SD	**0.5**	**0.2**	**0.5**	**0.3**	**0.1**	**0.2**	**9.0**
CV (%)	**2.4**	**2.2**	**1.2**	**1.9**	**3.1**	**6.1**	**10.9**

Table 9.8 Relative proportion (%) of NEFA component fatty acids in the validation samples.

FATTY ACID CONCENTRATION (µg/ml)							
Sample	PA	SA	OA	LA	ALNA	DHA	TOTAL (EXC *STD)
1	16.3	7.7	30.1	13.5	2.8	2.6	73.1
2	15.6	7.2	29.1	13.2	2.5	2.3	69.9
3	16.0	7.4	28.9	13.1	2.6	2.4	70.4
4	16.4	7.5	29.6	12.9	2.7	2.2	71.2
5	13.8	6.4	27.5	12.2	2.5	2.1	64.6
6	14.2	6.7	27.2	11.9	2.5	2.3	64.8
7	14.3	6.9	27.0	11.7	2.4	2.4	64.7
Mean	**15.2**	**7.1**	**28.5**	**12.7**	**2.6**	**2.3**	**68.4**
SD	**1.1**	**0.5**	**1.3**	**0.7**	**0.1**	**0.2**	**3.6**
CV (%)	**7.3**	**6.6**	**4.4**	**5.4**	**5.7**	**6.5**	**5.3**

Table 9.9 Absolute concentration (µg/ml) of NEFA component fatty acids in the validation samples.

Plasma CE

	PEAK AREA COUNTS										
Sample	PA	SA	OA	LA	GLA	ALNA	DGLA	ARA	EPA	DHA	TOTAL (EXC *STD)
1	15307.0	1142.4	7850.9	21696.7	268.8	230.6	213.8	2545.6	245.5	311.3	49812.6
2	18361.3	1677.8	29934.9	82715.5	1061.8	812.9	1029.7	9537.6	817.9	546.3	146495.7
3	18533.6	1717.2	30215.7	83241.7	1118.6	817.7	1046.9	9646.2	848.1	532.8	147718.5
4	17732.9	1554.4	28195.2	80033.3	980.0	773.0	1009.8	9272.9	805.9	598.0	140955.4
5	18559.4	1728.6	30419.6	82782.5	970.5	787.8	1008.3	9607.7	810.1	570.8	147245.3
6	18362.5	1582.8	29559.0	81535.2	931.5	788.8	1001.9	9467.7	811.4	590.3	144631.1
7	17405.9	1582.2	28404.6	78200.5	933.4	775.4	970.0	9024.0	784.7	556.0	138636.7
8	18033.5	1760.3	29508.1	80931.6	959.1	772.2	1103.5	9349.9	790.9	556.4	143765.5
9	18228.4	1725.5	29739.5	81273.8	1080.7	788.9	1027.6	9346.2	799.4	580.5	144590.5

Table 9.10 Plasma CE validation sample chromatogram fatty acid and standard peak area counts.

	FATTY ACID RELATIVE PROPORTION (%)									
Sample	PA	SA	OA	LA	GLA	ALNA	DGLA	ARA	EPA	DHA
1	12.5	1.1	20.4	56.5	0.7	0.6	0.7	6.5	0.6	0.4
2	12.5	1.2	20.5	56.4	0.8	0.6	0.7	6.5	0.6	0.4
3	12.6	1.1	20.0	56.8	0.7	0.5	0.7	6.6	0.6	0.4
4	12.6	1.2	20.7	56.2	0.7	0.5	0.7	6.5	0.6	0.4
5	12.7	1.1	20.4	56.4	0.6	0.5	0.7	6.5	0.6	0.4
6	12.6	1.1	20.5	56.4	0.7	0.6	0.7	6.5	0.6	0.4
7	12.5	1.2	20.5	56.3	0.7	0.5	0.8	6.5	0.6	0.4
8	12.6	1.2	20.6	56.2	0.7	0.5	0.7	6.5	0.6	0.4
Mean	**12.6**	**1.2**	**20.4**	**56.4**	**0.7**	**0.5**	**0.7**	**6.5**	**0.6**	**0.4**
SD	**0.1**	**0.0**	**0.2**	**0.2**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
CV	**0.4**	**3.8**	**1.0**	**0.3**	**6.1**	**1.5**	**3.5**	**0.5**	**1.7**	**5.1**

Table 9.11 Relative proportion (%) of CE component fatty acids in the validation samples.

9.1.2 POST-PROCESSING ANALYSIS

	Peak Area Counts		
Integration Number	**PA**	**DPA**	**DHA**
1	644629.0	25494.8	75931.2
2	644630.0	25550.9	76145.9
3	644661.0	25616.8	76110.2
4	644618.0	25477.2	75909.5
5	644531.0	25491.0	75720.3
6	644606.0	25489.1	76217.4
Mean	**644612.5**	**25520.0**	**76005.8**
SD	**43.9**	**54.0**	**185.3**
CV (%)	**<0.01**	**0.21**	**0.24**

Table 9.12 Repeated integration of 3 fatty acid peaks on a single chromatogram.

9.2 CALCULATION OF PLASMA INTERNAL STANDARD RECOVERIES

Internal standard recovery from the TAG and NEFA fractions was calculated as follows:

$$\frac{\text{Area of Internal Standard Peak}}{\text{Area of C23:0 Recovery Standard Peak}}$$

Multiplied by 100, giving a percentage.

Internal standard recovery from the PC fraction was calculated using a correction factor which accounts for the significant proportion of the internal standard molecule that is not fatty acid.

1 mole of PC 15:0 yields 2 moles of 15:0 FAME derivative. The molecular weights of PC 15:0 and 15:0 ME are 706.0 and 256.4, respectively, thus one mole of PC 15:0 and 15:0 ME have masses of 706.0 g and 256.4 g, respectively.

1 mole of PC 15:0 (706.0 g) yields 2 moles of 15:0 ME (512.8 g), thus:

Correction factor for PC 15:0 = (512.8/706.0)
= 0.73

The recovery of PC 15:0 internal standard was calculated from GC-FID chromatograms as follows:

$$\frac{(\text{Area of 15:0 Internal Standard Peak} / \text{Area of 23:0 Recovery Standard Peak})}{\text{Appropriate Correction Factor}}$$

Multiplied by 100, giving a percentage.

9.3 CALCULATION OF TOTAL PLASMA FATTY ACID CONCENTRATION

Subsequent to post-processing analysis, fatty acid and internal standard peak areas were used to calculate the concentration of fatty acid in the plasma PC fraction:

$$\frac{\text{Sum Peak Areas of Interest (Exc. All Standards Peaks)}}{\text{Area Internal Standard Peak}}$$

Multiplied by: mass of Internal Standard added to fraction (μg)

Equals: μg lipid per volume of extracted plasma (i.e. 1ml)

9.4 MATERIALS USED FOR PROCESSING OF HUMAN PLASMA LIPIDS FOR GAS CHROMATOGRAPHY - FLAME IONISATION DETECTOR ANALYSIS OF PHOSPATIDYLCHOLINE, TRIACYLGLYCEROL, NON-ESTERIFIED FATTY ACIDS AND CHOLESTEROL ESTERS.

MATERIALS	SUPPLIER
Blood Collection	
o 10ml Lithium heparin vacutainer	BD Vacutainer Systems, Plymouth, Devon
Solvents	
o Hexane o Trifluoroethanol o Chloroform o Methanol o Ethyl acetate	Fisher Scientific, Loughborough, Leicestershire

Lipid Standards	
○ Di-pentadecanoyl phosphatidylcholine (PC 15:0/15:0) ○ Heneicosanoic acid (C21:0) ○ Tri-heptadecanoin (1, 2, 3-triheptadecanoylglycerol) ○ Tricosanoic acid methyl ester (C23:0) ○ Fatty acid methyl esters	Sigma-Aldrich, Poole, Dorset

General Chemicals & Consumables	
○ Butyrated hydroxytoluene ○ Sodium chloride (NaCl) ○ Potassium carbonate (K_2CO_3) ○ Potassium bicarbonate ($KHCO_3$)	Sigma-Aldrich, Poole, Dorset
○ Sulphuric acid (H_2SO_4) ○ Acetic acid (glacial) (CH_3COOH)	Fisher Scientific, Loughborough, Leicestershire
○ Aminopropylsilica Bond Elut columns (100mg packed silica per 1.0ml cartridge)	Varian, Walton-on-Thames, Surrey

GC Equipment	
○ BPX70 fused silica column (50m x 320µm x 0.25µm)	SGE Europe, Milton Keynes, UK
○ Hewlett Packard 6890 autosampler ○ Hewlett Packard ChemStation software ○ Hewlett Packard 5890 Gas Chromatograph	Hewlett Packard, Wokingham, Berks, UK

9.5 OTHER MATERIALS

MATERIALS	SUPPLIER
Physical Assessment	
o Stadiometer	CMS Weighing Equipment Ltd
o Seca Alpha Digital Weighing Scales (model 770)	CMS Weighing Equipment Ltd
Data Analysis & Presentation	
o Microsoft Access, 2000 Professional Edition	Microsoft Corporation, USA
o SPSS for Windows, Version 14.0	SPSS Inc., Chicago, USA
o Microsoft Excel, XP 2002 Edition)	Microsoft Corporation, USA
o GraphPad Prism, Version 3	GraphPad Software Inc., USA

9.6 BLOOD LIPID CONCENTRATIONS IN THE COLECTOMY GROUP AND AN AGE MATCHED REFERENCE GROUP

Blood Lipid Measured	Age Group	Males		Females	
		HSFE	Colectomy	HSFE	Colectomy
Cholesterol (mmol/L)	25-34	-	NA	5.0±0.04	4.8±0.07
	35-44	5.8±0.04	5.2±1.29	5.4±0.03	4.9±0.00
	45-54	5.9±0.04	6.1±0.40	5.8±0.04	5.7±0.68
	55-64	5.8±0.04	5.0±0.47	6.3±0.04	6.9±0.33
	65-74	5.5±0.06	5.8±.57	6.2±0.05	5.5±1.45
HDL (mmol/L)	25-34	-	NA	1.6±0.02	1.6±0.29
	35-44	1.4±0.01	1.7±0.30	1.6±0.01	2.3±0.00
	45-54	1.4±0.01	1.7±0.37	1.7±0.01	2.2±0.34
	55-64	1.4±0.01	1.5±0.24	1.7±0.02	2.4±0.37
	65-74	1.4±0.02	1.4±0.22	1.7±0.02	2.6±0.98
LDL (mmol/L)	25-34		NA		-
	35-44	3.5±0.09	2.6±1.00	3.2±0.08	2.3±0.00
	45-54	3.7±0.10	4.2±0.63	3.5±0.11	3.3±0.57
	55-64	3.6±0.14	2.6±0.52	3.8±0.09	3.6±0.26
	65-74	3.7±0.09	3.6±0.51	3.9±0.14	2.4±0.38
TAG (mmol/L)	25-34		NA		-
	35-44	1.7±0.14	2.0±0.87	1.2±0.07	0.7±0.00
	45-54	1.8±0.16	3.4±1.47	1.3±0.07	1.4±0.69
	55-64	2.1±0.23	2.6±1.07	1.5±0.08	2.0±0.39
	65-74	1.7±0.10	1.5±0.29	1.6±0.10	1.0±0.20

Table 9.13 Concentration of cholesterol, HDL, LDL and TAG in blood in the colectomy patients and a HSFE reference population (Blake et al., 2004). Values are mean±SEM. Grey boxes indicate that no HSFE reference data are available. NA indicates no colectomy patients applicable to that age group.

9.7 FOOD FREQUENCY QUESTIONNAIRE

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Faculty of Medicine, Health and Biological Sciences

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STUDY TITLE: The Nutritional and Metabolic Problems of Patients with Ileostomies
(Ethics Submission No. 086/00)

Nutritional Intake Survey

Professor Dame Barbara Clayton
CBE

University of Southampton

FOOD INTAKE: EXAMPLE

Listed below are food items divided into sections according to food type. Please put a ✓ in the box to indicate how often, on average, you have eaten the specified amount of each food during the last 12 months.

Example: White bread, so if you eat 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
BREAD/SAVOURY BISCUITS	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
White bread & rolls	1	2	3	4	5	6	7	8	✓ 9	10

Example: For seasonal fruit such as strawberries, if you eat strawberries about once a week when in season you should put a tick in the column headed "once a week".

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
SEASONAL FRUIT	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Strawberries	1	2	3	✓ 4	5	6	7	8	9	10

If you make a mistake and put a tick in the wrong box just cross through the tick as shown below, and put another tick in the correct box.

Example: If you eat apples twice a week, but ticked "2-3 times per day" box instead, just cross this through as shown, and tick in the "2-4 per week" box instead.

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
FRUIT	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Apples	1	2	3	4	✓ 5	6	7	✗ 8	9	10

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
BREAD/SAVOURY BISCUITS (One slice or biscuit)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
White bread and rolls	1	2	3	4	5	6	7	8	9	10
Brown bread and rolls	1	2	3	4	5	6	7	8	9	10
Wholemeal bread and rolls	1	2	3	4	5	6	7	8	9	10
Chapatis, Naan, Paratha	1	2	3	4	5	6	7	8	9	10
Papadums	1	2	3	4	5	6	7	8	9	10
Pitta bread	1	2	3	4	5	6	7	8	9	10
Crispbread e.g. Ryvita	1	2	3	4	5	6	7	8	9	10
Cream crackers, cheese biscuits	1	2	3	4	5	6	7	8	9	10

BREAKFAST CEREALS (Medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Porridge, Readybrek	1	2	3	4	5	6	7	8	9	10
Sugar coated cereals e.g. Sugar Puffs	1	2	3	4	5	6	7	8	9	10
Non-sugar coated cereal e.g. Cornflakes, Rice Krispies	1	2	3	4	5	6	7	8	9	10
Muesli	1	2	3	4	5	6	7	8	9	10
All Bran, Bran flakes	1	2	3	4	5	6	7	8	9	10
Weetabix, Shredded Wheat	1	2	3	4	5	6	7	8	9	10
PASTA & RICE (Medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
White/ Green/ Red Pasta e.g. Spaghetti, Tagliatelle	1	2	3	4	5	6	7	8	9	10
Wholemeal Pasta e.g. Wholemeal Spaghetti	1	2	3	4	5	6	7	8	9	10
Macaroni cheese	1	2	3	4	5	6	7	8	9	10
White rice, wild rice	1	2	3	4	5	6	7	8	9	10
Brown rice	1	2	3	4	5	6	7	8	9	10
Bulgar Wheat	1	2	3	4	5	6	7	8	9	10
Couscous	1	2	3	4	5	6	7	8	9	10
Wheat Germ (tablespoon)	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
POTATOES (Medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Chips	1	2	3	4	5	6	7	8	9	10
Jacket Potato	1	2	3	4	5	6	7	8	9	10
Potatoes e.g. boiled, mashed	1	2	3	4	5	6	7	8	9	10
Roast Potatoes	1	2	3	4	5	6	7	8	9	10
Sweet Potato	1	2	3	4	5	6	7	8	9	10
Potato Salad	1	2	3	4	5	6	7	8	9	10

PULSES (include when used in recipes)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Baked Beans	1	2	3	4	5	6	7	8	9	10
Black-eyed beans	1	2	3	4	5	6	7	8	9	10
Butter beans/ broad beans	1	2	3	4	5	6	7	8	9	10
Chick Peas, Chanas	1	2	3	4	5	6	7	8	9	10
Hummus	1	2	3	4	5	6	7	8	9	10
Lentils, Dals	1	2	3	4	5	6	7	8	9	10
Mung beans & Red kidney beans	1	2	3	4	5	6	7	8	9	10

VEGETABLE DISHES	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Quorn	1	2	3	4	5	6	7	8	9	10
Soya - mince, sausages, burgers	1	2	3	4	5	6	7	8	9	10
Tofu (bean curd)	1	2	3	4	5	6	7	8	9	10
Nut Roast, Nut Cutlets,	1	2	3	4	5	6	7	8	9	10
Vegeburgers	1	2	3	4	5	6	7	8	9	10
Vegetable Chilli/Curry	1	2	3	4	5	6	7	8	9	10
Mixed Bean Casserole/Ratatouille	1	2	3	4	5	6	7	8	9	10
Vegetable - Lasagne/ Moussaka / filled pasta with sauce	1	2	3	4	5	6	7	8	9	10
Vegetable pizza	1	2	3	4	5	6	7	8	9	10
Vegetable pies/ samosas	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
VEGETABLES (Medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Aubergine, Okra	1	2	3	4	5	6	7	8	9	10
Beansprouts	1	2	3	4	5	6	7	8	9	10
Beetroot	1	2	3	4	5	6	7	8	9	10
Broccoli	1	2	3	4	5	6	7	8	9	10
Brussel Sprouts	1	2	3	4	5	6	7	8	9	10
Cabbage	1	2	3	4	5	6	7	8	9	10
Spring Greens, Kale	1	2	3	4	5	6	7	8	9	10
Spinach	1	2	3	4	5	6	7	8	9	10
Carrots	1	2	3	4	5	6	7	8	9	10
Cauliflower	1	2	3	4	5	6	7	8	9	10
Celery	1	2	3	4	5	6	7	8	9	10
Coleslaw	1	2	3	4	5	6	7	8	9	10
Courgettes, Marrow, Squash	1	2	3	4	5	6	7	8	9	10
Cucumber	1	2	3	4	5	6	7	8	9	10
Endives	1	2	3	4	5	6	7	8	9	10
Garlic	1	2	3	4	5	6	7	8	9	10
Green Beans, Runner Beans	1	2	3	4	5	6	7	8	9	10
Leeks	1	2	3	4	5	6	7	8	9	10
Lettuce	1	2	3	4	5	6	7	8	9	10
Mushrooms	1	2	3	4	5	6	7	8	9	10
Olives	1	2	3	4	5	6	7	8	9	10
Onions	1	2	3	4	5	6	7	8	9	10
Parsley - Flat leaf	1	2	3	4	5	6	7	8	9	10
Parsnips & Turnips	1	2	3	4	5	6	7	8	9	10
Peas, Mushy, Mange-tout	1	2	3	4	5	6	7	8	9	10
Peppers - Red, Green, Yellow	1	2	3	4	5	6	7	8	9	10
Swedes	1	2	3	4	5	6	7	8	9	10
Sweetcorn	1	2	3	4	5	6	7	8	9	10
Tomatoes - raw/canned/sauce	1	2	3	4	5	6	7	8	9	10
Watercress, Mustard & Cress	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
FRESH/FROZEN FRUIT (1 fruit or medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
For seasonal fruits please tick how often you have eaten these fruits when they are in season										
Apples	1	2	3	4	5	6	7	8	9	10
Apricots	1	2	3	4	5	6	7	8	9	10
Avocado	1	2	3	4	5	6	7	8	9	10
Bananas	1	2	3	4	5	6	7	8	9	10
Blackberries, Gooseberries	1	2	3	4	5	6	7	8	9	10
Blackcurrants	1	2	3	4	5	6	7	8	9	10
Cherries	1	2	3	4	5	6	7	8	9	10
Grapes	1	2	3	4	5	6	7	8	9	10
Guavas	1	2	3	4	5	6	7	8	9	10
Kiwi Fruit	1	2	3	4	5	6	7	8	9	10
Mangoes	1	2	3	4	5	6	7	8	9	10
Melon	1	2	3	4	5	6	7	8	9	10
Nectarines	1	2	3	4	5	6	7	8	9	10
Oranges, Satsumas, Grapefruit	1	2	3	4	5	6	7	8	9	10
Papaya/Paw Paw	1	2	3	4	5	6	7	8	9	10
Peaches	1	2	3	4	5	6	7	8	9	10
Pears	1	2	3	4	5	6	7	8	9	10
Pineapple	1	2	3	4	5	6	7	8	9	10
Plums	1	2	3	4	5	6	7	8	9	10
Raspberries	1	2	3	4	5	6	7	8	9	10
Redcurrants	1	2	3	4	5	6	7	8	9	10
Rhubarb	1	2	3	4	5	6	7	8	9	10
Strawberries	1	2	3	4	5	6	7	8	9	10

TINNED FRUIT	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
All varieties e.g. Peaches, Pears, Pineapple	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
DRIED FRUIT	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Apricots	1	2	3	4	5	6	7	8	9	10
Dates	1	2	3	4	5	6	7	8	9	10
Figs	1	2	3	4	5	6	7	8	9	10
Prunes	1	2	3	4	5	6	7	8	9	10
Currants, Raisins, Sultanas	1	2	3	4	5	6	7	8	9	10
Other dried fruits e.g. Apples, Pears, Mangoes	1	2	3	4	5	6	7	8	9	10

MEAT (Medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Beef e.g. Roast, Steak	1	2	3	4	5	6	7	8	9	10
Beef Stew/ Casserole/ Mince/ Curry	1	2	3	4	5	6	7	8	9	10
Beefburger/Hamburger	1	2	3	4	5	6	7	8	9	10
Pork e.g. Roast, Chops	1	2	3	4	5	6	7	8	9	10
Pork Stew/Casserole	1	2	3	4	5	6	7	8	9	10
Lamb e.g. Roast, Chops	1	2	3	4	5	6	7	8	9	10
Lamb Stew/Casserole	1	2	3	4	5	6	7	8	9	10
Chicken/Turkey Roast, Slices	1	2	3	4	5	6	7	8	9	10
Breadcrumbed e.g. Chicken Nuggets/Kievs	1	2	3	4	5	6	7	8	9	10
Chicken/Turkey in a creamy sauce, Curry	1	2	3	4	5	6	7	8	9	10
Bacon	1	2	3	4	5	6	7	8	9	10
Ham	1	2	3	4	5	6	7	8	9	10
Corned Beef, Spam, Luncheon Meats	1	2	3	4	5	6	7	8	9	10
Sausages e.g. Beef, Pork	1	2	3	4	5	6	7	8	9	10
Pies/Pasties/Sausage Rolls/ Meat Samosas	1	2	3	4	5	6	7	8	9	10
Offal e.g. Liver, Kidney	1	2	3	4	5	6	7	8	9	10
Liver Pâté/Sausage, Salami	1	2	3	4	5	6	7	8	9	10
Meat - Lasagne/Moussaka/ Ravioli/filled pasta with sauce	1	2	3	4	5	6	7	8	9	10
Meat Pizza	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
FISH (Medium serving)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Fish Fingers/Cakes	1	2	3	4	5	6	7	8	9	10
Fried Fish in batter/breadcrumbs	1	2	3	4	5	6	7	8	9	10
White Fish not fried e.g. Cod, Haddock, Plaice, Sole	1	2	3	4	5	6	7	8	9	10
Fresh Oily Fish e.g. Kippers, Herring, Mackerel, Salmon, Trout, Tuna	1	2	3	4	5	6	7	8	9	10
Tinned Oily Fish e.g. Tuna, Pilchard, Sardine, Salmon	1	2	3	4	5	6	7	8	9	10
Shellfish e.g. Crab, Prawns, Mussels	1	2	3	4	5	6	7	8	9	10
Fish Roe, Taramasalata	1	2	3	4	5	6	7	8	9	10
Fish Pie, Fish Pasta/Lasagne	1	2	3	4	5	6	7	8	9	10

EGGS/EGG DISHES	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Boiled/ Poached Egg (1 egg)	1	2	3	4	5	6	7	8	9	10
Omelette/ Scrambled Egg (2)	1	2	3	4	5	6	7	8	9	10
Fried Egg (1 egg)	1	2	3	4	5	6	7	8	9	10
Quiche	1	2	3	4	5	6	7	8	9	10

MARGARINES/BUTTERS (enough for 1 slice of bread)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Butter	1	2	3	4	5	6	7	8	9	10
Block Margarine e.g. Stork, Krona, NOT in tub	1	2	3	4	5	6	7	8	9	10
Polyunsaturated Margarine e.g. Flora, Sunflower in tub	1	2	3	4	5	6	7	8	9	10
Other Soft Margarine, Dairy Spreads e.g. Blue Band, Clover, in tub	1	2	3	4	5	6	7	8	9	10
Low fat spread e.g. Outline, Gold, Flora Lite in tub	1	2	3	4	5	6	7	8	9	10
Very low fat spread, e.g. St Ivel Lowest fat Spread	1	2	3	4	5	6	7	8	9	10
Monounsaturated Margarine e.g. Mono, Olivio	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
DAIRY & NON-DAIRY PRODUCTS	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Thick & Creamy Yoghurt	1	2	3	4	5	6	7	8	9	10
Low fat Yoghurt	1	2	3	4	5	6	7	8	9	10
Diet Yoghurt	1	2	3	4	5	6	7	8	9	10
Greek Yoghurt	1	2	3	4	5	6	7	8	9	10
Fromage Frais/ Creme Fraiche	1	2	3	4	5	6	7	8	9	10
Dairy Desserts	1	2	3	4	5	6	7	8	9	10
Single/Sour Cream	1	2	3	4	5	6	7	8	9	10
Double/Clotted Cream	1	2	3	4	5	6	7	8	9	10
Ice Cream	1	2	3	4	5	6	7	8	9	10
Milk Puddings	1	2	3	4	5	6	7	8	9	10
Cheddar type, low fat cheese	1	2	3	4	5	6	7	8	9	10
Cheese e.g. Cheddar, Brie, Edam	1	2	3	4	5	6	7	8	9	10
Cottage Cheese	1	2	3	4	5	6	7	8	9	10
Cheese & Onion Pastie	1	2	3	4	5	6	7	8	9	10
Soya Cheese	1	2	3	4	5	6	7	8	9	10
Soya Yoghurt	1	2	3	4	5	6	7	8	9	10

NUTS & SAVOURY SNACKS (small handful unless indicated otherwise)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Crisps (1 bag)	1	2	3	4	5	6	7	8	9	10
Other Fried snacks e.g. Wotsits (1 bag)	1	2	3	4	5	6	7	8	9	10
Bombay Mix	1	2	3	4	5	6	7	8	9	10
Almonds	1	2	3	4	5	6	7	8	9	10
Brazil Nuts	1	2	3	4	5	6	7	8	9	10
Cashew Nuts	1	2	3	4	5	6	7	8	9	10
Peanuts	1	2	3	4	5	6	7	8	9	10
Pistachio Nuts	1	2	3	4	5	6	7	8	9	10
Walnuts, Pecan Nuts	1	2	3	4	5	6	7	8	9	10
Mixed nuts and raisins	1	2	3	4	5	6	7	8	9	10
Sunflower / Sesame Seeds	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
SWEET SNACKS (1 bar)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Cereal Bars/Flapjacks	1	2	3	4	5	6	7	8	9	10
Chocolate Snack Bars e.g. Mars, KitKat, Snickers	1	2	3	4	5	6	7	8	9	10
Milk Chocolate e.g. Galaxy, Cadbury's Dairy Milk, Flake	1	2	3	4	5	6	7	8	9	10
Plain Chocolate e.g. Cadbury's Bourneville	1	2	3	4	5	6	7	8	9	10
High Cocoa Content Chocolate (70% cocoa solids) e.g. Lindt Excellence	1	2	3	4	5	6	7	8	9	10
Boiled Sweets, Toffees, Mints	1	2	3	4	5	6	7	8	9	10

BISCUITS AND PUDDINGS	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Plain Biscuits e.g. Marie, Nice, Digestive (one)	1	2	3	4	5	6	7	8	9	10
Chocolate Biscuits (one)	1	2	3	4	5	6	7	8	9	10
Sandwich/Cream Biscuits (1)	1	2	3	4	5	6	7	8	9	10
Fruit Cake (1 slice)	1	2	3	4	5	6	7	8	9	10
Sponge Cakes (1 slice)	1	2	3	4	5	6	7	8	9	10
Buns/Pastries e.g. Croissants, Doughnuts, Tray Bakes (one)	1	2	3	4	5	6	7	8	9	10
Scones/Pancakes/Muffins/ Crumpets (1)	1	2	3	4	5	6	7	8	9	10
Fruit Pies, Tarts, Crumbles	1	2	3	4	5	6	7	8	9	10
Sponge Puddings	1	2	3	4	5	6	7	8	9	10

SAUCES & SOUPS	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Mayonnaise, Salad Cream	1	2	3	4	5	6	7	8	9	10
French Type Dressing	1	2	3	4	5	6	7	8	9	10
Sauces e.g. white/cheese/'Cook In'/curry	1	2	3	4	5	6	7	8	9	10
Tomato Ketchup	1	2	3	4	5	6	7	8	9	10
Pickles/Chutney/Pesto Sauce	1	2	3	4	5	6	7	8	9	10
Soups - Meat / Vegetable	1	2	3	4	5	6	7	8	9	10
Low Calorie Soups	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

PLEASE TICK ONE NUMBER ON EVERY LINE E.G. 1 2✓ 3

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
SPREADS (enough for 1 slice of bread)	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Chocolate Spread	1	2	3	4	5	6	7	8	9	10
Jam/ Marmalade/ Honey	1	2	3	4	5	6	7	8	9	10
Marmite/Bovril	1	2	3	4	5	6	7	8	9	10
Peanut Butter	1	2	3	4	5	6	7	8	9	10
Vegetable/ Nut Pâté	1	2	3	4	5	6	7	8	9	10

BEVERAGES	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Tea	1	2	3	4	5	6	7	8	9	10
Herbal Tea	1	2	3	4	5	6	7	8	9	10
Coffee - instant, ground	1	2	3	4	5	6	7	8	9	10
Coffee - decaffeinated	1	2	3	4	5	6	7	8	9	10
Coffee substitute e.g. Caro	1	2	3	4	5	6	7	8	9	10
Cocoa, Hot Chocolate	1	2	3	4	5	6	7	8	9	10
Horlicks, Ovaltine	1	2	3	4	5	6	7	8	9	10
Low Calorie/Low fat Horlicks, Ovaltine, Chocolate	1	2	3	4	5	6	7	8	9	10
Orange Juice (Pure fruit)	1	2	3	4	5	6	7	8	9	10
Tomato Juice	1	2	3	4	5	6	7	8	9	10
Other Pure Fruit Juices	1	2	3	4	5	6	7	8	9	10
Fruit Squash/Cordial	1	2	3	4	5	6	7	8	9	10
Fizzy soft drinks e.g. Coke	1	2	3	4	5	6	7	8	9	10
Low Calorie/Diet Soft Drinks	1	2	3	4	5	6	7	8	9	10

ALCOHOLIC BEVERAGES	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
Beer, Lager, Cider (½ pint)	1	2	3	4	5	6	7	8	9	10
Port, Sherry, Vermouth, Martini, Liqueurs (1 glass)	1	2	3	4	5	6	7	8	9	10
Spirits e.g. Whisky, Gin, Vodka, Brandy (Single measure)	1	2	3	4	5	6	7	8	9	10
White Wine (1 glass)	1	2	3	4	5	6	7	8	9	10
Red Wine not Chianti (1 glass)	1	2	3	4	5	6	7	8	9	10
Chianti Wine (1 glass)	1	2	3	4	5	6	7	8	9	10

PLEASE CHECK THAT YOU HAVE A TICK ON EVERY LINE

FOOD FREQUENCY QUESTIONAIRE

MILK

a. How much milk do you drink each day, including milk with tea or coffee, milky drinks, cereal etc?

None	1	¾ Pint	4
¼ Pint	2	1 Pint	5
½ Pint	3	More Than 1 Pint	6

b. What type of milk do you use **most often**?

Full Cream	1	Sterilised Milk	5
Semi-skimmed	2	Dried Milk	6
Skimmed	3	Soya	7
Channel Islands	4	None	8

c. If you use soya milk please describe the brand and type. _____

SUGAR

a. Do you **usually** take sugar on your breakfast cereal? Yes 1 No 2

If YES: How many teaspoons? teaspoons

b. Do you **usually** take sugar or honey in tea, herbal tea, coffee or coffee substitute? Yes 1 No 2

If YES: Please write down the number of teaspoon per cup?

Sugar/honey in tea	teaspoons
Sugar/honey in herbal tea	teaspoons
Sugar/honey in coffee	teaspoons
Sugar/honey in coffee substitute	teaspoons

c. Do you use sweeteners instead of sugar or honey? Yes 1 No 2

Which brand of sweetener do you use? Please specify. _____

If YES: How many tablets per day, or teaspoons of powder per day?

USE OF FATS

a. Do you spread butter or margarine on your bread?

Always 1 Never 2 Sometimes 3

b. How many slices of bread/rolls/crackers do you have **with spread** each day?

PLEASE CHECK THAT YOU HAVE ANSWERED EVERY QUESTION

c. How much spread do you use?

Just a scrape/thinly spread	1
Medium	2
Thickly spread	3

d. What kind of fat do you use most often for frying/ roasting/ grilling etc?

Tick more than once if necessary

Butter	1
Lard/ dripping	2
Vegetable oil	3
Solid white vegetable fat	4
Margarine	5
None	6

If you use **vegetable oil** please give the type you use most often
Eg. Sunflower, olive, corn _____

If you use margarine, please give the brand name of the one you use most often
Eg. Stork, Flora _____

d. What kind of fat do you use most often for baking cakes etc?

Tick more than one if necessary.

Butter	1
Lard/ dripping	2
Vegetable oil	3
Solid white vegetable fat	4
Margarine	5
None	6
Do not bake	7

USE OF SALT

a. How often do you add salt to food while cooking?

Always	1	Usually	2	Sometimes	3
Rarely	4	Never	5		

b. How often do you add salt to food at the table?

Always	1	Usually	2	Sometimes	3
Rarely	4	Never	5		

c. Do you use a salt substitute eg. Losalt Yes 1 No 2

If YES: Which brand? _____

PLEASE CHECK THAT YOU HAVE ANSWERED EVERY QUESTION

Chapter 10

References

References

(2001). National Food Survey 2000: annual report on food expenditure, consumption and nutrient intakes.

Ainley, C.C., Keeling, P.W.N., and Thompson, R.P.H. (1983). Tissue zinc depletion in Crohn's disease. *Gut.* **24**;A463-

Arab, L. (2003). Biomarkers of fat and fatty acid intake. *Journal of Nutrition.* **133**;925S-932S.

Aslan, A. and Triadafilopoulos, G. (1992). Fish oil fatty acid supplementation in active ulcerative colitis: a double blind, placebo-controlled, cross study. *American Journal of Gastroenterology.* **87**;432-437.

Assmann, G., Schulte, H., von Eckardstein, A., and Huang, Y. (1996). High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis.* **124 (Suppl)**;S11-S20.

Baylin, A., Kabagambe, E.K., Siles, X., and Campos, H. (2002). Adipose tissue biomarkers of fatty acid intake. *American Journal of Clinical Nutrition.* **76**;750-757.

Belluzzi, A., Brignola, C., Campieri, M., Pera, A., Boschi, S., and Miglioli, M. (1996). Effect of an enteric-coated fish oil preparation on relapses in Crohn's disease. *The New England Journal of Medicine.* **334**;1557-1560.

Billiar, T., Bankey, P., Svingen, B., Curran, R.D., West, M.A., Holman, R.T., Simmons, R.L., and Cerra, F.B. (1988). Fatty acid uptake and Kupffer cell function: Fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery.* **104**;343-349.

Bingham, S.A., Cummings, J.H., and McNeil, N.I. (1982). Diet and health of people with an ileostomy. 1. Dietary assessment. *British Journal of Nutrition.* **47**;399-406.

Bjerve, K.S., Brubakk, A.M., Fougner, K.J., Johnsen, H., and Midthjell, K. (1993). Omega-3 fatty acids: essential fatty acids with important biological effects, and serum phospholipid fatty acids as markers of dietary ω3-fatty acid intake. *American Journal of Clinical Nutrition.* **57**;801S-806S.

Blake, M., Chaudhury, M., Deverill, C., Doyle, M., Erens, B., Falaschetti, E., Hirani, V., Moody, A., Prescott, A., Primatesta, P., Shelton, N., Stamatakis, E., and Wardle, H. (2004). Health Survey for England 2003: Risk Factors for Cardiovascular Disease. **Volume 2**;1-337.

Box, G.E.P. (1953). Non-normality and tests on variances. *Biometrika.* **40**;318-335.

Brenner, R.R. (1981). Nutritional and hormonal factors influencing desaturation of essential fatty acids. *Journal of Lipid Research.* **20**;41-47.

Burdge, G.C. (1998). The role of docosahexaenoic acid in brain development and fetal alcohol syndrome. *Biochemical Society Transactions.* **26**;246-252.

Burdge, G.C., Jones, A.E., Wright, P., Ware, L., and Wootton, S.A. (2001). α-linolenic acid metabolism in adult men: evidence for synthesis of eicosapentaenoic and docosapentaenoic acids, but not docosahexaenoic acid. *Proceedings of the Nutrition Society.* **60**;22A-

Burdge, G.C., Wright, P., Jones, A.E., and Wootton, S.A. (2000a). A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid phase extraction. *British Journal of Nutrition.* **84**;781-787.

Burdge, G.C., Wright, P., Jones, A.E., and Wootton, S.A. (2000b). A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid phase extraction. *British Journal of Nutrition.* **84**;781-787.

Burdge, G.C., Finnegan, Y.E., Minihane, A.M., Williams, C.M., and Wootton, S.A. (2003). Effect of altered dietary *n*-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [^{13}C]α-linolenic acid to longer-chain fatty acids and partitioning towards β-oxidation in older men. *British Journal of Nutrition.* **90**;311-321.

Burley, V., Cade, J., Margetts, B., Thompson, R., and Warm, D. (2000). Consensus document on the development, validation and utilisation of food frequency questionnaires.1-62.

Cabré, E., Periago, J.L., Mingorance, M.D., Fernández-Bañares, F., Abad, A., Esteve, M., Gil, A., Lachica, M., González-Huix, F., and Gassull, M.A. (1992). Factors relating to the plasma fatty acid profile in healthy subjects, with special reference to antioxidant micronutrient status: a multivariate analysis. *American Journal of Clinical Nutrition.* **55**;831-837.

Caesar, P.A., Wilson, S.J., Normand, C.S., and Postle, A.D. (1988). A comparison of the specificity of phosphatidylcholine synthesis by human fetal lung maintained in either organ or organotypic culture. *Biochemical Journal.* **253**;451-457.

Calder, P.C. (1996b). Bioactive lipids in foods. *Biochemical Society Transactions.* **24**;814-824.

Calder, P.C. (1997). n-3 polyunsaturated fatty acids and cytokine production in health and disease. *Annals of Nutrition and Metabolism.* **41**;203-234.

Calder, P.C. (2001). N-3 polyunsaturated fatty acids, inflammation and immunity: pouring oil on troubled waters or another fishy tale? *Nutrition Research.* **21**;309-341.

Calder, P.C. (1996a). Bioactive lipids in foods. *Biochemical Society Transactions.* **24**;814-824.

Calder, P.C. and Field, C.J. (2002) Fatty acids, inflammation and immunity. In *Nutrition and Immune Function*, eds. Calder, Field, & Gill, pp. 57-92. CABI Publishing, Wallingford, UK.

Cantwell, M.M., Gibney, M.J., Cronin, D., Younger, K.M., O'Neill, J.P., Hogan, L., and Flynn, M.A. (2005). Development and validation of a food-frequency questionnaire for the determination of detailed fatty acid intakes. *Public Health Nutrition.* **8**;97-107.

Carey, M.C., Small, D.M., and Bliss, C.M. (1983). Lipid digestion and absorption. *Annual Review of Physiology.* **45**;651-677.

Carlier, H., Bernard, A., and Caselli, C. (1991). Digestion and absorption of polyunsaturated fatty acids. *Reproduction, Nutrition and Development.* **31**;475-500.

Caspary, W.F. (1992). Physiology and pathophysiology of intestinal absorption. *American Journal of Clinical Nutrition.* **55**;299S-308S.

Caughey, G.E., Mantzioris, E., Gibson, R.A., Cleland, L.G., and James, M.J. (1996). The effect on human tumor necrosis factor α and interleukin 1β production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *American Journal of Clinical Nutrition.* **63**;116-22.

Cawood, A.L., Carroll, M.P., Wootton, S.A., and Calder, P.C. (2005). Is there a case for n-3 fatty acid supplementation in cystic fibrosis? *Current Opinion in Clinical Nutrition and Metabolic Care.* **8**;153-159.

Christl, S.U. and Scheppach, W. (1997). Metabolic consequences of total colectomy. *Scandinavian Journal of Gastroenterology.* **32**;20-24.

Cooper, R.A. (1970). Lipids of human red cell membrane: normal composition and variability in disease. *Seminars in Hematology.* **7**;296-322.

Cullis, P.R., Fenske, D.B., and Hope, M.J. (1996) Physical properties and functional roles of lipids in membranes. In *Biochemistry of lipids, lipoproteins and membranes*, eds. Vance & Vance, pp. 1-33. Elsevier Science, Vancouver.

Cunnane, S.C., Ainley, C.C., Keeling, P.W.N., Thompson, R.P.H., and Crawford, M.A. (1986). Diminished phospholipid incorporation of essential fatty acids in peripheral blood leucocytes from patients with Crohn's disease: Correlation with zinc depletion. *Journal of the American College of Nutrition.* **5**;451-458.

Department of Health (1991). Dietary Reference Values for Food, Energy and Nutrients for the United Kingdom.

Department of Health (1994b). Nutritional Aspects of Cardiovascular Disease. Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy.

Department of Health (1994a). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom: report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy.

Devereux, G. (2002) The Immune System: An Overview. In *Nutrition and Immune Function*, eds. Calder, Field, & Gill, pp. 1-20. CABI Publishing, Wallingford, UK.

Ekbom, A. (1999) Epidemiology of inflammatory bowel disease. In *Inflammatory Bowel Diseases*, eds. Bistrian & Walker-Smith, pp. 7-21. Nestec Ltd, Basel.

Elson, C.O. (1988) The Immunology of Inflammatory Bowel Disease. In *Inflammatory Bowel Disease*, eds. Kirsner & Shorter, pp. 97-164. Lea & Febiger, Philadelphia.

Emken, E.A., Adolf, R.O., Duval, S.M., and Nelson, G.J. (2002). Effect of dietary docosahexaenoic acid on desaturation and uptake in vivo of isotope-labeled oleic, linoleic, and linolenic acids by male subjects. *Lipids.* **34**;785-791.

Endres, S., Ghorbani, R., Kelley, V.E., Georgilis, K., Lonnemann, G., van der Meer, J.M.W., Cannon, J.G., Rogers, T.S., Klempner, M.S., Weber, P.C., Schaeffer, E.J., Wolff, S.M., and Dinarello, C.A. (1989). The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *The New England Journal of Medicine.* **320**;265-271.

Erlinger, S. (1987) Physiology of Bile Secretion and Enterohepatic Circulation. In *Physiology of the Gastrointestinal Tract*, eds. Johnson, pp. 1557-1580. Raven Press, New York.

Esteve, M., Navarro, E., Klaassen, J., Abad-Lacruz, A., González-Huix, F., Cabré, E., Ramos, E., Condom, E., Fernández-Bañares, F., Pastor, C., Humbert, P., Martí-Ragué, J., and Gassull, M.A. (1998). Plasma and mucosal fatty acid pattern in colectomized ulcerative colitis patients. *Digestive Diseases and Sciences.* **43**;1071-1078.

Esteve-Comas, M., Núñez, M.C., Fernández-Bañares, F., Abad-Lacruz, A., Gil, A., Cabré, E., González-Huix, F., Bertrán, X., and Gassull, M.A. (1993). Abnormal plasma polyunsaturated fatty acid pattern in non-active inflammatory bowel disease. *Gut.* **34**;1370-1373.

Esteve-Comas, M., Ramírez, M., Fernández-Bañares, F., Abad-Lacruz, A., Gil, A., Cabré, E., González-Huix, F., Moreno, J., Humbert, P., Guilera, M., Boix, J., and Gassull, M.A. (1992). Plasma polyunsaturated fatty acid pattern in active inflammatory bowel disease. *Gut.* **33**;1365-1369.

Färkkilä, M.A., Tilvis, R.S., and Miettinen, T.A. (1987). Plasma fatty acid composition in patients with ileal dysfunction. *Scandinavian Journal of Gastroenterology.* **22**;411-419.

Field, C.J. and Clandinin, M.T. (1984). Modulation of adipose-tissue fat composition by diet: a review. *Nutrition Research.* **4**;743-755.

Fisher, M., Levine, P.H., Weiner, B.H., Johnson, M.H., Doyle, E.M., Ellis, P.A., and Hoogasian, J.J. (1990). Dietary n-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *American Journal of Clinical Nutrition.* **51**;804-808.

Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry.* **226**;497-509.

Frayn, K.N. (1996d) Digestion and intestinal absorption. In *Metabolic Regulation: A Human Perspective*, eds. Frayn, pp. 27-47. Portland Press

Frayn, K.N. (1996b) Metabolic characteristics of the organs and tissues. In *Metabolic Regulation; A Human Perspective*, eds. Frayn, pp. 49-85. Portland Press, London.

Frayn, K.N. (1996a) Lipoprotein Metabolism. In *Metabolic Regulation: A Human Perspective*, eds. Frayn, pp. 197-217. Portland Press, London.

Frayn, K.N. (1996c) Some Important Concepts. In *Metabolic Regulation: A Human Perspective*, eds. Frayn, pp. 1-26. Portland Press, London.

Frayn, K.N. (1996e) Diabetes mellitus. In *Metabolic Regulation: A Human Perspective*, eds. Frayn, pp. 219-232. Portland Press, London.

Frayn, K.N. (1996f) Integration of carbohydrate, fat and protein metabolism in the whole body. In *Metabolic Regulation: A Human Perspective*, eds. Frayn, pp. 103-141. Portland Press, London.

Gallai, V., Sarchielli, P., Trequattrini, A., Franceschini, M., Floridi, A., Firenze, C., Alberti, A., Di Benedetto, D., and Stragliotto, E. (1993). Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. *Journal of Neuroimmunology.* **56**;143-153.

Garland, M., Sacks, F.M., Colditz, G.A., Rimm, E.B., Sampson, L.A., Willett, W.C., and Hunter, D.J. (1998). The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. *American Journal of Clinical Nutrition.* **67**;25-30.

Geerling, B.J., Badart-Smook, A., van Deursen, C., van Houwelingen, A.C., Russel, M.G., Stockbrugger, R.W., and Brummer, R.J.M. (2000). Nutritional supplementation with N-3 fatty acids and antioxidants in patients with Crohn's disease in remission: effects on antioxidant status and fatty acid profile. *Inflammatory Bowel Diseases.* **6**;77-84.

Geerling, B.J., Brummer, R.J.M., Badart-Smook, A., Stockbrugger, R.W., and Houwelingen, A.C.V. (1999). Fat intake and fatty acid profile in plasma phospholipids and adipose tissue in patients with crohn's disease, compared with controls. *American Journal of Gastroenterology.* **94**;410-417.

Gensini, G.F., Comeglio, M., and Colella, A. (1998). Classical risk factors and emerging elements in the risk profile for coronary artery disease. *European Heart Journal.* **19**;A53-A61.

Gerster, H. (1998). Can adults adequately convert α-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:3n-3) and docosahexaenoic acid (22:6n-3). *International Journal of Vitamin and Nutrition Research.* **68**;159-173.

Goldsby, R.A., Kindt, T.J., and Osborne, B.A. (2000a) Cytokines. In *Kuby Immunology*, eds. Goldsby, Kindt, & Osborne, pp. 303-327. Freeman

Goldsby, R.A., Kindt, T.J., and Osborne, B.A. (2000b) Overview of the Immune System. In *Kuby Immunology*, eds. Goldsby, Kindt, & Osborne, pp. 3-26. Freeman

Griffin, G.E. and Northfield, T.C. (1994) Consequences of small intestinal resection. In *Diseases of the Gut and Pancreas*, eds. Misiewicz, Pounder, & Venables, pp. 639-648. Blackwell Scientific, London, UK.

Gurr, M.I. (1988). Lipid metabolism in man. *Proceedings of the Nutrition Society.* **47**;277-285.

Hawthorne, A.B., Daneshmend, T.K., and Hawkey, C.J. (1992). Treatment of ulcerative colitis with fish oil supplementation: a prospective 12 month randomised controlled trial. *Gut.* **33**;922-928.

Healy, D.A., Wallace, F.A., Miles, E.A., Calder, P.C., and Newsholme, P. (2000). Effect of low-to-moderate amounts of dietary fish oil on neutrophil lipid composition and function. *Lipids.* **35**;763-768.

Hellerstein, M.K. (1999). De novo lipogenesis in humans: metabolic and regulatory aspects. *European Journal of Clinical Nutrition.* **53(suppl 1)**;553-565.

Henderson, L., Gregory, J., Irving, K., and Swan, G. (2003). National Diet and Nutrition Survey: adults aged 19 to 64 years. Energy, protein, carbohydrate, fat and alcohol intake. **Volume 2**;

Henry, M.M. (1994) Surgery of Ulcerative Colitis. In *Diseases of the Gut and Pancreas*, eds. Misiewicz, Pounder, & Venables, pp. 709-715. Blackwell Scientific, London.

Holman, R.T., Smythe, L., and Johnson, S. (1979). Effect of sex and age on fatty acid composition of human serum lipids. *American Journal of Clinical Nutrition.* **32**;2390-2399.

Jeppesen, P.B., Hoy, C.E., and Mortensen, P.B. (2000). Deficiencies of essential fatty acids, vitamin A and E and changes in plasma lipoproteins in patients with reduced fat absorption or intestinal failure. *European Journal of Clinical Nutrition.* **54**;632-642.

Johansson, C., Walldius, G., and Rössner, S. (1987). Fatty acid composition in serum lipids and adipose tissue in patients with morbus Crohn after ileal resection. *Digestion.* **37**;171-177.

Kamm, M.A. (1999a) Mechanisms of Inflammation. In *Inflammatory Bowel Disease*, eds. Kamm, pp. 9-13. Martin Dunitz, London.

Kamm, M.A. (1999b) Surgery and endoscopic therapy. In *Inflammatory Bowel Disease*, eds. Kamm, pp. 60-66. Martin Dunitz, London.

Kelley, D.S., Taylor, P.C., Nelson, G.J., Schmidt, P.C., Ferretti, A., Erickson, K.L., Yu, R., Chandra, R.K., and Mackey, B.E. (1999). Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids*. **34**;317-324.

Knutsen, S.F., Fraser, G.E., Beeson, W.L., Lindsted, K.D., and Shavlik, D.J. (2003). Comparison of adipose tissue fatty acids with dietary fatty acids as measured by 24-hour recall and food frequency questionnaire in black and white Adventists: the Adventist Health Study. *Annals of Epidemiology*. **13**;119-27.

Lee, T.H., Hoover, R.L., Williams, J.D., Sperling, R.I., Ravalese III, J., Spur, B.W., Robinson, D.R., Corey, E.J., Lewis, R.A., and Austen, K.F. (1985). Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *The New England Journal of Medicine*. **312**;1217-1224.

Loeschke, K., Ueberschaer, B., and Pietsch, A. (1996). n-3 fatty acids retard early relapse in ulcerative colitis in remission. *Digestive Diseases and Sciences*. **41**;2087-2094.

Lokesh, B., Hsieh, H.L., and Kinsella, J.E. (1986). Peritoneal macrophages from mice fed dietary (n-3) polyunsaturated fatty acids secrete low levels of prostaglandins. *Journal of Nutrition*. **116**;2547-2552.

Lokesh, B.R., Sayers, T.J., and Kinsella, J.E. (1990). Interleukin-1 and tumor necrosis factor synthesis by mouse peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Immunology Letters*. **23**;281-286.

Lorenz, R., Weber, P.C., Szimnau, P., Heldwein, W., Strasser, T., and Loeschke, K. (1989). Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease: a randomised, placebo-controlled, double-blind cross-over trial. *Journal of Internal Medicine*. **225(suppl)**;225-232.

Lorenz-Meyer, H., Bauer, P., and Nicolay, C. (1996). Omega-3 fatty acids and carbohydrate diet for maintenance of remission in Crohn's disease. *Scandinavian Journal of Gastroenterology*. **31**;778-785.

MacLean, C.H., Mojica, W.A., Newberry, S.J., Pencharz, J., Garland, R.H., Wenli, T., Hilton, L.G., Gralnek, I.M., Rhodes, S., Khanna, P., and Morton, S.C. (2005). Systematic review of the effects of n-3 fatty acids in inflammatory bowel disease. *American Journal of Clinical Nutrition*. **82**;611-619.

Margetts, B.M., Cade, J.E., and Osmond, C. (1989). Comparison of a food frequency questionnaire with a diet record. *International Journal of Epidemiology*. **18**;868-873.

McCall, T.B., O'Leary, D., and Bloomfield, J. (1989). Therapeutic potential of fish oil in the treatment of ulcerative colitis. *Alimentary Pharmacology and Therapeutics*. **3**;415-424.

Meydani, S.N., Endres, S., Woods, M.M., Goldin, B.R., Soo, C., Morrill-LaBrode, A., Dinarello, C., and Gorbach, S.L. (1991). Oral (n-3) fatty acid supplementation suppresses cytokine production, and lympthocyte proliferation: comparison between young, and older women. *Journal of Nutrition*. **121**;547-555.

Meydani, S.N., Lichtenstein, A.H., Cornwall, S., Meydani, M., Goldin, B.R., Rasmussen, H., Dinarello, C.A., and Schaeffer, E.J. (1993). Immunologic effects of national cholesterol

education panel step-2 diets with and without fish-derived n-3 fatty acid enrichment. *Journal of Clinical Investigation*. **92**;105-113.

Mingrone, G., Greco, A.V., Benedetti, G., Capristo, E., Semeraro, R., Zoli, G., and Gasbarrini, G. (1996). Increased resting lipid oxidation in Crohn's Disease. *Digestive Diseases and Sciences*. **41**;72-76.

Mossmann, T.R. and Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today*. **17**;138-146.

Müller, M.J., Schmidt, L.U., Körber, J., Mühlen, A.V.Z., Canzler, H., and Schmidt, F.W. (1993). Reduced metabolic efficiency in patients with crohn's disease. *Digestive Diseases and Sciences*. **38**;2001-2009.

NACC (2006a). *www nacc org uk*.

NACC (2006b). *www nacc org uk*.

Nissinen, M.J., Gylling, H., Järvinen, H.J., and Miettinen, T.A. (2004). Ileal pouch-anal anastomosis, conventional ileostomy and ileorectal anastomosis modify cholesterol metabolism. *Digestive Diseases and Sciences*. **49**;1444-1453.

Patton, J.S. (1981) Gastrointestinal lipid digestion. In *Physiology of the Intestinal Tract*, eds. Johnson, pp. 1123-1146. Raven Press, New York.

Pawlosky, R.J., Hibbeln, J.R., Novotny, J.A., and Salem Jr, N. (2001). Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *Journal of Lipid Research*. **42**;1257-1265.

Ralph, A. (1993) Methods for dietary assessment. In *Human Nutrition and Dietetics*, eds. Garrow & James, pp. 777-781. Churchill Livingstone, London.

Reed, R.G., Kris-Etherton, P.M., Stewart, P.W., and Pearson, T.A. (2000). Variation of lipids and lipoproteins in premenopausal women compared with men and postmenopausal women. *Metabolism*. **49**;1101-1105.

Rhodes, J., Mayberry, J.F., Roberts, G.M., Williams, G.T., Dew, M.J., and Harries, A.D. (1994a) Clinical features of Crohn's disease. In *Diseases of the Gut and Pancreas*, eds. Misiewicz, Pounder, & Venables, pp. 717-740. Blackwell Scientific, London.

Rhodes, J.M., Ryder, S.D., and Tsai, H.H. (1994b) Clinical features of Ulcerative Colitis. In *Diseases of the Gut and Pancreas*, eds. Misiewicz, Pounder, & Venables, pp. 677-697. Blackwell Scientific, London.

Roitt, I.M., Brostoff, J., and Male, D. (1998a) Cell-Mediated Immune Reactions. In *Immunology*, eds. Roitt, Brostoff, & Male, pp. 121-138. Mosby

Roitt, I.M., Brostoff, J., and Male, D. (1998b) Cells Involved in the Immune Response. In *Immunology*, eds. Roitt, Brostoff, & Male, pp. 13-30. Mosby

Roitt, I.M., Brostoff, J., and Male, D. (1998c) Introduction to the Immune System. In *Immunology*, eds. Roitt, Brostoff, & Male, pp. 1-12. Mosby

Saleh, J., Sniderman, A.D., and Cianflone, K. (1999). Regulation of plasma fatty acid metabolism. *Clinica Chimica Acta.* **286**;163-180.

Salomon, P., Asher, A., Kornbluth, P., and Janowitz, H.D. (1990). Treatment of ulcerative colitis with fish oil n-3 fatty acid: an open trial. *Journal of Clinical Gastroenterolgy.* **12**;157-161.

Schmidt, E.B., Varming, K., Møller, J.M., Pedersen, I.B., Madesen, P., and Dyerberg, J. (1996). No effect of a very low dose of n-3 fatty acids on monocyte function in healthy humans. *Scandinavian Journal of Clinical and Laboratory Investigation.* **56**;87-92.

Shaheen, S.O., Sterne, J.A.C., Thompson, R.L., Songhurst, C.E., Margetts, B.M., and Burney, P.G.J. (2001). Dietary antioxidants and asthma in adults. *American Journal of Respiratory and Critical Care Medicine.* **164**;1823-1828.

Shiau, Y.F. (1987) Lipid Digestion and Absorption. In *Physiology of the Gastrointestinal Tract*, eds. Johnson L R, pp. 1527-1556. Raven Press, New York.

Siguel, E.N. and Lerman, R.H. (1996). Prevalence of essential fatty acid deficiency in patients with chronic gastrointestinal disorders. *Metabolism.* **45**;12-23.

Simons, L.A., Simons, J., and Jones, A.S. (1984). The interactions of body weight, age, cigarette smoking and hormone usage with blood pressure and plasma lipids in an Australian community. *The Australian and New Zealand Journal of Medicine.* **14**;215-221.

Spector, A.A. (1999). Essentiality of fatty acids. *Lipids.* **34**;S1-S3.

Sperling, R.I., Benincaso, A.I., Knoell, C.T., Larkin, J.K., Austen, K.F., and Robinson, D.R. (1993). Dietary ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation, and chemotaxis in neutrophils. *Journal of Clinical Investigation.* **91**;651-660.

Staggers, J.E., Fernando-Warnakulasuriya, G.J.P., and Wells, M.A. (1981). Studies on fat digestion, absorption and transport in the suckling rat. *Journal of Lipid Research.* **22**;675-679.

Stryer, L. (1995) Membrane Structure and Dynamics. In *Biochemistry*, eds. Stryer, pp. 263-290. Freeman

Teitelbaum, J.E. and Walker, W.A. (2001). Review: the role of omega 3 fatty acids in intestinal inflammation. *Journal of Nutritional Biochemistry.* **12**;21-32.

Thomson, A.B.R., Keelan, M., Garg, M.L., and Clandinin, M.T. (1988). Intestinal aspects of lipid absorption: in review. *Canadian Journal of Physiology and Pharmacology.* **67**;179-191.

Tougaard, L., Giese, B., Hojlund Pedersen, B., and Binder, V. (1986). Bile acid metabolism in patients with crohn's disease in terminal ileum. *Scandinavian Journal of Gastroenterology.* **21**;627-633.

Trebble, T., Arden, N.K., Stroud, M.A., Wootton, S.A., Burdge, G.C., Miles, E.A., Ballinger, A.B., Thompson, R.L., and Calder, P.C. (2003a). Inhibition of tumour necrosis factor-α and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *British Journal of Nutrition.* **90**;405-412.

Trebble, T., Wootton, S.A., Miles, E.A., Mullee, M.A., Arden, N.K., Ballinger, A.B., Stroud, M.A., Burdge, G.C., and Calder, P.C. (2003b). Prostaglandin E_2 production and T cell function after

fish-oil supplementation: response to antioxidant cosupplementation. *American Journal of Clinical Nutrition*. **78**;376-382.

Trebble, T.M., Arden, N.K., Wootton, S.A., Mullee, M.A., Calder, P.C., Burdge, G.C., Fine, D.R., and Stroud, M.A. (2004). Peripheral blood mononuclear cell fatty acid composition and inflammatory mediator production in adult Crohn's disease. *Clinical Nutrition*. **23**;647-655.

Uauy, R., Mena, P., and Rojas, C. (2000). Essential fatty acids in early life: Structural and functional role. *Proceedings of the Nutrition Society*. **29**;3-15.

van Staveren, W.A., Deurenberg, P., Katan, M.B., Burema, J., de Groot, C.P.G.M., and Hoffmans, M.A.D.F. (1986). Validity of the fatty acid composition of subcutaneous fat tissue microbiopsies as an estimate of the long-term average fatty acid composition of the diet of separate individuals. *American Journal of Epidemiology*. **123**;455-463.

Yaqoob, P. and Calder, P.C. (1995). Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cellular Immunology*. **163**;120-128.

Yaqoob, P., Pala, H.S., Cortina-Borja, M., Newsholme, E.A., and Calder, P.C. (2000). Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *European Journal of Clinical Investigation*. **30**;260-274.