Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

NUTRITIONAL STUDIES IN

UPPER GASTROINTESTINAL CANCER

A Thesis for the degree of Doctor of Philosophy (Ph.D)

at

University of Dublin, Trinity College

by

Aoife Ryan

2008

Departments of Clinical Nutrition & Surgery
St. James's Hospital
Dublin 8

DECLARATION

This thesis is submitted by the undersigned to Trinity College, University of Dublin for the degree of Ph.D. I certify that none of the work in this thesis has been submitted for any degree or diploma at this, or any other university, and that all the work in this thesis is entirely my own. I agree that the Library may lend or copy this thesis upon request.

Aoife Ryan

Aoife Ryan

12/02/08

Date

ACKNOWLEDGEMENTS

I would like to thank my supervisor Professor John V. Reynolds for his support and enthusiasm for this research, and the expert guidance he gave me over the four and a half years I spent at the Department of Surgery, St. James's Hospital. I could not have asked for a more enthusiastic and helpful supervisor. I am also hugely grateful to Dr. Patrick Byrne for the help and advice he gave me during this time.

I would like to thank my manager, Ms. Philomena Flood in the Department of Clinical Nutrition for her constant support and hard work in securing funding for this research, and allowing me to be released from my clinical duties.

A huge thanks to the team of researchers I worked with over the years, who provided great advice, help and support to me: Ms. Laura Healy, Dr. Miriam Byrne, Dr. Seamus Murphy, Ms. Aoife Murphy, Ms. Sinead Murphy, Dr. Nicola Miller, Dr. Ruth Pritchard, Dr. Aileen Cunningham & Dr. Vinnie Malik. I am also very grateful to Ms Suzanne Rowley who established and managed the Upper Gastrointestinal Cancer database at St. James's Hospital and allowed me to access much of the clinical data I required for a large majority of this thesis. Thank you also to Dr. Tony Fitzgerald and Ms Aine Hearty for statistical advice given for several of the studies.

I would like to thank Professor Mike Gibney and Dr Sinead Mc Carthy of the Irish Universities Nutrition Alliance (IUNA) for allowing me access to the raw data on BMI for the healthy controls under the age of 65 I used for chapter 2. Without this data this epidemiological study would not have been possible. Thank you also to the staff and clients of the several day centres for the elderly in South Dublin I visited to collect BMI data on the over 65 year old healthy controls I used for this study.

I am indebted to the 200 or so patients suffering from gastro-oesophageal reflux disease (GORD) or Barrett's oesophagus who volunteered to take part in the metabolic syndrome study in chapter 3. I would like to thank the staff in the Upper GI Function Unit – Patricia and Tracey; and consultant Gastroenterologists Dr Keeling, Prof Kelleher and Dr Norris for allowing me access their patients with Barrett's oesophagus and GORD. Thanks also to Adrienne Lennon, Laura Tier and Kathleen Gibbons, who gave me much needed expert secretarial support in organising the many Barrett's research

clinics for this study. I would also like to thank Sinead Murphy, Dr Miriam Byrne and Laura Healy for helping me with all the phlebotomy and laboratory work, and the staff in the Central Pathology Laboratory at St. James's for performing all the routine assays used in several chapters of this thesis.

I am also grateful to Fresenius Kabi Ireland for providing financial assistance to complete the two studies on nutrition support in chapter 6.

I am indebted to Abbott Laboratories, both the Dublin and Chicago branches, for so generously providing financial assistance to carry out the randomised trial in chapter 7. This trial was a huge undertaking, and I am grateful to so many people for helping me. In particular I would like to thank Ms. Sandra Stanley, Senior Dietitian with Abbott Laboratories for her assistance in securing funding and organising the blinding of the study product. This study took over a year of preparation, and without her help, would not have been possible. A special thanks to Laura Healy who was involved so closely in patient recruitment and data collection and was such a fantastic friend and colleague to me. I would also like to acknowledge the team of clinical dietitians who I worked so closely with over the duration of the trial – their diligence and record keeping helped me hugely – thank you to Deirdre McCormack, Aisling Mc Hugh, Caitriona Caulfield, Niamh Brannelly and Arun Fenton. I am also very grateful to the staff in the immunology department in SJH - especially Dr. Eleanor Wallace and Dr John Jackson for their patience and time in teaching me the immunology techniques used in this trial, and to and Mr. Christian Coates for his help in running the specialised immunological assays in Chapter 7 and Elisa tests for chapter 3. Thank you also to all the nursing Staff on the Surgical Wards at St. James's Hospital – High Dependency Unit, Intensive Care Unit, Edward Halloran Bennett Ward, and Private 2, for their helpful co-operation - without their diligence this study would not have been possible. I am also very grateful to Caroline, Heather and Ellen, staff nurses in the Out-Patient Department, for their help and co-operation over the past 2 years. Also thanks to Mary Martin, chief phlebotomist for the expert training on phlebotomy she gave me.

A very special thank you to my friend and colleague Jennifer Moore, Upper GI Cancer Co-ordinator and nurse specialist, who constantly informed me of patients' treatment plans to ensure optimal recruitment to all the studies. I would like to say a special thank you to Dr. Miriam Byrne for her endless help with all the phlebotomy over the years and

for her help with the cytokine assays in Chapter 7 – without her help my life would have been a lot more difficult! I am also very grateful to the staff in the Cancer Clinical Trials office – Ms Jennifer Feighan, Ms Rachael Griffin, Ms Ingrid Kiernan for their help and support of this trial and to Marion Bruce in the Haughton Institute for all her advice. Thank you also to Ms Fionnuala King, Chief Oncology Pharmacist who was involved in the dispensing of the study product.

I would like to say a huge thank you to my parents, for their listening ear and constant encouragement to write up my work. A very special thanks to my new husband Derek for putting up with my long working hours, and for the support he always gives me. Thank you.

Finally, ***and most especially***, thank you to all of the patients suffering from cancer, so many of whom I got to know so well throughout their cancer journey, and sadly many of whom have passed away. I was so often moved by their enormous generosity and wholehearted willingness to participate in these research studies, in the hope they might improve our understanding of their disease process, and perhaps benefit the lives of future cancer patients. Mile Buiochos O Croi.

This work was undertaken with the approval of the ethics committee of St. James's Hospital and Federated Dublin Hospitals, according to the Helsinki agreement. All of the patients who agreed to take part in the studies gave their full informed consent.

SUMMARY

Carcinoma of the oesophagus, gastro-oesophageal junction and stomach represent aggressive diseases with a poor prognosis even in patients undergoing curative resection. Where squamous cell histology once predominated, the incidence of oesophageal adenocarcinoma has risen dramatically in Western countries over the past three decades. Nutrition plays a key role in both the epidemiology of upper GI cancers and throughout the cancer journey. It is a key risk factor, with obesity thought to be fuelling the dramatic increase in incidence observed recently in the Western World. It also impacts on Gastro-oesophageal reflux disease and Barrett's Oesophagus, and the metabolic abnormalities found in obesity are thought to be responsible for this. Once diagnosed with Upper GI cancer, profound changes occur to the patients' nutritional status. Again nutritional therapy plays a key role on patients' performance status, quality of life and immunological and physical well-being. This thesis describes several studies of the impact of nutrition on upper GI cancer –from its aetiology to treatment outcomes.

PART I of this Thesis examined the nutritional epidemiology of upper GI cancer and its precursor lesion, Barrett's Oesophagus. **Chapter 2**, a case control study of 760 cancer cases and 893 healthy controls showed that obesity was an independent risk factor for adenocarcinoma of the oesophagus, oesophago-gastric junction and gastric cardia. Obesity increased the risk 11-fold in obese men compared to healthy controls. This study was the first Irish report on obesity and cancer. To investigate plausible mechanisms whereby obesity relates to adenocarcinoma, 102 Barrett's and 78 patients with Gastro-oesophageal reflux disease were screened for abdominal obesity and metabolic syndrome in **Chapter 3**. 46% of Barrett's patients had metabolic syndrome compared with 32% of GORD ($p < 0.05$). In the long segment Barrett's cohort, 92% were centrally obese, 60% had metabolic syndrome and a pro-inflammatory state from adipo-cytokine production was demonstrated. The prevalence of metabolic syndrome in an unselected Barrett's as well as GORD cohort far exceeded population norms. This association suggests both potential pathways in the progression of Barrett's metaplasia and novel therapeutic approaches.

PART II of this thesis examined the effect of major upper gastrointestinal surgery on morbidity and mortality following multimodality treatment or surgery alone and examined the role of albumin in predicting post operative complications. **Chapter 4** established the model of oesophagectomy as a severe surgical operation with high morbidity. We performed a non-randomised comparison of 148 patients undergoing neoadjuvant therapy or surgery alone, and reported that neoadjuvant therapy

was associated with increased respiratory and septic complications. In an attempt to identify early makers of postoperative morbidity we chose to examine the role of serum albumin as a predictor of complications in 200 patients undergoing oesophagectomy in **Chapter 5.** We observed that patients with an albumin <20 g/L on the first post-operative day were twice as likely to develop post-operative complications, had a significantly higher rate of Adult Respiratory Distress Syndrome, respiratory failure and in-hospital mortality than those with an albumin >20 g/L. We conclude that serum albumin concentration on the first post-operative day is a better predictor of surgical outcome than many other pre-operative risk factors. **Chapter 6** then examined the impact of Upper GI malignancy on Nutritional Status. In **chapter 6A** we reviewed our institutions experience of jejunostomy feeding post oesophagectomy over an 8-year period. The records of 205 consecutive cases were reviewed and showed that jejunostomy feeding was an effective method of providing nutritional support post-oesophagectomy, and allowed home support for the subset that fail to thrive. Serious complications were very rare. **Chapter 6B** examined the role of Total Parenteral Nutrition (TPN) versus Intravenous fluids only on nutritional status post total gastrectomy for malignancy in 90 patients. This study showed that there is a high prevalence of malnutrition in gastric cancer patients undergoing surgery. Total Gastrectomy is associated with dramatic weight loss, with patients losing an average of 15.5 kgs by 3-month follow up. Provision of TPN post-operatively significantly reduced in-hospital weight loss, and also helped to attenuate further weight loss post discharge.

PART III of this thesis concerned a randomised controlled trial with peri-operative immuo-nutrition. **Chapter 7** investigated possible modulation of the immuno-inflammatory response to oesophagectomy using an enteral supplement enriched with eicosapentaenoic acid (EPA). In a double blinded randomised design we investigated the effect of EPA on post operative complications, stress response, immune function, and body composition. 53 patients were prospectively recruited over 2.5 years. Peri-operative administration of an enteral formula enriched with 2.2 g EPA per day for 5 days pre op and 21 days post op was associated with preservation of lean body mass and nutritional status, lower body temperature and a lesser pro-inflammatory response to surgery than standard enteral nutrition. In addition there was less suppression of immune function.

The conclusions are summarised in **Chapter 8**. These studies on Nutrition in Upper Gastrointestinal cancer add to our understanding of obesity's aetiological role, and also add to our knowledge of the effectiveness of nutrition support after major cancer surgery. The results have treatment implications for many other solid tumours.

TABLE OF CONTENTS

PART II:

EFFECT OF UPPER GASTROINTESTINAL MALIGNANCY ON NUTRITIONAL WELLBEING, IMMUNO-INFLAMMATORY RESPONSE, AND MORBIDITY AND MORTALITY FOLLOWING SURGICAL TREATMENT

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6A

CHAPTER 6B

CHAPTER 7

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6A

CHAPTER 6B

CHAPTER 7

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACA	Adenocarcinoma
ANOVA	Analysis of Variance
ARDS	Adult respiratory Distress Syndrome
ASA	American Society of Anaesthesiology
BD	Becton Dickinson
BIA	Bioelectrical Impedance Analysis
BMI	Body mass index
BO	Barrett's Oesophagus
BP	Blood Pressure
CARS	Compensatory anti-inflammatory response syndrome
CCK	Choleytokinin
CI	Confidence Interval
cm	centimetres
CRP	C-Reactive Protein
CT	Chemotherapy
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic Acid
EBAO	Ethidium bromide-acridine orange
ECOG	Eastern Co-operative Oncology Group
EDTA	Ethylene Diamine Tetraacetic Acid
EGF	Epidermal Growth Factor
ELISA	Enzyme Linked Immuno Sorbent Assay
EN	Enteral nutrition
EPA	Eicosapentaenoic Acid
EUS	Endoscopic Ultrasound
ESR	Erythrocyte sedimentation rate
FEV	Forced expiratory volume
FFA	Free Fatty Acid
FFM	Fat Free Mass
FITC	Fluorescein isotiocyanate
FVC	Forced volume capacity
GC	Gas Chromotography

GI	Gastrointestinal
GIST	Gastrointestinal Stromal Tumour
GOR	Gastro-Oesophageal Reflux
GORD	Gastro-Oesophageal Reflux Disease
Gy	Gray
H Pylori	Helicobactor pylori
HBSS	Hank's Balanced Salt Solution
HDL	High Density Lipoprotein
HDU	High Dependency Unit
HH	Hiatus Hernia
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
HR	Hazard Ratio
ICU	Intensive Care Unit
IgE	Immunoglobulin E
IGF-1	Insulin Like Growth Factor
IgG	Immunoglobulin G
IL	Interleukin
INF	Interferon
IQ	Interquartile
IVF	Intra venous fluids
Kg	Kilogram
LDL	Low Density Lipoprotein
LMF	Lipid Mobilising Factor
LnOS	Length of Stay
LOS	Lower Oesophageal Sphincter
LSB	Long Segment Barrett's
LSS	Lymphocyte Sub Sets
LTB4	Leukotriene B4
MCP	Monocyte Chemoattractant Protein
MHC	Major Histo-compatibility Complex
MIP	Macrophage inflammatory Protein
ml	millilitre
mm	millimetre
mmHg	millimetres Mercury
mmol	millimole

MODS	Major Organ Dysfunction Syndrome
MOF	Multiple Organ Failure
n	number of patients
N_2	Nitrogen
NCEP-ATP	National Cholesterol Education Programme – Adult Treatment Panel
NCJ	Needle Catheter Jejunostomy
ng	nano grams
NKC	Natural Killer Cells
NRI	Nutrition Risk Index
ns	non-significant
NS	Nutrition Support
OG	Oesophago-gastric
OR	Odds Ratio
PAF	Platelet Activating Factor
PAI	Plasminogen Activator Inhibitor
PATS	Patient Analysis and Tracking System
PBMC	Peripheral Blood Mononuclear Cell
pCR	Pathological Complete Response
PET	Positive Emission Tomography
PGE2	Prostaglandin E2
pH	Hydrogen Ion Concentration
PIF	Proteolysis Inducing Factor
PNI	Prognostic Nutrition Index
POD	Post operative day
POD	Post operative Day
Pre-op	Pre-operatively
PUFA	poly unsaturated fatty acid
QOL	Quality of life
RIP	Rest in Peace
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RR	Relative Risk
RR	Relative Risk
RT	Radiotherapy
RTI	Respiratory Tract Infection

SAA	serum amalyoid A
SAT	Subcutaneous Adipose Tissue
SCC	Squamous Cell Carcinoma
SD	Standard Deviation
SGA	Subjective Global Assessment
SHBG	Sex Hormone Binding Globulin
SIM	Specialised Intestinal Metaplasia
SIRS	Systemic inflammatory response syndrome
SPSS	Statistical Package for Social Sciences
SSB	Short Segment Barrett's
TAG	Triglycerides
Tis	Tumour in situ
TNF	Tumour Necrosis Factor
TNM	Tumour Staging (T-Tumour, N-Nodes, M-Metastases)
TPN	Total Parenteral Nutrition
UK	United Kingdom
US	United States
VAT	Visceral Adipose Tissue
VEGF	Vascular Endothelial Growth Factor
WCC	White Cell Count
WCC	White Cell Count
WHO	World Health Organisation

LIST OF PUBLICATIONS ARISING FROM THIS WORK

"Adenocarcinoma of the Oesophagus and Gastric Cardia: Male preponderance in association with overweight and obesity"

Aoife Ryan, Suzanne P Rowley, Anthony P Fitzgerald, Narayanasami Ravi, John V Reynolds. *European Journal of Cancer* (2006) May; 42(8):1151-1158

"Prevalence of Central Adiposity, Metabolic Syndrome, and a Pro-inflammatory state in Barrett's Esophagus"

Aoife Ryan, Laura A Healy, Derek G Power, Miriam Byrne, Sinead Murphy, Patrick J Byrne, Napoleon Keeling, Dermot Kelleher, John V Reynolds. *(Accepted for publication Annals of Surgery Oct 2007, In Press)*

"Neoadjuvant chemoradiation may increase the risk of respiratory complications and sepsis after transthoracic esophagectomy"

John V Reynolds, Narayanasami Ravi, Donal Hollywood, Michael J Kennedy, Suzanne P Rowley, **Aoife Ryan ,** Niall Hughes, Martin Carey, Patrick Byrne.
Journal Thoracic and Cardiothoracic Surgery (2006) Sept; 132(3):549-55.

"Hypoalbuminaemia on the first post operative day post Oesophagectomy may predict short-term adverse outcomes"

Aoife Ryan, Áine Hearty, Ruth S Prichard, Aileen Cunningham, Suzanne P Rowley, John V Reynolds.
Journal of Gastrointestinal Surgery (2007) Oct; 11(10):1355-1360.

"Post Oesophagectomy Early Enteral Enteral Nutrition via a needle catheter jejunostomy: 8-year experience at a specialist unit"

Aoife Ryan, Suzanne P Rowley, Laura A Healy, Philomena M Flood, Narayanasami Ravi, John V Reynolds.
Clinical Nutrition (2006) Jun; 25(3):386-93

"Short-term Nutritional Implications of Total Gastrectomy for Malignancy, and the Impact of Parenteral Nutritional Support"

Aoife M Ryan, Laura A Healy, Suzanne P Rowley, John V Reynolds.
Clinical Nutrition, (2007)26; 718-727

CHAPTER 1:
INTRODUCTION & LITERATURE REVIEW

1.1 Oesophageal Cancer

- 1.1.1 Incidence Rates/Demographic Trends
- 1.1.2 Pathology
- 1.1.3 Sex, Race, Age Distribution
- 1.1.4 Pathologic Process

1.2 Epidemiology of Oesophageal Cancer

- 1.2.1 Gastro-Oesophageal Reflux Disease
- 1.2.2 Specialised Intestinal Metaplasia/Barrett's Oesophagus
- 1.2.3 Alcohol
- 1.2.4 Smoking
- 1.2.5 Diet and Nutrition
- 1.2.6 Helicobacter Pylori Infection
- 1.2.7 Socio-economic status
- 1.2.8 Heredity

1.3 Nutritional Epidemiology of Oesophageal Adenocarcinoma: Obesity

- 1.3.1 Obesity: definition & scale of the problem
- 1.3.2 Obesity and Cancer
- 1.3.3 Obesity and Oesophageal Cancer: Epidemiological evidence to date
  - 1.3.3.1 North America/Canada
  - 1.3.3.2 European Studies
  - 1.3.3.3 Australia and Asia
- 1.3.4 Obesity and Adenocarcinoma of Gastric Cardia
- 1.3.5 Obesity and Oesophageal Squamous Cell Carcinoma
- 1.3.6 Mechanism of Altered Cancer Risk
- 1.3.7 Abnormal body composition: Abdominal Obesity
- 1.3.8 Obesity as an inflammatory condition: Adipo-cytokines

1.3.9 Leptin, Adiponectin and Resistin

1.3.10 Metabolic Syndrome

1.3.11 Metabolic Syndrome and Cancer

1.3.12 Pathophysiological mechanisms whereby Metabolic Syndrome/central obesity promotes Oesophageal Cancer development

1.3.12.1 Obesity and GORD

1.3.12.2 Obesity and Barrett's Oesophagus

1.4 Presentation, Diagnosis & Management of Upper Gastrointestinal Cancer

1.4.1 Presentation of Upper GI Cancer

1.4.2 Diagnosis and staging of Oesophageal Cancer

1.4.3 Management of localised Oesophageal Cancer

1.4.4 Surgery

1.4.5 Post Operative Complications

1.4.6 Pro-inflammatory response to surgery

1.4.6.1 Acute Phase Proteins

1.4.6.2 C-Reactive Protein

1.4.6.3 Cytokines

1.4.6.4 Negative Acute Phase Proteins

1.4.7 Anti-Inflammatory response

1.4.8 Systemic Inflammatory Response Syndrome and MODS

1.4.9 Multimodal Treatment of Oesophageal Cancer

1.4.9.1 Neoadjuvant Chemo-radiotherapy and post-operative complications

1.5 Impact of Upper GI Malignancy on Nutritional Status

1.5.1 Malnutrition and cancer

1.5.2 Malnutrition and Upper GI Cancer

1.5.3 Changing profile of malnutrition in Cancer patients

1.5.4 Pathophysiology of cancer-associated malnutrition and cachexia

1.5.5 Nutrition Screening Methods

1.5.6 Effects of Upper Gastrointestinal cancer on nutritional status

1.5.7 Nutritional consequences of Oesophagectomy

1.5.8 Nutritional consequences of total gastrectomy

1.5.8.1 Reduced Intake

1.1 OESOPHAGEAL CANCER

Oesophageal Cancer is associated with a dismal prognosis (Enzinger & Mayer, 2003, Lagergren, 2005). During the past three decades dramatic changes have occurred in the epidemiologic patterns associated with this disease. These changes differ across the two principle histologic types of oesophageal cancer, squamous cell carcinoma (SCC) and adenocarcinoma, as well as across race, gender and country (Holmes & Vaughan, 2007). In addition to epidemiologic changes, recent advancements in the diagnosis, staging, and treatment of this neoplastic condition have lead to small but significant improvements in survival. However, mortality from oesophageal cancer is high and the response to treatments for advanced-stage disease is poor, suggesting that an effective method for mortality reduction may be through early intervention on modifiable risk factors (Kubo & Corley, 2006).

1.1.1 Incidence Rates and Demographic Trends

Oesophageal cancer is the third most common cancer of the digestive tract and the sixth leading cause of cancer-related deaths worldwide, with approximately 400,000 cases diagnosed each year (Blackstock, 2007; Pisani et al, 1999). The incidence of oesophageal cancer has been increasing markedly in several countries, up to 400% during the past three decades, reflecting the most rapid rate of increase of any cancer in the Western World (Kubo & Corley, 2006). Several population-based studies from the United States and Western Europe have confirmed the rising incidence of oesophageal adenocarcinoma and oeophago-gastric junction adenocarcinoma (Pera et al, 2005; Blot et al, 1993; Blot et al, 1991; Devesa et al, 1998; Hansson et al, 1993; Pera et al, 1993; Yang & Davis, 1988). In the United States, the incidence of adenocarcinoma increased 4-fold between 1973 to 1982 and 1993 to 2002, from 0.5 to 2.1 per 100,000. This increase was observed in all race and gender groups but most dramatically in white males, who showed nearly a 5 fold rise (Holmes & Vaughan, 2007). Approximately 14,500 new cases of Oesophageal cancer are diagnosed annually in the US and 13,770 deaths from this cancer occur (Jemal et al, 2006). In Europe approximately 43,700 cases are diagnosed each year and 39,500 deaths occur (Boyle & Ferlay, 2005). In Ireland, oesophageal cancer is the 13th most common cancer in women, and the 8th most common cancer in men with approximately 408 cases diagnosed each year (Ireland-Northern Ireland National Cancer Institute, 2001). Ireland has a higher incidence of oesophageal cancer than the European average for both men and women. In women, the incidence

rate is 6.1 per 100,000 women in the Republic compared with a European Union average of 2.2 per 100,000. In men the rate is 11.7 per 100,000 in the Republic compared with an EU average of 9.5 per 100,000 (Ireland-Northern Ireland National Cancer Institute, 2001).

1.1.2 Pathology

More than 90 percent of oesophageal cancers are either squamous cell carcinomas (SCC) or adenocarcinomas (Daly et al, 2000). Other more rare forms of cancers that can develop in the oesophagus include melanomas, leiomyosarcomas, carcinoids, and lymphomas. Approximately three quarters of all adenocarcinomas are found in the distal oesophagus, whereas squamous-cell carcinomas are more evenly distributed between the middle and lower third (Daly et al, 2000; Siewert et al, 2001).

1.1.3 Age, Sex and Race Distribution

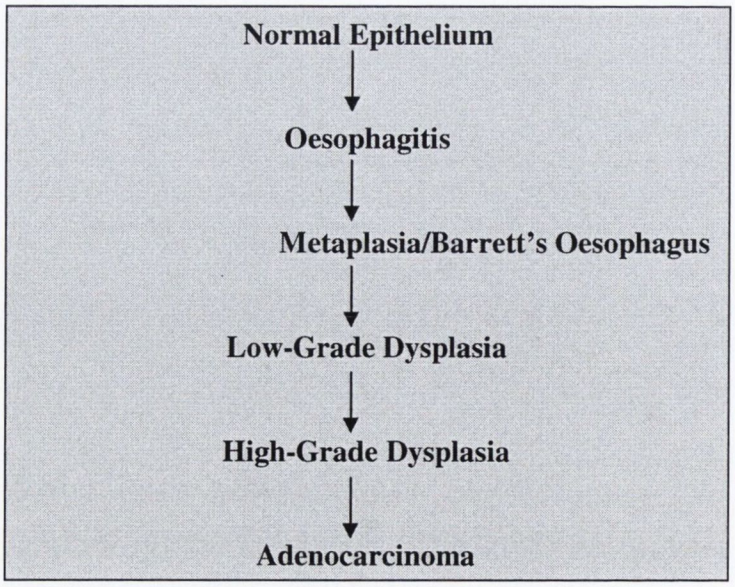
The risk of oesophageal cancer increases with increasing age, with a median age at diagnosis of 60-75 years (Lagergren, 2005; Holmes & Vaughan 2007). An unexplained feature of the incidence of this cancer is the striking male predominance. This observation has been similar in all populations studied and across both histological subtypes (Blot et al, 1991; Powell & McConkey, 1992; Hansson et al, 1993, Holmes & Vaughan, 2007).

For adenocarcinoma, the incidence is substantially higher in males than females with a ratio of 7:1, and in whites compared with blacks (ratio 4:1), (Blot & McLaughlin, 1999; Crew & Neugut, 2004; Lagergren, 2005; Pera et al, 2005; Blot et al, 1991; Devesa et al, 1998). For SCC, the incidence is also higher in males than females in most countries, and higher in black men than in white men in the United States. Among blacks in the US, rates of SCC are still substantially higher than adenocarcinoma, however the overall incidence of SCC in the US has dropped from 3.0 per 100,000 in 1973 to 1982 to 2.1 in 1993 to 2002, with declines seen in all race and gender groups (Holmes & Vaughan, 2007). The incidence of SCC peaked in black males in the late 1970's and early 80s at about 21 per 100,000 and has decreased by over 60% since then to 7.6 per 100,000 in 2002. Among Asians SCC still predominates, and adenocarcinomas – particularly those at the OG junction are less common (Pera et al, 2005).

1.1.4 Pathologic Process

The pathogenesis of oesophageal cancer remains unclear (Enzinger & Mayer, 2003). Data from animal studies suggests that oxidative damage from factors including smoking, alcohol and gastro-oesophageal reflux, which cause inflammation, oesophagitis, and increased cell turnover, may initiate the carcinogenic process (Terry et al, 2000). Adenocarcinoma is thought to arise during a metaplasia-dysplasia-carcinoma sequence (Jankowski et al, 1999). Specialised Intestinal Metaplasia (SIM), also known as Barrett's Oesophagus, is defined as metaplastic columnar epithelium containing goblet cells in a biopsy specimen obtained anywhere within the tubular oesophagus above the anatomic oesophago-gastric junction (Pera, 2003). Progression to dysplasia is considered a pre-neoplastic condition, whereby cellular changes have become encoded into the genome and are passed onto daughter cells during cellular replication. It is thought that this sequence of events begins with chronic acid and bile damage to the oesophagus which is normally rapidly healed by restitution or cellular replication, however, in 10% of cases chronic damage to the epithelial stem cells allows rapid clonal replacement by lineages with a growth advantage containing p53 mutations, and appearances of dysplasia occurs. In 1 in 100 cases, aneuploidy (the occurrence of one or more extra or missing chromosomes) and errors in DNA repair represent final pathways which disrupt invasion suppressor genes (Jankowski et al, 1999), *See Figure 1.1.*

Figure 1.1: Metaplasia-Dysplasia-Carcinoma Sequence in Oesophageal Adenocarcinoma



High grade dysplasia has been reported in 2-24% of individuals with Barrett's metaplasia and these individuals have a four-fold to eight-fold greater risk of developing cancer (Morales et al, 1997) compared with individuals with low grade dysplasia (Wright, 1997; Altorki et al, 1991; Levine et al, 1995).

Once cancer develops, it can spread rapidly to adjacent lymph nodes (Siewert et al, 2001; Collard et al, 2001). At the time of diagnosis of oesophageal cancer, more than 50 percent of patients have either unresectable tumours or radiologically visible metastases (Enzinger & Mayer, 2003; Layke & Lopez, 2006). Mortality rates for SCC and adenocarcinoma are similar because most patients are diagnosed at a late stage of disease. Five year survival is still about 10% in most Western countries, although there have been recent improvements in survival, albeit small, reflecting improvements in endoscopic detection, and in surgical and medical therapy for early stage disease (Pera et al, 2005). Because the mortality from these cancers is high, and the response to treatments for advanced stage disease is poor, an effective method for mortality reduction may be through early intervention on modifiable risk factors (Daly et al, 1996). These risk factors include Gastro-oesophageal reflux disease, Barrett's oesophagus, diet, smoking, alcohol intake and obesity.

1.2 EPIDEMIOLOGY OF OESOPHAGEAL CANCER: ADENOCARCINOMA & SCC

1.2.1 Gastro-Oesophageal Reflux Disease

Frequent symptoms of Gastro-Oesophageal Reflux (GOR) affect between 10 and 30% of the adult population (Hampel et al, 2005; Spechler, 1994). A recent systematic review of 31 articles including 77,671 patients, reported that in Western populations, 25% of people report having heartburn at least once a month, 12% at least once per week, and 5% describe daily symptoms (Moayyedi & Axon, 2005). The pathophysiology of GOR is complex, since it is influenced by many factors, and many mechanisms remain incompletely understood. Physiological abnormalities of the anti-reflux barrier, hiatus hernia, oesophageal body emptying, and gastric factors including acid, volume, and emptying all play a role in the pathogenesis of GORD (Katz, 2001). Asymptomatic oesophagitis is also common but the natural history remains unknown (Moayyedi & Talley, 2006). In a random sample of the Swedish adult population, reflux symptoms

were reported in 40% and oesophagitis was diagnosed in nearly 16%; however, 37% of those with oesophagitis had no symptoms of GORD (Shaheen & Provenzole, 2003).

The role of gastro-oesophageal reflux in the development of oesophageal adenocarcinoma has been investigated in four recent large epidemiological studies (Chow et al, 1995; Lagergren et al, 1999, Ye et al, 2001; Farrow et al, 2000). Chow et al (1995) performed a medical record-based case-control study of 196 patients with adenocarcinoma of the oesophagus and gastric cardia in the United States. They reported a significant two-fold increased risk amongst persons with a recorded history of gastro-oesophageal reflux disease, hiatus hernia, oesophagitis/oesophageal ulcer, or difficulty in swallowing. In a Swedish population-based, nationwide case-control study carried out by Lagergren et al (1999), information on subjects' history of gastro-oesophageal reflux was collected during personal interviews with 189 patients with oesophageal adenocarcinoma and 820 control subjects. Amongst persons with recurrent symptoms of reflux occurring at least once per week, the risk of oesophageal adenocarcinoma was increased eightfold. The more frequent, more severe, and longer lasting the symptoms of reflux, the greater the risk. Amongst persons with longstanding and severe symptoms of reflux, the odds ratio was 43.5 for oesophageal adenocarcinoma (Lagergren et al, 1999). In a case control study of similar design in the United States by Farrow and colleagues (2000), there was also a dose-response relationship between reflux symptoms and oesophageal adenocarcinoma; however, the relative risk estimates were not as high as in the Swedish study. A recent population based cohort of 65,000 male patients with a discharge diagnosis of heartburn, hiatus hernia, or oesophagitis was performed by Ye et al (2001) to investigate the relationship between GORD and oesophageal adenocarcinoma. Virtually complete follow up was attained through record linkage with several nationwide registers, and 37 cases of oesophageal adenocarcinomas were identified. There was a nine-fold increased risk of oesophageal adenocarcinoma amongst patients with an endoscopically verified oesophagitis. The risk estimates increased with increasing follow up time. Based on all these four studies, it is possible to establish that reflux is a major risk factor for oesophageal adenocarcinoma. This may be through the development of Barrett's oesophagusor/Specialised Intestinal Metaplasia (SIM) which is thought to develop in 8 – 14% of patients with chronic GORD (Kim et al, 1997), and is a metaplastic precursor to oesophageal adenocarcinoma (Reid et al, 1996; Mayne et al, 2002), but whether the inflammation to adenocarcinoma sequence can bypass the development of SIM is unknown.

1.2.2 Specialised Intestinal Metaplasia/Barrett's Oesophagus

The strongest risk condition for oesophageal adenocarcinoma is Barrett's oesophagus (Lagergren, 2005). Barrett's Oesophagus or specialised intestinal metaplasia (SIM), a complication of long-standing gastro-oesophageal reflux disease, is the only known precursor lesion for adenocarcinoma of the oesophagus (Nilsson et al, 2003). Like adenocarcinoma of the oesophagus, the prevalence of Barrett's oesophagus has also been rising in Europe and North America (Cameron & Lomboy, 1992). The risk of adenocarcinoma amongst patients with Barrett's oesophagus has been estimated to be 30 to 60 times that in the general population (Spechler et al, 1984; Cameron et al, 1985; Van der Veen et al, 1989; Drewitz et al, 1997).

It is now well established that Barrett's Oesophagus is a complication of severe and long-standing gastro-oesophageal reflux and is found in 10-16% of such patients at endoscopy (Winters et al, 1987). Pathophysiological studies have shown that patients with Barrett's oesophagus show a higher proportion of lower oesophageal sphincter failure, and peristaltic dysfunction than patients with erosive oesophagitis, and over 90% have an associated hiatal hernia (Stein et al 1992). Barrett's Oesophagus is also associated with higher levels of acid exposure than erosive oesophagitis and duodeno-gastro-oesophageal exposure as measured by Bilitec monitoring, particularly in the presence of complications (Attwood et al, 1993; Kauer et al, 1995). Therefore, patients with Barrett's are at the extreme end of the pathophysiological spectrum of gastro-oesophageal reflux disease (Caygill et al, 2004).

Short segment Barrett's oesophagus (SSB), i.e. Barrett's mucosa less than 3cm in length, is found in 8-20% of adult individuals, making it more prevalent than long-segment Barrett's oesophagus (1% adult prevalence) (Morales et al, 1997; Chalasani et al, 1997, Johnston et al, 1996). Despite this fact, only 35% of oesophageal adenocarcinomas arise in short segment Barrett's oesophagus; therefore, the true cancer risk in SSB is presently unclear but probably lies between 0.03-1% (Morales et al, 1997). To date no treatments have been shown to reverse the progression of Barrett's oesophagus completely or to alter its natural history once it has developed (Barr et al, 1996). Even after prolonged high-dose proton-pump inhibition or successful anti-reflux surgery, fewer than 10% of Barrett's cases regress, and progression to cancer may occur over a short period of 3 years (Prach et al, 1997; Sagar et al, 1995). Five-year survival rates of 35-45 % have

been reported for cancers detected in endoscopic surveillance, thus highlighting the importance of Barrett's surveillance (Jankowski et al, 1999).

There are very few studies on lifestyle factors and Barrett's Oesophagus thus making it impossible to say anything concrete at this stage. The available evidence suggests that neither alcohol consumption nor tobacco use have an effect (Caygill et al, 2004). One study (Logan & Riddick, 1990) found past smoking to be moderately connected with Barrett's Oesophagus development, possibly as a result of the effect of smoking on promoting gastro-oesophageal reflux. Another study (Caygill et al 2002) suggested a role for obesity in young Barrett's Oesophagus patients. In this context it is of interest that Barrett's Oesophagus occurs as a complication of long standing GORD (Winters et al 1987) which, itself, is a complication of obesity.

1.2.3 Alcohol

Both alcohol consumption and smoking are strong established risk factors for SCC. There is consistent epidemiologic evidence for elevated risk of SCC with alcohol consumption, and the risk increases with the amount of alcohol consumed (Blot & McLaughlin, 1999). Among heavy drinkers ($\geq$ 12 drinks/week), relative risks range from 2.9 - 7.4 (Blot & McLaughlin, 1999; Gammon et al, 1997; Bahmanyar & Ye, 2006). Several studies have reported a dose response relationship between alcohol intake and risk of SCC (Linblad et al, 2005; Lee et al, 2005); with some authors reporting reduction in risk of SCC several years after quitting drinking. It is thought that alcohol may act as a direct irritant to the oesophageal epithelium, may increase susceptibility to other carcinogens, or may contribute to dietary deficiencies that predispose to SCC (Holmes & Vaughan, 2007). Other authors have suggested that alcohol consumption is likely related to socio-economic status in some populations, which is inversely related to SCC risk (Tran et al, 2005).

A synergistic effect of alcohol and smoking on the risk of SCC has also been reported (Crew & Neugut, 2004). A case-control study from Italy and Switzerland addressed SCC with a family history of cancer as the primary exposure, and smoking and alcohol together as the secondary exposures (Garavello et al, 2005). For those without a family history of oesophageal cancer, the odds ratio for current smokers consuming $\geq$49 drinks per week was 15.5, compared with non-smokers consuming less then this amount of

alcohol. For subjects with a family history of oesophageal cancer and the same smoking and alcohol histories, the OR for SCC increased to 107 (Garavello et al, 2005).

Alcohol does not appear to be an important risk factor for adenocarcinoma of the oesophagus (Blot & McLaughlin, 1999; Brown & Devesa, 2002; Crew & Neugut, 2004; Lagergren, 2005; Lindblad et al, 2005, Zhang et al 1997; Menke-Pluymers et al, 1993). Three large population based case-control studies have probably provided the most reliable results hitherto concerning the influence of alcohol on the risk of adenocarcinoma of the oesophagus (Levi et al, 1990; Gao et al, 1994; Lagergren et al, 2000; Gammon et al 1997; Wu et al, 2001). Based on data from all these studies, it may be concluded that alcohol is not associated with an increased risk of oesophageal adenocarcinoma (Lagergren, 2005).

1.2.4 Smoking

Smoking greatly increases the risk of SCC. Prospective epidemiologic data show that smokers have a 5-fold higher risk than non-smokers, with a risk of heavy smokers of nearly 10-fold (Blot & McLaughlin, 1999). However the risk of SCC decreases rapidly after smoking cessation, with a substantial decline within 5-10 years (Blot, 1999; Brown & Devessa, 2002; Crew & Neugut, 2004). In contrast to alcohol where the *intensity* of consumption seems to be important in SCC, the *duration* of smoking seems to be of greater importance than the amount of tobacco smoked (Lee et al, 2005; Tran et al, 2005).

Smoking increases the risk of adenocarcinoma, although not nearly as strongly as for SCC (Holmes & Vaughan, 2007). Eight case control studies have reported a moderately increased risk of oesophageal adenocarcinoma and/or gastric cardia among tobacco smokers, with ORs of generally about 1.5-2.8 (Vaughan et al, 1995; Wu et al 2001; Kabat et al, 1993; Li et al, 1989; Wu-Williams et al, 1990; Brown et al, 1994; Gonzalez et al, 1994). However some studies have found no association between smoking and adenocarcinoma (Lagergren et al, 2000; Gao et al 1994; Levi et al, 1990). Taken together, any association with smoking seems to be of moderate strength (Lagergren, 2005). In addition, data that supports a role for tobacco as an aetiologic risk factor for adenocarcinoma does not explain the rising incidence of this cancer at a time when SCC is stable or decreasing in incidence, considering recent reductions in the prevalence of cigarette smoking in the general population (Zhang et al, 1997, Pera et al, 2005).

1.2.5 Diet and Nutrition

The potential role of dietary factors in the aetiology of oesophageal cancer has attracted considerable attention in previous epidemiologic studies (Pera et al, 2005). The majority of published studies relate to SCC, with little information available for adenocarcinoma. High intakes of fresh fruits and vegetables, especially if eaten raw, and of antioxidants are associated with decreased risk of both major types of oesophageal cancer (Holmes & Vaughan, 2007; Terry et al, 2001). Case-control studies worldwide have shown lower intakes of fruit and vegetables in subjects with SCC and adenocarcinoma, with an average two-fold increase in risk with low intakes (Tran et al, 2005; DeStefani et al, 2005; Navarro et al, 2004). A high intake of calories and fat has also been associated with strongly increased risk (Zhang et al, 1997; Chen et al, 2002; Mayne & Navarro, 2002) as well as dietary cholesterol and animal protein (Mayne et al, 2001; Navarro et al, 2004). Several studies have shown that some foods and nutrients are protective factors against adenocarcinoma including fruit and vegetables, lutein, niacin, beta-carotene, folate, iron, zinc and vitamins B6, B12 and C (Brown et al, 1995; Chen et al, 2002; Terry et al, 2001; Takesaki et al, 2001; Zhang et al, 1997). Antioxidants such as Vitamin C, beta-carotene and alpha-tocopherol have the potential to neutralise the harmful effects of DNA-damaging free radicals, such as those produced by smoking. A protective effect of dietary fibre on the risk of adenocarcinoma has also been reported (Terry et al, 2000; Brown et al, 1995; Mayne & Navarro, 2002; Zhang et al 1997). However, data concerning diet and oesophageal adenocarcinoma remains limited and susceptible to bias, particularly the role of confounding by dietary variables, which is a source of error that is difficult to reliably adjust for. Therefore, more well-designed studies are needed to establish new dietary risk factors (Lagergren, 2005).

1.2.6 Helicobacter Pylori Infection

Helicobacter Pylori (H. Pylori) is an important risk factor for non-cardia gastric adenocarcinoma, however it is not associated with increased risk of cancer of the oesophagus or oesophago-gastric junction (Huang et al, 1998). In fact, an inverse relationship between CagA+ strains of H. Pylori infection and the risk of adenocarcinoma and OG junction adenocarcinoma has been reported (Chow et al, 1998; Wu et al 203; Ye et al, 2004; Graham & Yamaoka, 1998; Vieth et al, 2000; Weston et al, 2000; Siman et al, 2001). Infection with Helicobacter Pylori reduces the risk of oesophageal adenocarcinoma by 60-80% (Ye et al, 2004; Chow et al, 1998). The postulated mechanism for the protective effect of Helicobacter Pylori is through it's

ability to cause atrophic gastritis, and possibly by increasing intra-gastric ammonia production which results in a higher pH in gastric juice and refluxate (Richter et al, 1998). However other recent studies have shown that the inverse association remains unaffected after adjustment for gastric atrophy (Ye et al, 2004).

1.2.7 Socio-economic status

Low Socio-economic status (SES), measured by income, education, occupation and other variables, is associated with a higher risk of SCC (Holmes & Vaughan, 2007). It has been suggested that low SES may be the underlying factor explaining the association between poor nutrition and low BMI with SCC (Tran et al, 2005). However, in studies where SES risk has been adjusted for smoking, alcohol and intake of fruit and vegetables, the ORs were not attenuated, suggesting that SES did not act through these mechanisms (Janson et al, 2005). For adenocarcinoma, low SES also appears to be a risk, but the effect does not appear to be as strong as for squamous cell carcinoma (Brown & Devessa, 2002). In contrast to SCC, when studies assessing the risk of adenocarcinoma based on SES were adjusted for other risk factors such as reflux, BMI and smoking, the trend is no longer significant suggesting that for adenocarcinoma, SES acts largely through these established risk factors (Jansson et al, 2005).

1.2.8 Heredity

Although familial clustering of both Barrett's oesophagus and oesophageal adenocarcinoma occurs (Romero et al, 1997; Chak et al, 2002; Jochem et al, 1992; Eng et al, 1993; Poynton et al, 1996), the influence of genetic factors in the aetiology of oesophageal adenocarcinoma seems to be of limited importance (Lagergren, 2005). In three population based studies of familial occurrence, no evidence of family history of digestive cancer among cases of oesophageal adenocarcinoma was found (Zhang et al, 1996; Lagergren et al, 2000; Dhillon et al, 2001). Hence, the aetiology of oesophageal adenocarcinoma is dominated by non-genetic risk factors. Moreover, the recent increase in incidence of oesophageal adenocarcinoma is not related to hereditary factors because a change of gene pool in 20–30 years is unlikely (Lagergren, 2005).

1.3 NUTRITIONAL EPIDEMIOLOGY OF OESOPHAGEAL ADENOCARCINOMA: OBESITY

1.3.1 Obesity: Definition and Scale of the Problem

The relationship between excess body weight and mortality has long been recognised and is well established in the literature (Manson et al, 1995; Willett et al, 1995; Stevens et al, 1998; Lindsted et al, 1998; Calle et al, 1999; Kopelman, 2000). Obesity has recently come to light as an important risk factor for many cancers, including oesophageal cancer (Calle et al, 2003), and is thought to be a driving force behind recent epidemic increases in adenocarcinoma of the oesophagus (Holmes & Vaughan, 2007). Obesity is diagnosed using Body Mass Index (BMI) criteria - this is computed as weight in kilograms divided by height in meters squared (kg/m^2). Cut-offs used by the World Health Organisation (1998) are: 20-25 kg/m^2 normal, overweight 25-29.9 kg/m^2, and obese >30 kg/m^2. Obesity is further classified as Grade I (BMI 30-35) Grade II (35-40) or Grade III (BMI > 40).

Obesity has reached epidemic proportions globally, with more than 1.7 billion adults overweight and 300 million clinically obese (WHO, 1998). Currently 64.5% of U.S. adults age 20 years or over are overweight (approx 127 million) and 30.5 % are obese (60 million obese and 9 million severely obese). In Europe, about half the adult population are currently estimated to be either overweight or obese (WHO 2002). The Republic of Ireland, like other Western countries, has witnessed a marked increase in the prevalence of obesity since the early 1990s, the prevalence increasing by 67%. Sixty seven percent of men and 75% of women over 51 years of age are either overweight or obese (McCarthy et al, 2002).

1.3.2 Obesity and Cancer

Although the associations between obesity and diabetes, cardiovascular disease, and various digestive and musculoskeletal disorders are well documented, the relationship of obesity with overall cancer and site-specific cancers has only been examined in the last 30 years. There is growing evidence that overweight and obesity are associated with many cancer sites (Bianchini et al, 2002). In fact, obesity is one of the strongest emerging risk factors for many cancers in Western Countries (Kubo & Corley, 2006).

In the largest prospective cohort investigation of the role of overweight and obesity and cancer mortality, Calle et al (2003) followed over 900,000 American adults for 16 years. Analyses were adjusted for many potential confounding variables, including smoking status, physical activity, alcohol use, fat consumption and vegetable consumption. Compared with men whose BMI was in the normal range, men with obesity had significant increases in cancer mortality from oesophageal, colorectal, liver, gallbladder, pancreatic, prostate, kidney, non-Hodgkin's lymphoma, multiple myeloma and leukaemia. Among women, high BMI was associated with greater mortality from colorectal, liver, gallbladder, pancreatic, breast (post-menopausal), uterine, cervical, ovarian and kidney cancers, and from non-Hodgkin's lymphoma and multiple myeloma. The heaviest members of the cohort (BMI>40) had death rates from all cancers that were 52% higher for men and 62% higher for women than the rates in men and women of normal weight (Calle et al, 2003). Taken together, the authors estimate that obesity is responsible for up to 14% of all deaths from cancer in men, and 20% of all deaths from cancer in women in the US – a staggering 90,000 cancer deaths may thus potentially be avoided annually if BMI was kept below 25 kg/m^2 (Calle et al, 2003). Similar figures are not available for Europe, but it is estimated that 36,000 cancer cases could be avoided annually by halving the prevalence of overweight & obesity (Bergstrom et al, 2001).

1.3.3 Obesity & Oesophageal Cancer: Epidemiological Evidence

Increasing epidemiological evidence strongly links obesity with the incidence of oesophageal adenocarcinoma (Calle et al 2003, Lagergren et al 1999; Chow et al 1998; Vaughan et al 1995; Brown et al 1995; Engel et al, 2003) and it is thought to be a responsible for up to 40 per cent of cases (Engel et al, 2003). There are 17 studies in the scientific literature addressing BMI and Oesophageal Cancer - 8 from North America/Canada, 5 from Europe, 3 from China and 1 from Australia (they are summarised in **Table 1.1**).

Table 1.1: Published Studies on Obesity and Oesophageal Adenocarcinoma

Author	Design	Country	No.cases/ Controls	Adjusted for	Exposure Definitions BMI Reference	BMI overwt & obese	Results OR(95% CI)	
NORTH AMERIAN/CANADIAN STUDIES								
Brown (1995)	Case-Control	USA	174/750	A,C,E,L,T,S	<23.1	≥26.6	OR (OA)	3.1 (1.8-5.3)
Vaughan (1995)	Case-control	USA	298/724	A,E,T,R,S	<25	>30	OR(OA)	2.5 (1.2-5.6)
							OR(CA)	1.6 (0.8-3.0
-Chen (2002)	Case Control	USA	124/449		<25	≥25	OR (OA)	1.1(1.04-1.18)/BMI unit increase
-Chow (1998)	Case Control	USA	493/695	A,G,L,R,T	<23	≥27	OR (OA)	2.9(l1.8-4.7) p<0.0001
							OR(CA)	1.6(1.1-2.6) p=0.008
-Wu (2001)	Case Control	USA	499/1,356	A,G,R,S,T,Y	<23	≥28	OR (OA)	2.8 (1.7-4.4) p<0.0001
							OR(CA)	2.1 (1.4-3.2) p=0.0016
-Kabat (1993)	Case Control	USA	173/4,544	A,D,E,L,S,T	<22	≥28	OR (OA&CA)	1.2(0.6-2.4) p=ns
-Zhang (1996)	Case Control	USA	95/132	A,B,D,E,G,H,K,R,S,T	<25	≥25	OR (OA&CA)	0.93 (0.8-1.03)p=ns
Calle (2003)	Cohort	USA	1,065/900,053	A,P,S,M,E,T,R,D	<25	>35	OR (OA&SCC)	1.63 (0.95-2.8) p=0.008
Veugelers (2006)	Case Control	Canada	57/102	T,E,V,C,	<20	≥30	OR(OA)	4.67(1.27-17.9)P=0.001
EUROPEAN STUDIES								
-Incarbone (2000)	Case Control	Italy	262/262		<25	≥25	OR (OA)	OR not calculated
Engeland (2004)	Cohort	Norway	575/2 million	A	<25	≥30	OR (OA)	2.58 (1.81-3.68) p=0.001
-Cheng (2000)	Case Control	UK	74/74	F,D	<19.5	≥22.7	OR (OA)	6.04 (1.3-28.5) p= 0.002
-Lagergren (1999)	Case Control	Sweden	451/820	A,C,D,E,G,HPST	<22.3	≥25.6	OR (OA)	16.2(6.3-41.4) p=0.0001
							OR(CA)	4.3(2.1-8.7) p=0.0001
Linblad (2005)	Case Control	UK	287/10,000	A,G,S,E,H	20-25	≥25	OR (OA)	1.93(1.24-3.01) p=0.005
							OR(CA)	1.46(0.84-2.54) p=0.04
AUSTRALASIAN STUDIES								
MacInnis (2006)	Cohort	Australia	30/41,295	Y,G,P,S	<25	>30	OR (OA&CA)	3.7(1.1-12.4)P=0.03
Tran (2005)	Cohort	China	1089/29,584	A,G	<20	≥23	OR(CA)	0.95(0.8-1.13)p=ns*
-Ji (1997)	Case-Control	China	185/1451	A, T, E, S, H	<19.5	≥22.2	OR (CA)	3.0(1.7-5.4)p=0.01
-Zhang (2003)	Case Control	China	300/258		18.5-24	≥24	OR(CA)	0.16(0.05-0.44)P<0.005

A=Age, B=Barrett's, C=energy intake, D=Diet, E=Alcohol, F=breast-feeding, G=gender, H=History of ulcer, reflux or gastric disease, J=family history, K=History of hypertension, L=Location, area, hospital, M=marital status, P=physical activity, R=Race, S=SES, education, T=Tobacco, V=Vitamin intake, Y=birthplace, Z= time between interview & disease, OA=Oesophageal Adenocarcinoma, CA=Cardia adenocarcinoma, SCC=squamous cell carcinoma, OR=Odds Ratio, UK=United Kingdom, USA=United States of America, ns=non-significant.

1.3.3.1 North America/Canada

The first population-based case-control study to investigate dietary and nutritional risk factors for adenocarcinoma of the oesophagus was carried out by Brown and colleagues (Brown et al, 1995) in 1995: 174 males with adenocarcinoma and 750 control subjects in three areas of the US were studied from 1985 to 1989, and they reported an increased risk (OR 3.1) in the heaviest quartile compared with the lightest quartile. Vaughan and colleagues (Vaughan et al 1995), in a case control of 404 cases of oesophageal cancer (298 adenocarcinoma and 106 SCC) and 724 healthy controls, reported that patients in the highest decile of BMI had the greatest risk of oesophageal adenocarcinoma (OR 2.5, 95% CI 1.2-5.6), and gastric cardia adenocarcinoma (OR 1.6(95%CI, 0.8-3.0) and the risk of SCC was inversely related to BMI (OR 0.2 95%CI, 0.1-1.0). Another study, by Chow and colleagues (Chow et al 1998) examined anthropometric risk factors in a population-based case control study of 589 cases of SCC and 554 cases of adenocarcinoma of the oesophagus and gastric cardia, along with 695 healthy control subjects. The risk of adenocarcinoma rose with increasing BMI (OR 2.9 (95% CI, 1.8-4.7, p=0.0001 for highest quartile), and the magnitude of the association was greatest among the younger age groups and among non-smokers.

In a case-control study of 499 cancer cases and 1,356 healthy controls, Wu et al (2001) reported an odds ratio for oesophageal adenocarcinoma of 2.8 (95% CI, 1.7-4.4, $p<0.0001$) for subjects with a BMI $\geq$ 28 kg/m^2 versus < 23 kg/m^2 and an odds ratio for gastric cardia adenocarcinoma of 2.1 (95%CI, 1.4-3.2, p=0.0016). In a study that mainly addressed dietary risk factors for oesophageal and gastric malignancy, Chen and colleagues (2002) studied 124 cases of adenocarcinoma and 449 healthy controls in a case-control study. They found that BMI was linearly, and positively, associated with the risk of oesophageal adenocarcinoma: odds ratio of 1.1(95% CI, 1.04-1.18) per unit increase in BMI. Calle and colleagues (2003) studied over 900,000 US adults over 16 years. From the total population 876 deaths from oesophageal cancer in males and 189 deaths in women occurred. A significant association between BMI and death from oesophageal cancer was seen in males, with a relative risk of 1.63 (0.95-2.8, p=0.008), but no effect was observed in women. This study did not differentiate between adenocarcinoma and SCC. The only negative study from the United States was a very early study published in 1993 by Kabat and colleagues. The main focus of this study (173 cases of oesophageal/gastric cardia adenocarcinoma, 136 SCC, 4,544 hospital controls) was to examine the effect of tobacco, alcohol intake and diet as risk factors.

When the authors examined the effect of BMI they used a cut off of >28 kg/m^2 as the referent value compared to individuals with BMI <22 kg/m^2, (in contrast to more recent studies). They found that the risk of oesophageal and gastric cardia adenocarcinoma was inversely related to BMI (OR 1.2 (0.6-2.4) although this result was not statistically significant. Another study by Zhang et al (1996) on 95 cases of oesophageal and gastric cardia adenocarcinoma and 132 controls reported a non-significant relationship between BMI and risk of cancer, odds ratio of 0.93 (95%CI, 0.83-1.03). It is not clear from this paper how the odds ratio was calculated nor was the effect of BMI the main focus of the study.

The only Canadian study to examine the effect of BMI on oesophageal adenocarcinoma was performed by Veugelers et al (2006). In this prospective hospital based case-control study 57 cases of oesophageal adenocarcinoma were compared to 142 proven GORD cases, 130 Barrett's cases and 102 healthy controls. The OR for oesophageal adenocarcinoma for BMI > 30 kg/m^2 versus normal was 4.67 (95% CI, 1.27-17.9).

1.3.3.2 European studies

The first European study was published by Lagergren and colleagues in 1999 who conducted a nationwide, population-based case-control study in Sweden of 189 cases of adenocarcinoma of the oesophagus, 262 cases of gastric cardia adenocarcinoma, 167 cases of oesophageal SCC, and 820 controls, and reported a significant dose-dependent relationship between BMI and oesophageal adenocarcinoma. The adjusted odds ratio was 7.6 amongst persons in the highest BMI quartile compared to persons in the lowest quartile. This study was followed by an Italian study carried out by Incarbone et al (2000). Here, 262 patients with adenocarcinoma of the oesophagus, gastric cardia and stomach were compared with 262 control subjects, and 138 GORD cases. This study did not calculate the odds ratio for cancer according to BMI as previous studies had done, but simply reported that adenocarcinoma cases were significantly heavier than SCC cases but not significantly heavier than controls. Unfortunately this study did not exclude non-cardia gastric adenocarcinoma cases from the analysis.

In the UK, Cheng and colleagues (2000) conducted a small case-control study of 74 women with adenocarcinoma of the oesophagus and showed that a high BMI at the age of 20 years and low consumption of fruit was associated with increased risk (odds ratio 6.04 for highest BMI quartile versus lowest). Engeland and colleagues (2004) in a cohort study followed over 2 million Norwegians for an average of 23 years and

recorded 575 cases of oesophageal adenocarcinoma. This study did not distinguish between histological sub-site nor did it control for other well known risk factors such as alcohol intake, diet, age, or socio economic status, but reported that obese men had a relative risk of death from adenocarcinoma of the oesophagus 2.58 times that of normal weight men (95% CI, 1.81-3.68). The fifth and most recent European study was reported by Linblad and colleagues (2005) who conducted the second UK case-control study using the General Practitioner Research Database. During follow up of 4,340,207 person-years, 287 cases of oesophageal adenocarcinoma and 196 gastric cardia adenocarcinoma were identified and compared to 10,000 controls. A dose-dependent relationship between BMI and oesophageal (OR 1.67, 95% CI 1.22-2.3) and gastric cardia (1.46, 95% CI 0.98-2.18) adenocarcinoma was identified, the association being independent of reflux symptoms.

1.3.3.3 Australia and Asia

In a prospective cohort study from Australia, McInnis et al (2006) followed 41,295 subjects for 11 years. Detailed body composition information from bioelectrical impedance analysis was performed at baseline. By follow up 11 cases of lower oesophageal adenocarcinoma, 19 gastric cardia tumours were identified - too few cases to perform separate analysis for individual sites. The Hazard ratio (HR) of adenocarcinoma of the lower oesophagus and gastric cardia for individuals with a BMI > 30 kg/m^2 compared with a BMI of < 25 kg/m^2 was 3.7 (95% CI, 1.1-12.4). For every 10cm increase in waist circumference the HR was 1.46 (95% CI, 1.05 – 2.04) and for every 10kg increase in fat free mass the HR was 2.06 (95% CI, 1.15-3.69).

All of the epidemiological studies from Asia are from Chinese studies which solely looked at BMI and the risk of gastric cardia adenocarcinoma (Tran et al, 2005; Ji et al, 1997; Zhang et al, 2003). Several authors note that great differences exist in genetic background, lifestyles, dietary habits, and smoking and alcohol consumption between Chinese and Westerners (Zhang et al, 2003) and these factors are important considerations when comparing obesity's relationship to cancers across different Continents. Only one Chinese study - that of Ji et al (1997) has reported a relationship between obesity and risk of gastric cardia adenocarcinoma. In this population-based case-control study in Shanghai of 1,124 cancers of the cardia (185) and stomach (939), Ji et al (1997) reported significantly elevated risk for adenocarcinoma of the gastric cardia of 3.0 (95% CI, 1.7-5.4) for the highest BMI quartile versus the lowest – but the risk was unique to males only. Zhang and colleagues (2003) studied 330 cases of gastric cardia

adenocarcinoma, and 258 controls in Northern China, and reported an inverse relationship between obesity and risk of gastric cardia adenocarcinoma. In another population based prospective study of 29,584 adults followed for 15 years in Linxian China, Tran et al (2005) reported on 1,089 cases of gastric cardia malignancy and found no association between BMI and risk of gastric cardia cancer – Odds Ratio 0.95 (95%CI, 0.8-1.13) for individuals with a BMI > 23 kg/m^2 versus < 23 kg/m^2. Of note in this study is the very low socio economic status and poor levels of education reported as well as median BMIs that are significantly lower than studies in Western countries, possibly reflecting poor nutritional status in this area of China.

1.3.4 Obesity and Adenocarcinoma of the Gastric Cardia

Obesity has been shown to be a risk factor for adenocarcinoma of the gastric cardia but the relationship is weaker than that of oesophageal adenocarcinoma (Wu et al, 2001; Lagergren et al, 1999; Chow et al, 1998; Linblad et al, 2005; Kubo & Corley, 2006). Meta-analysis of studies examining BMI and risk of adenocarcinoma of the oesophagus and gastric cardia suggests that the BMI-cancer association is strongest for oesophageal adenocarcinomas that are >2cm from the gastro-oesophageal junction (Kubo & Corley, 2006) – the risk increases with increasing distance from the gastro-oesophageal junction. Obesity does not seem to have any role in the pathogenesis of non-cardia gastric adenocarcinoma (Mc Innis et al, 2006; Linblad et al, 2005).

1.3.5 Obesity and Oesophageal Squamous Cell Carcinoma

The available evidence to date suggests that obesity is inversely related to squamous cell carcinoma of the oesophagus (Lagergren et al, 1999; Incarbone et al, 2000; Chow et al, 1998) however the mechanisms remain poorly understood but may relate to lower BMI observed in heavy smokers and drinkers.

1.3.6 Mechanism of Altered Cancer Risk

Despite epidemiological evidence, the precise biological mechanism by which obesity increases the risk of oesophageal cancer remains unknown. Adipose tissue has long been considered to be metabolically passive and primarily responsible for energy storage. However, recent scientific advances have dramatically altered our understanding of the function of this tissue. Abdominal adipose tissues are metabolically active, secreting a variety of biologically active substances that are important in the pathogenesis of insulin

resistance, dyslipidaemia, glucose intolerance, hypertension, hypercoagulable state, and cardiovascular risk (Ahima & Flier, 2000; Fruhbeck et al 2001).

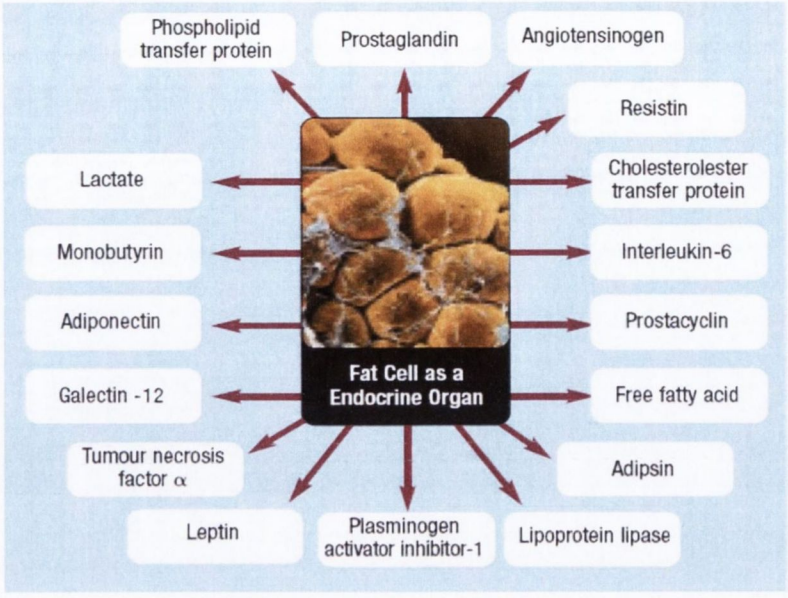


Figure 1.2: Fat cells as an endocrine organ

1.3.7 Abnormal Body Composition: Abdominal Adiposity

Crucial to the obesity-cancer risk is the effect of body composition and fat distribution. Adipocyte anatomy (size), physiology (growth, catecholamine sensitivity, lipolysis, insulin action), and biochemistry (leptin, plasminogen activator inhibitor-1, cytokines, rennin-angiotensin system) are reported to be site-specific, highlighting unique roles of regional adipose tissue depots. It has been shown that among equally overweight or obese individuals, those characterised by an increase in abdominal fat (waist circumference >102 cm in males, >88 cm in females) are at increased risk of Type II diabetes, Cardiovascular Disease, and certain cancers (Ohlson et al 1985; Rexrode et al, 1998; Macinnis et al, 2006; Giovannucci et al, 1995; Bray, Lancet, 1998). Computerised Tomography (CT) assessment of visceral adiposity shows that those individuals with an excess of visceral adipose tissue are characterised by the most substantial adverse alterations in their metabolic risk profile (Pouliot et al, 1994) (See figure 1.3). Visceral adipose tissue depots are the most metabolically active and appear to be important for the pathogenesis of insulin resistance, dyslipidaemia, glucose intolerance, hypertension, hypercoagulable state, and cardiovascular risk. It is known that for the same BMI, the distribution of body fat tends to be more visceral than truncal in Caucasians (compared with African-Americans), and men (compared with women) (Weinsier et al, 2001). Males deposit fat preferentially in the intra-abdominal region at all ages in contrast to

females who deposit sub-cutaneous adipose tissue predominantly in youth, and only post middle age do females tend to deposit intra-abdominal adipose tissue preferentially (Misra & Vikram et al, 2003).

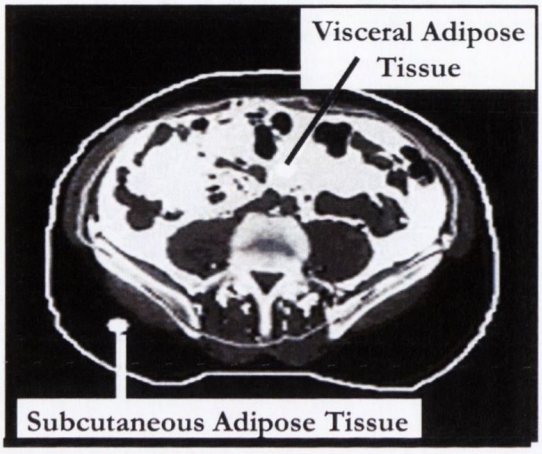


Figure 1.3: Abdominal CT scan at level of L6 showing deposits of subcutaneous and visceral adipose tissue

The physiological mechanism linking adiposity to cancer risk may be through alterations in endogenous hormone metabolism including insulin, bio-available sex steroids, insulin- like growth factor I (IGF 1) and IGF binding proteins (IGFBPs) (Figure 1.4). A metabolic consequence of obesity, and specifically the accumulation of intra-abdominal fat, is the development of insulin resistance, which leads to an increase in secretion of insulin from the pancreas. Chronically increased insulin concentrations reduce the synthesis of IGF-binding protein-1 and 2 (IGFBP1 and 2). As a result, IGF1 activity increases predominantly in the liver, which is the main source of circulating IGF1 and IGFBPs, and is the only source of sex-hormone binding globulin (SHBG). Insulin and IGF1 both inhibit the synthesis of SHBG—the major carrier protein for testosterone and oestradiol in the plasma—and may lead to an increase in the amount of unbound sex-steroid available for bioactivity. Furthermore, in men and postmenopausal women, adipose tissue is a major site for the synthesis of oestrogens (oestrone, and oestradiol) from androgenic precursors. Adiposity also leads to increased peripheral formation of oestrogen from androgen precursors with higher oestrogen concentrations in men and postmenopausal women. These changes are important because sex steroids (androgens, oestrogen, and progesterone) and insulin are known to regulate the balance between cellular differentiation, proliferation, and apoptosis, and alterations in their metabolism may favour the selective growth of pre-neoplastic and neoplastic cells (Dickson et al,

1990). Insulin and IGF1 also strongly stimulate cell proliferation, inhibit apoptosis, and can enhance angiogenesis (Khandwala et al, 2000).

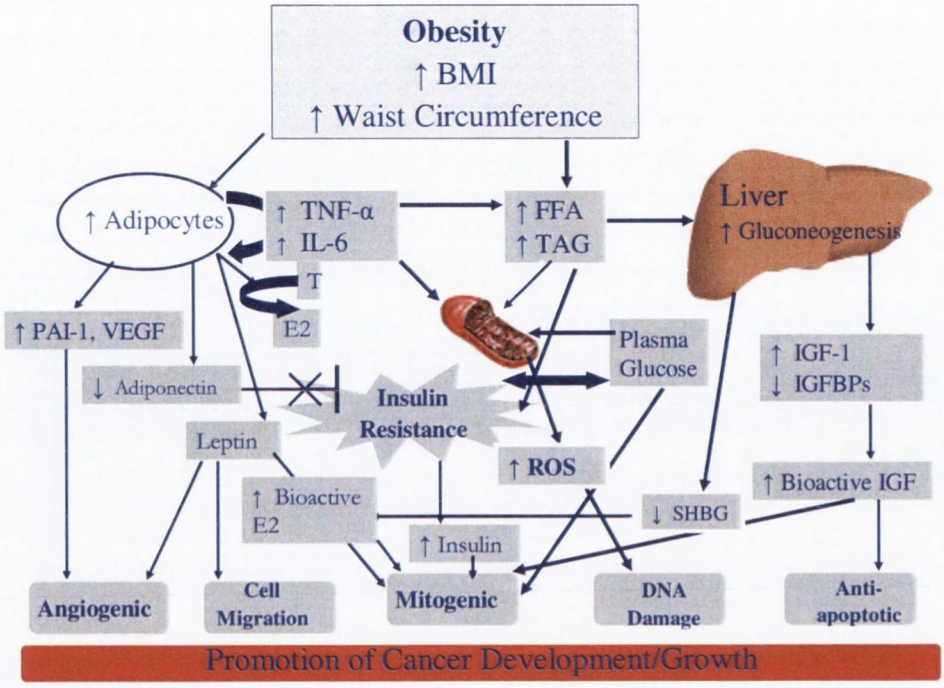


Figure 1.4: Plausible mechanisms linking central obesity and insulin resistance to Cancer Development

TNF=tumour necrosis factor, IL=interleukin, FFA=Free Fatty Acids, TAG=Triglyceride, IGF=Insulin Like Growth factor, IGFBP=Insulin like growth factor binding proteins, SHBP=sex hormone binding globulins, PAI = Plasminogen activator inhibitor, ROS=reactive oxygen species, BMI=body mass index

1.3.8 Obesity as an Inflammatory condition: Adipocytokines

Recent studies have shown that fat cells exert a number of important endocrine and immune functions. These are achieved predominantly through release of adipocytokines, which include several novel and highly active molecules released abundantly by adipocytes like leptin, resistin, adiponectin as well as some more classical cytokines released by inflammatory cells infiltrating fat, like Tumour Necrosis Factor alpha (TNF-α), Interleukin (IL)-1, IL-6, IL-8, IL-10 Monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1 (MIP 1) (Guzik et al, 2006; Cowey & Hardy, 2006). Adipose tissue expresses pro-inflammatory cytokines such as Interleukin-6, releases it into circulation (Fried et al, 1998) and is responsible for the production of about 25% of systemic IL-6 in vivo (Mohamed-Ali-V et al, 1997; Guzik et al, 2006). IL-6 in turn stimulates acute phase protein production in the liver, inducing a state of low-grade systemic inflammation in persons with excess body fat (Visser et al, 1999). A proinflammatory state is recognised by elevated C-reactive protein (CRP) levels and is

commonly present in people who are centrally obese (Yudkin, 2003). A significant relationship has been reported between plasma CRP levels and measures of adiposity and of insulin resistance (Lemieux et al 2001).

1.3.9 Adipokines – Leptin, Resistin and Adiponectin

Fat cells also secrete a number of adipokines. Leptin, a protein produced by adipose tissue is secreted in proportion to adiposity. Leptin exerts its effects through interaction with a specific cell membrane localized receptor (Ogunwobi et al, 2006). The effects of leptin on body weight and peripheral energy expenditure are well described (Friedman et al, 1998), but it has become apparent that leptin has a plethora of other activities including the regulation of angiogenesis, wound healing, fertility, immune function, and renal and lung functions (Sierra-Honigmann et al, 1998; Lord et al, 1998; Tsuchiya et al, 1999). Recent studies have shown that leptin stimulates proliferation of several cancer cell lines causing growth potentiation in breast, oesophagus, colon and prostate cancer in vitro (Somasundar et al, 2003). Other studies have demonstrated that leptin can stimulate proliferation of insulin-secreting tumour cell lines by suppression of apoptosis (Okuya et al, 2001). Recent research has shown that Barrett's epithelium and adenocarcinoma have functional leptin receptors *in vivo* through which leptin can exert the biological effects of inhibition of apoptosis, stimulation of proliferation, and increased COX-2 mRNA. (Ogunwobi et al, 2006). While research is in the very early stages, both leptin and leptin antagonism may have potential efficacy in cancer therapy based on cellular origin.

In contrast to Leptin, adiponectin seems to have several beneficial and protective effects including anti-inflammatory, vasculo-protective and anti-diabetic effects (Guzik et al, 2006). Adiponectin exerts insulin-sensitising effects and levels are decreased in obese subjects. Adiponectin is inversely related to breast, endometrial and gastric cancer risk (Ishikawa et al, 2005) by inhibiting inflammation and insulin resistance, both of which are involved in cancer progression (Cowey & Hardy, 2006). Resistin is another key adipocytokine that exerts pro-inflammatory effects. It conveys resistance to insulin and levels are increased in obesity. Release of resistin is stimulated by inflammation, LPS, Il-6, hyperglycaemia and growth and gonadal hormones – when released within fat tissue resistin acts on adipocytes themselves leading to insulin resistance (Guzik et al, 2006).

It is still unclear why adipocytes produce so many pro-inflammatory factors in the obese condition (Cowey & Hardy, 2006). These cytokines secreted by adipose tissue are known to promote insulin resistance and increase circulating triglycerides, features of what has become knows as the Metabolic Syndrome (Shoelson et al, 2006).

1.3.10 Metabolic Syndrome

The combination of metabolic disturbances now known as the metabolic syndrome was first described by Kylin in the 1920's as the clustering of hypertension, hyperglycaemia, and gout. Two decades later, Vague noted that upper body adiposity (android or male-type obesity) was the type most often associated with the metabolic abnormalities seen with diabetes and cardiovascular disease (Vague et al, 1947). Over the last 50 years metabolic syndrome has been referred to as "Syndrome X", "The deadly quartet", and "The insulin resistance syndrome" (Reaven et al, 1988; Kaplan et al, 1989; Haffner et al, 1992). It is now agreed that the well-established term "metabolic syndrome", remains the most useful and widely accepted description of this cluster of metabolic abnormalities, which are related to CVD and predict a high risk of developing diabetes.
General features of the metabolic syndrome include: abnormal body fat distribution, insulin resistance, atherogenic dyslipidaemia, elevated blood pressure, and a proinflammatory and prothrombotic state.

Today there are several definitions of the metabolic syndrome. The most widely used are those produced by the World Health Organisation (WHO), The European group for the study of insulin resistance (EGIR) and the national cholesterol education programme-Third adult treatment panel (NCEP ATP III) (WHO 1999; Balkau & Charles 1999; NCEP 2001). In 2006, the International Diabetes Federation published a consensus statement on Metabolic syndrome which considered *all* previous definitions and outlined a new world-wide set of criteria to define metabolic syndrome (Alberti et al, 2006), *see table 1.2.* The new definition differs from the ATP III definition in that it requires evidence of central obesity for the diagnosis of Metabolic syndrome.
It is estimated that approximately 24% of US adults have the metabolic syndrome (Moller & Kaufman, 2005). In an Irish cohort of 1018 adults studied by Villegas et al (2004) the prevalence of metabolic syndrome was 21%.

Table 1.2: International Diabetes Federation metabolic syndrome world wide definition

Central Obesity	Waist Circumference ≥ 94cm European males, ≥ 80 cm European females plus any two of the following:
Raised Triglycerides	≥ 1.7mmol/l or specific treatment for this lipid abnormality
Reduced HDL	<1.03 mmol/l in males or <1.29mmol/l in females
Raised blood pressure	Systolic: ≥ 130mmHg or Diastolic ≥85 mmHg or treatment of previously diagnosed hypertension
Raised Fasting glucose	Fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed Type II Diabetes.

1.3.11 Metabolic syndrome and Cancer

In addition to cardiovascular disease, individual components of the metabolic syndrome have been linked to several processes, including insulin resistance, aromatase activity, adipokine production, angiogenesis, elevated CRP, glucose utilisation, and oxidative stress/DNA damage, which can work together to increase cancer risk beyond that of the individual components alone (Cowey and Hardy, 2006). While there are many studies showing an independent correlation between a single risk factor of the metabolic syndrome and cancer, recent epidemiological evidence has emerged indicating the clustering of the components of the metabolic syndrome increases the risk of colorectal cancer mortality compared with the individual components alone (Colangelo et al, 2002; Trevisan et al, 2001). There are currently no studies in the literature addressing metabolic syndrome and upper GI cancers.

1.3.12 Pathophysiological Mechanisms whereby Metabolic Syndrome/Central Obesity promotes Oesophageal Cancer development

1.3.12.1 Obesity and GORD

Central or android adiposity may increase GORD (La Vecchia et al, 2002; Rigaud et al, 1995). Obese subjects compared with non-obese subjects have elevated intra-abdominal and intra-gastric pressures (Barak et al, 2002, El Serag et al, 2005), an increase of

transient relaxations of the lower oesophageal sphincter (O Brien 1980; Orlando 2001), slower oesophageal transit and abnormal diaphragmatic pinchcock and phreno-oesophageal membrane anatomy (Mathys-Vliegen & Tygat, 1996). Obese individuals are over four times more likely than lean individuals to have a hiatus hernia (HH), and have an overall prevalence of HH of 40% versus 12.6% for the general population (Wilson et al, 1999).

There are numerous studies supporting a positive association between obesity and GORD (Locke et al, 1999; Murray et al 2003; Nilsson et al, 2003; Delgado-Aros et al, 2004; Nandurkar et al, 2004; Diaz-Rubio et al, 2004; Wilson et al, 1999; Chang et al, 1997; Ruhl & Everhart, 1999; Jacobson et l, 2006). Moreover the relationship between obesity and GOR symptoms remains significant when other factors such as presence of HH, smoking, race, gender, family history of GORD, or dietary fat intake are controlled for (Wilson et al, 1999; El-Serag et al, 2005; Jacobson et al, 2006; Nandurkar et al, 2004; Hampel et al, 2005).

A recent meta-analysis of obesity and gastro-oesophageal reflux disease showed that six out of 9 studies found significant associations between BMI and GORD symptoms (Hampel et al, 2005). There was a trend towards a dose-response relationship, with an increase in the pooled adjusted odds ratios for GORD symptoms of 1.43 (95% CI, 1.158 to 1.774) for BMI of 25 kg/m^2 to 30 kg/m^2, and 1.94 (CI, 1.468 to 2.566) for BMI greater than 30 kg/m^2. Six out of 7 studies found significant associations with erosive oesophagitis. The pooled adjusted odds ratio for erosive oesophagitis for BMI of 25 kg/m^2 or higher was 1.76 (CI, 1.156 to 2.677; p=0.0004). The effect of BMI on GORD-related disorders was independent of dietary intake (Hampel et al, 2005).

In a re-analysis of Swedish nationwide case control data, Lagergren (2000) estimated the number of endoscopies needed to identify one oesophageal or cardia adenocarcinoma in people with various combinations of both obesity and reflux. Risk of oesophageal adenocarcinoma increased dose dependently with increasing BMI and reflux severity. The risks combined in a multiplicative manner. Among obese people (BMI >30 kg/m^2) with reflux symptoms, the odds ratio was 184 (95% CI 36 to 949) for oesophageal adenocarcinoma compared with lean people (BMI <22 kg/m^2) without reflux.

Interestingly, the link between body mass and reflux is much stronger in women than in men, at least in the studies that did sex-specific analysis (Nilsson et al, 2003; Nilsson et al, 2002; Lagergren et al, 2000), and the association is augmented by high exposure to oestrogen suggesting a potential role for hormonal factors related to adiposity in the pathogenesis of GORD (Jacobson et al, 2006). In a study of 10,545 women, BMI had a greater association with symptoms of GORD than waist to hip ratio, suggesting that risk of symptoms of GOR rises more with the percentage body fat, than with the distribution of body fat.

Chronic GOR in combination with adverse metabolic effects of adipokines are likely to play a crucial role in oesophageal carcinogenesis. However further work is needed to establish the link between obesity, reflux, and oesophageal adenocarcinoma, and, in particular, the potential pro-inflammatory and pro-tumorigenic pathways facilitated through the altered immunological, metabolic and endocrine milieu in obesity, in particular male obesity.

1.3.12.2 Obesity and Barrett's Oesophagus

The association between obesity and the presence and length of Barrett's oesophagus has been largely un-investigated (Stein et al, 2005). Furthermore the effect of body fat distribution on the risk of Barrett's Oesophagus is not well known.

In a retrospective cross sectional study of 65 cases of Barrett's oesophagus cases and 385 non-cases without Barrett's oesophagus obesity was shown to be associated with a 2.5 fold increase in the risk of BO – for each ten pound increase in weight, or five-point increase in BMI, there was a 10% and 35% increase in the risk of BO, respectively (Stein et al, 2005). In a population-based study of 167 cases of Barrett's Oesophagus and 261 matched controls Smith et al (2005) reported that obese people with self-reported symptoms of acid reflux had markedly higher risks of BO (OR 34.4, 95% CI 6.3-188) than people with reflux alone (OR, 9.3; 95% CI 1.4-62.2) or obesity alone (OR 0.7, 95% CI 0.2 – 2.4). This finding suggests that obesity plays a further role in the development of BO, over and above it's role in promoting acid reflux.

The first study to examine the role of body fat distribution and the risk of BO was carried out by El-Serag et al (2005). This was a retrospective case-control study of 36 BO cases and 93 controls that underwent abdominal CT scan where the surface of visceral adipose

tissue (VAT) and subcutaneous adipose tissue (SAT) at the level of inter-vertebral disc between L4 and L5 were calculated. This study found that a greater BMI was a significant risk factor for BO but VAT was an even stronger independent risk factor for BO. Visceral fat is recognised as metabolically active, and has been strongly associated with elevated serum levels of several pro-inflammatory cytokines (adipocytokines) including interleukin-6 and tumour necrosis factor-α (Xu et al, 2003; Cannon et al, 1993; Weinsier et al, 2001). The latter two cytokines have been shown in multiple studies to be over-expressed in erosive oesophagitis and Barrett's Oesophagus (El-Serag, et al, 2005).

It has been postulated that the effects of increased BMI are largely manifested early in the pathogenesis of oesophageal adenocarcinoma, that is in the development of the specialised intestinal metaplasia that characterizes Barrett's oesophagus (Vaughan at al, 2002), possibly by increasing the risk of developing a Hiatus hernia, with resulting increased frequency and severity of reflux (Wilson et al, 1999). In contrast central obesity may be more important later in the pathogenesis, that is, in the development of cell cycle and genetic abnormalities that mark the progression of Barrett's oesophagus towards cancer (Vaughan et al, 2002). This might occur through alterations in growth factors, hormones or other metabolic factors that affect regulation of cell growth. It is possible that the metabolic consequences of central obesity also include accelerated rates of division and proliferation of Barrett's epithelium (Vaughan et al, 2002). A recent study demonstrated increasing risk for cell-cycle (aneuploidy) and genetic abnormalities (17p loss) in Barrett's oesophagus with increasing waist: hip ratio, supporting the notion that distribution of body fat (central obesity) may be more important than BMI (Vaughan et al, 2002).

There are currently no studies in the scientific literature describing obesity related metabolic abnormalities, namely the metabolic syndrome and their association with Barrett's oesophagus. It is likely that obesity and lifestyle factors interact to modulate individual susceptibility for progression to oesophageal adenocarcinoma in patients with severe GORD and Barrett's oesophagus (Veugelers et al, 2006); however these factors remain poorly understood and demand further investigation.

1.4 PRESENTATION, DIAGNOSIS & MANAGEMENT OF UPPER GI MALIGNANCY

1.4.1 Presentation of Upper GI cancer

Progressive dysphagia (difficulty swallowing) or odynophagia (pain on swallowing food and liquids) are the most common presenting symptoms in oesophageal cancer (Enzinger & Mayer, 2003; Layke & Lopez, 2006). These symptoms are usually present for several months before medical treatment is sought. Patients can also present with un-intentional weight loss which often tends to be greater than 10% of body mass. Later signs and symptoms include chest or back pain when swallowing, supra-clavicular adenopathy, persistent sub-sternal chest pain unrelated to swallowing, sudden onset of hiccups, or severe dyspepsia (Layke & Lopez, 2006).

1.4.2 Diagnosis and Staging of Oesophageal Cancer

The diagnostic evaluations of oesophageal adenocarcinoma and SCC are identical – patients initially undergo a barium swallow, endoscopy and biopsy. Once a tumour is identified and the histopathology is established, evaluation of the extent of invasion is necessary for staging and selecting appropriate treatment options. This work-up includes computed tomography (CT) of the neck, thorax and abdomen, and ^{18}-F-deoxyglucose Positron Emission Tomography (PET) scanning (a recent development in imaging which allows for more accurate staging - in particular in identifying suitable potential operable tumours). Using CT and PET imaging, mediastinal and left gastric nodes can be classified as N_1 (invaded) if the maximal transverse diameter of these nodes are larger than 1 cm. Resectable disease is often defined as $T_{1\text{-}3}$, $N_{0\text{-}1}$. Tumours at the oesophago-gastric junction can be further classified as Type I, II or III, as per Siewert et al (1998): Type I is adenocarcinoma of the distal oesophagus, usually arising in specialised intestinal metaplasia; Type II is a true adenocarcinoma of the cardia arising immediately at the oesophago-gastric junction; and Type III is a subcardial gastric carcinoma infiltrating the oesophago-gastric junction and distal oesophagus from below.

For patients being considered for surgery it is necessary to evaluate pulmonary function by Pulmonary Function Tests (PFTs) and chest X-ray; cardiac function by ECG or ECHO in those with cardiovascular disease, and blood tests to assess liver and renal function as well as blood count to rule out anaemia. Additional investigations may include endoscopic ultrasound of the oesophagus, or staging laparoscopy.

1.4.3 Management of Localised Oesophageal Cancer

Treatment options for localised oesophageal cancer include surgery, chemotherapy, and radiation therapy. These therapies can be used individually or in combination.

1.4.4 Surgery

Localised oesophageal cancer is most commonly resected with the use of either a right transthoracic or a transhiatial approach (Enzinger & Mayer, 2003). The right transthoracic approach combines a laparotomy and a right-sided thoracotomy, leading to an oesophago-gastric anastamosis either in the upper chest (2-stage oesophagectomy), or in the neck (3-stage oesophagectomy). The transhiatial approach avoids a thoracotomy and uses a laparotomy with blunt dissection of the thoracic oesophagus and places the anastamosis in the neck (Cunningham et al, 2005).

Oesophagectomy carries a very significant risk of postoperative morbidity and mortality. Earlam and Cunho-Melo (1980) reported an overall mortality rate of 29% from papers published between 1960 and 1979, and Muller (1990) reported a postoperative mortality rate of 13% from papers published between 1980-1988. In a review by Jamieson et al (2004) of 70,756 patients covering the period 1990-2000, the reported mortality rate was 6.7 per cent. Bailey et al (2003) reviewed 1,777 patients with oesophageal cancer who underwent resection at 109 Veterans Affairs hospitals between 1991 and 2000, and reported an approximate 50% major morbidity rate, and 10% mortality rate. In the United Kingdom, McCullogh et al (2003) reported a 14% in-hospital mortality rate from a multi-centre review of 365 patients.

1.4.5 Post Operative Complications

The high morbidity rates observed following oesophagectomy reflect the profound changes in the endocrine, neuroendocrine and immune system as well as significant changes in organ function that occur following this type of operation (Desborough, 2000). In fact, recent literature has highlighted the unassailable fact that there is no common elective surgical procedure that carries the same operative risks (Van Lanschot et al, 2001; Begg et al, 1998).

The insult of oesophagectomy sets in motion a systemic pro-inflammatory host immune response which is then followed by a counter-inflammatory reaction, that may leave the patient highly susceptible to opportunistic infections and subsequent infections. These

two responses are referred to respectively as the systemic inflammatory response syndrome (SIRS) and the compensatory anti-inflammatory response syndrome (CARS). The latter syndrome is a cytokine antagonist cascade, which results in an immunosuppressed and/or lymphopenic state and has been recognized for over 40 years. The balance between pro-and anti-inflammatory responses is frequently lost (Muller Kobold AC et al, 2000; Windsor ACJ et al, 1995).

1.4.6 Pro Inflammatory Response to surgery

The pro-inflammatory response to surgery is characterised by a number of hormonal changes initiated by neuronal activation of the hypothalamic-pituitary-adrenal axis (Desborough, 2000). Principle endocrine changes that occur in response to surgery include increased secretion of Growth Hormone, Thyroid Stimulating Hormone, adreno-corticotrophic hormone (corticotrophin), arginine vasopressin, cortisol, aldosterone; and a decrease in the production of insulin, glucagons and thyroxine.

The net effect of the endocrine response to surgery is an increased secretion of catabolic hormones resulting in catabolism of carbohydrates, fat and protein. The usual mechanisms that maintain glucose homeostatsis are ineffective in the perioperative period. Hyperglycaemia persists because catabolic hormones such as cortisol and catecholamines stimulate hepatic glycogenolysis and glyconeogenesis. In addition peripheral use of glucose is decreased, a situation further hampered by post operative lack of insulin as well as insulin-resistance. Protein catabolism is also stimulated by cortisol leading to the breakdown of skeletal muscle and often resulting in marked weight loss and muscle wasting. Breakdown of body fat stores also occurs in response to cortisol, catecholamines and growth hormone as well as a relative lack of insulin. The net result of protein and fat breakdown is the release of amino acids and triglyceride respectively which are used as substrates for gluconeogenesis, or provide substrates for the production of acute phase proteins in the liver.

1.4.6.1 Acute Phase Proteins

The molecules collectively referred to as acute phase proteins are one of the three main soluble components of the innate immune system. An acute-phase protein has been defined as one whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 25 percent during inflammatory disorders (Gaby et al, 1999). C-reactive protein (CRP) was the first acute phase protein

to be described, as early as 1930, and is an exquisitely sensitive systemic marker of inflammation and tissue damage (Pepys & Hirschfield, 2003; Tillet & Francis, 1930). Other acute phase proteins include serum amyloid A, proteinase inhibitors and coagulation proteins (Delves & Roitt 2000).

1.4.6.2 C-reactive protein

CRP is a member of an old and stable family of plasma proteins, the pentraxin family, the other main member of which is serum amyloid A. The normal plasma CRP concentration in healthy adults is <0.8 mg/l, the 90th centile is 3.0 mg/l and the 99th centile is 10mg/l (Shine B et al, 1981). It is produced by hepatocytes, and its production is regulated by IL-6. Synthesis occurs rapidly following stimulus – serum concentrations rise after 6 hours and can peak at 48 hours; the half-life is approximately 19 hours, plasma concentrations will therefore fall rapidly after cessation of the stimulus (Vigushin et al, 1993). In most, though not all, diseases, the circulating value of CRP reflects ongoing inflammation and/or tissue damage much more accurately than do other laboratory parameters of the acute-phase response, such as the erythrocyte sedimentation rate (ESR). Importantly, there is no diurnal or post-prandial variation, and while liver failure impairs production, no other pathologies and few drugs affect CRP values, unless they also affect the underlying cause of the CRP elevation. CRP is therefore useful as a screening tool for organic pathology, as a marker to monitor disease progression or response to treatment.

1.4.6.3 Cytokines

Cytokines also have a major role to play in the inflammatory response to surgery and trauma. They are a group of low-molecular weight proteins, which include the interleukins and interferons. They are produced from activated leucocytes, fibroblasts and endothelial cells as an early response to tissue injury and have a major role in mediating immunity and inflammation (Sheeran & Hall, 1997; Blok et al, 1996). Cytokines have local effects of mediating and maintaining the inflammatory response to tissue injury, and also initiate some of the systemic responses such as the acute phase response (Desborough, 2000). After major surgery the major cytokines released are interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), both of which are released from activated macophages and monocytes in damaged tissue, which in turn stimulates the production and release of further cytokines, in particular interleukin-6 (IL-6), the main cytokine responsible for the acute phase response. The acute phase response is

characterised by fever, production of acute phase proteins in the liver (C-Reactive Protein, fibrinogen, α_2-macroglobulin, serum amyloid A), changes in serum concentrations of transport proteins (decrease in transferrin, albumin and α_2-macroglobulin, increase in ceruloplasmin), and granulocytosis. The early phases of the acute phase response are local and serve to check bleeding, to demarcate damaged tissues and to recruit cells for the subsequent reparative phase, which requires a systemic response (Blok et al, 1996). This second, systemic phase involves fever, anorexia, leukocytosis and metabolic changes that include an increased flow of amino acids from muscle to liver and a rearrangement of the pattern of protein synthesis in the liver: albumin production decreases and acute phase proteins such as fibrinogen, C-reactive protein and serum amyloid A are synthesised. Their appearance in plasma contributes to elevated erythrocyte sedimentation rate (Blok et al, 1996).

1.4.6.4 Negative Acute Phase Proteins

The release of pro-inflammatory mediators causes endothelial dysfunction with severe capillary leakage, massive loss of protein, and fluid shift from the intravascular space into the interstitium. Serum albumin levels decrease in acute illness and injury, as the liver reprioritizes protein synthesis from visceral proteins to acute phase reactant proteins, hypoalbuminaemia thus acts as a marker of underlying systemic disease and is referred to as a 'negative acute phase protein'(Soeters et al, 1990; Spanga et al, 1985; Dowd & Heatly 1984). Albumin decreases rapidly in critically ill/surgical patients. This is a result of a combination of factors including haemodilution during fluid resuscitation, and capillary leakage into the interstitial space. The degree of capillary hyper-permeability is proportional to the inflammatory response mounted by the patient, and therefore those with the greatest rate of vascular permeability are associated with the highest mortality. The development and degree of hypoalbuminaemia thus relates to the severity of the underlying traumatic insult and therefore to the ultimate outcome. A reduction in the serum albumin has also been positively associated with impaired immunological function and a reduction in the resistance to post-operative nosocomial infections (Schwartz et al, 2000; Rey-Ferro et al, 1997; Bone et al, 1992; Gibbs et al, 1999).

Table 1.3: Cytokines and their functions

Cytokine	Source	Major Function
IL-1	Monocytes	Production of Acute Phase Proteins, Fever, Activates T, B and NK Cells
IL-2	CD4+ T-Cells	T Cell growth, differentiation & activation, B Cell growth, activates monocytes, neutrophils & NK cells
IL-3	T & NK Cells, Mast Cells	Growth factor for haemopoetic cells
IL-4	CD4+ T cells Mast cells	Growth factor T_H2 CD4+ Cells, B cell growth & stimulation Promotes IgG & IgE synthesis
IL-6	T & B cells	B-cell stimulation, T-Cell activation & IL-2 production, production of acute phase proteins, hemopoetic cell growth
IL-8	T Cells, monocytes, Endothelial cells	Chemotaxis, activation of leucocytes
IL-9	T cells	T cell & mast cell growth & proliferation
IL-10	T_H2 Cells, Macrophages	Inhibition of cytokine production of T_H1 Cells & macrophages. B-cell stimulation
IL-12	B cells & Macrophages	Activates NK cells, promotes generation of T_H1 Cells
TNF-α	Monocytes	Cell proliferation & apoptosis enhances cytolytic activity of NK Cells
TNF-β	Lymphocytes	Growth & differentiation of numerous cells
INF-α	Lymphocytes Monocytes	Regulates class I MHC expression, Induces viral resistance
INF-γ	T & NK cells	Activates macrophages, B & T-cells, NK Cells.

IL=Interleukin, INF=Interferon, NK=Natural killer, MHC=Major Histo-compatibility Complex, TNF=Tumour Necrosis Factor

1.4.7 Anti-Inflammatory response

In order to restore homeostasis it is important to mount an anti-inflammatory response by the release of anti-inflammatory mediators such as IL-10, production of cytokine receptor antagonists, secretion of glucocorticoids and down regulation of nuclear factor kappa β activation by enhancement of antioxidants defences. However, the balance between pro-and anti-inflammatory responses to major trauma is frequently lost (Muller Kobold et al, 2000; Windsor et al, 1995). In a subset of patients, overwhelming stress response can leads to the systemic inflammatory response syndrome (SIRS) which is often associated with adverse outcomes such as organ dysfunction and failure (Fogler & Lindsey, 1998; Pepys & Hirschfield, 2003; Karaylannakis et al, 1997).

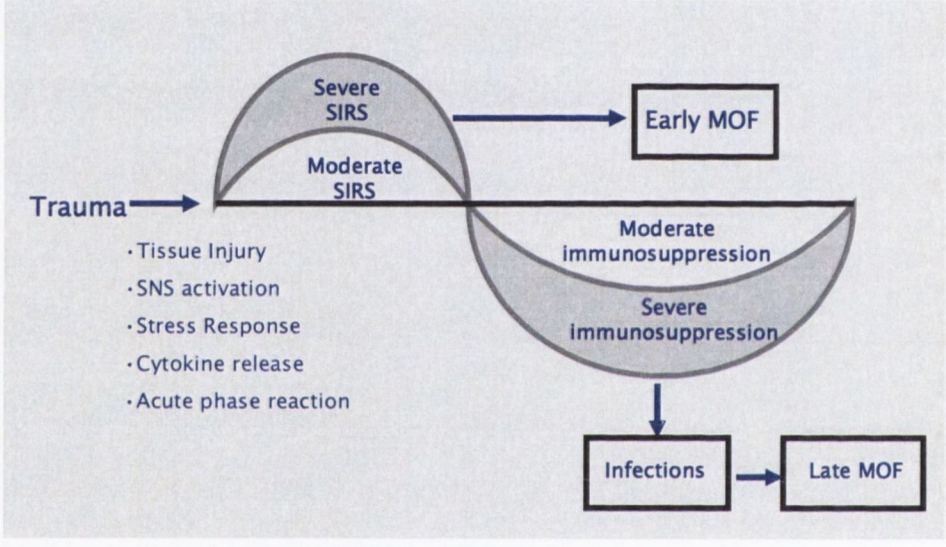


Figure 1.5: Operative trauma: Two hit model of SIRS, sepsis, and Multiple organ failure

1.4.8 Systemic Inflammatory Response Syndrome (SIRS)

The SIRS response is manifested by two or more of the following conditions: Temperature > 38°C or <36°C ; Heart rate >90 beats per minute ; Respiratory rate >20 breaths per minute or PaCO2 <32 mmHg; White blood cell count >12,000mm^3, <4,000mm^3 or >10% immature (band) forms (Bone et al, 1992). SIRS can also arise during sepsis - the clinical syndrome of systemic inflammation resulting from invasive infection. It is now understood that a similar or identical response can arise in response to other stimuli, such as trauma and pancreatitis, in the absence of infection. The American College of Chest Physicians and the Society of Critical Care Medicine have since defined this clinical immune response as systemic inflammatory response

syndrome (SIRS), independent of its cause. SIRS therefore is defined as a clinical syndrome whose differential diagnosis includes infection as well as a number of non-infectious causes (Bone et al, 1992; Nathens et al, 1996; Rangel-Frausto et al, 1995).

SIRS can lead to multiple organ dysfunction syndrome (MODS) - a condition that has become recognized in the last three decades as a major cause of surgical morbidity and mortality. It is estimated that it is responsible for 50-80% of all surgical intensive care deaths (Deitch, 1992; Moore et al, 1996). The detection of altered organ function in the acutely ill patient is termed multiple organ dysfunction syndrome (MODS) and this is the more severe end of the spectrum of severity of illness in SIRS/sepsis. This may develop as the immediate result of trauma (primary) or as a consequence of the host response (secondary). Secondary MODS therefore usually develops some time after the initial insult to the body. MODS has become recognized as a leading cause of death in trauma and post-operative patients. Although infection/sepsis is a major course of MODS, the syndrome can develop as a result of SIRS in the absence of infection in up to 50% of cases. Despite the high percentage of ICU deaths attributable to this condition, much of our treatment to date remains supportive and research is ongoing to understand the pathophysiology of this condition. Like sepsis and SIRS, it is not clear that MODS is a distinct clinical syndrome, but it does provide a convenient framework for describing morbidity in critical illness. Whatever the initial cause, the progress of MODS is generally uniform, commencing with the lungs and then followed by hepatic, intestinal and renal failure, usually in that order. Coagulopathies and myocardial failure may also occur (Deitch, 1992; Nathens et al, 1996; Tilney et al, 1973).

As MODS usually develops in organs unrelated to the initial disease, it is hypothesized that MODS is a systemic process, caused by disruption of normal homeostatic mechanisms in the immune system. This can be caused by a number of mechanisms, infection being the most readily recognized. Trauma, pancreatitis or shock can also induce SIRS and MODS. There are a number of interrelated hypothesis as to why SIRS develops, but it is clear that cytokines and other mediators produced by the immune system in response to infection or major surgery can cause the inflammatory response seen in SIRS (Deitch, 1992; Goris, 1996; Rixen et al, 1996; Saadia et al, 1996). SIRS and MODS are of significant prognostic value in determining oucomes following oesophageal surgery and to date no treatments, except supportive care, have been shown to reverse these conditions should they arise.

1.4.9 Multimodal Treatment of Oesophageal Cancer

Despite the fact that surgical resection remains the standard of care for most oesophageal surgeons, even with en-bloc resections and with radical 2 or 3 field lymphadenectomy, 3-year survival rarely exceeds 40 per cent (Siewert et al, 2001; Altorki et al, 2002; Lerut et al, 1999). The disappointing outcomes from surgery alone have resulted in considerable interest in multimodal approaches, either neoadjuvant chemotherapy alone or combined with radiation therapy (Enzinger & Mayer, 2003). However, the interpretation of randomized clinical trials to date is controversial.

The concept of giving pre-operative chemotherapy, combined with radiation therapy emerged in the 1990's in an attempt to down-stage tumours or preferably achieve a complete pathological response to treatment i.e. no tumour seen on final pathology specimen post oesophagectomy. The regime uses the radio-sensitising effects of chemotherapy to reduce tumour size and maximise local control (Herskovic et al, 1992; Gebski et al, 2007). However, analysis of trials of combination chemotherapy and radiation therapy prior to surgery (Nygard et al, 1992; Le Prise et al, 1994; Walsh et al, 1996; Bosset et al, 1997; Urba et al, 2001; Law et al, 1998; Burmeister et al, 2005), is difficult for several reasons: few studies appear adequately powered with over 200 patients; there is a mix of pathologic types: adenocarcinoma and squamous cell cancer, the total dose of radiation therapy administered, and treatment fractions, is different across trials; and limited cross-sectional imaging in preoperative staging in some trials.

Despite these problems, a recent meta-analysis of 10 randomised controlled trials of chemo-radiotherapy versus surgery alone, incorporating 1,209 patients showed a survival benefit for the neoadjuvant arm – the Hazard Ratio for mortality was 0.81 (95%CI, 0.7-0.93, p=0.002) corresponding to a 13% absolute difference in survival at 2 years (Gebski et al, 2007). Neo-adjuvant chemotherapy prior to surgery showed benefits across both histological subtypes – SCC and adenocarcinoma.

1.4.9.1 Neoadjuvant Chemo-radiotherapy and post-operative complications

However despite the survival advantage shown in this meta-analysis concerns have been expressed that neoadjuvant chemoradiotherapy prior to surgery increases post-operative complications (Reynolds et al, 2006; Bailey et al, 2003; Bosset et al, 1997). This added operative risk of multimodal therapy has received little direct attention in the literature. Bailey et al (2003) in a study of 1,777 oesophagectomy cases carried out at 109 Veterans

Affairs Hospitals reported that neoadjuvant therapy was independently associated with perioperative mortality. In a recent meta-analysis of randomized controlled trials, Fiocia et al (2004) reported increased postoperative mortality; 6% for patients treated with surgery alone versus 12% for patients treated with multimodal therapy (odds ratio 2.1, CI 1.18-3.7, p=0.001). In a study of adenocarcinoma patients only, Walsh and associates (1996) found an increase in perioperative mortality (10.7 versus 3.7%) in the multimodal group, and Nygaard et al (1992) observed a 1.8 fold (24% versus 13%) increase in the multimodal group. The short term postoperative risk of multimodal therapy was highlighted in the multi-centre randomized controlled trial in France of Bosset and co-workers (1997). In an adequately powered study of 297 patients with oesophageal squamous cell cancer, this group reported that 17 of 138 patients with multimodal therapy died after surgery, compared with 5 of 137 in the surgery only group, and this difference was due to respiratory failure and mediastinal infection. This study was criticized for larger fractions of radiation, with a fractional dose of 3.7-Gy compared with 1.8-2.67Gy per fraction in other trials. The best surgical outcomes in multimodal regimens was achieved in a small randomized trial of Urba et al (2001), who used a hyperfractionated regimen, with 1.67 Gy per fraction, and just one of 47 patients died following transhiatial oesophagectomy. The fractional dose administered in this study was 2 to 2.67Gy, and no significant pattern of difference has emerged between these two regimens.

Normal tissue radiation response is a dynamic process involving inflammatory responses, tissue repair processes, altered cell-cell communication, changes in cytokines, and radiation fibrosis (Lee et al, 2003). The genetic characteristics of the host, moreover, can impact on the radiation response. It is impossible to spare the lung from preoperative treatment planning, but whether idiopathic ARDS or respiratory failure, relates to priming or sensitizing of immunoinflammatory cells in the lung to the further effect of one-lung anaesthesia and the trauma of surgery, or acts through alternate mechanisms, requires further study. The role of the immunosuppressive effect of chemotherapy is unclear. Heidecke et al (2002) reported defective proliferation of T cells after chemoradiotherapy, when compared to patients undergoing oesophagectomy alone. Whether neutrophils, lymphocytes and other cells actually function normally in the blood following multimodality therapy, lungs and other tissues remains unknown (Reynolds et al, 2006)

Notwithstanding the controversy whether oncologic benefit accrues from multimodal regimens, informed decision-making requires better information on other end-points, including quality of life outcomes, toxicity of neoadjuvant regimens, and operative complications. Intuitively, the administration of chemotherapy and radiation therapy prior to major surgery presents an added challenge, both through treatment-related immunosuppression and direct tissue toxicity from radiation.

1.5 EFFECT OF UPPER GI MALIGNANCY ON NUTRITIONAL STATUS

1.5.1 Malnutrition and cancer

Malnutrition is common in patients with cancer. Estimated prevalence rates vary according to tumour site, disease stage, the type of treatment used and the methods used to identify malnutrition (Elia et al, 2006) and can range from 9% in urological cancers, to 46% in lung cancer, and up to 85% in pancreatic, gastric and oesophageal cancer (Stratton et al, 2003; Von Meynfeldt, 2005). During the course of the disease, weight loss greater than 10% of pre-illness body weight may occur in up to 45% of all affected patients (Stratton et al, 2003).

1.5.2 Malnutrition in Upper GI cancer

Weight loss is a common feature in Upper GI malignancy (Chate, 2006) and can be caused by reduced food intake secondary to a number of factors including: systemic effects of the disease (e.g. anorexia, hyper metabolism, nausea, vomiting, alterations in taste and smell, pain); local effects of the tumour (e.g. dysphagia, odynophagia, early satiety); psychological factors (e.g. fear, depression, and anxiety) and the side effects of treatment (Fearon, 2001a, Grant & Kravitis, 2000; Mutlu, 2000; Nitenberg & Raynard, 2000; Rivadeneira et al, 1998; Ravasco et al, 2003).

Malnutrition in cancer patients has been shown to adversely affect quality of life, reduce the effectiveness of chemotherapy (Andreyev et al, 1998; Langer et al 2001; Persson & Glimelius, 2002), increase the risk of chemotherapy-induced toxicity, reduce performance status, increase the risk of post-operative complications, and reduce overall survival (Andreyev et al, 1998; Bauer et al, 2002; Brauanschweig et al, 2000; Dickson et al, 1999; Iida et al, 1999; Jagoe et al, 2001; Rey-Ferro et al, 1997; Van Cutsem & Arends, 2005). In addition, nutrient deficits have specific adverse effects on immune competence, including decreased lymphocyte response to mitogens, impaired cell-mediated immunity, phagocytic dysfunction, impaired inflammatory response, and impaired cytotoxic T-cell activity (Langer et al, 2001).

1.5.3 Changing Profile of Malnutrition in Cancer Patients

Despite the well know affects of malnutrition on clinical outcome, diagnosing malnutrition has become increasingly difficult as the nutritional profile of surgical oncology patients has changed over the past 15 years. While studies in the early 1990's

reported incidences of malnutrition of 40% on admission to hospital (McWhirter & Pennington, 1994), more recent studies in British and Irish hospitals (Harrison et al, 1997; Corish et al, 2000; Edington et al, 2000) have reported incidence rates between 6% and 20%. Surgeons are now seeing an alarming increase the prevalence of overweight and obese hospitalised patients, estimated to be between 35 - 40% (Choban & Flanchbaum, 2000).

The term 'malnutrition' therefore no longer applies to only those underweight but also to those over nourished. *Table 1.4* lists factors to identify malnutrition.

Identification of Undernutrition	**Identification of Over-nutrition**
• Low BMI : <16 kg/m^2 = severely malnourished 16-19 kg/m^2 = underweight <24 kg/m^2 in elderly[6]	High BMI: >30kg/m^2 obese > 40kg/m^2 morbidly obese
ALSO : • Significant and/or rapid weight loss: >5% usual weight in 1 month, >7.5% usual weight in 3 months >10% usual weight 6 months • Evidence of depletion of muscle mass • Evidence of poor nutritional intake	

Table 1.4: Identification of malnutrition, over- and under- nutrition

While BMI of <20 kg/m^2 is generally used to detect risk of under-nutrition related complications, BMI <24 kg/m^2 has been proposed as the cut off point that should be used for older patients (Beck & Ovessen, 1998). A high BMI also carries significant operative risks (*see table 1.5).*

Consequences of Under-nutrition	**Consequences of Over-nutrition**
• Immune dysfunction • Decreased respiratory muscle strength • Impaired wound healing • Impaired gut barrier function • Muscle wasting leading to decreased functional ability • Increased length of hospital stay • Death	• Longer operative times • Abnormal cardiorespiratory function • Metabolic derangements • Abnormal haemostasis • Impairments of immunity • Higher incidence of post operative complications – wound dehiscence, nosocomial infections, respiratory complications, delayed cardiac recuperation • Higher peri-operative weight loss

Table 1.5: Consequences of under- and over nutrition

With the secular increase in BMI in both the general population and hospitalised population, it has been suggested that the thresholds for classifying patients as

undernourished need to be reviewed. Recent weight loss and functional status may be more appropriate variables to use in the evaluation of nutritional status on admission to hospital than BMI alone (Harrison, 1997). The relationship between percentage weight loss and reductions in organ function, immune status, wound healing and muscle strength have been well described (Allison, 1992).

The degree of weight loss believed to be clinically significant was first described by Blackburn et al (1977), see table 1.6. Clinically significant changes begin to appear with rapid weight loss in association with disease at somewhere between 5 and 10% loss of usual body weight (Kinney et al, 1988). Unintentional weight loss greater than 10% within the previous 6 months is a good prognostic indicator of poor outcome (Klein et al, 1997). In fact, the rate and timing of weight loss is postulated to be a more important predictor for the development of post-operative complications than the underlying diagnosis (Detsky et al, 1987b).

Duration	**Clinically significant weight loss**	**Clinically Severe weight loss**
1 week	1-2%	>2%
1 month	5%	>5%
3 months	7.5%	>7.5%
6 months	10%	>10%

Table 1.6: Influence of time-span on the severity of weight loss (adapted from Blackburn et al, 1977).

Obese patients should be assessed using the same approach as non-obese patients (Choban & Flanchbaum, 2000). An involuntary recent weight loss is concerning in the obese population and may indicate a loss of lean body mass, placing these individuals at increased risk for complications related to infection and healing because of malnutrition (Choban & Flanchbaum, 2000). The odds of complications are significantly greater for patients who decline nutritionally regardless of nutritional status on admission, *versus* patients who do not decline in nutritional status (Braunschweig et al, 2000).

1.5.4 Pathophysiology of cancer-associated malnutrition and cachexia

In a subset of cancer patients malnutrition arises as a result of alterations in metabolism as a direct consequence of malignant cells. Profound weight loss can occur and has been referred to as "Cancer Cachexia". Several factors have been implicated in the pathogenesis of cancer cachexia, including those derived from the tumour and those produced as a result of the host response to the tumour, including hormones and mediators of inflammation.

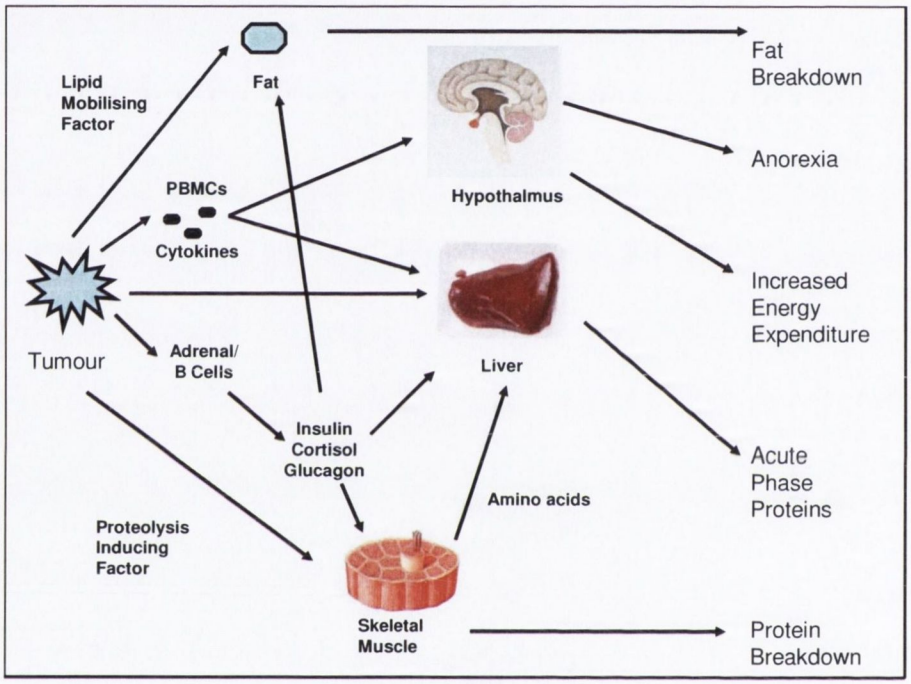


Figure 1.6: Pathophysiology of cancer cachexia (adapted from Barber et al, 2000)

The presence of abnormal, rapidly proliferating cancer cells can induce an inflammatory immune response, which is thought to start early in the disease and may contribute to the development of malnutrition (Argiles et al, 2003). Release of acute phase proteins is associated with hypermetabolism, accelerated weight loss (Falconer et al, 1994; Staal-van den Brekel et al, 1995) and poor survival (Blay et al, 1992; Falconer et al, 1995) in patients with advanced cancer, forming part of the innate immune response to tumours. This immune response can result in chronic inflammation.

The presence and magnitude of a systemic inflammatory response has been correlated with poor outcome and survival in some patients with cancer (Mc Millan et al, 2001; Caruse et al, 2004). The inflammatory cytokines interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-α and interferon (IFN)-γ can promote angiogenesis and tumour growth and survival (Robinson and Coussens, 2005), and also alter nutrient metabolism:

there is an increasing amount of evidence to support a role for chronic production of these factors in the pathogenesis of cancer-associated malnutrition (Espat et al, 1994; Espat et al, 1995; Strassmann and Kambayashi, 1995). Inflammatory cytokines, particularly IL-1, may also act centrally and induce anorexia (Plata-Salaman, 2000), thus reducing nutritional intake. Although an exclusive role for any single cytokine in cancer-associated malnutrition has not been defined, there is growing acceptance that the host inflammatory response to a tumour supports the progression to cachexia.

It has also been suggested that altered hormone levels have a role in cancer-associated malnutrition. An increase in the ratio of catabolic to anabolic hormones has been described, resulting in elevated catabolism, and failure to accumulate lean body mass, even when nutritional intake is normal (Knapp et al., 1991; Huang et al., 2005).

Tumour-derived catabolic factors have also been implicated in the pathogenesis of cancer-associated malnutrition (Argilés et al., 2003a). Lipid-mobilising factor (LMF) and proteolysis-inducing factor (PIF) have recently been isolated from cachexia-inducing tumours (Todorov et al., 1996) and from the urine of malnourished patients with cancer (Groundwater et al., 1990; Cabal-Manzano et al., 2001). LMF increases lipid mobilisation and metabolism promoting loss of body fat, and PIF has been shown to induce skeletal muscle wasting and weight loss in mice (Todorov et al., 1999). These findings suggest that the tumour itself produces and releases factors that can cause malnutrition and cachexia, irrespective of nutritional intake.

1.5.5 Nutrition Screening Methods

Although a variety of nutritional indices have been found to be valuable in predicting patient outcome when used alone, there is no consensus on the best method for assessing the nutritional status of hospitalized patients (Kuzu et al, 2006). A number of nutrition screening methods are available and include nutrition risk index (NRI), the Prognostic Nutrition Index (PNI) and subjective global assessment (SGA). The NRI was developed by the Veterans Affairs Total Parenteral Co-operative Study Group (1991) and relies on serum albumin levels and usual weight loss. It is calculated by the equation:

NRI = 1.519 x serum albumin (g/l) + 0.417 x (current weight/usual weight) x 100

A score <83.5 is severe nutritional risk; a score 83.5-97.5 is mild nutritional risk; a score 97.5 – 100 is borderline risk; and a score >100 is no nutritional risk. The NRI has been used in a number of studies where the effects of undernutrition (Reynolds et al, 1996) or

nutritional intervention has been investigated (Keele et al, 1997). It has been shown to predict post-operative complications in surgical patients (Veterans Affairs Total Parenteral Co-operative Study Group, 1991).

A second well known method of nutritional screening is the subjective global assessment (SGA). This technique involves assessment of weight loss in the previous six months, dietary intake in relation to usual intake, presence of gastrointestinal symptoms, and functional capacity. Physical examination assesses loss of subcutaneous fat, muscle wasting and/or presence of peripheral oedema. SGA divides patients into 3 categories: well nourished, mild-moderate malnutrition or severely malnourished (Detsky & Smalley, 1994). The pattern of weight loss seems to be of more importance than the underling diagnosis in determining outcome and it has been shown to have a high specificity in predicting infections in surgical patients (Detsky & Smalley, 1994).

A third, but perhaps less common method of nutritional screening in surgical patients is the Prognostic Nutrition Index (PNI) as described by Buzby et al (1980). It is calculated by the formula:
PNI = 158-16.6(Albumin g/dl)–0.78(triceps Skinfold (mm)-0.2(transferrin)–5.8 (lymphocyte count). The higher the score the higher the risk of infection.

The relationship between the pre-operative nutritional condition and the outcome of surgical treatment in patients with oesophageal carcinoma has been investigated using some of these methods. In a study of 258 oesophagectomy patients, the correlation of pre-operative values of prognostic nutritional index (PNI) with the incidence of post-operative complications and prognosis of the patients was investigated by Nozoe et al (2002). The results showed the mean pre-operative value of PNI in patients with post-operative complications (41.8+/-5.4) was significantly lower than that in patients without post-operative complications (46.5+/-5.3; $P<0.0001$). The survival in patients with higher PNI value was significantly more favourable than that in patients with lower PNI value ($P=0.0001$). In a study of 400 patients who underwent oesophageal resection for malignancy Han Geurtz et al (2006) examined preoperative nutritional status by body mass index, prognostic nutritional index (PNI), nutritional risk index (NRI) and weight loss. They reported that preoperative nutritional status established by PNI, NRI, body mass index and weight loss has limited value in predicting complications following oesophageal resection. Kuzu et al, (2006) studied 460 patients who underwent major

elective surgery using the Nutritional Risk Index (NRI), Subjective Global Assessment (SGA) and other methods to determine the best possible nutrition screening system in surgical practice. The odds ratio for morbidity between the well nourished and malnourished patients was 3.09 [95% confidence interval (CI), 1.96-4.88] using SGA and 3.47 (95% CI, 2.12-5.68) using NRI. Sungurteki et al (2004) also demonstrated that SGA and NRI were predictive for malnutrition and post operative complications in patients undergoing major elective surgery. At present it is clear that further investigations are needed, and much effort must be given to find the best method for assessing nutritional status.

Another nutritional marker commonly used in the assessment of nutritional status is serum proteins such as albumin. It is considered the single best serum marker of malnutrition in an otherwise stable patient and has a half life of 21days. Serum levels of albumin are influenced by synthesis rates, degradation rates and vascular losses into the interstitium, and losses through the gut or kidney. Albumin levels drop in inflammation, trauma, sepsis, peritonitis or burns because high levels of IL-6 stimulate acute phase protein production, thus decreasing production of transport protein production (Kudsk, 1994).Hypoalbuminaemia pre-operatively or pre-trauma is independently associated with the development of post-operative complications, especially the development of infective complications (Pepys & Hirschfield, 2003; Gibbs et al, 1999; Schwartz et al, 2004; Dewar et al, 1992; Rey-Ferro et al, 1997). In upper gastrointestinal cancer surgery, low preoperative serum albumin levels have significantly correlated with anastomotic leak as well as major morbidity and in-hospital mortality (Buzby et al, 1980; Detsky et al, 1987; Kudsk et al, 2003).

1.5.6 Effects of Upper Gastrointestinal Surgery on Nutritional Status

The surgical treatments used for Upper GI cancer can also have a profound effect on nutritional status. However, very often the effects of surgery on nutritional status are overlooked and referred to infrequently in the scientific literature – authors preferring to report on surgical outcomes and any nutritional morbidity that occurs has been deemed unimportant in the face of curing malignant disease (Saito et al, 2001).

Though many researchers consider malnutrition an unavoidable consequence of upper GI surgery (Adashek et al, 1989), there have been few studies on the nutritional consequences of oesophagectomy or total gastrectomy. However, the importance of

nutritional status which influences quality of life, morbidity and mortality cannot be ignored (Bae et al, 1998).

1.5.7 Nutritional consequences of Oesophagectomy

There is a paucity of published literature on nutritional outcomes after oesophagectomy. Most of the published literature on nutrition and oesophagectomy concerns surgical reports of needle catheter jejunostomy feeding and the majority of these reports make no reference to nutritional outcomes whatsoever.

It is somewhat intuitive that the procedure of oesophagectomy results in significant nutritional consequences. Endocrine, physiological and immune cell response to surgery also contributes to post-operative catabolism after major oesophageal resections, and marked weight loss is normally observed. This is usually observed on a background of pre operative weight loss, with oesophageal cancer being associated with the highest level of malnutrition compared with other digestive and extra-digestive cancers (Larrea, 1992). In addition, reconstruction of the oesophagus by gastric pull up results in patients almost uniformly experiencing early satiety, post prandial fullness, poor appetite, lack of hunger sensations and nausea, irregular bowel habit, and or reflux (see figure 1.7).

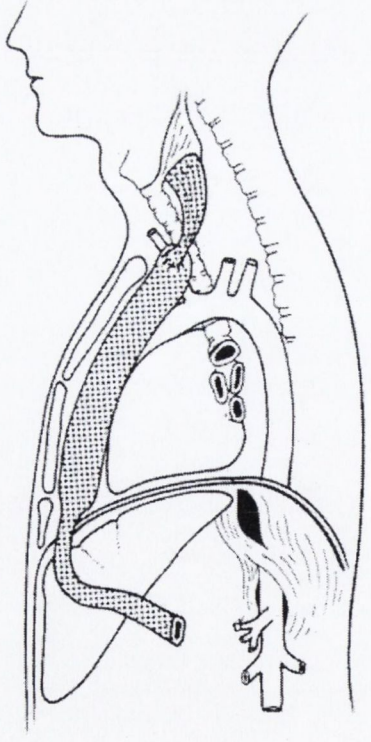


Figure 1.7: Anatomy following oesophagectomy

In the months following oesophagectomy, patients can drastically alter their diet preferring soft/semisolid consistency foods and reducing the volumes consumed at each meal. Previous reports have estimated that the mean time required to achieve what patients considered to be a socially acceptable diet was six months and that a significant amount of adjustment and experimentation with diet is necessary in the first three months following surgery (Ludwig et al, 2001). While the "normal" level of weight loss is not known post oesophagectomy, weight loss has been shown to persist for six months after surgery with a mean loss of 10kg, it is thought that after six months over half of patients are able to gain weight (Ludwig et al, 2001). Weight loss however is associated with a reduction in Karnofsky performance status (a quality of life measure) and poorer survival in patients with oesophageal cancer (O'Gorman, 1999; Christein, 2002).

1.5.8 Nutritional Consequences of Total Gastrectomy

A subset of patients with tumours of the distal oesophagus (Type III OG junction tumours) and patients with true non-cardia gastric adenocarcinoma require total gastrectomy. In fact, radical surgery offers the only possibility for cure in patients with gastric cancer (Scutru et al, 2005; Liedman, 1999). Roux-en-Y oesophago-jejunal anastamosis remains the most commonly used type of reconstruction following total gastrectomy (Espat & Karpeh, 1998; Scurtu et al, 2005) (see figure 1.8).

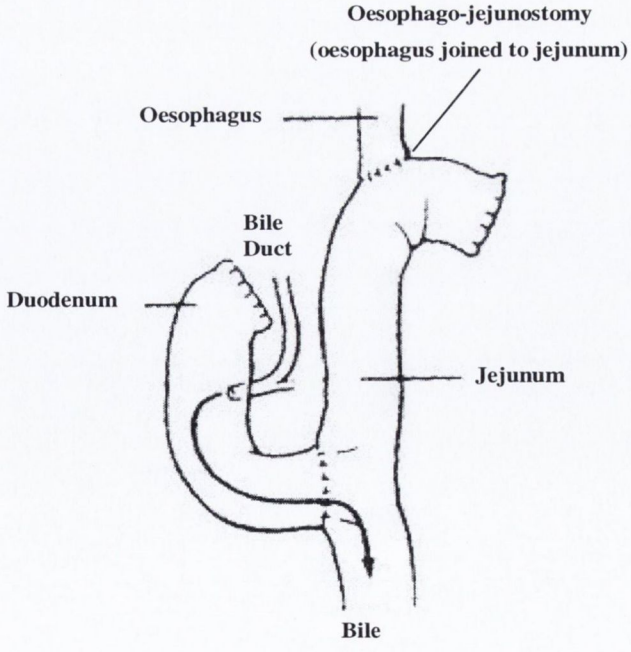


Figure 1.8: Anatomy following Roux-en-Y total Gastrectomy

In contrast to the lack of published data on nutritional outcomes post oesophagectomy, there are several reports of nutritional outcomes post total gastrectomy. In fact, malnutrition is considered one of the main complications following surgery for gastric cancer (Bradley et al, 1975; Braga et al, 1988; Powell-Tuck, 1988). Total gastrectomy, by altering the physiology of digestion and damaging the delicate mechanisms at the gastro-oesophageal junction, is generally believed inevitably to produce malnutrition (Sategna-Guidetti & Bianco, 1998; Liedman, 1999). Weight loss has also been shown to be a significant survival related factor post gastrectomy (Sanchez-Bueno et al, 1998). However a common problem when studying such a highly malignant disease as gastric cancer is that the effect of gastrectomy and the effect of recurrent malignant disease are mixed in a way difficult to survey (Liedman, 1999).

Amongst the mechanisms involved in the aetiology of protein-energy malnutrition are included anorexia, iatrogenic starvation, inadequate efforts at oral feeding, malabsorption, maldigestion, loss of reservoir function, dyspepsia, altered intestinal motility and shortened intestinal transit time (Bae et al, 1998; Liedman, 1999). This can manifest as severe weight loss compromising the quality of life, often leading to an unnecessary loss of muscle mass with impaired mobilisation and increased morbidity (Kusche et al, 1987). Some patients can become nutritionally crippled with marked weight loss and poor tolerance to regular diets (Saito et al, 2001).

1.5.8.1 Reduced intake

Voluntary reduction in calorie intake due to anorexia, absence of hunger sensations and post-prandial abdominal discomfort occurs in many patients. The reduction in calorie intake may be drastic and is considered the main factor responsible for weight loss. Several studies have reported on severe weight loss - which can be between 18-29 kgs post op and can span up to 4 years. Loss of up to 40% of body fat stores has been reported in the 6 months following surgery (Liedman et al, 1997). Bae et al, (1998) reported an average pre operative BMI of 22.2 kg/m^2 and post-operative BMI of 18.9 kg/m^2. They found no significant correlation between the time since surgery and the magnitude of the weight loss. This group reported that weight loss increased until 4.2 years after total gastrectomy and then weight loss tended to decrease. Bozetti et al (1990) reported on 44 disease-free patients 3 years post total- and subtotal gastrectomy. They found that weight loss after both total gastrectomy and subtotal gastrectomy reaches its nadir at the 15^{th} post-operative month.

In a study of 108 patients who underwent gastrectomy Kiyama et al (2005) examined body compositional changes using bioelectrical impedance analysis. Results showed that body protein mass was lost preferentially (rather than fat mass) in the first 14 days following surgery. Loss of body protein continued until 6 months post, but from 6 months to 1 year post op weight loss was from fat mass alone with no change in body protein. Total gastrectomy patients lost an average of 8.9 kg(std dev 5) in the first 6 months and a further 4 kg (std dev 3.4) in the second six months following surgery (Kiyama et al, 2005). These results are consistent with those of Liedman et al (1997) who showed that weight loss (10% of pre operative weight) occurs early after total gastrectomy and body fat decreased by 40% during the first 6 months post op. The selective wasting of body fat and sparing of lean body mass is probably an adequate adaptation to the new situation where eating is not as comfortable as before (Liedman, 1999).

1.5.8.2 Eating related Symptoms

Gastrectomy patients have been shown to have an average of three eating related symptoms (Liedman, 1999). Amongst the most frequently cited symptoms are: 48% reporting early satiety, 26% epigastric fullness, 26% epigastric pain, 43% reflux, 22% diarrhoea and 17% nausea. Total Gastrectomy can dramatically reduce the reservoir into which patients can eat. Innervation of the stomach is also damaged leading to small stomach syndrome. Patients can become very selective in their choice of foods avoiding hyperosmolar, starchy and high volume foods. Diarrhoea commonly occurs after total gastrectomy and is probably partly caused by the vagotomy and possible lack of gastric hormones, but also partly from defective fat absorption due to pancreatic insufficiency, bacterial overgrowth, or short small-bowel transit time (Liedman, 1999; Armbreht et al, 1988; Friess et al, 1996).

1.5.8.3 Maldigestion and Malabsorption

Malabsorption of dietary fat has been proposed as a major contributor to weight loss post total gastrectomy. Up to 50 g fat per day can be lost in stools – almost 7 times that of healthy controls (Bae et al, 1998; Cristallo et al, 1986). Malabsorption of amino acids has also been reported following gastrectomy resulting in a state of persistent proteolysis of lean tissue mass for long periods after surgery (Saito et al, 2001). It has been suggested that this malabsorption is cause by relative pancreatic insufficiency (Bragelmann et al, 1996). It is important to note that with regard to energy balance a

small difference in faecal energy loss amounting to 50 kcal/day would have the potential to result in a weight difference of 10kg body fat in 5 years if allowed to work in one direction (Liedman, 1999).

1.5.8.4 Impact of Malnutrition on post operative outcome following total gastrectomy

Grossmann et al, 2002 reported on morbidity and mortality after gastrectomy for cancer on 708 patients, of whom 234 underwent a total gastrectomy. They reported a post-operative complication rate of 38% and a 30-day mortality rate of 7.7%. On multivariate logistic regression analysis a weight loss >10% in the six months prior to surgery was predictive of 30-day mortality. This increased mortality rate was also observed by Sitges-Serra et al (1988) who showed that patients who lost >20% of their body weight had a significantly higher mortality rate than those losing <20% of their body weight (23% versus 7%, $p<0.05$). Similar results were observed by Rey-Ferro et al, (1997) who reported a 19% weight loss in patients who died post-operatively versus 9% weight loss in those who survived. Hill (1992) correlated weight loss with post operative morbidity and mortality rates and showed that weight loss of >20% and associated functional alterations presented a rate of complications 3-5 times greater, increasing the hospitalisation stay by 4-6 days (Hill, 1992; Windsor & Hill, 1988).

Other means of assessing nutritional status such as the Nutritional Risk Index (NRI) and Prognostic Nutritional Index (PNI) have also been shown to be of value in terms of predicting post operative complications (Rey-Ferro et al, 1997; Sitges-Serra et al, 1988). By associating weight loss with albumin levels in the NRI as described by Buzby (1980), Rey-Ferro et al (1997) found a greater correlation between severe malnutrition (NRI<83.5) and post operative mortality and cellular immunosuppression. They reported a 42.5% incidence of moderate malnutrition and 15% incidence of severe malnutrition in patients with gastric cancer according to the NRI - a post-operative mortality rate of 33% was observed in the severely malnourished group and 6.5% in the moderately malnourished group. These groups also displayed cellular immunosuppression with poor CD4/CD8 ratios and this was related to post operative mortality (Rey-Ferro et al, 1997). Sitges-Serra et al (1988) reported that patients with a PNI below 50% have a lower mortality rate than those with a PNI of at least 50% and it has been postulated that this difference is related to the lower resistance to infection in patients who are most malnourished – their sepsis related death rates have been reported to be five times higher than well-nourished patients.

Pre operative albumin level has also been shown to be inversely related to post operative complications and mortality following gastrectomy (Grossmann et al, 2002; Rey-Ferro et al, 1997).

1.5.9 Post operative Artificial Nutrition Support: Enteral and Parenteral Feeding

There is an emerging consensus that early postoperative nutritional support benefits the surgical patient at high risk of complications by decreasing septic morbidity, maintaining immunocompetence and improving wound healing (Baigrie et al, 1996). An increasing body of literature indicates functional advantages of early postoperative enteral feeding in ameliorating the stress response and in diminishing the risk of major postoperative infections (Myers et al, 1995; Beier-Holgersen & Boesby, 1996; Kudsk et al, 1992). A recent meta-analysis of 11 prospective randomised controlled trials containing 837 patients who underwent gastrointestinal surgery, early enteral nutrition was associated with a significantly lower incidence of infections and a reduced length of stay versus starvation (Lewis et al, 2001).

Early enteral feeding impacts positively on whole body protein metabolism, and the hyperinsulinaemia induced by feeding, decreases endogenous fat oxidation (Hochwald et al, 1997). Nutritional fluid given enterally is completely absorbed even immediately following highly invasive oesophageal surgery. It has been suggested that this gut-directed therapy also modulates post surgical inflammatory responses, and encourages faster recovery of lymphocyte counts and attenuated levels of bilirubin and C-reactive protein compared with gut starvation after oesophagectomy (Aiko et al, 2001), although other studies fail to show a clear association between enteral nutrition and immune parameters (Reynolds et al, 1997). EN post oesophagectomy is associated with a significant increase in the levels of serum total protein and albumin (Yagi et al, 1999), and has also been shown to significantly attenuate gut permeability when compared to intravenous fluids only and is associated with significantly fewer post operative complications (Carr et al 1996). Postoperative starvation after oesophagectomy has been shown to be associated with poor nitrogen balance, poor gut mucosal integrity, a slower recovery in immune function and a more exaggerated inflammatory response (Aiko et al, 2001; Carr et al 1996).

1.5.10 Enteral Feeding post oesophagectomy: Jejunostomy Feeding

Needle catheter jejunostomy (NCJ) was first described in 1973 (Delaney et al, 1973). It is useful after oesophagectomy as normal food intake is delayed until any concerns about anastamotic healing and gastric emptying are abated, the average being approximately the 10^{th} postoperative day. It has been demonstrated that the small bowel is able to absorb nutrients almost immediately after surgery (Martin et al, 2007). NCJ allows provision of nutrition, fluid and electrolytes early after surgery and permits a safe means of administering many medications that might otherwise require central venous access or monitoring if given intravenously (Sarr et al, 1988). Once some oral feeding is permitted, patients almost uniformly experience early satiety and tend to eat smaller meals. Because it take several months for patients to adjust to their new anatomy (Ludwig et al, 2001), the presence of a NCJ provides a useful back up for patients who require supplementary enteral nutrition during this period of adjustment.

The use of the NCJ is not without risk, however, and as an adjunct to oesophageal resection serious complications, sometimes life-threatening, are well described and include Small bowel obstruction, small bowel perforation or Jejunal Intussusception (Han-Geurts et al, 2004; Biffi et al, 2000). However, the most alarming reports of serious complications are products of small series and probably represent a learning curve. In reports of series containing greater than 150 patients serious complications occur in less than 3% of patients. Gerndt et al (1994) reported in 523 oesophagectomy cases fed by NCJ that 11% required prolonged feeding for more than 3 weeks, and that the major complication rate associated with NCJ feeding was just 2%. These authors recommended the routine use of NCJ post oesophagectomy. Also recommending routine NCJ feeding were McCarter et al (1997) who reported no major complications or mortality associated with NCJ in 167 upper GI patients. Braga et al (2002) in a report of 402 NCJ and 248 nasojejunal tubes reported a serious complication rate of 1.7% and a 0.1% mortality rate. Similarly Sica et al (2005) in a report of 262 NCJ cases reported a 1.5% serious complication rate and 0% mortality rate. The only large report in the literature not to recommend routine NCJ feeding post oesophagectomy was that of Hans-Geurtz et al (2004) who reported on 1,166 oesophagectomy cases with a 1.1% re-operation rate and a 0.4% mortality rate. It is quite surprising that none of these reports make any reference to nutritional outcomes which is perhaps the main reason the NCJ is inserted in the first place. One can probably assume from other studies on enteral nutrition that NCJ feeding benefits the patient by decreasing weight loss and improving

recovery (Heylen et al, 1997), although these have yet to be properly examined in the setting of oesophageal surgery.

1.5.11 Parenteral Nutrition post gastrectomy

Patients with gastric cancer who undergo total gastrectomy usually receive TPN for 7 to 10 days after surgery because of concern over the integrity of the oesophagojejunal anastamosis (Kamei et al, 2005; Sand et al, 1997). There are very few reports published on early enteral feeding after total gastrectomy either by nasojejunal tube feeding or percutaneous catheter jejunostomy (Braga et al, 1996; Juhani et al, 1997; Sand et al, 1997).

Enteral nutrition is considered superior to parenteral nutrition based on several grounds including reduced costs; lower incidence of intra-abdominal and systemic sepsis (Kudsk et al, 1996; Moore et al, 1992); preservation of mucosal barrier by presence of enteric nutrients (McClave et al, 1992; Wilmore et al, 1988); maintenance of immune function (Kirby et al, 1995; Kudsk et al, 1994); faster recovery, shorter ICU and hospital stay (Gabor et al, 2005). However parenteral feeding is often the route of choice of surgeons who fear for the safety of enteral nutrients passing directly over a new anastamosis.

1.5.11.1 Further Studies on enteral feeding post oesophagectomy

Because it is safe and feasible to provide immediate post operative enteral nutrition post operatively, oesophagectomy provides an ideal model for studies on nutrition support. Not only is it a major homogenous insult with predictable alterations of immune cell function and metabolism, it carries a high risk of septic complications, weight loss and compromised quality of life. An improvement in nutrition support and immune function in the perioperative period could bring meaningful clinical benefits, and unsurprisingly a new range of products, so called immunonutrition or nutrient immuno-modulation is targeted on this premise. Glutamine, arginine, RNA and structured lipids, for instance, and hormones such as anabolic steroids, insulin and growth hormone, have been studied with varying degrees of promise in this context.

1.6 NUTRITIONAL MODULATION OF IMMUNE FUNCTION

1.6.1 The concept of 'Immunonutrition'

The interrelationship between nutrition and the immune system has become the focus of ever increasing attention with many substrates being recently identified as having an immune-modulating function. At present various enteral formulas are available containing substrates assumed to be beneficial e.g. glutamine, arginine, nucleotides and n-3 fatty acids, as well as selenium, vitamins E, C and β-carotene, at various concentrations (Suchner et al, 2000). The clinical significance of these immuno-modulating nutrients has only been recognised since the early 1980's – administered in doses far exceeding the amount used in a simple prevention of deficits, 'pharmacological' dosing with these nutrients has been shown to have many clinical effects. The application of nutrients for this purpose is referred to as 'immunonutrition' which has been defined as 'modulation of the activities of the immune system, and the consequences on the patient of immune activation, by nutrients or specific food items fed in amounts above those normally encountered in the diet' (Grimble, 2001).

Numerous clinical studies have been published examining the effects of immunonutrition using a variety of formulations and doses of immunonutrients as well as different types of operations/trauma. Several different commercial formulas are available with varying concentrations of individual immunonutrients. Unfortunately it is impossible to dissect the role of each individual component and combination since both the component and its concentration are relevant to their effect (Kudsk, 2006). In addition to this, the contradictory results in immunonutrition trials in the early 1990's possibly reflected the varying degrees of nutritional status in patients studied, as well as huge variations in the severity of the operative insult. It is now accepted that mild-moderate pre-existing malnutrition does not affect outcome in patients undergoing lesser surgical procedures, but pre-existing nutritional deficits increase post-operative complications as the surgical stress increases from minor to moderate to high (e.g. hernia to colectomy to pancreatectomy to oesophagectomy) (Kudsk, 2006).

A recent large meta-analysis of 12 studies containing over 1400 patients receiving enteral immunonutrition, showed a significant reduction in infectious complications and reduced overall length of stay in patients with critical illness and GI cancer (Beale et al, 1999). The majority of trials on immunonutrition have used enteral formulations containing arginine, glutamine, omega-3 fatty acids, nucleotides (RNA) and branched

chain amino acids. In 2006 the European Society of Parenteral and Enteral Nutrition recommended preoperative immunonutrition in patients with elective GI cancer surgery with an "A" level of evidence in their guidelines on Enteral Nutrition and Surgery (Braga 2007).

1.6.2 Arginine, Glutamine, Nucleotides (RNA), and Branched Chain Amino Acids

Arginine becomes an essential amino acid during periods of stress due to its use in tissue repair and due to up regulation of arginase following trauma. Depletion of arginine reduces wound healing and Kupfner cell function (Kudsk, 2006). Supplementation promotes proliferation of T cells *in vitro* and increases natural killer cell cytotoxicity, macrophage tumour cytotoxicity, and cytolytic T-cell activity (Kirk & Barbul, 1990). *Glutamine,* the most abundant free amino acid in the cytosol also becomes an essential amino acid during periods of stress (Kudsk, 2006). In these situations it becomes a major metabolic fuel for T-lymphocytes, enterocytes and other rapidly proliferating cells. In randomised controlled trials the administration of glutamine (either as a dipeptide during TPN to surgical patients or as a glutamine enriched enteral feed to trauma patients), resulted respectively in improved nitrogen retention (less tissue depletion) and reduced length of stay; reduced inflammation and a lower incidence of infective complications, suggesting improved immune function (Grimble, 2001; Morlion et al, 1998). *Nucleotide* deprivation inhibits T-cell and macrophage function and increases susceptibility to sepsis with Staphylococcus aureus and Candida albicans. Administration of RNA has been shown to reverse these effects in animal models (Grimble, 2001). *Branched Chain Amino Acids* provide the primary fuel for skeletal muscle during stress and sepsis. Addition of leucine, isoleucine and valine to formulas provides a metabolic source to supplement skeletal muscle metabolic needs during these metabolic states (Grimble, 2001).

1.6.3 Fatty Acids

It has been known since the 1970s that consuming diets high in fat tends to suppress immune responses such as phagocytosis and infectious disease resistance (Palmblad & Gyllenhammer, 1988). PUFAs can remodel the composition of cellular membranes especially of T cells and this is associated with diminished signalling through the T cell receptor (Fan et al, 2004; Stulnig, 2003). The most abundant PUFA in immune cell membranes is arachidonic acid (AA). Eicosanoids are derived from AA and act as mediators of inflammation and immune cell function. PUFA are also capable of

modulating cytokine production by immune cells as well as altering membrane fluidity. Modulation of dietary fatty acids can therefore potentially have an impact on many immune processes such as proliferation, phagocytosis, cytotoxicity and cytokine production (Fritshce, 2006). Most of the research on this topic has focused on one class of fatty acids, omega-3 polyunsaturated fatty acids (n-3 PUFAs). Fish oil derived n-3 PUFA have well established cardio-protective effects, but also purportedly possess anti-inflammatory and immunosuppressive activity. In addition to their potential immunomodulationg role in the setting of major surgery, n-3 PUFA have proven very promising in reversing aspects of the inflammatory response seen in cancer cachexia (Fearon et al, 2001b). For these reasons they may hold several promising effects in future research studies on cancer patients undergoing major surgery.

1.6.3.1 Mechanism of Action

With increasing enteral or parenteral intake of long chain n-3 PUFA, the ratio of n-3:n-6 PUFA in the phospholipids spectrum of the cell membrane in various tissues changes in favour of n-3 PUFA (Palombo et al, 1993; Morlion et al, 1996). In fact long chain (i.e. C20) dietary fatty acids of the omega 3 series are rapidly incorporated into cell membranes - incorporation of EPA into cell membranes has been achieved in 5 days with oral administration whereas incorporation is much faster (hours) with IV administration (Carpentier et al, 1997; Senkal et al, 2005).

This profoundly influences biologic responses, particularly during stress. These lipids influence membrane stability, membrane fluidity, cell mobility, the formation of receptors, binding of ligands to their receptors, activation of intracellular signalling pathways either directly or through the formation of eicosanoids, gene expression, and cell differentiation (Senkal et al, 2005).

Alterations in membrane phospholipids directly influences the synthesis of lipid-derived mediators such as eicosanoids, phosphatidic acid, platelet-activating factor and the secondary messengers, diacylglycerol and ceramide (Ross, 1999; Suchner et al, 2000), see figure 1.9. By the action of the enzyme phospholipase A2, PUFA can be released from the membrane phospholipids and either act as a secondary messenger or alternatively serve as a precursor for the cyclo-oxygenase pathway (Suhner et al, 2000). The latter pathway metabolises arachidonic acid to the 2-series of prostaglandins, especially prostaglandins E2 and F2alpha and thromboxane A2. These products are vasoconstrictive and induce platelet aggregation (Kudsk, 2006). These immunosuppressive products impair cytotoxic T-cell function, cytokine secretion,

leukocyte migration, and reticuloendothelial system function. In contrast, EPA derived thromboxane A3 is less active in platelet aggregation than thromboxane A2; EPA is converted to leukotriene B5 which results in decreased chemotactic migration and endothelial cell adherence, therefore EPA exerts major effects on the synthesis of leukotrienes by promoting an anti-inflammatory action.

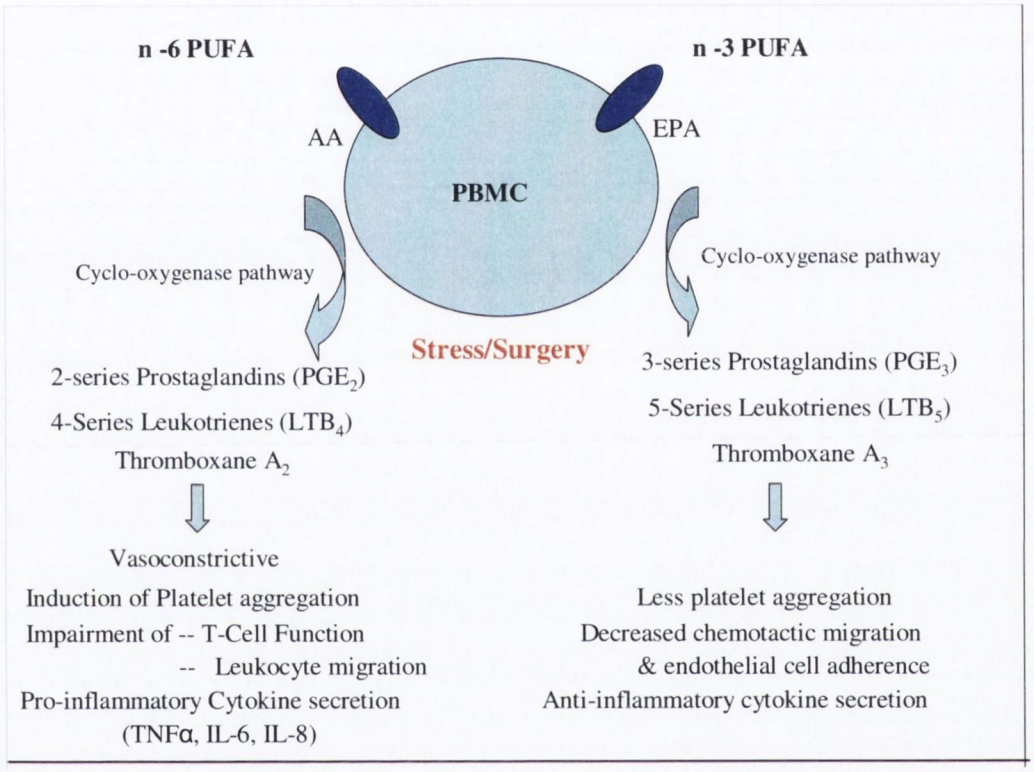


Figure1.9: Alterations in membrane phospholipids and subsequent alterations in the synthesis of lipid-derived mediators such as eicosanoids, leukotrienes and platelet-activating factors following major surgery.

The mechanisms underlying the differential effects of omega 6 and omega 3 lipids on cytokine synthesis are relatively unknown (Aiko et al, 2005). Several studies have demonstrated that there is a close relationship between the release and metabolism of AA from cell membranes and the generation of platelet activating factor (PAF). PAF is known to have a wide range of pro-inflammatory properties including increased chemotaxis, adherence, and aggregation of human neutrophils and monocytes. PAF also induces these cells to produce pro-inflammatory cytokines such as TNFα, IL-6 and IL-8 (Ruis et al, 1991). These cells in turn are capable of inducing cyclo-oxygenase 2 which metabolises AA and provides PGE2. These proinflammatory cytokines can also act as

pyrogens through increased production of PGE2 which can act directly on the brain causing an upward shift in thermo regulation (Coceani & Akarus, 1998). By substituting AA in cell membranes with EPA, PGE3 is preferentially formed and thus a more anti-inflammatory milieu arises with less risk of fever (see Figure 1.9). Thus, the provision of immunomodulating nutrients may promote restoration of normal tissue function post operatively and prevent the occurrence of SIRS (O'Flaherty, 1999).

1.6.3.2 Eicosapentaenoic Acid & Cancer Cachexia

Providing patients with cancer-associated malnutrition with nutritional support has been associated with a better response to therapy and fewer treatment-related complications (Nayel et al., 1992; Heys et al., 1999), as well as improved immune function (den Broeder et al., 2000; Bozzetti, 2001b), performance status, outcome and quality of life (den Broeder et al., 1998 and den Broeder et al., 2000; Barber et al., 1999a; McCarthy and Weihofen, 1999; Roberge et al., 2000; van Bokhorst-de van der Schueren et al., 2000). However, as cancer-associated malnutrition is a multifactorial condition, increasing nutrient intake may not be sufficient to reverse or prevent nutritional decline. Accumulating evidence supporting a role for inflammatory mediators in the pathogenesis of cancer-associated malnutrition has led to the suggestion that dietary supplements with anti-inflammatory properties may be beneficial (McCarthy, 2003). EPA has been shown to affect several factors, both systemic and tumour-derived, which have been implicated in the development of cancer cachexia. EPA supplementation in cancer patients has been shown to down-regulate the production of pro-inflammatory cytokines (IL-6, IL-1, and TNF) and attenuate progression of the acute phase protein response e.g. C-Reactive Protein (Grimble, 2003; Gogos et al, 1998; Barber et al, 1998; Barber et al, 1999a; Barber et al, 2001; Wigmore et al, 1997; Furukawa et al, 1999; Calder, 2002; Calder, 2003). Alterations in hormone levels, resulting in a more anabolic state, and weight gain, have also been reported (Barber et al., 2001). In preclinical studies, EPA has been shown to attenuate the catabolic effects of Lipid Mobilising Factor and Proteolysis Inducing Factor (PIF) (Beck et al., 1991; Tisdale, 1996; Lorite et al., 1997; Islam-Ali et al., 2001). Furthermore, EPA has been shown to reduce urinary PIF levels (Barber et al., 2001), attenuate cachexia (Wigmore et al., 1996; Barber et al., 1997) and has also been associated with the halting or reversal of weight loss (Barber et al, 2000; Barber et al, 1999b; Barber et al., 1999c; Fearon et al, 2001b; Wigmore et al, 2000), improvements in physical functioning and quality of life and a prolongation of survival (Gogos et al, 1998).

Improved immune function (Furukawa et al., 1999) and elevated effector T-cell numbers (Gogos et al., 1998) have also been reported in cancer patients receiving EPA supplements. EPA may also reduce treatment-related immunosuppression (Takagi et al., 2001) and the incidence of treatment-related complications (Kenler et al., 1996; Swails et al., 1997; Takatsuka et al., 2001). Thus EPA may also have a positive effect on the patient's immune system. Consistent with these observations, EPA has been associated with improved outcome in both malnourished and nourished patients with cancer, including increased survival (Gogos et al., 1998; Takatsuka et al., 2001).

Experimental studies suggest that EPA may also have direct effects on tumour growth and metastatic spread in vitro and in vivo (Lai et al., 1996; Jho et al., 2002; Tevar et al., 2002). Although in vivo inhibition of tumours may reflect effects on the host immune system, it has also been suggested that EPA and related n-3 PUFAs may act via reduction of prostaglandin and angiogenic growth factor levels or oncogene expression (Lai et al., 1996). This suggests that EPA may provide additional benefits to patients with cancer, beyond those achieved through improving nutritional status by interfering with pathways that underlie the development of cancer-associated malnutrition.

1.6.3.3 Eicosapentaenoic Acid & Gastrointestinal Surgery

A recent meta-analysis of 13 randomised controlled trials involving 1269 patients undergoing gastrointestinal surgery and who were given perioperative immunonutrition (nutrients including glutamine, arginine, omega-3 fatty acids and ribonucleic acids) showed that immunonutrition had no significant effect on post operative mortality, but had a positive effect on postoperative infection rate (OR 0.41, $p<0.00001$, length of hospital stay (less 3.5 days, $p<0.00001$) and improved immune function by increasing total lymphocytes, CD4 levels, IgG levels, and decreasing IL-6 levels, with no effect on CD8, IL-2 and CRP (Zheng et al, 2007).

A five day course of oral administration of EPA seems to be long enough for incorporation of omega 3 fatty acids into the cell membranes (Senkal et al, 2005) in contrast to IV administration, where the incorporation into cell membranes has been shown to be much faster (Carpentier et al, 1997). Senkal et al (2005) studied 40 patients who underwent esophagectomy (8), gastrectomy (23) or Duodenohemipancreatectomy (9) all for malignancy. Patients were randomised to receive a PUFA enriched (1g omega

3 PUFA/day) enteral diet (IMPACT) for 5 days pre operatively or an isocaloric diet. The enriched diet also contained arginine and RNA. Results showed that the PUFA enriched study arm had significantly increased levels of EPA in liver tissue, gut mucosa and tumour tissue

1.6.3.4 Concept of "pre loading" cells with Peri-operative EPA

As with any other substance with supposed pharmacologic action, immunonutrients should reach suitable tissue and plasma concentrations to be active (Gianotti et al, 2002). Some authors, believing that even with early postoperative immunonutrition it is not possible to prevent the immunosuppression produced immediately after surgery, have advocated the initiation of nutrition before surgery (Gianotti et al, 1999). This theory came about from results of trials with postoperative immunonutrition where the modulation of immune parameters in cancer patients took place after 5-7 days (Kemen et al, 1995) and this was accompanied by clinical effects (such as reduced infection rates), which were apparent after 5 days (Senkal et al, 1997). By contrast, administration of omega-3 fatty acids and other immunonutrients for 7 days preoperatively *and* in the post operative period resulted in significant reductions in post operative infections and length of hospital stay (Beale et al, 1999; Braga et al, 1999). These data lead to the concept of "pre-loading" of cell membrane phospholipids with active precursors of desirable immune modulators as it is beneficial for patient recovery independent of pre-operative nutritional status (Tsekos et al, 2004). Pre-loading cell membranes with EPA is associated with a reduced production of proinflammatory cytokines like IL-1α, IL-1β, IL-6 and TNFα in response to an inflammatory stimulus (Caughey et al, 1996; Senkal et al, 2005). Therefore authors recommend anticipation of the provision of immunonutrients before surgery to obtain adequate levels at the time of surgical stress when the need for stimulation of the immune system is maximised (Gianotti et al, 2002).

1.6.3.5 Evidence for EPA in upper GI cancer Surgery

Although several studies have demonstrated the beneficial effects of imunonutrition on immune competence and patient outcome, controlled clinical trials focusing on the use of perioperative enteral EPA alone are scarce. Three trials on enteral EPA following oesophageal cancer surgery have recently been published. Furkawa et al (1999) reported that the postoperative administration of Eicosapentaenoic acid at a dose of 1.8 g/d either orally or enterally in combination with TPN reduces the stress response to

oesophagectomy and stress-induced immune dysfunction compared to TPN alone. EPA supplementation significantly reduced the level of serum IL-6, and significantly improved the lymphocyte proliferation and Natural Killer cell activity on postoperative day 21 compared to TPN alone.

Takagi et al, (2001) studied the effects of EPA on immune suppression induced by postoperative chemo-radiation therapy in a small cohort of 15 patients who underwent thoracic oesophagectomy and received post operative chemotherapy. For 1 week before surgery and 2 weeks after patients were fed by total parenteral nutrition – group 1 (n=5) were given TPN with 1.8 g/day of EPA and group 2 (n=10) were given TPN alone. The authors assessed Phytohemagglutin- and concanavalin-A stimulated lymphocyte proliferation, Natural Killer Cell activity and total lymphocyte count 3 weeks after surgery and after post operative chemo-radiotherapy. The results revealed significantly less inhibition of cell mediated immunity in EPA treated patients undergoing thoracic oesophagectomy.

In the first and only study of enteral EPA after oesophagectomy for malignancy to date, Aiko et al (2005) performed a retrospective study of 27 patients and investigated whether supplementation of enteral nutrition with 2.25 g EPA/day affected platelet aggregation, coagulation activity and inflammatory response compared to standard enteral nutrition. Seventeen patients received an enteral formula for 7 days post operatively containing 2.25g EPA/day, the remaining 11 patients received a standard enteral formula for 7 days containing 0.7 g EPA/day. The results showed that administration of EPA in enteral nutrition significantly inhibited the post-operative decrease in platelet count, attenuated D-dimer levels and significantly decreased levels of the proinflammatory cytokine, IL-8, on days 1 and 3 post operatively. The anti-inflammatory effects of EPA were confirmed by the clinical findings of lower body temperature. There was also a decrease in the duration of fever in a number of patients. While these results seem promising it does seem surprising however that these authors were able to demonstrate decreased levels of pro-inflammatory cytokines and lower body temperature at day 3 post operatively as the patients had only received <1 g EPA on post operative day 1 and 1.1 gs and 2.2gs respectively on post operative days 2 and 3. Further studies with peri-operative administration of EPA are warranted to confirm these findings. There also is a need for long term studies addressing the issues of perioperative EPA supplementation on body composition and quality of life outcomes post major upper gastrointestinal surgery.

1.7 HYPOTHESES FOR THIS PhD THESIS

- There is currently no Irish data, and very little European data on obesity and oesophageal cancer. The first hypothesis of this thesis is that obesity is related to oesophageal adenocarcinoma in an Irish population. To prove his hypothesis we will examine the incidence of obesity prior to cancer development in a large population of patients who presented to St. James's Hospital over the past 10 years and compare these patients to healthy controls in a case-control study design.

- Metabolic syndrome has recently been identified as high risk state for cancer. The second hypothesis of this thesis is that metabolic abnormalities of obesity are related to precursor lesion of oesophageal adenocarcinoma, Barrett's Oesophagus. We will perform detailed nutritional studies on Barrett's patients and compare them to patients who have Gastro intestinal Reflux disease only. We will attempt to examine for the first time whether central obesity and it's metabolic consequences namely the "metabolic syndrome" is related to Barrett's oesophagus.

- Once diagnosed with upper GI cancer patients can be treated with multimodal therapy or surgery alone. We will examine which treatment pathway is related to higher morbidity - multimodality therapy or surgery alone. Then we will look for nutritional markers to help predict morbidity after this surgery.

- We will look at methods of nutrition support and how they can improve nutritional status. Finally we will use the model of oesophagectomy to investigate for the first time if intervention with an immunomodulating enteral feed enriched with Eicosapentaenoic acid peri-operatively can impact on post operative complications body composition, and immuno-inflammation.

CHAPTER 2:

OBESITY AS A RISK FACTOR FOR CANCER OF THE OESOPHAGUS, OESOPHAGO-GASTRIC JUNCTION, GASTRIC CARDIA AND STOMACH.

Published in: ***The European Journal of Cancer*** **(2006) May; 42(8):1151-1158**

"Adenocarcinoma of the Oesophagus and Gastric Cardia: Male preponderance in association with overweight and obesity".

Aoife Ryan, Suzanne P Rowley, Anthony P Fitzgerald, Narayanasami Ravi, John V Reynolds.

2.1 SUMMARY

Background Recent evidence links obesity with the rising incidence of oesophageal adenocarcinoma. In Ireland between 1995-2004, the incidence of oesophageal adenocarcinoma increased by 38 per cent, and this coincided with a 67% increase in the prevalence of obesity. The objective of this study was to assess the impact of Ireland's obesity trends on incidence rates of oesophageal cancer.

Design & Setting A case control study was undertaken in 760 patients presenting to a tertiary centre between 1994-2004 with a diagnosis of either cancer of the oesophagus, gastric cardia or stomach to investigate the prevalence of obesity prior to disease. Data were compared with 893 healthy controls. Multivariate logistic regression models were used to calculate the odds ratio (OR) of developing either cancer type according to quartiles of body mass index (BMI).

Results: Based on pre illness BMI, 82% of patients who developed adenocarcinoma of the oesophagus were either overweight or obese compared with 59% of the healthy control population ($p<0.001$). Males who developed adenocarcinoma of the oesophagus were significantly more overweight and obese than both patients with squamous cell cancer (SCC) and healthy controls ($p<0.001$). A dose-dependent relationship existed between BMI and oesophageal adenocarcinoma in males. The adjusted odds ratio was 4.3 (95%CI: 2.3 to 7.9) among males in the highest BMI quartile compared with males in the lowest quartile ($p<0.001$ for trend). Using common cut off points for BMI, the OR of adenocarcinoma of the lower oesophagus was 11.3 times higher (95% CI, 3.5 to 36.4) for males and females with a BMI > 30 kg/m^2 versus individuals with a BMI < 22kg/m^2 ($p<0.001$ for trend). For adenocarcinoma of the gastric cardia, a significant but weaker association with obesity was found; males in the top quartile of BMI had an OR of 3.5 (95% CI, 1.3 to 9.4) compared with the lowest quartile ($p=0.03$ for trend). A significant association was not observed in females. There was no association between obesity and adenocarcinoma of the stomach in males or females. A significant ($p < 0.001$) inverse relationship between BMI and oesophageal SCC was observed (males and females).

Conclusion: The odds ratio for adenocarcinoma of the oesophagus, the oesophago-gastric junction and gastric cardia rises significantly with increasing BMI. For tumours of the lower oesophagus obesity increases the risk 10.9 fold. The increased risk is significant in males only.

2.2 INTRODUCTION

The patterns of oesophageal cancer in Europe and North America are changing rapidly. The incidence of oesophageal adenocarcinoma is increasing by 5-10% per year (Enzinger & Mayer, 2003). The striking increased trends seen in adenocarcinoma of the oesophagus and gastric cardia are thought to result from several modifiable and interrelated risk factors, including chronic gastro-oesophageal reflux disease, poor diet, *H. pylori* eradication, and obesity (Enzinger & Mayer, 2003; Engel et al, 2003; Chow et al, 1998). A recent study of population attributable risks for oesophageal adenocarcinoma linked being overweight to 41% of cases (Engel et al, 2003).

The Republic of Ireland has witnessed a marked increase in the prevalence of obesity since the early 1990s. Sixty seven per cent of men, and 75% of women over the age of 51 are now overweight or obese (McCarthy et al, 2002). During this same period there was a 38% increase in the number of cases of oesophageal adenocarcinoma registered by the National Cancer Registry of Ireland (National Cancer Registry of Ireland http://www.ncri.ie). The incidence rate of oesophageal cancer in Ireland is amongst the highest in the Western world with 11.7 cases per 100,000 males and 6.1 cases per 100,000 females compared with a European Union average of 9.5/100,000 for males and 2.2/100,000 for females.

2.3 AIMS OF STUDY

The aim of this study was to examine the impact of Body Mass Index (BMI) and obesity on the risk of upper gastrointestinal cancer in Irish subjects.

2.4 PATIENTS AND METHODS

All histologically confirmed cases of adenocarcinoma and squamous cell carcinoma (SCC) of the oesophagus, oesophago-gastric junction, gastric cardia, and stomach, diagnosed or treated at the Oesophageal Unit of St James's Hospital, Dublin between 1994 and 2004 were included. This unit treats approximately 35% of patients in the Republic of Ireland with tumour at these sites, and approximately 50% of referrals can be treated with curative intent.

Cancer cases were identified from the St. James's Upper Gastrointestinal Cancer database, which uses the Patient Analysis and Tracking System (PATS) ^{TM} software from Dendrite Clinical Systems, UK. Cancer cases were selected by tumour location, which was based on endoscopic and radiological assessment. Tumours at the oesophagogastric junction were designated after pathological resection as Type I, II or III, as per Siewert and colleagues (WHO 1998): Type I was adenocarcinoma of the distal oesophagus involving the junction, usually arising in specialised intestinal metaplasia; Type II tumours are centred at the oesophagogastric junction; and Type III is a gastric carcinoma infiltrating the oesophagogastric junction and distal esophagus from below. In this study, Types I and II represent the O-G junction tumours, and Type III denotes a gastric cardia tumour.

The dietetic record cards of these cancer patients were then located and the nutritional details of all the patients were entered onto the PATS ^{TM} system. Each cancer patient had been assessed individually by a registered dietitian who gathered information on anthropometric measurements including height, weight at diagnosis, Body Mass Index (BMI) at diagnosis, pre-illness weight (at least one year prior to diagnosis), and pre illness BMI. For cancer cases, the patient's pre illness BMI was calculated from reported usual adult weight. Adiposity was estimated by BMI, computed as weight in kilograms divided by height in meters squared (kg/m^2). BMI was defined using the World Health Organisation definitions, with a BMI of 20-25 kg/m^2 normal, overweight 25-29.9 kg/m^2, and obese >30 kg/m^2.

The medical, dietetic and histopathology records of the cancer cases were recorded on a computerised upper Gastrointestinal Cancer Database (Patient Analysis and Tracking System ^{TM}, Dendrite Clinical Systems, UK). Data recorded concerned age, sex, tumour site, pathology, smoking and alcohol intakes, co-morbid disease, socio-economic status, reflux symptoms, medications, and the presence or absence of Barrett's oesophagus. *H.pylori* status was available in a small percentage of cases.

Anthropometric data on 893 healthy controls were used for comparison - controls under the age of 65 years were obtained from nationwide data as part of the North/South Ireland Food Consumption Survey (McCarthy et al, 2002), and over 65 year-old controls were interviewed and nutritionally assessed by a registered Dietitian (the author) at several day centres for the elderly in Dublin. Weight and height were measured, and BMI calculated, and data regarding cigarette and alcohol consumption, and socio-economic status gathered. Controls with a previous history of any cancer were excluded

from the study. Information on reflux history and *H.pylori* status was not available in the control group, and therefore could not be considered in this study.

2.5 STATISTICAL ANALYSIS

Pre Illness BMI was grouped into quartiles for analysis based on BMI distributions amongst the control subjects. Relative risks according to anthropometric status were expressed as odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression models. We tested for linear trend by including BMI as a continuous risk factor in the logistic regression. We investigated the linearity of any possible association between BMI and risk of cancer using a flexible model fitting approach that used a restricted cubic spline model with knots at the 25^{th}, 50^{th}, and 75^{th} percentiles of BMI in the controls. All analyses were adjusted for the effects of age sex, cigarette consumption (current smoker, ex-smoker, never smoker), and heavy alcohol (>14 units/week women, >21 units week men). Data was analysed in STATA^{TM} (version 8.2, Stata-Corp LP). The logistic regression analysis was also repeated excluding patients with Barrett's Oesophagus and gastro-oesophageal reflux disease

2.6 RESULTS

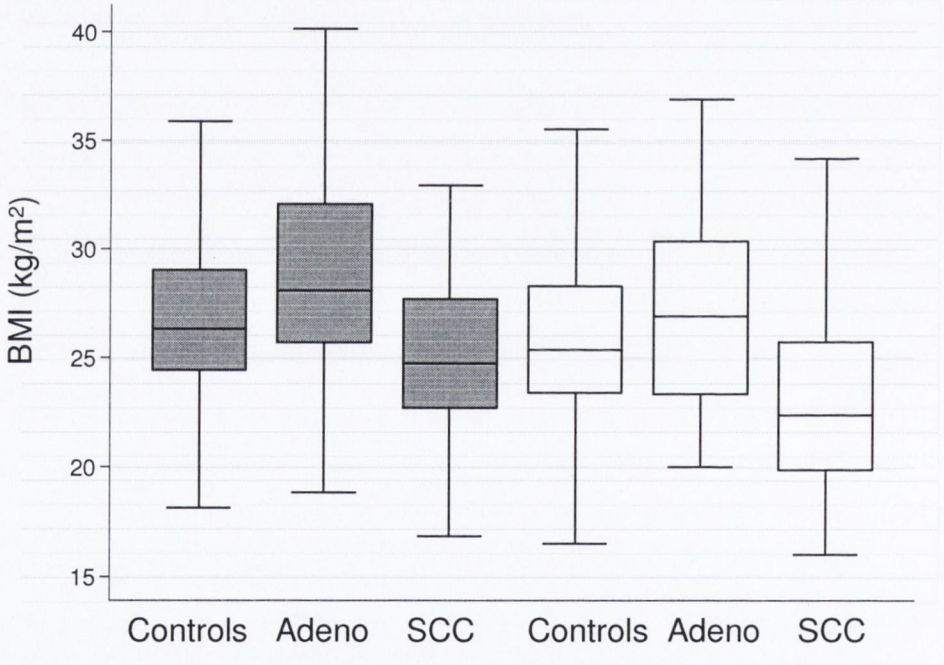
2.6.1 *Patient Demographics*

The sample population consisted of 239 females and 521 males, 508 patients had adenocarcinoma and 252 had SCC of the oesophagus. Tumour sites were lower oesophagus (n=279), mid oesophagus (n=91), oesophago-gastric junction (n=142), upper oesophagus (n=26), gastric cardia (n=65), and fundus, body or distal stomach (n=157).

2.6.2 Nutritional Status Pre Illness (Adenocarcinoma & SCC all tumour sites)

Based on pre illness BMI, 82% of adenocarcinoma patients were overweight or obese versus 35% of patients with SCC ($p<0.001$). The median pre illness BMI of males who developed ACA of the oesophagus was 28 kg/m^2 (IQ 25.5-31.8 kg/m^2) versus 24 kg/m^2 (IQ 21.25-26.56 kg/m^2) for SCC ($p<0.001$). Males who developed ACA of the oesophagus were significantly heavier than healthy controls that had a median BMI of 25.84 kg/m^2 (IQ 23.76-28.66 kg/m^2), $p<0.001$. SCC cases had a pre illness BMI that was significantly lighter than healthy controls and ACA cases ($p<0.001$), (*see Figure 2.1*).

Figure 2.1: Median BMI & (upper and lower quartiles) for males (Black) and females (White) with Adenocarcinoma and SCC versus healthy controls ($p<0.001$ for trend across groups). Error bars are 95% Confidence Intervals



2.6.3 *Adenocarcinoma of the Oesophagus and Oesophago-gastric junction (Table 2.1, Figure 2.2)*

The median pre illness BMI for patients who developed adenocarcinoma of the oesophagus was 27.96 kg/m^2 (IQ 25.53-31.79 kg/m^2). This was significantly greater than healthy controls (median BMI 25.84 kg/m^2, IQ 23.76-28.66 kg/m^2, p<0.001). Patients with tumours in the lower oesophagus and the oesophago-gastric junction were the heaviest (28.1 kg/m^2, IQ 25.9-32.14 kg/m^2; 27.8 kg/m^2, IQ 25.14-31.1 kg/m^2 respectively). Subjects were divided into 4 categories using the 25th, 50th, and 75th centiles of BMI among healthy controls. (<23.8 kg/m^2 quartile I, 23.8-25.8 kg/m^2 quartile II, 25.8-28.7 kg/m^2 quartile III, and >28.7 kg/m^2 quartile IV). Forty five percent of patients with adenocarcinoma of the oesophagus had a pre illness BMI in the top quartile (i.e. >28.7 kg/m^2). The OR for oesophageal adenocarcinoma rose significantly with increasing BMI. When compared to the first quartile the OR increased from 1.0 (95% CI, 0.6 to 1.7) for the second, to 1.9 (95% CI, 1.1 to 3.3) and 3.0 (95% CI, 1.8 to 5.0) in the third and fourth quartile respectively (p <0.001 for trend). When this analysis was broken down by gender, a significant increase was only observed for men who had an OR of 4.3 (95% CI, 2.3 to 7.9) for the top quartile versus quartile 1 (p=0.001 for trend), (*see table 2.1*).

The association between pre-illness BMI and the risk of cancer is illustrated in figure 2.2. When looking at oesophageal adenocarcinoma the spline model did not yield a significant improvement in fit, compared to the linear model, for men or women (p=0.57 and p=0.38 respectively). For adenocarcinoma of the lower oesophagus alone the improved fit was of borderline significance (p=0.10 and p=0.07 for men and women respectively).

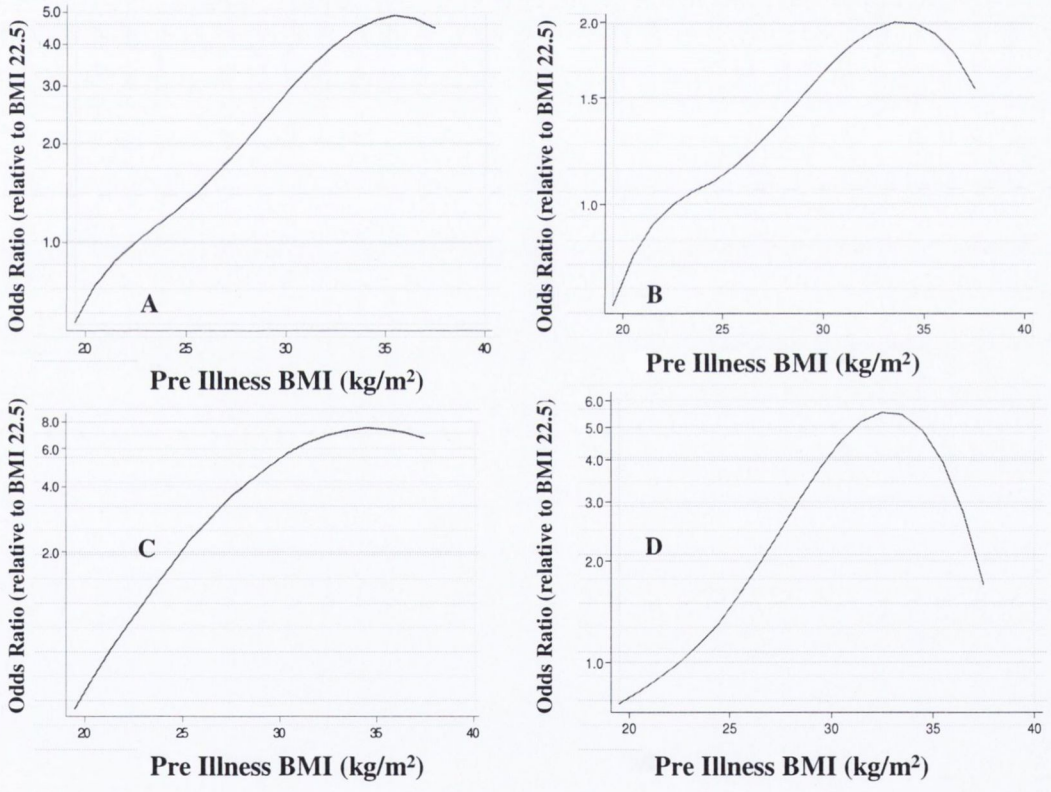
Table 2.1: Odds Ratios (OR) and 95% confidence intervals (CI s) associated with pre illness body mass index (BMI (kg/m^2)) by sex * for Adenocarcinoma$^{\Psi}$

	Oesophageal Adenocarcinoma		Lower Oesophageal Adenocarcinoma		OG Junction Adenocarcinoma	
Factor	Case/ controls	OR (95% CI)	No	OR (95% CI)	No	OR (95% CI)
Males and Females						
Pre Illness BMI						
Quartile 1	40/223	1.0 (referent)	13/223	1.0(referent)	24/223	1.0(referent)
Quartile 2	43/222	1.0 (0.6-1.7)	24/222	2.2(0.9-5.3)	19/222	0.7(0.3-1.4)
Quartile 3	74/225	1.9 (1.1-3.3)	44/225	5.0(2.1-11.4)	28/225	1.3(0.7-2.5)
Quartile 4	131/223	3.0 (1.8-5.0)	74/223	7.2(3.2-16.2)	54/223	2.2(1.2-4.0)
Test for Trend		p<0.001		p<0.001		p=0.001
Males Only						
Pre Illness BMI						
Quartile 1	27/74	1.0 (referent)	9/74	1.0(referent)	17/74	1.0(referent)
Quartile 2	38/96	1.4 (0.7-2.8)	22/96	3.2(1.25-8.9)	16/96	0.8(0.4-1.9)
Quartile 3	60/115	2.3 (1.3-4.6)	35/115	5.9(2.1-16.1)	25/115	1.7(0.8-3.7)
Quartile 4	114/110	4.3 (2.3-7.9)	62/110	9.1(3.4 -24.3)	49/110	2.9(1.4-6.1)
Test for Trend		p=0.0001		p=0.0001		p=0.001
Females Only						
Pre Illness BMI						
Quartile 1	13/149	1.0 (referent)	4/149	1.0(referent)	7/149	1.0(referent)
Quartile 2	5/126	0.4 (0.1-1.3)	2/126	0.6(0.1-4)	3/126	0.5(0.1-1.9)
Quartile 3	14/110	1.4(0.5-3.5)	9/110	3.2(0.8-13.8)	3/110	0.5(0.1-2.5)
Quartile 4	17/113	1.3 (0.6-3.2)	12/113	3.6(1.0-14.4)	5/113	0.8(0.24-3.0)
Test for Trend		P=0.34		p=0.09		p=0.86
Common cut-off						
Points for BMI**						
I –low	17/99	1.0 (referent)	6/99	1.0(referent)	9/99	1.0(referent)
II	35/267	1.0 (0.4-2.1)	13/267	1.3 (0.4-4.5)	21/267	1.1 (0.4-2.8)
III	133/376	2.7 (1.3-5.5)	77/376	6.8 (2.2-21.4)	54/376	2.0 (0.8-5.0)
IV – high	103/151	4.5 (2.2-9.5)	59/151	11.3 (3.5-36.4)	41/151	3.4 (1.4-8.7)
Test for Trend		P=0.001		p=0.001		p=0.001

*Cut off points for pre illness BMI (kg/m^2): I – first Quartile (<23.8), II (23.8 - 25.8), III (25.8-28.7), IV (>28.7).

** Standard cut off Points for BMI (kg/m^2): I (<22), II, 22-24.9), III (25-29.9), IV (>30)
Comparison of the trends in men and women: p=0.008 (oesophageal adenocarcinoma), p=0.11 (Lower Oesophageal adenocarcinoma), p=0.04 (Oesophago-gastro junction adenocarcinoma)
Ψ Data adjusted for age, sex, smoking, and alcohol intake.

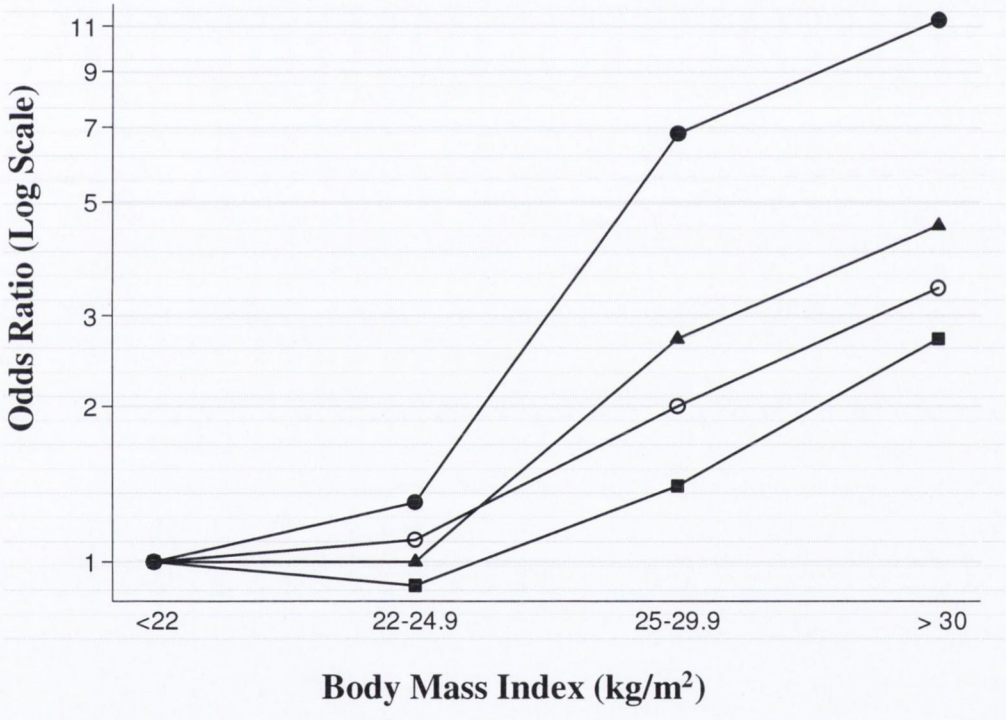
Figure 2.2: Spline Curves plotting relationship between Pre Illness BMI (kg/m^2) and risk of oesophageal adenocarcinoma for males only (A), oesophageal adenocarcinoma females only (B), lower oesophageal adenocarcinoma males only (C), lower oesophageal adenocarcinoma females only (D). Odds ratios are relative to a BMI of 22.5 kg/m^2.



When the heaviest quartile was divided in two (28.7-30.5 kg/m^2 and > 30.6 kg/m^2) the pattern became more striking. For males, the OR for oesophageal adenocarcinoma was 6.3 (95%CI, 3.2 to 21.6) for BMI >30.6 kg/m^2 versus the lowest quartile (< 23.7 kg/m^2) ($p<0.001$). For adenocarcinoma of the lower oesophagus alone, males with a BMI >30.63 kg/m^2 had an OR of 12.9 (95% CI, 4.6 to 36.6) versus males with a BMI <23.7 kg/m^2, $p=0.001$. Males with a BMI between 28.7-30.5 kg/m^2 had an OR of 5.8 (95% CI, 1.9 to 17.1) versus males with a BMI < 23.7 kg/m^2 ($p=0.002$ for trend). The odds ratios did not change significantly when patients with a history of gastro-oesophageal reflux disease and Barrett's oesophagus were excluded from the analysis (data not shown).

Analysed by common cut off points for BMI as defined by the World Health Organisation (WHO 1998), the risk of adenocarcinoma of the lower oesophagus was 11.3 for obese men and women (95% CI, 3.5 to 36.4) versus individuals with a BMI < 22 kg/m^2 ($p<0.001$ for trend). For Adenocarcinoma at the oesophago-gastric junction the OR rose significantly with increasing BMI, OR 2.2 (95% CI, 1.2 to 4.0) for top quartile versus lowest quartile ($p<0.001$ for trend). When the analysis was broken down by gender, a significant increase was only observed for males who had an OR of 2.9 (95% CI, 1.4 to 6.1) for top quartile versus lowest quartile ($p<0.001$ for trend), (*see Figure 2.3*).

Figure 2.3: Odds ratio for Adenocarcinoma of Oesophagus (all sites: ▲), lower oesophagus (●), oesophagus gastric junction (○) and gastric cardia (■) for common BMI groups relative to the BMI<22 group. Odds ratios shown on a log-scale.



2.6.4 Adenocarcinoma of Gastric Cardia (Table 2.2)

The median pre illness BMI for patients who developed adenocarcinoma of the gastric cardia was 26.6 kg/m^2 (IQ 24.3-30.1). This was not significantly heavier than healthy controls, with a median of 25.84 kg/m^2 (IQ 23.79-28.66). Using multivariate logistic regression adjusted for age, gender, smoking, and alcohol, the adjusted odds ratio for cardia adenocarcinoma for males and females together was 2.29 (95% CI, 1.0 to5.3) among persons in the highest BMI quartile compared to those in the lowest BMI quartile. This trend was not significant (p=0.14). When the analysis was repeated for each gender the association with obesity and gastric cardia adenocarcinoma was significant only in men. The OR for males in the top BMI quartile was 3.5 (95%CI, 1.3 to 9.4) versus males in the lowest BMI quartile (p=0.03 for trend) (*see table 2.2*).

2.6.5 Non-Cardia Gastric Adenocarcinoma (Table 2.3)

No relationship between BMI and non-cardia adenocarcinoma of the stomach was observed by either univariate or multivariate analysis. When the analysis was repeated using common cut off points for BMI the results remained insignificant (*see table 2.3).*

2.6.6 Oesophageal Squamous Cell Carcinoma (Table 2.4)

Prior to illness 50% of patients with SCC of the oesophagus had a normal BMI, 24% were overweight, 11% obese, and 15% underweight. The median pre illness BMI for SCC cases was 24 kg/m^2 (IQ: 21.25 – 26.7 kg/m^2) and this was significantly lower compared with both controls and adenocarcinoma cases (p<0.001), (*see figure 1).* Using multivariate logistic regression analysis, adjusting for age, gender, smoking, alcohol intake, an inverse association between pre illness BMI and risk of SCC was found (p<0.001). This inverse association was only significant for females. With increasing BMI the OR of SCC fell significantly, with an OR of 0.2 for the top quartile compared with the lowest quartile (95% CI, 0.1 to 0.4, p<0.001 for trend). A comparison of the trends between males and females showed a significant difference in risk pattern (p=0.02). When the analysis was repeated using common cut off points for BMI the inverse association remained highly statistically significant (*see table 2.4).*

Table 2.2: Anthropometric Indices and Risk of Gastric Cardia Adenocarcinoma$^{\Psi}$

Variable	Case-Patients/ Control, n/n	Multivariate adjusted Odds Ratio (95%CI)	*P* Value for trend
Pre Illness BMI			
Quartile 1	14/223	1.0 (referent)	
Quartile 2	14/222	0.8 (0.34 – 2.0)	
Quartile 3	14/225	1.7 (0.7-4.1)	p=0.14
Quartile 4	22/223	2.3 (1.0-5.3)	
Males Only			
Quartile 1	10/74	1.0 (referent)	
Quartile 2	12/96	1.2 (0.4-3.2)	p=0.03
Quartile 3	11/115	2.0 (0.7-5.7)	
Quartile 4	20/110	3.5 (1.3-9.4)	
Females Only			
Quartile 1	4/149	1.0 (referent)	
Quartile 2	2/126	0.3 (0.2-0.3)	p=0.70
Quartile 3	3/110	1.3 (0.3-6.3)	
Quartile 4	2/113	0.7 (0.1- 4.6)	
Common Cut-off			
*Points BMI***			
I – Low	8/99	1.0 (referent)	
II	14/267	0.9 (0.3 - 2.7)	p=0.14
III	25/376	1.4 (0.5 - 4.1)	
IV – High	17/151	2.7 (0.9 - 8.0)	

*Cut off points for pre illness BMI (kg/m^2): I – first Quartile (<23.76), II (23.76-25.84), III (25.84-28.7), IV (>28.7).
** Standard cut off Points for BMI (kg/m^2): I (<22), II, 22-24.9), III (25-29.9), IV (>30)
Comparison of the trends in men and women: p=0.10
Ψ Data adjusted for age, sex, alcohol, smoking.

Table 2.3: Anthropometric Indices and Risk of Gastric Adenocarcinoma (non-Cardia)[Ψ]

Variable	Case-Patients/ Control, n/n	Multivariate adjusted Odds Ratio (95%CI)	*P* Value for trend
Pre Illness BMI			
Quartile 1	46/223	1.0 (referent)	
Quartile 2	31/222	0.6 (0.3 – 1.1)	
Quartile 3	37/225	1.0 (0.6 – 1.7)	p=0.63
Quartile 4	42/223	0.9 (0.5 – 1.6)	
Males Only			
Quartile 1	26/74	1.0	
Quartile 2	21/96	0.7 (0.3 – 1.4)	p=0.20
Quartile 3	24/115	1.0 (0.4 – 2.1)	
Quartile 4	30/110	1.3 (0.6 – 2.6)	
Females Only			
Quartile 1	20/149	1.0	
Quartile 2	10/126	0.6 (0.3 – 1.4)	p=0.66
Quartile 3	13/110	1.1 (0.5 – 2.5)	
Quartile 4	12/113	0.6 (0.3 – 1.5)	
*Common Cut-off Points BMI***			
I – Low	16/99	1.0 (referent)	
II	50/267	1.3 (0.6-2.7)	p=0.63
III	57/376	1.2 (0.6-2.5)	
IV – High	33/151	1.5 (0.7-3.2)	

*Cut off points for pre illness BMI (kg/m^2): I – first Quartile (<23.76), II (23.76-25.84), III (25.84-28.7), IV (>28.7).
** Standard cut off Points for BMI (kg/m^2): I (<22), II, 22-24.9), III (25-29.9), IV (>30)
Comparison of the trends in men and women: p=0.21
Ψ Data adjusted for age, sex, alcohol, smoking.

Table 2.4: Anthropometric Indices and Squamous Cell Carcinoma$^{\Psi}$

Variable	Case-Patients/ Control, n/n	Multivariate adjusted Odds Ratio (95%CI)	*P* Value for trend
*Pre Illness BMI**			
Males and Females			
Quartile 1	116/223	1.0 (referent)	
Quartile 2	59/222	0.5 (0.3-0.8)	
Quartile 3	43/225	0.6 (0.3-0.9)	p=0.001
Quartile 4	33/223	0.3 (0.2-0.6)	
Males Only			
Quartile 1	45/74	1.0 (referent)	
Quartile 2	38/96	0.8 (0.4-1.6)	p=0.1
Quartile 3	24/110	0.8 (0.4 – 1.6)	
Quartile 4	21/110	0.6 (0.3 – 1.2)	
Females Only			
Quartile 1	71/149	1.0 (referent)	
Quartile 2	21/126	0.4 (0.2 – 0.7)	p=0.001
Quartile 3	24/110	0.4 (0.2-0.9)	
Quartile 4	21/110	0.2 (0.1-0.4)	
*Common Cut-off Points BMI***			
I – Low	79/99	1.0 (Referent)	
II	85/267	0.4 (0.3-0.7)	
III	60/376	0.3 (0.2-0.5)	p=0.001
IV – High	27/151	0.2 (0.1-0.4)	

*Cut off points for pre illness BMI (kg/m^2):: I – first Quartile (<23.76), II (23.76-25.84), III (25.84-28.7), IV (>28.7).
** Standard cut off Points for BMI (kg/m^2): I (<22), II, 20-24.9), III (25-29.9), IV (>30)
Comparison of the trends in men and women: p=0.02
Ψ Data adjusted for age, sex, alcohol, smoking.

2.7 DISCUSSION

Obesity is the strongest emerging risk factor associated with the marked increase in oesophageal adenocarcinoma in western societies. The incidence of adenocarcinoma of the oesophagus amongst white US males increased by over 350% from the mid 1970's to the mid 1990's (Devessa et al, 1998), and being overweight is thought to be linked to approximately 40% of cases (Engel et al, 2003). A recent study estimates that overweight/obesity, smoking, chronic gastro-oesophageal reflux, and low fruit and vegetable intake account for almost 80% of cases of adenocarcinoma of the oesophagus, and smoking and overweight account for 56% of cases of adenocarcinoma of the cardia (Engel et al, 2003). Increasing BMI has not been associated with SCC of the oesophagus, whereas alcohol, tobacco and low fruit and vegetable intake are associated with 90% of cases (Engel et al, 2003).

This study of an Irish population firmly supports the link between rising BMI and the risk of both adenocarcinoma of the oesophagus and gastric cardia. Obesity (BMI > 30 kg/m^2) was associated with a four-fold risk of adenocarcinoma of the oesophagus compared to males and females with a normal BMI. When the analysis was split for individual tumour sites the risks associated with high BMI were strikingly more marked for males compared with females. Males with a BMI in the top quartile had an OR of adenocarcinoma of the lower oesophagus over nine times that of males in the lowest quartile. For females the O.R was 3.6 for the top quartile compared with the lowest quartile but this trend was not significant. When common cut off points for BMI[7] were used, individuals with a BMI >30kg/m^2 had over eleven times increased risk for adenocarcinoma of the lower oesophagus compared with those with a normal BMI. This risk of adenocarcinoma was independent of the presence of reflux symptoms and the presence of Barrett's oesophagus did not affect the strength of the association with BMI. For adenocarcinoma of the oesophago-gastric junction the relationship between obesity and cancer was only significant for males with the risk being three-fold higher for males in the top quartile versus the lowest quartile.

Several studies report a relationship between obesity and adenocarcinoma of the oesophagus. The first population-based case-control study to investigate dietary and nutritional risk factors for adenocarcinoma of the oesophagus was carried out by Brown and colleagues (Brown et al, 1995) in 1995: 174 males with ACA and 750 control

subjects in three areas of the US were studied from 1985 to 1989, and they reported an increased risk (OR 3.1) in the heaviest quartile compared with the lightest quartile. Vaughan and colleagues (Vaughan et al 1995), in a case control of 404 cases of oesophageal cancer (298 adenocarcinoma and 106 SCC) and 724 healthy controls, reported that patients in the highest decile of BMI had the greatest (OR 1.9) risk of adenocarcinoma, and the risk of SCC was inversely related to BMI. Another study, by Chow and colleagues (Chow et al 1998) examined anthropometric risk factors in a population-based case control study of 589 cases of SCC and 554 adenocarcinoma cases, along with 695 healthy control subjects. The risk of adenocarcinoma only rose with increasing BMI, and the magnitude of the association was greatest among the younger age groups and among non-smokers. The largest European study is from Lagergren and colleagues (Lagergren et al 1999) who conducted a nationwide, population-based case-control study in Sweden of 189 cases of adenocarcinoma of the oesophagus, 262 cases of gastric cardia adenocarcinoma, 167 of SCC of the oesophagus, and 820 controls, and reported a significant dose-dependent relationship between BMI and oesophageal adenocarcinoma. The adjusted OR was 7.6 among persons in the highest BMI quartile compared to persons in the lowest quartile. In the UK, Cheng and colleagues (Cheng et al, 2000) conducted a case control study of 74 women with adenocarcinoma of the oesophagus and showed that a high BMI at the age of 20 years and low consumption of fruit was associated with increased risk (OR 6.04 for highest BMI quartile versus lowest). A recent study by Engeland and colleagues (Engeland et al 2004) reported on 2245 cases of oesophageal cancer from Norway. This study did not control for smoking, alcohol intake or diet, but again reported that obese men had a relative risk of death from adenocarcinoma of the oesophagus 2.58 times that of normal weight men.

In the largest prospective examination of the influence of overweight and obesity on deaths from cancer, Calle and colleagues (Calle et al 2003) studied over 900,000 US adults over 16 years. From the total population 876 deaths from oesophageal cancer in males and 189 deaths in women occurred. A significant association BMI and death from oesophageal cancer was seen in males, RR 1.63 (0.95-2.8, p=0.008), but no effect was observed in women. This study did not differentiate between adenocarcinoma and SCC.

This study demonstrates a strikingly greater link between BMI and adenocarcinoma of the oesophagus or cardia in men compared with women. An explanation for this is unclear. One possible mechanism links the gender specific different patterns of adipose

tissue distribution between males and females, and the well-described association of chronic gastro-oesophageal reflux disease (GORD) and adenocarcinoma. Males deposit fat preferentially in the intra-abdominal region at all ages in contrast to females who deposit sub-cutaneous adipose tissue predominantly in youth, and only post middle age do females tend to deposit intra-abdominal adipose tissue preferentially (Misra & Vikram et al, 2003). This central or android adiposity may increase GORD (La Vecchia et al, 2002; Rigaud et al, 1995). Obese subjects compared with non-obese subjects have elevated intra-abdominal and intra-gastric pressures, an increase of transient relaxations of the lower oesophageal sphincter, slower oesophageal transit and abnormal diaphragmatic pinchcock and phreno-oesophageal membrane anatomy (Mathys-Vliegen & Tygat 1996). Obese individuals are over four times more likely than lean individuals to have a hiatus hernia (HH), and have an overall prevalence of HH of 40% versus 12.6% for the general population (Wilson et al, 1999). The central adiposity of obese men may be associated with the risk of neoplastic progression in Barrett's oesophagus and may account for the male predominance of Barrett's oesophagus and adenocarcinoma (Vaughan et al 2002). With increasing duration and severity of reflux symptoms, and with increasing BMI the risk of adenocarcinoma increases in a dose dependant manner (Lagergren et al, 1999). When combined, reflux symptoms and obesity entails a greatly increased risk and relative risk exceeding 100 compared with persons with neither reflux symptoms nor obesity (Kershaw & Flier, 2004).

This mechanical thesis may be plausible, but since Calle and colleagues (Calle et al 2003) highlighted the link between obesity and death rates from not only oesophageal but many cancer types, other mechanisms are likely to be relevant. The pleiotropic properties of the adipocyte have come under scrutiny, in particular adipocytes deposited centrally, more typical of males, as these cells may have endocrine, paracrine and immunological properties. This may be manifested in the metabolic syndrome which is a constellation of metabolic risk factors consisting of atherogenic dyslipidaemia, elevated blood pressure, elevated blood glucose associated with insulin resistance, prothrombic and proinflammatory state. Adipose tissue is a complex and highly active metabolic and endocrine organ, expressing and secreting several endocrine hormones such as leptin, adiponectin, cytokines, complement components, plasminogen-activator inhibitor-1, proteins of the renin-angiotensin system and resistin. It is also a major site for the metabolism of sex steroids and glucocorticoids (Kershaw & Flier, 2004). The important endocrine function of adipose tissue is emphasised by adverse metabolic

consequences of adipose tissue excess. In the metabolic syndrome insulin resistance induces compensatory hyper-insulinaemia with increased insulin-like growth factor-1 (IGF-1 production), sex hormones and unbound sex hormone level, and these may interfere with cellular differentiation, proliferation and apoptosis thus increasing the risk of pre-neoplastic and neoplastic cell growth (Calle & Kaaks, 2004). Leptin, a protein produced by adipose tissue has recently been shown to increase proliferation of several cancer cell lines in vitro (Somasundar et al, 2003) and further studies are required relating adipocyte function with cancer biology. Why certain tumours could be promoted by the endocrine properties of adipocytes demands further study, in particular whether leptin and other growth factor receptors are differentially expressed in Barrett's and oesophageal adenocarcinoma compared with squamous epithelium.

The authors recognise that this study, like most comparable studies, used pre-illness reported weights, and recall bias is possible. The link however between body mass and cancer risk was unknown to the patients, and the patients were unaware of the histological subtype of their tumours, and thus any impact of recall bias should be similar for SCC and adenocarcinoma. Moreover, other studies indicate that overweight and obese individuals under-report their weight to a greater extent than lean individuals, and that BMI from self-reported weights underestimates the true prevalence of overweight and obesity (Flood et al, 2000; Kuczmarski et al, 2001). If this assumption is accepted, we can have confidence in the prevalence of obesity reported in this study and it may even understate the association.

2.8 CONCLUSIONS

Body Mass Index and adenocarcinoma of the oesophagus and gastric cardia are directly related in an Irish population. Males are especially sensitive to the increased risk of this cancer posed by obesity. The prevalence of obesity in Ireland and in Western countries could be important in understanding the increasing incidence of this tumour. Further research into oesophageal and gastric cardia adenocarcinoma is needed to clarify the risk factors and mechanisms responsible for the upward trends as well as the racial and gender disparities. Further work should establish the link between obesity, reflux, and oesophageal adenocarcinoma, and, in particular, the potential pro-inflammatory and pro-tumourigenic pathways facilitated through the altered immunological, metabolic and endocrine milieu in obesity, in particular male obesity.

CHAPTER 3

PROSPECTIVE INVESTIGATION OF THE INCIDENCE OF CENTRAL ADIPOSITY, METABOLIC SYNDROME, INSULIN RESISTANCE AND ADIPO-CYTOKINE SECRETION AMONGST PATIENTS WITH GASTRO-OOESOPHAGEAL REFLUX DISEASE AND BARRETT'S OESOPHAGUS.

Accepted for Publication in *Annals of Surgery* (Oct 2007), In Press.

"Barrett's Esophagus: Prevalence of Central Adiposity, Metabolic Syndrome, and a Pro-inflammatory state".

Aoife Ryan, Laura A Healy, Derek G Power, Miriam Byrne, Sinead Murphy, Patrick J Byrne, Dermot Kelleher, John V Reynolds.

3.1 SUMMARY

Background: Obesity is a risk factor for oesophageal adenocarcinoma, with inflammation and metaplasia secondary to obesity-related acid reflux the dominant hypothesis. The pro-inflammatory impact of adipo-cytokines associated with the metabolic syndrome of central adiposity may also be relevant. The primary aim of this study was to perform a detailed nutritional assessment on a patient population with Barrett's oesophagus, to screen for the metabolic syndrome, and to measure adipokines and cytokines that may have relevance to inflammation and tumour development.

Methods: Patients with Barrett's oesophagus or non-Barrett's Gastro Oesophageal Reflux Disease (GORD) were selected from hospital databases and invited to attend for metabolic and nutritional assessment. Studies performed included anthropometry, segmental body composition analysis by bioelectrical impedance, fasting lipids, insulin, glucose, and C-reactive protein in all patients, and measurement of a panel of adipokines and cytokines in the Barrett's population.

Results: 180 patients (102 Barrett's, 78 GORD) were studied. Seventy eight percent of Barrett's patients and 75% of GORD patients were overweight or obese according to BMI (p=ns). 46% of Barrett's patients had metabolic syndrome compared with 32% of GORD ($p = 0.04$), and the number of features of metabolic syndrome was significantly ($p = 0.03$) increased in the Barrett's cohort. Within the Barrett's cohort, metabolic syndrome was significantly associated with an adverse metabolic profile including increased trunk fat, a 10cm greater waistline, elevated CRP, leptin, insulin resistance, hypertension, and decreased adiponectin. Moreover, in patients with long-segment metaplasia (>3cms), 60% had metabolic syndrome and 92% were centrally obese compared with 23.8% and 62% respectively (p=0.007 and 0.005) in patients with short segment Barrett's (<3cms). Long-Segment Barrett's also had significantly higher IL-6 levels than short segment Barrett's (4 versus 0.56, p=0.03).

Conclusions: We report herein, for the first time, a high prevalence of obesity and metabolic syndrome in a cohort of Barrett's Oesophagus, which far exceeds population norms and also an association between central fat, metabolic syndrome, and the adipocytokine profile in patients with long-segment Barrett's compared with short-segment Barrett's oesophagus, suggesting a pathway that may be important to the continuum of metaplasia within the Barrett's cohort.

3.2 INTRODUCTION

The pathologic phenotype of specialised intestinal metaplasia (SIM) defines Barrett's oesophagus (Sampliner, 1998), and SIM is the sole recognized precursor of adenocarcinoma of the oesophagus. Patients with Barrett's oesophagus have a 30-40 fold increased risk of developing oesophageal adenocarcinoma. The existing consensus is that long-standing acid and bile reflux results in chronic inflammation and SIM over a variable length of the oesophagus, and that SIM may progress through dysplasia to adenocarcinoma. The factors that determine why an individual with reflux develops SIM remain unclear, and information on factors determining the length of SIM within a Barrett's oesophagus as well as what governs progression from SIM to dysplasia and cancer is unknown.

There has been a marked recent increase in the incidence of oesophageal adenocarcinoma in the Western world, and this has been paralleled by an increased prevalence of obesity. Epidemiological evidence strongly links obesity with oesophageal adenocarcinoma, and obesity may be a factor in up to 40 percent of cases (Ryan et al, 2006; Calle et al 2003; Lagergren et al 1999; Chow et al 1998; Vaughan et al 1995; Brown et al, 1995; Engel et al 2004). Although obesity promotes gastro-oesophageal reflux disease (GORD) and this presents one possible pathway to adenocarcinoma, the association of obesity with the intermediate steps, including the development and extent of Barrett's oesophagus, and the progression of Barrett's metaplasia to dysplasia and adenocarcinoma is poorly understood. Moreover, obesity is positively associated with the prevalence and death rates of many other cancers, and therefore other mechanisms may be important, in particular the systemic inflammatory state consequent on the altered metabolism in obese patients, and the associated impact of adipokines, cytokines, and pro-coagulant factors released by adipocytes, particularly central fat. This may be manifest in the Metabolic Syndrome, best described in association with cardiovascular disease and type II diabetes, where the usual screening variables are waist circumference, circulating levels of triacylglycerols and high-density lipoprotein cholesterol, fasting glycaemia, and blood pressure (Despres & Lemieu, 2006; Guzik et al, 2006). It is estimated that approximately 24 percent of US adults have metabolic syndrome, and approximately 12 percent in Europe. There is no national reference data in Ireland for the prevalence of metabolic syndrome in the normal population, but a recent study of over

1000 individuals aged between 50 and 69 reported a prevalence of metabolic syndrome of 21 percent (Villegas et al, 2006).

The primary aim of this study was to perform a detailed nutritional assessment on a patient population with Barrett's oesophagus, to screen for the metabolic syndrome, and to measure adipokines and cytokines that may have relevance to inflammation and tumour development. A contemporaneous cohort of patients with non-Barrett's GORD was also studied. We report herein a high prevalence of obesity and metabolic syndrome, as well as an association between central fat, metabolic syndrome, and the adipocytokine profile in patients with long-segment compared with short-segment Barrett's oesophagus, suggesting metabolic alterations in obesity that may be important in the progression of Barrett's.

3.3 PATIENTS AND METHODS

The study was approved by the hospital ethics committee for research involving human subjects according to the Helsinki agreement. Informed consent was obtained from all patients prior to participation (See appendices for patient information leaflet and consent forms).

3.3.1 Patients

Cases were identified from the electronic endoscopic records kept at St. James's Hospital. Cases were eligible who were diagnosed with SIM or endoscopically evident Barrett's oesophagus since 2005. The pathological records of patients were also checked to confirm the findings of SIM on biopsy. In addition to this, the records of patients who attended the Upper Gastrointestinal Function Unit for pH manometry were searched for suitable cases of GORD and/or Barrett's. Cases identified in this manner were cross checked for pathological evidence of SIM in the case of Barrett's and for significant acid reflux (> 14.92 De Meester score) identified by 24 hr pH study for GORD cases (Scarpulla et al, 2007). Long Segment Barrett's (LSB) was defined as Barrett's of 3cm or greater, and Short Segment Barrett's (SSB) was defined as <3 cms. Every suitable patient identified during tis time frame were invited by letter to attend one of several Oesophageal Research Clinics with a view to undergoing an assessment of nutrition and metabolism. At the clinic patients met with a consultant upper gastrointestinal surgeon who explained the nature of the study and obtained informed consent. Patients were

excluded from the study if they had adenocarcinoma of the oesophagus or junction; had undergone anti-reflux surgery or were suffering from any chronic inflammatory disorder or other form of malignancy. Once consented, patients attended an assessment with a research dietitian who carried out several measurements as detailed below and also asked about reflux symptoms, medication use, medical history, alcohol and tobacco use and physical activity (see appendix for case report form).

3.3.2 Venous Blood Sampling

After a 12-hour overnight fast, venous blood samples were taken for the measurement of plasma concentration of glucose (fasting); total-, HDL- and HDL- cholesterol, triglycerides, fasting insulin levels and C reactive protein (CRP). Serum from patients with Barrett's oesophagus was also frozen at –80°C for later measurement of adipokines as well as cytokines and growth factors. The degree of insulin resistance was estimated by Homeostatic model assessment (HOMA) according to the method described by Matthews et al (Matthews et al 1985), an insulin resistance score (HOMA-IR) was computed with the formula: *Fasting plasma glucose (mmol/l) X fasting serum insulin (mU/l) divided by 22.5.* Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity (insulin resistance).

3.3.3 Anthropometry, Segmental Body Composition Analysis & Blood Pressure

Weight was measured using a digital scales to within 0.1kg, without heavy outdoor clothing or shoes. Height was measured barefoot using a portable stadiometer (Seca) to within 0.5cm. Waist circumference (to within 1mm) was measured using a plastic tape at the midpoint between the lowest rib and the iliac crest with the subjects standing, after gentle expiration. Segmental body composition was analysed using the Tanita BC 418 MA bioelectrical impedance analyzer (Tanita UK Ltd, Middlesex, UK) which gives precise information on the amount of lean and fat tissue in the trunk area and in each limb, as well as overall body composition and hydration status. Blood pressure was measured with a digital sphygmomanometer on the left arm after at least 10 minutes of rest. The mean blood pressure result was determined from three independent measurements.

3.3.4 Metabolic Syndrome Classification

Metabolic syndrome was diagnosed according to the criteria set out by the International Diabetes Federation (Alberti et al 2006): central obesity (waist circumference ≥ 94cm

European males, ≥ 80 cm European females) *plus any two of the following*: raised Triglycerides ≥ 1.7mmol/l or specific treatment for this lipid abnormality; reduced HDL <1.03 mmol/l in males or <1.29mmol/l in females; raised blood pressure: systolic: ≥ 130mmHg or Diastolic ≥85 mmHg, or treatment of previously diagnosed hypertension; fasting plasma glucose ≥ 5.6 mmol/l or previously diagnosed Type II Diabetes. Patients were also classified according to the National Cholesterol Education Programme (NCEP) Adult Treatment Panel III definition of Metabolic Syndrome (NCEP 2001).

3.3.5 Quantification of Serum Adipokines

Leptin, Adiponectin, and Resistin levels in serum were determined by standard ELISA techniques (Linco Research Inc, Missouri, USA). Briefly, the assay is a sandwich ELISA, based sequentially on concurrent capture of Human adiponectin, leptin or resistin molecules from samples to the wells of a microtitre plate coated with a monoclonal antibody to the captured molecules, washing of unbound materials from samples, binding of conjugate to the immobilised biotinylated antibodies, washing of excess of free enzyme conjugates and quantification of immobilised antibody-enzyme conjugates. The enzyme activity was measured spectrophotometrically by the increased absorbance at 450nm-590nm after acidification of formed products, and concentrations calculated from a reference curve.

3.3.6 Quantification of Serum Cytokines

A panel of cytokines and growth factors were measured using the Randox Evidence Investigator (Randox Laboratories, Belfast, U.K). These included IL-2, IL-4, IL-6, IL-8, IL-10, TNFα, IL-1α, IL-1β, INFγ, VEGF, EGF and monocyte chemotactic protein-1 (MCP-1) as described previously (Fitzgerald et al, 2005).

3.4 STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS® Version 14.0 for Windows™ (SPSS® Inc., Chicago, IL). Mean (± standard deviation) were compared with each other using paired samples t-tests. Cross-tabulation was used to compare differences between groups for categorical variables. Significant differences were tested using Pearson Chi-square analysis. Differences in mean laboratory data and anthropometric data across categories were evaluated using one-way analysis of variance (ANOVA). Where statistically

significant effects were encountered ($p<0.05$), comparisons of means were made using Scheffe *post-hoc* multiple comparisons test.

3.5 RESULTS

There was a 70% response rate from Barrett's patients and a 68% response rate from GORD patients to the invitation to attend the Oesophageal Clinic. In total 188 patients attended the clinics and all gave informed consent. Eight patients did not meet the inclusion/exclusion criteria and were excluded due to the following pre-existing inflammatory diseases (e.g. Arthritis (2), Crohns Disease (1), Cellulitis (1), Alcoholic Liver Disease (2), Breast Cancer (2)). One hundred and eighty patients were included, 115 males and 65 females, 102 with proven Barrett's Oesophagus and 78 with confirmed GORD from pH studies.

3.5.1 *Anthropometry, Biochemistry and Prevalence of Metabolic Syndrome in Barrett's versus GORD (Table 3.1)*

The mean age at assessment was 56 years (±12.5) in Barrett's and 52 years (± 11.5) for GORD (p=0.001). Barrett's cases had a significantly higher DeMeester score compared with GORD (67 versus 42, p=0.007). Seventy eight percent of Barrett's patients and 75% of GORD patients were overweight or obese according to BMI (p=ns). When classified according to central obesity cut-off points (>80cm for women and >94cm for males), 78% of Barrett's and 74.5% of GORD were centrally obese (p=ns). There was no significant difference in any of the segmental body composition analysis results between the two groups. Twenty four percent of Barrett's patients were on anti hypertensive medication versus 9% of GORD (p=0.006), and 13% were on cholesterol lowering medication versus 9% in GORD group (p=0.301). Thirty three percent of Barrett's and 27% of GORD patients reported heavy alcohol consumption (>14 units/week for females and > 21 units week for males). Thirty seven percent of Barrett's and 37% of GORD patients had a gamma GT level above the normal reference range (i.e. 40 IU/L). There was no significant difference in the reported physical activity levels at work-time, however, Barrett's patients were significantly less active in their leisure time than GORD cases (p=0.006).

The prevalence of Metabolic Syndrome was 46% in Barrett's cases and 32% in GORD cases (p= 0.04) using the International Diabetes Federation definition (Alberti et al, 2006). There was also a significant difference in the number of features of the Syndrome

between the two groups, with 46% of patients in the Barrett's cohort having 3 or more features of the metabolic syndrome compared with 32% in the GORD cohort (p = 0.03). The same pattern was also confirmed when the NCEP ATP III definition of Metabolic syndrome was used. Barrett's cases were significantly more likely to have hypertension than GORD patients (68% versus 43%, p=0.001) or to be on treatment for hypertension (24.5% versus 9%, p=0.006); the mean systolic BP was 144 mmHg (± 20) for Barrett's and 138 mmHg (± 16) for GORD (p=0.02), the mean diastolic BP was 90 mmHg for Barrett's (±18) and 84 mmHg (±10) for GORD, p=0.003. The mean ± SD CRP level in Barrett's patients was 6.3(12) compared with 5.7(10) in the GORD cohort (p = ns). Barrett's patients however were more likely to have a CRP above the normal range (i.e. 10mg/L) versus GORD (15.8% versus 5.7%, p=0.054). There was no significant difference in fasting insulin levels or in the HOMA-insulin resistance scores.

3.5.2 Prevalence of Metabolic Syndrome in Patients with Barrett's Oesophagus (Table 3.2)

There were 47 patients (46%) in the Barrett's cohort with the metabolic syndrome. Compared with patients without the Metabolic Syndrome, Barrett's patients with the Syndrome were significantly heavier and had a higher BMI, had significantly greater waist circumference (96cms versus 106 cms, p=0.0001) with higher trunk fat as a percentage (27.6 versus 32%, p=0.006) and in kg (12 kg versus 15 kg, 0=0.003). Barrett's patients with Metabolic syndrome had significantly higher levels of insulin (p=0.006), HOMA-IR (p=0.007), fasting glucose (p = 0.006), CRP (8.4 versus 4.5, p=0.019), and leptin (p=0.006). Differences in adiponectin levels approached significance (p=0.096), with lower levels in the cohort with the metabolic syndrome. There was no significant difference in resistin between both groups. When we compared the mean serum cytokine levels (IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, INFγ, TNFα, IL-1α, IL-1β, MCP1, or EGF) of Barrett's patients with Metabolic Syndrome to those without Metabolic Syndrome, there was no significant difference between the groups (data not shown).

The mean levels of leptin, adiponectin and insulin per BMI group in the Barrett's cohort are shown in Figure 3.1. As BMI increased the levels of leptin (p=0.037) and insulin (p=0.0001) increased significantly and the levels of adiponectin decreased significantly (p=0.005).

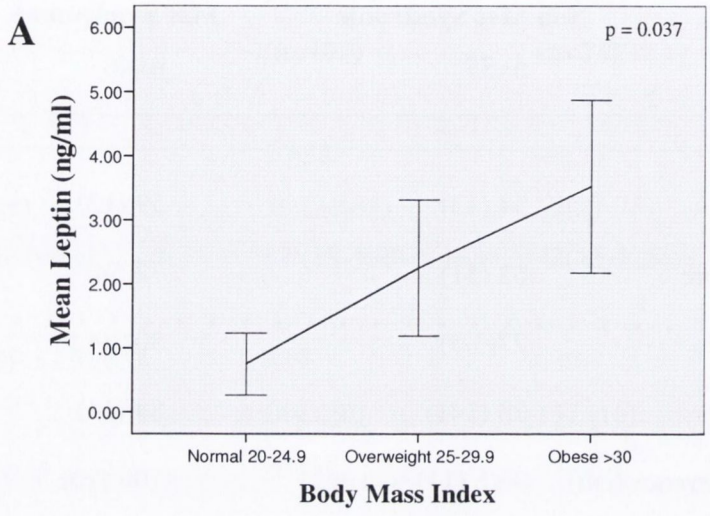
Table 3.1: Anthropometric and metabolic features of Barrett's versus GORD patients. Values shown as mean (standard deviation) or percentage.

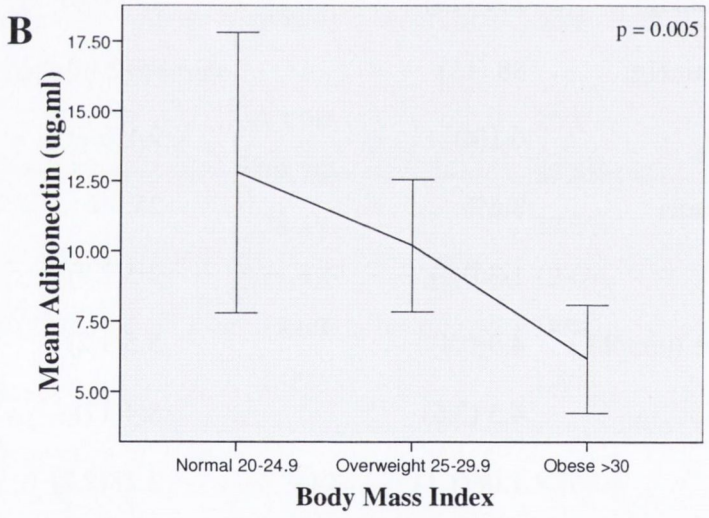
	Barrett's (n=102)	**GORD (n=78)**	**P**
Male: Female	70:32	45:33	0.007
Mean Age (range)	56 (26-83)	52(27-75)	0.001
DeMeester Score (range)	67 (18-196)	42(15-115)	0.007
Hypertensive (BP >130/85)	68%	43%	0.001
Systolic BP	144 (20)	137 (16)	0.02
Diastolic BP	90 (18)	84 (10)	0.003
Metabolic Syndrome	46%	32%	0.04
Number features of Met Syndrome			
0	12.9%	20%	
1	12.7%	25.5%	
2	28.4%	22.5%	
3	31.4%	12.6%	0.03
4	13.6%	18%	
5	1.0%	1.4%	
CRP (mg/L)	6.3(12)	5.7(10)	ns
High CRP (>10 mg/L)	15.8%	5.7%	0.054
Insulin (mU/L)	7.5 (5.5)	8.5 (6)	ns
Hyperinsulinaemia	16%	17%	ns
HOMA-IR	1.9(2.1)	1.9(1.5)	ns
Weight (kg)	81(14)	81 (18)	ns
BMI kg/m^2(mean)	28.5(4)	28.7(6)	ns
Waist Circumference(cm)	101(12)	98(14)	ns
Central Obesity (yes)	78.4%	74.5%	ns
Trunk Fat %	29.6(8)	29.3(9)	ns
Trunk Fat Mass (kg)	13.5(5)	13.1(6)	ns

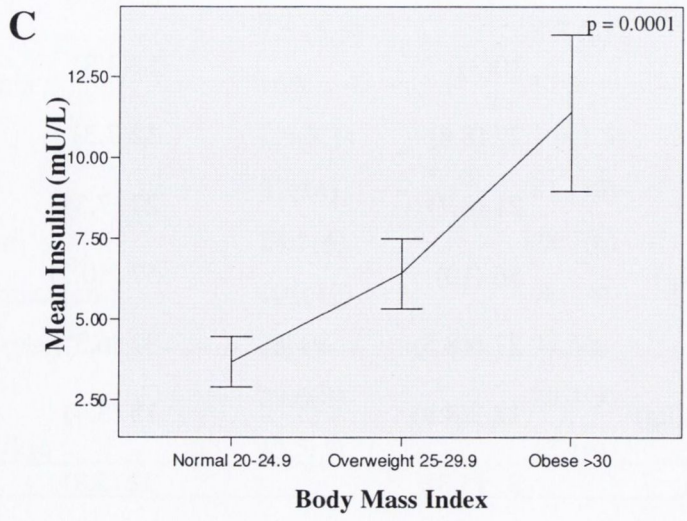
Table 3.2: Anthropometric and metabolic features of Barrett's patients according to Metabolic Syndrome Classification (mean & standard deviation)

	Barrett's Oesophagus		
	Non Met Syndrome n=55	Met Syndrome n=46	P
Age (years)	54 (13)	59 (12)	0.039
DeMeester Score	63 (51)	72(54)	0.546
BMI (kg/m^2)	27(3.5)	30.2(4.5)	0.0001
Weight (kg)	78 (14)	84 (14)	0.02
Waist Circumference (cm)	96.6 (11)	106 (10)	0.0001
Systolic BP (mmHg)	139 (19)	150 (88)	0.006
Diastolic BP (mmHg)	88 (15)	93(20)	ns
Insulin (mU/L)	6.1(4)	9.1(6.7)	0.006
Hyperinsulinaemia	8.6%	25.4%	0.009
HOMA-IR	1.3(0.9)	2.5(2.9)	0.007
Fasting Glucose (mmol/L)	4.9(0.4)	5.5(1.2)	0.0001
CRP(mg/L)	4.5 (3.6)	8.4(17)	0.019
Leptin (ng/ml)	1.06(1.1)	3.18(2.2)	0.006
Adiponectin (μg/ml)	11(5.5)	8.1(4.0)	0.096
Resistin (ng/ml)	10(3)	12(3.8)	ns
Fat %	27 (8.6)	32(7.3)	0.006
Fat Mass (kg)	21 (8.7)	27 (7.3)	0.002
Fat Free Mass (kg)	56 (10)	57 (10.7)	ns
Trunk Fat %	27.6(8.5)	32 (6.3)	0.006
Trunk Fat Mass (kg)	12.3(4.9)	15 (3.4)	0.003
Trunk FFM (kg)	31 (5.3)	32 (5.8)	ns

Figure 3.1: (A) Serum Leptin levels (ng/ml), (B) Adiponectin levels (µg/ml) and (C) fasting Insulin levels (mU/L) in Barrett's patients according to BMI group (normal = 20-24.9 kg/m^2, n=22, overweight = 25-29.9 kg/m^2 n=45, obese= >30 kg/m^2 n=35).







3.5.3 Obesity, Metabolic Syndrome and Length of Barrett's (Table 3.3; Figure 3.2)

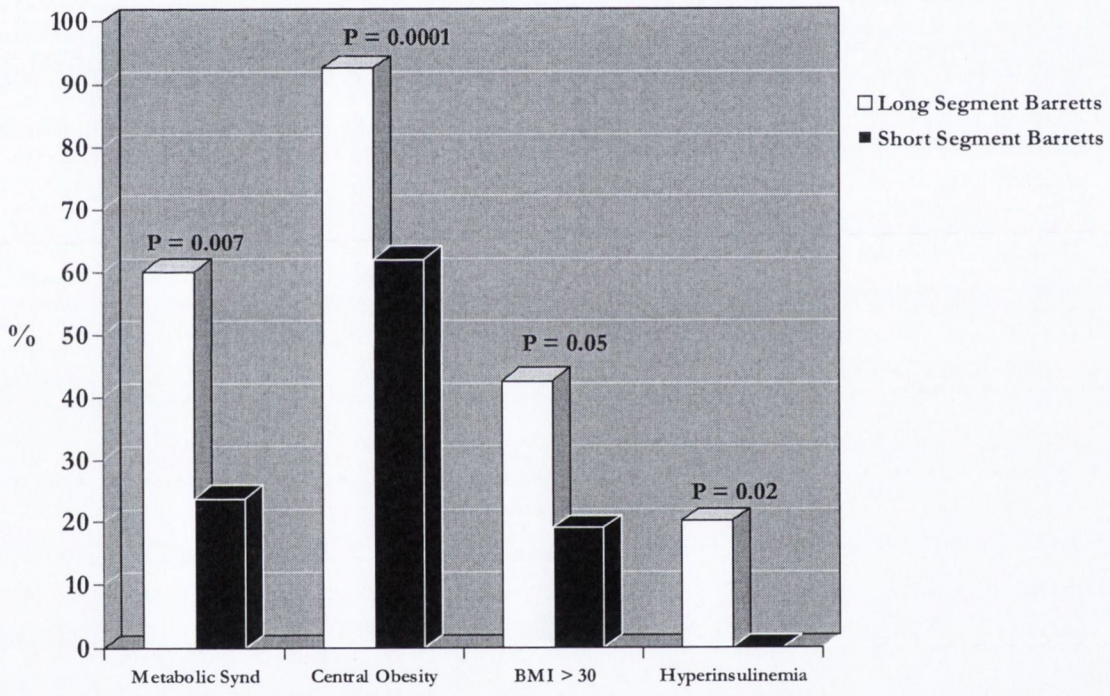
Precise data concerning the length of Barrett's was available for 79 Barrett's cases. There were 49 patients with long segment Barrett's (LSB), and 30 with short segment Barrett's (SSB). There was no significant difference in age between the two groups. There were significantly ($p<0.05$) more obese patients (43%) with LSB compared with SSB (19%). Fifteen percent of the LSB cohort had a normal BMI compared with 33% of the SSB cohort ($p=0.01$). Ninety three percent of LSB patients were centrally obese compared with 62% of SSB patients ($p=0.005$) and LSB was significantly associated with a greater waistline (106cm versus 95cm, $p=0.0001$), and greater trunk fat (14.7 kg versus 12.5 kg, $p=0.049$) compared with SSB.

There was no difference in the incidence of hypertension (33% versus 22.5%, $p=0.56$), use of statins (4.8% versus 15%, p=ns), or any of the lipid profile or incidence of hypertriglyceridaemia (19% versus 30%, p=ns) parameters between short and long segment Barrett's. Sixty percent of LSB patients had the metabolic syndrome, compared with 24% in patients with SSB ($p=0.007$), and this was also associated with a higher incidence of hyperinsulinaemia (20% versus 0%, $p=0.049$), a trend towards higher HOMA-IR scores in LSB cases (1.8 versus 1.2, $p=0.075$) and significantly higher serum IL-6 levels (4.0 versus 0.6, $p=0.03$). There was no difference in any of the other cytokines, growth factors or adipokines between the groups.

Table 3.3: Anthropometric and metabolic features of Long Segment Barrett's (>3cms) versus Short Segment Barrett's (<3cms). Means (standard deviation).

	Short Segment (n=30)	Long Segment (n=49)	P
Age (years)	60(11.5)	54.8(12.5)	0.092
Length of Barrett's (cm)	1.2(0.8)	6.5(3.2)	0.0001
Metabolic Syndrome	23.8%	60%	0.007
No. features Met Syndrome	1.76(1)	2.5(1.1)	0.01
Insulin	5.3(2.8)	7.5(4.4)	0.049
Hyperinsulinaemia (>12)	0%	20%	0.026
HOMA-IR	1.2(0.7)	1.8(1.2)	0.075
Anthropometry			
Waist Circumference	95(11)	106(10.8)	0.0001
Central Obesity	62%	92.5%	0.005
BMI	26.8(4.4)	29.5(4.5)	0.033
Overweight (25-30)	48%	42.5%	ns
Obese (>30)	19%	42.5%	0.05
Fat Mass (kg)	21.8(7.7)	25.8(8.4)	0.091
Trunk Fat Mass (kg)	12.5(4)	14.7(4)	0.049
Adipo-cytokines			
CRP (mg/L)	5.6(6.3)	7.7(18)	ns
Leptin (ng/ml)	0.8(0.7)	2.7(2.5)	ns
Adiponectin (μg/ml)	8.4(4.1)	8(4.1)	ns
Resistin(μg/L)	13.3(2.2)	11.2(3.7)	ns
IL2 (pg/ml)	0(0)	1.01(1.68)	ns
IL4 (pg/ml)	1.23(1.1)	1.5(1.56)	ns
IL6 (pg/ml)	0.56(0.4)	4.0(6.1)	0.03
IL8 (pg/ml)	8(3.1)	6.7(3.9)	ns
IL10 (pg/ml)	0.7(0.6)	0.4(0.5)	ns
VEGF (pg/ml)	99.7(56.7)	163(149)	ns
INFγ (pg/ml)	0(0)	0.1(0.5)	ns
IL-1α (pg/ml)	0(0)	0.3(0.4)	ns
IL-1β (pg/ml)	0(0)	0.2(0.5)	ns

Figure 3.2: Long Segment Barrett's (>3cms) versus Short Segment Barrett's (<3cm): Features of the Metabolic Syndrome and Anthropometry.



3.6 DISCUSSION

This is the first study to our knowledge to explore obesity and related metabolic abnormalities in patients with Barrett's oesophagus, and to document adipokine and cytokine responses in this patient population. In an unselected cohort, the incidence of metabolic syndrome at 46% for Barrett's patients, and 62% in patients with long-segment Barrett's oesophagus, far exceeds available U.S., European and Irish data where a prevalence of approximately 20 percent may have been anticipated (Villegas et al, 2006; Moller & Kaufman 2005; Ford et al, 2002). In this study, the metabolic syndrome was also associated with a systemic immuno-inflammatory response, evident by increased levels of C-reactive protein and IL-6, and a relative insulin resistance through raised insulin and lower adiponectin levels. Body composition analysis highlighted the unique role of central fat, particularly in patients with long-segment Barrett's, where 92.5% were centrally obese and had a mean 11 cm greater waistline than the cohort with short segment Barrett's.

Obesity is a known independent risk factor for oesophageal adenocarcinoma (Ryan et al, 2006; Calle et al 2003; Lagergren et al 1999; Chow et al 1998; Vaughan et al 1995; Brown et al 1995; Engel et al 2003). In Chapter 2 of this thesis data is given from the first Irish study on obesity and cancer, reporting an almost 10-fold increase in the odds ratio of oesophageal adenocarcinoma with obesity compared with a normal BMI (Ryan et al 2006). Numerous studies also support a positive association between obesity and GORD (Locke et al, 1999; Nilsson et al 2003; Delgado-Aros et al, 2004; Nandurkar et al, 2004; Wilson et al 1999; Chang et al 1997; Ruhl & Everhart 1999; Jacobson et al, 2006). In a recent meta-analysis of nine reports, an association between BMI and erosive oesophagitis was reported in 6 of 7 studies (Hampel et al, 2005). The evidence is also that the link between obesity and reflux symptoms remains significant when other factors such as the presence of hiatus hernia, smoking, race, gender, family history of GORD, or dietary fat intake are controlled (Nandurkar et al, 2004; Wilson et al 1999; Jacobson et al 2006; El-Serag et al 2005).

The simplest disease construct is that obesity promotes reflux, and that chronic inflammation and Barrett's metaplasia predispose to adenocarcinoma. In view of the pivotal position of SIM in this disease model, and the large evidence-base associating obesity and GORD, and obesity and adenocarcinoma, it is surprising that there are few

studies examining the association between obesity and Barrett's oesophagus. In a retrospective review including 65 cases of Barrett's and 385 non-Barrett's refluxers, Stein et al (2005) reported that obesity was associated with a 2.5 fold increase in the risk of Barrett's oesophagus– for each ten pound increase in weight, or five-point increase in BMI, there was a 10% and 35% increase in the risk of Barrett's oesophagus, respectively. Smith et al (2005) in a population-based study of 167 cases of Barrett's Oesophagus and 261 matched controls reported that obese people with self-reported symptoms of acid reflux had higher risks of Barrett's oesophagus (OR 34.4, 95% CI 6.3-188) than patients with reflux alone (OR, 9.3; 95% CI 1.4-62.2) or obesity alone (OR 0.7, 95% CI 0.2 – 2.4).

An intriguing link may exist between the pattern of fat distribution and the risk of Barrett's oesophagus and adenocarcinoma. In a retrospective case-control study of 36 patients with Barrett's oesophagus and 93 controls that underwent abdominal CT scan where the surface of visceral adipose tissue and subcutaneous adipose tissue at the level of inter-vertebral disc between L4 and L5 were calculated, visceral fat was an even stronger independent risk factor for Barrett's oesophagus than BMI (El-Serag et al 2005). Although central or visceral fat may predispose to hiatal hernia, increase transient relaxations of the lower oesophageal sphincter, and decrease oesophageal peristalsis, visceral fat may also be metabolically active and be associated with the metabolic syndrome. In addition to the well characterised features of hypertension, altered glucose metabolism, insulin resistance, and dyslipidaemia, the syndrome is associated with a pro-inflammatory state from the release of adipokines and cytokines which theoretically could promote tissue inflammation and tumourigenesis (Xu et al 2003; Cannon et al 1993; Weinsier et al 2001). Since obesity may be linked to death rates from not only oesophageal, but many other cancer sites (Calle et al 2003), research into Barrett's oesophagus and oesophageal adenocarcinoma should also explore the relevance of these pathways in both the development of SIM and it's progression to dysplasia and cancer.

In this study, the incidence of obesity in an unselected Barrett's and GORD cohort was high, at 34 and 38 percent respectively. Both cohorts had proven significant acid reflux, and the greater level of reflux in Barrett's is anticipated (Attwood et al 1993; Kauer et al 1995). This is consistent with a recent report of 751 patients with reflux, 22% of whom had Barrett's oesophagus, where the mean BMI was 27.8 overall but there was no

difference between Barrett's and non-Barrett's groups (Gerson et al 2007). In this study the pattern of fat deposition was predominantly central in both cohorts, with an estimated trunk fat mass of between 13 and 14 kg in both groups. In the overall group the vast majority of patients (76%) were centrally obese, and 78% were overweight or obese on BMI alone. Forty nine percent had dyslipidaemia, 16% fasting hyperinsulinaemia, 18% fasting hyperglycaemia and 30% reported heavy alcohol consumption. Because the mean age in the Barrett's group was older than the GORD cohort, at 56 and 50 respectively, it is unclear whether the development of SIM is a continuum with increasing BMI, or whether the response pattern or oesophagitis, non-erosive disease, or SIM, is pre-determined, perhaps genetically, as has been suggested (Quigley, 1997; Fitzgerald & Farthing 2000).

The primary focus of the study was on the Barrett's cohort, and these patients underwent detailed assessment of adipokine and cytokine production in addition to nutritional assessment. Barrett's patients with metabolic syndrome were significantly older than those without metabolic syndrome, had a higher BMI, body weight, waist circumference, fat mass, trunk fat mass, and had significantly higher fasting glucose, triacylglycerol, CRP, and leptin levels and significantly higher rates of fasting hyperinsulinaemia and poorer insulin sensitivity as confirmed by elevated HOMA-IR levels. Adiponectin and resistin and serum cytokines were not significantly different between cohorts. The relevance of metabolic syndrome and insulin resistance to the progression of Barrett's will demand long-term prospective studies, but the association of metabolic and immunoinflammatory changes with the length of SIM may be an important observation. Long-segment Barrett's oesophagus is associated with a greater risk of adenocarcinoma than short-segment Barrett's, but whether this relates solely to the quantity of at-risk epithelium, or other factors in combination, is unknown. In this study, patients with long-segment SIM were significantly more obese than patients with short segment changes (42.5% versus 19%, p=0.05), and just 15% of patients with long-segment disease had a normal BMI compared with 33% of patients with short segment disease (p=0.01). The majority (93%) of long segment Barrett's patients had central obesity, and on segmental body composition analysis this cohort had greater fat in their trunk than the short segment cohort (14.7 kg versus 12.5 kg, p=0.049). Moreover, metabolic syndrome was present in 60 percent compared with 24 percent in long and short –segment cohorts respectively; hyperinsulinaemia was evident in 20 percent of the long-segment patients

as well as a trend towards poorer insulin sensitivity, and systemic immunoinflammation as measured by IL-6 and CRP was more evident in the long-segment group.

The implications of metabolic syndrome and the associated adipocytokine response with respect to the length of metaplasia and the risk of progression demands further study. A recent study demonstrated increasing risk for cell-cycle (aneuploidy) and genetic abnormalities (17p loss) in Barrett's oesophagus with increasing waist: hip ratio, in keeping with the thesis that distribution of body fat (central obesity) may be more important than BMI (Vaughan et al, 2002). Focused studies are now required that address whether the metabolic syndrome and the documented systemic response impact on key regulators of inflammation and tumourigenesis in the oesophagus, including Nuclear Factor kappa B, Mitogen Activated Protein kinases, Cyclo-oxygenase 2 (Cox 2), Tumour Necrosis Factor-alpha, and Interleukin-8. The role of leptin in the oesophagus may also be relevant, as leptin stimulates proliferation and inhibits apoptosis in Barrett's oesophageal adenocarcinoma cells (Ogunwobi et al 2006). The analysis of whether the pattern of expression of leptin receptors and other growth factors is altered in Barrett's epithelium in the presence of the metabolic syndrome also demands analysis.

While this study has yielded novel findings in the area of obesity and Barrett's oesophagus future studies are needed to expand the patient numbers particularly in the Barrett's cohort as this would allow for better age and sex matching. Another limitation of this study is the reliance on historical data on the prevalence of metabolic syndrome in the "normal" population. Extended studies in this area should consider recruitment of healthy subjects to act as a control arm. Ideally these subjects should also have upper GI endoscopy and pH studies to rule out asymptomatic oesophagitis which is estimated to be present in 37% of patients with oesophagitis (Shaheen & Provenzole, 2003).

3.7 CONCLUSION

In conclusion, this study demonstrates a surprisingly strong association between obesity, the metabolic syndrome, central adiposity, and reflux manifest as GORD or Barrett's oesophagus. The prevalence of metabolic syndrome in the Barrett's cohort, and particularly the relationship between the length of Barrett's and altered leptin, insulin, and pro-inflammatory markers, suggests that changes in Barrett's may be a continuum that is impacted upon by the metabolic changes induced by adipokines and cytokines.

This thesis needs validation in further studies, as well as prospective follow-up data on disease progression, and molecular studies on the inflammatory milieu in the metaplastic epithelium itself that may uncover mechanisms of progression. The study is consistent with studies linking obesity and GORD, and obesity and adenocarcinoma. The data in the Barrett's cohort at minimum highlights a target population that need general health advice, and suggests a potential added value of prevention and treatment in this cohort may be a reduction in the progression of metaplasia and further consequences.

CHAPTER 4

A COMPARISON OF POSTOPERATIVE MORBIDITY, MORTALITY AND SURVIVAL OF NEO-ADJUVANT THERAPY VERSUS SURGERY ALONE FOR OESOPHAGEAL CANCER

Published in *Journal Thoracic and Cardiothoracic Surgery* (2006); 132(3):549-55.

"Neoadjuvant chemoradiation may increase the risk of respiratory complications and sepsis after transthoracic esophagectomy".

John V Reynolds, Narayanasami Ravi, Donal Hollywood, Michael J Kennedy, Suzanne P Rowley, **Aoife Ryan**, Niall Hughes, Patrick J Byrne.

4.1 SUMMARY

Background: The role of neo-adjuvant chemotherapy and radiation therapy prior to resection in oesophageal cancer remains controversial. Operative risks may be increased, but this has not been systematically addressed in published trials or reports. The aims of this study were to investigate the incidence of postoperative morbidity and mortality amongst patients who underwent curative oesophagectomy after neo-adjuvant therapy or surgery alone.

Methods: Medical records of 213 consecutive patients undergoing oesophagectomy for oesophageal cancer by a single consultant surgeon from 1996 to 2004 were reviewed. Patients were included in the study if they were of curative intent to surgery and had clear margins in final pathology (R0). This represented 148 cases, 70 of which underwent neo-adjuvant therapy and 78 surgery alone. Data on diagnosis, stage of disease, surgical approach, patient co-morbidities, technical complications, and postoperative medical complications and outcomes including length of stay and overall survival were determined. The primary predictor was surgical complications and the primary outcome was survival. Multivariate logistic regression models were used to identify the odds ratio of postoperative morbidity and mortality. Actuarial survival was calculated from the date of positive histological diagnosis by the Kaplan-Meier method, and comparisons between the two groups were made by the log rank test.

Results: Multimodal therapy was associated with increased respiratory and septic complications compared with a surgery–only cohort undergoing the equivalent surgery. The odds ratio for sepsis, respiratory failure, Adult respiratory Distress Syndrome and Renal Dysfunction was significantly increased with neo-adjuvant therapy. There was a concerning trend towards increased in-hospital mortality in the neo-adjuvant group. No significant difference in median survival, and the 1, 2 and 3-year survival rates in neo-adjuvant therapy versus surgery alone were found.

Conclusions: In this non-randomised comparison of neo-adjuvant therapy and surgery alone in patients undergoing curative R0 resection, neo-adjuvant therapy was associated with increased postoperative complications and no improvement in survival. These data suggest that efforts should be made to limit radiation lung exposure in multimodal regimens, and to understand and modulate the local and systemic effects of preoperative chemo-radiation.

4.2 INTRODUCTION

Carcinoma of the oesophagus and gastro-oesophageal junction represent aggressive diseases with a poor prognosis even in patients undergoing curative resection (Enzinger & Mayer, 2003; Daly, 2000). Where squamous cell histology once predominated, the incidence of oesophageal adenocarcinoma in the western world has risen dramatically over the past three decades (Blot et al, 1991; Pera et al, 1993). Oesophagectomy remains the gold standard treatment for resectable oesophageal cancer. However the proximity of the oesophagus to vital mediastinal structures may compromise a complete curative (R0) resection, and micrometastatic disease is often present at the time of diagnosis. Even with en-bloc resections and with radical 2 or 3 field lymphadenectomy, 3-year survival rarely exceeds 40 per cent (Altorki et al, 2002; Lerut et al, 1999; Siewert et al, 2001).

The disappointing outcomes from surgery alone have resulted in considerable interest in multimodal approaches, either neo-adjuvant chemotherapy alone or combined with radiation therapy (Enzinger et al, 2003). Neo-adjuvant therapy offers early treatment of micrometastatic disease by down staging tumours and may facilitate a complete curative resection.

Analysis of trials of combination chemotherapy and radiation therapy prior to surgery (Nygard et al, 1992; Burmeister et al, 2005), and meta-analysis (Siewert et al 1998; Bone et al, 1992), is difficult, for several reasons: only 2 of 8 studies (Bosset et al, 1997; Law et al, 1998), both negative, appear adequately powered with over 200 patients; there is a mix of pathologic types (adenocarcinoma and squamous cell carcinoma) in all but one study (Walsh et al, 1996); the total dose of radiation therapy administered, and treatment fractions, is different across trials; and the interpretation of the one trial showing a benefit for multimodal therapy (Walsh et al, 1996), undertaken in patients with adenocarcinoma at St. James's Hospital between 1990 and 1995, is complicated by relatively small numbers, limited cross-sectional imaging in preoperative staging, and an outcome in the surgery alone arm below standard benchmarks. The most recent trial, an adequately powered Australasian study of 256 patients, 61% of whom had adenocarcinoma, failed to show a survival benefit from neo-adjuvant chemo-radiotherapy (Burmeister et al, 2005).

Notwithstanding the controversy whether oncologic benefit accrues from multimodal regimens, several studies have shown marked survival advantages for the subset of patients who achieve a histological complete response (pCR) as a result of neo-adjuvant

therapy (Stahl et al., 1996, Ganem et al., 1997, Forastiere et al., 1997). Three-year survival rates of more than 60% have been reported in these patients. According to several phase II and some phase III studies a pCR can be expected in 20-30% of patients (Urba 2001, Bosset 1997) regardless of the applied protocol, type of histology, and tumour stage.

Oesophageal resection in itself is associated with high rates of perioperative morbidity and mortality, reported at 50% and 10% respectively (Bailey 2003). Furthermore, concerns have been raised that pre operative chemo-radiation therapy may further increase complication rates. There has been some controversy in the literature as to whether neo-adjuvant chemoradiotherapy is associated with an increased rate of complications when compared to that in patients undergoing immediate oesophagectomy (Walsh, 2002; Fink, 1995; Bosset, 1997; Heidecke, 2002). Many have claimed that patients with carcinoma of the oesophagus exhibit a higher postoperative morbidity and mortality after preoperative chemoradiotherapy (Fink, 1995). Unfortunately this morbidity affects *all* patients, including those who show only a partial response; or no response at all, to chemo-radiotherapy.

4.3 AIMS AND OBJECTIVES

The aims of this study were to (1) investigate the incidence of post-operative morbidity and mortality amongst patients who underwent curative (R0) oesophagectomy (2) to compare multimodal patients to surgery alone (3) estimate the odds ratio for post-operative complications in multimodality versus surgery alone and (4) compare survival in both groups.

4.4 PATIENTS AND METHODS

From 1996 to 2004, 213 oesophagectomy procedures were performed by a single consultant surgeon at St. James's Hospital. A complete review of the medical, surgical and pathological records of these patients showed that 148 had curative intent to surgery and had clear margins on final pathology (R0). 70 of these patients underwent neo-adjuvant therapy and 78 surgery alone.

4.4.1 Inclusion and Exclusion Criteria

The inclusion criteria for multimodal therapy of oesophageal adenocarcinoma or squamous cell cancer at this centre is as follows: age $\leq$ 77; satisfactory performance status and medical fitness for surgery; a leucocyte count greater than 3500 per cubic millimetre, a platelet count above 100,000 per cubic millimetre, a serum creatinine less than 124μmol per litre; and no previous chemotherapy or radiation therapy. Patients receiving this treatment regimen were compared with patients treated with surgery alone who also fulfilled these same criteria.

Patients with any one of the following were excluded from this analysis: age > 80, high-grade dysplasia or carcinoma-in-situ; emergency oesophagectomy following oesophageal rupture; surgery determined preoperatively to be palliative (based on tumour extent or patient performance); patients who achieved clear margins (R0) but did not undergo a lymphadenectomy; had microscopic (R1) or macroscopic (R2) residual disease on final pathology reports; had evidence of bronchial invasion based on CT imaging and bronchoscopy; or had a tumour classified as T_4, N_{any} by the multidisciplinary oesophageal panel.

4.4.2 Tumour Staging

All patients underwent upper gastrointestinal endoscopy with biopsy and staging chest x-ray, and a further percentage additionally underwent endoscopic ultrasound (15%, n=22). Computed tomography (CT) scans of the neck, thorax and abdomen were performed on all patients (Donington, 2005)- using CT-criteria, the mediastinal and left gastric nodes were classified as N1 (invaded) if the maximal transverse diameter of these nodes were larger than 1 cm. Resectable disease was defined as T_{1-3}, N_{0-1}. All tumours at the oesophago-gastric junction were assigned as Type I, II or III, as per Siewert et al (1998): Type I was adenocarcinoma of the distal oesophagus, usually arising in specialised intestinal metaplasia; Type II is a true adenocarcinoma of the cardia arising immediately at the oesophago-gastric (OG) junction; and Type III is a subcardial gastric carcinoma infiltrating the oesophago-gastric junction and distal oesophagus from below. Seven percent of patients (n=11) underwent ^{18}-F-deoxyglucose PET scans (now routine), as these only became available in mid-2003 (Ott et al, 2006; Donington, 2005). Nine percent (n=14) underwent a staging laparoscopy (9%, n=14). Pulmonary function was evaluated by pulmonary function tests (PFTs) in all patients (Abou-Jawde et al, 2005); cardiac function by echocardiography, and liver and renal function was assessed by

laboratory tests (Donington, 2005). All 148 patients were discussed at multidisciplinary CT conferences and were of curative intent.

Every patient with localized disease (T_{2-3},N_{0-1}; predicted R0 resection) of the oesophagus or junction (Type I and II) were offered the option of either surgery alone or the multimodal regimen, patients with Type III OG junction tumours had surgery alone. Patients with more locally advanced disease were treated with radical radiation therapy and chemotherapy.

Pre-operative co-morbid disease such a diabetes, cardiovascular disease or major organ disease was documented as was smoking and alcohol intake (heavy consumption was defined as >14 units/week for females and >21 units/week for males). Baseline nutritional status was documented (weight, height, BMI, weight loss at diagnosis, % weight loss in the six months prior to diagnosis) as well as several routine bloods including a full blood count and serum albumin levels.

Performance status was measured using the Eastern Co-operative Oncology Group (ECOG) grades of performance (Oken et al, 1982) where 0=fully active and able for all pre disease performance, 1= restricted I physically strenuous activities but ambulatory and able to carry out light work of a sedentary nature, 2= ambumatory and capable of self care but unable to carry out any work activities, 3- capable of only limited self-care, confined to bed or chair for >50% of waking hours, 4=completely disabled, cannot self care, bed bound. In addition the American Society of Anaesthesiologists (ASA) physical status classification was documented where ASA I=normal healthy patient, ASA II=mild systemic disease with no functional limitation, ASA III=moderate systemic disease with finctional limitations, ASA IV=severe systemic disease that is a constant threat to life, ASA V=moribund patient with life expectancy <24 hours without surgery (Lee et al, 1998). Performance was also assessed by Karnofsky score, a measure of quality of life (Grieco & Long 1984). Results above 80% indicated normal activity with few symptoms or signs of disease, results below indiated the need for help with activities of daily living and symptoms of disease requiring regular medical care.

4.4.3 Neo-adjuvant Therapy

Patients who were in the neo-adjuvant treatment arm were given a standard protocol of chemo-radiotherapy consisting of: Radiotherapy 40 Gy/15 fractions on days 1 to 5, 8-12 and 15-19, and concurrent chemotherapy consisting of: 5-Fluorouracil 15mg/kg was given on days 1-5 and Cisplatin 75mg/m2 given on day 7 (Walsh et al, 1996). Chemotherapy was repeated on week 6. Patients were restaged by CT and

oesophagoscopy at week 8 and scheduled for surgery on week 9. Surgery took place if the neutrophil count was > $2x10^6/ml^{-1}$, if performance status had not significantly deteriorated, and if there was no evidence of local or systemic progression of disease on imaging.

4.4.4 Surgical Procedure

All patients had a thoracotomy as a component of their surgical management, either combined with an abdominal and neck exploration (3-stage) for mid and upper-oesophageal cancers, or cancer arising in long-segment Barrett's oesophagus, or with an abdominal exploration (2-stage) for most lower third and junctional tumours, or combined with a total gastrectomy for junctional tumours with significant gastric extension (Type III) (Benzoni et al, 2007). A 2-field lymphadenectomy (abdominal and thoracic) was performed in all cases. All patients were extubated immediately following surgery and managed in a high dependency unit (HDU). All patients with a gastric remnant had a pyloroplasty (Benzoni et al, 2007), and patients were fed enterally from 12 hours postoperatively via a needle catheter jejunostomy (8 French, Argyle, Tullamore, Ireland) (Sica et al, 2005). A gastrograffin contrast study was routinely performed on postoperative day 7 or 8 before initiating oral fluids (Timaksiz et al, 2005).

4.4.5 Postoperative Complications

All complications from surgery to discharge from hospital were prospectively documented. Respiratory failure was defined as the requirement for mechanical ventilation beyond 24 hours after surgery. Adult Respiratory Distress Syndrome (ARDS) and multiple organ failure (MOF) were defined as per Bone et al (1992), sepsis required evidence of Systemic Inflammatory Response Syndrome (SIRS) (Lever et al, 2007) with microbiological evidence of infection, and the diagnosis of pneumonia required either positive sputum cultures or clear clinical and radiographic evidence of consolidation (Drakopanagiotakis et al, 2008).

4.4.6 Follow Up

Once the patient was discharged from hospital, follow up consisted of an out patient visit at one, three, six months and then every six months. Extensive evaluation for recurrence depended on clinical evolution. A complete follow up was available for the entire group at the end of the present study. Details regarding cause of death and date of death were recorded from hospital/GP records and where unavailable the national death registry

(Civil Registration Office, Lombard Street, Dublin) was consulted. Survival was defined as the time between the date of positive histological diagnosis and the date of most recent follow up or for patients who died, the date of death.

4.5 STATISTICAL ANALYSIS

Statistical analysis was performed using STATA™ statistical package, version 8.2 for Windows. Quantative data are expressed as median and 95% confidence intervals. Qualitative data are described as percentages. Medians were compared using Kruskal Wallis rank sum tests. Uni-variate and multivariate logistic regression models were used to identify the odds ratio of postoperative morbidity and mortality. Operative mortality was defined as any death within the hospital after surgery. Actuarial survival was calculated from the date of positive histological diagnosis by the Kaplan-Meier method, and comparisons between the groups were made by the log rank test (Pallant, 2007).

4.6 RESULTS

4.6.1 Patient characteristics pre-operatively

148 patients were operated on for oesophageal cancer: 70 had neo-adjuvant therapy and 78 surgery alone (44 females and 104 males). Patient characteristics are described in table 4.1. The median age at diagnosis was 59.5 years for neo-adjuvant group and 63 for surgery alone (p=0.03). There was a trend towards more females in the surgery only group (p=0.08) and there was a significant difference in the morphology with more adenocarcinoma cases in the neo-adjuvant group (p=0.009).

Neo-adjuvant patients had significantly better Karnofsky performance scores (p=0.03), ECOG performance scores (p=0.001) and pulmonary function tests (p=0.01). There was no significant difference in ASA grades (p=0.57) or co-morbid disease (p=0.4). There was a trend towards more weight loss at diagnosis in the neo-adjuvant group (p=0.07) but no difference in nutritional status according to BMI (p=0.18). There were significantly more heavy drinkers (> 14 units/week for females and > 21 units/week for males) in the neo-adjuvant group, but no difference in smoking habits. However, pre operatively, neo-adjuvant patients had significantly lower counts of neutrophils, lymphocytes and white cell counts, but no difference in albumin, haemoglobin or platelets pre operatively.

4.6.2 Clinical & Pathological Staging

Table 4.2 describes the clinical staging (on pre op CT scan) and tumour sites prior to treatment and at final pathological staging (resected specimen pathological staging). The T size refers to the tumour size, N refers to nodal involvement and M refers to metastatic disease. The majority of patients had stage III and stage 2a disease. T3 N0 was the most common staging both clinically and on final pathology. Seventeen out of 70 (24%) patients in the neo-adjuvant arm had T0 tumours on final pathology indicating a complete pathological response to treatment (pCR).

Table 4.3 describes the operative approach and postoperative support patients received. Ninety-nine 2 stage oesophagectomy and jejunostomy tube insertion procedures were carried out, (46 in surgery only group and 53 in Multimodal group), 39 3-stage oesophagectomy (23 Surgery only, 16 MM), 9 Total gastrectomy and distal oesophagectomy (8 Surgery only group and 1 multimodal group) and 1 patient in surgery only group had a transhiatial oesophagectomy. There was no significant difference in the amount of blood lost or blood transfused, days ventilated, length of stay in the high dependence unit or intensive care unit. The median length of stay in hospital post surgery was 19 days (range 4-98) for the neo-adjuvant group versus 20 days (range 5-73) for the surgery alone group (p=0.46).

Table 4.4 describes the pathological findings of resected tumours. Patients in the neo-adjuvant group had significantly smaller tumours at the time of resection (p=0.0001), had significantly less total nodes resected (p=0.0007) and significantly less positive nodes on final pathology (p=0.0004). The complete pathological response rate (pCR) was 2.6% for surgery only patients and 24% for neo-adjuvant patients (p=0.001).

Table 4.1: Patient Characteristics pre operatively, neo-adjuvant versus surgery only, R0 resections for adenocarcinoma and SCC, n= 148

Characteristic	Neo-adjuvant (n=70)	Surgery only (n=78)	P
Age (yrs)	59.5(Q 53-64)	63 (Q 54-74)	0.03
Male/ Female	54 /16	50/28	0.08
Histology			
Adenocarcinoma	56(81%)	46(62%)	0.009
Squamous	12(20%)	30(38%)	
Performance Status			
Karnofsky Score >/=90%	70/70 (100%)	73/79(93%)	0.03
ASA Grade 1 or 2	65/70 (100%)	71/79 (93%)	0.57
ECOG 1 Fully active	58/67(87%)	47/78 (60%)	
ECOG 2 Restricted	9/67 (13%)	29/78(37%)	0.001
ECOG 3 unable	0/67 (0%)	1/78(1%)	
Nutritional Status			
% Weight Loss	6%(0-35%)	4.4%(0-21%)	0.07
>10% weight Loss	19/56	14/58	0.3
Body Mass Index (kg/m^2)	25.65	24.75	0.18
Smoking and Alcohol			
Current Smoker	31%(22/70)	27%(21/79)	0.7
Heavy Drinker	31%(20/70)	9%(7/79)	0.001
Degree of Dysphagia			
1 Able to eat anything	38%(29)	16%(11)	
2 soft food only	28%(19)	19%(15)	
3 unable to eat all solids	43%(29)	30%(22)	
4 liquids only	10.5%(7)	9%(7)	
5 complete dysphagia	1.5%(1)	4%(3)	
Pulmonary Function Tests			
FEV 1 (L)	2.9	2.57	0.01
FVC (L)	3.8	3.325	0.001
FEV1/FVC (%)	77	79	0.3
Co-morbid Disease	33% (22/68)	39% (31/79)	0.4
Cardiovascular Disease	19% (13/68)	23% (18/79)	0.69
Respiratory Disease	12% (8/68)	16%(13/79)	0.48
Vascular Disease	3% (2/68)	6% (5/79)	0.45
Bloods			
Neutrophils($X10^9/L$)	3.0	3.6	0.01
Lymphocytes($X10^9/L$)	0.45	1.7	0.0001
Platelets ($X10^9/L$)	221	230	ns
WCC ($X10^9/L$)	4.8	6.2	0.0001

ASA=American Society of Anaesthesiologists; ASA Grade I =Healthy patient, Grade II =Mild Systemic Disease, ECOG=Eastern Co-operative Oncology Group; FEV=Forced Expiratory Volume; FVC=Forced Volume capacity; WCC=White cell Count, NS=Non Significant. Heavy Alcohol=>14 units/week for females and > 21 units/week for males

Table 4.2: Clinical (Pre op staging) & Pathological Staging (final staging on resected specimen) of Tumours for R0 resections in neo-adjuvant treatment arm and surgery only arm, adenocarcinoma and SCC, n=148.

	Clinical		Pathological	
	Neo-adjuvant	*Surgery*	*Neo-adjuvant*	*Surgery*
Stage				
0	0	5	16	4
I	2	2	14	9
II	0	1	0	0
III	19	15	11	28
IV	0	0	0	0
1b	0	2	0	2
2a	47	42	17	15
2b	1	2	10	10
3a	0	4	0	6
Unknown	0	0	1	0
T0	0	1	17	2
T1	3	4	16	9
T2	2	20	10	21
T3	64	45	23	39
T4	0	1	0	1
Tis	0	3	1	2
Tx	0	0	3	0
N0	47	52	45	31
N1	19	20	23	43
Nx	3	2	1	0
M0	67	72	20	18
Mx	2	2	49	56

Stage 0 = tumour in situ; Stage I = T1N0M0; Stage IIa= T2N0M0 or stage T3N0M0; Stage IIb=T1N1M0 or stage T2N1M0; Stage 3 = T3N1M0 or T4 any N M0; Stage 4= Any T, Any N, M1. N0=no nodes involved, N1=nodes involved, Nx=unable to assess, M0=no distant metastasis, M1=distant metastases, Tis=Tumour in situ, Tx= unable to assess.

Table 4.3: Operative approach and postoperative support in neo-adjuvant versus surgery only, adenocarcinoma and SCC (n=148)

Characteristic	Neo-adjuvant Therapy	Surgery only	P
2 Stage Oesophagectomy	53(75%)	46(58%)	
3 Stage Oesophagectomy	16(23%)	24(30%)	0.102
TGast/Dist oesophagectomy	1(1%)	8(10%)	
Transhiatial	0	1(1%)	
Blood Loss	1100mls (0-4300)	850mls (0-4100)	0.12
Length of operation	5.5 (3.5-8)	5 hours (2.5-9.5)	0.02
Days in HDU	4 days (0-15)	3 days (0-11)	0.33
Days in ICU	0 (0-36)	0 (0-15)	0.1
Return to Theatre	7(10%)	6(7.6%)	0.77
Days Ventilated	0(0-32)	0(0-11)	0.38
Return to ICU	14%(10/70)	9%(7/79)	0.44
Days in Hospital Post Surgery	19(4-98)	20(5-73)	0.46

ICU=Intensive Care Unit

HDU=High Dependency Unit

Table 4.4: Pathological Findings of resected tumours in neo-adjuvant versus surgery only, adenocarcinoma and SCC, n=148

	Neo-adjuvant	Surgery Only	P
Tumour Length (cms)	2 (0-7.3)	3 (0.4-9)	0.0001
Tumour Width (cms)	1.25 (0-7.5)	2 (0.3-8)	0.005
Number of Nodes	10(0-30)	15 (2-41)	0.0007
Number positive nodes	0(0-9)	1(0-9)	0.0004
pCR Rate	24%(17/70)	2.6%(2/78)	0.001

pCR = complete pathological response

4.6.3 Post operative complications pre treatment approach

Table 4.5 describes the incidence of post-operative complications per treatment group. Although there was no difference in the overall postoperative morbidity rate between the two treatment groups (p=0.1), a significantly greater number of patients in the neo-adjuvant group developed sepsis (19% versus 4%, p=0.006), respiratory failure (14% versus 1.3%, p=0.003), adult respiratory distress syndrome (ARDS) (11.4% versus 0%, p=0.002), and renal dysfunction (13% versus 2.5%, p=0.03). No significant difference in any other post-operative complication was found.

Multivariate logistic regression was used to calculate the odds ratio of post-operative complications in neo-adjuvant group versus surgery group *(see table 4.6).* The odds ratio for sepsis was 15.5 (p=0.002), respiratory failure 14.9 (p=0.02) and renal dysfunction was 16.3 (p=0.02) and a concerning trend towards increased in hospital mortality (OR 4.6, p=0.07) was found for the neo-adjuvant group.

The operative (in hospital) mortality rate in this series was 6% (9 patients) due to: sepsis, ARDS, and multiple organ failure (2), sepsis and MOF (1), sepsis and renal failure, heart failure, and anastamotic leak (1), sepsis and renal failure (2), sepsis, ARDS and respiratory failure (2), sepsis, ARDS, Renal failure and respiratory failure (1). There was a trend towards increased in-hospital mortality in the neo-adjuvant group (7 deaths versus 2 in surgery alone group) (p=0.08).

Table 4.7 shows the median pre operative and post-operative days 1, 3 and 7 values of neutrophils, WCC, lymphocytes, albumin, haemoglobin and platelets. Neo-adjuvant patients had significant suppression of white blood cells, neutrophils, haemoglobin, and lymphocytes both preoperatively and on post-operative days 1, 3 and 7. When compared to surgery only patients, neo-adjuvant patients had significantly lower counts of neutrophils, lymphocytes, WCC and haemoglobin pre-operatively (see table 4.7). There was no significant difference in pre operative levels of platelets or serum albumin. Post operatively neo-adjuvant patients continued to show significantly lower counts of neutrophils, lymphocytes and platelets on post operative day 1, and by day 3 and day 7 post op also had significantly lower counts of WCC and platelets (see table 4.7 and figures 4.1A, 4.1B and 4.1C).

Table 4.5: Post operative Complications[$] by treatment approach in neo-adjuvant versus surgery only, adenocarcinoma and SCC n=148

	Neo-adjuvant	Surgery Only	P value
	n=70	n=78	
Post op Complication (yes)*	39 (56%)	43 (54%)	0.1
Sepsis	13 (19%)	3 (4%)	0.006
Respiratory Failure	10 (14%)	1 (1.3%)	0.003
ARDS	8 (11.4%)	0 (0%)	0.002
Mortality	7 (10%)	2 (2.6%)	0.08
Heart Failure	4 (6%)	1 (1.2%)	0.19
Pneumothorax	9 (13%)	4 (5%)	0.14
Haemothorax	0 (0%)	0 (0%)	1.0
Arrhythmia	9 (13%)	4 (5%)	0.14
Wound infection	3 (4%)	4 (5%)	1.0
Anastamotic Leak	4 (6%)	1 (1.2%)	0.19
Renal Dysfunction	9 (13%)	2 (2.5%)	0.03
Pneumonia	9 (13%)	8 (10%)	0.62
RTI	2 (3%)	2 (2.5%)	1.0
Pleural Effusion	22 (31%)	21 (27%)	0.59
Pulmonary Embolism	1 (1.4%)	0 (0%)	1.0
MOF	2 (3%)	1 (1.2%)	0.6
Atelactasis	3 (4%)	2 (2.5%)	0.67
Seizures	0 (0%)	1 (1.2%)	1.0
Chylothorax	3 (4%)	1(1.2%)	0.47

ARDS = adult respiratory distress syndrome, RTI = respiratory tract infection, MOF = multiple organ failure. [$] = several patients suffered one or more post op complication, *=all compliations

Table 4.6: Odds Ratio of Post-operative Complications for Neo-adjuvant versus surgery, adenocarcinoma and SCC, n=148

	Odds Ratio	95%CI	P Value
Sepsis	15.5	2.8 – 87	0.002
Mortality	4.6	0.88 – 24	0.07
Respiratory Failure	14.9	1.6 – 141	0.02
Renal Dysfunction	16.3	1.6 – 164	0.02

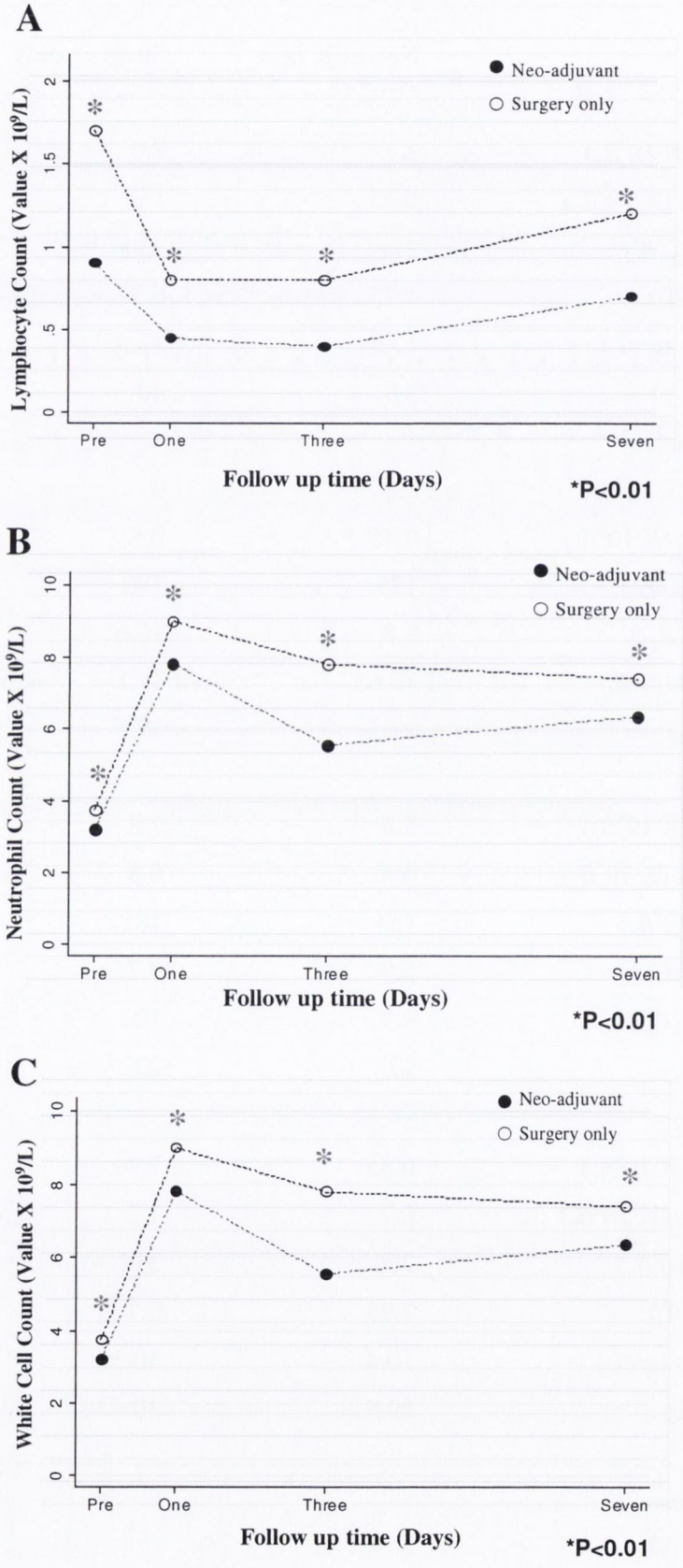
Data adjusted for age, gender, smoking, alcohol intake, pulmonary function

Table 4.7: Median Pre operative and post-operative days 1, 3 and 7 values of neutrophils, WCC, lymphocytes, albumin, haemoglobin and platelets.

	Neo-adjuvant	Surgery only	P
Pre op			
-Neutrophils (X 10^9/L)	3.7	7.8	0.004
-Lymphocytes (X 10^9/L)	0.45	1.7	0.0001
-Platelets (X 10^9/L)	221	230	0.18
-WCC (X 10^9/L)	4.75	6.2	0.0001
-Haemoglobin (g/dL)	12.5	13.3	0.002
-Albumin (g/L)	39	40	0.99
Day 1 Post op			
-Neutrophils (X 10^9/L)	7.8	9	0.008
-Lymphocytes (X 10^9/L)	0.45	0.8	0.0001
-Platelets (X 10^9/L)	175	180	0.34
-WCC (X 10^9/L)	8.8	10.6	0.0001
-Haemoglobin (g/dL)	10.65	11.1	0.37
-Albumin (g/L)	27.5	27	0.85
Day 3 Post op			
-Neutrophils (X 10^9/L)	5.6	7.8	0.001
-Lymphocytes (X 10^9/L)	0.4	0.8	0.0001
-Platelets (X 10^9/L)	154	181	0.004
-WCC (X 10^9/L)	6.9	9.7	0.0001
-Haemoglobin (g/dL)	9.9	10	0.38
-Albumin (g/L)	27	27	0.79
Day 7 Post op			
-Neutrophils (X 10^9/L)	6.35	7.4	0.001
-Lymphocytes (X 10^9/L)	0.7	1.2	0.0001
-Platelets (X 10^9/L)	266	314	0.0005
-WCC (X 10^9/L)	7.95	9.7	0.008
-Haemoglobin (g/dL)	10.4	10.35	0.45
-Albumin (g/L)	29.5	30	0.31

WCC = White cell count

Figure 4.1: Peri-operative (A) Lymphocyte counts (B) Neutrophil count (C) White Cell Count in neo-adjuvant versus surgery R0 Adenocarcinoma and SCC, n=148



7.6.4 Survival

Figure 4.2 illustrates the overall survival in this R0 oesophagectomy series. The overall survival for both groups was 77% at one year, 58% at two years and 43% at three years, giving a median survival of 2.3 years (table 4.14 gives numbers at risk i.e the number of patients alive at the beginning of each year that were followed up).

Figure 4.3 and table 4.15 illustrates the survival estimates of R0 cases per treatment approach. The median follow up among survivors for the multimodality group was 2.4 years and 2.3 years for the surgery only group (P=NS). The median survival for the multimodality group was 2.43 years and 2.25 years for surgery only (Log Rank 0.55). At three years 37% of multimodality patients were alive and 49% surgery only patients were alive, although this was not significant.
The numbers at risk (i.e the number of patients alive at the beginning of each year that were followed up) in both the neo-adjuvant group alone and the surgery group alone are shown in tables 4.16 and 4.17.

When the R0 group was analysed per morphology no significant difference was found between the 1, 2 and 3 year survival rates of adenocarcinoma versus Squamous cell carcinoma, log rank test = 0.14 (see figure 4.4 and table 4.18).

Figure 4.2: Kaplan Meier Survival Estimates for total population (adenocarcinoma and SCC) R0 resections, neo-adjuvant therapy and surgery together, n=148.

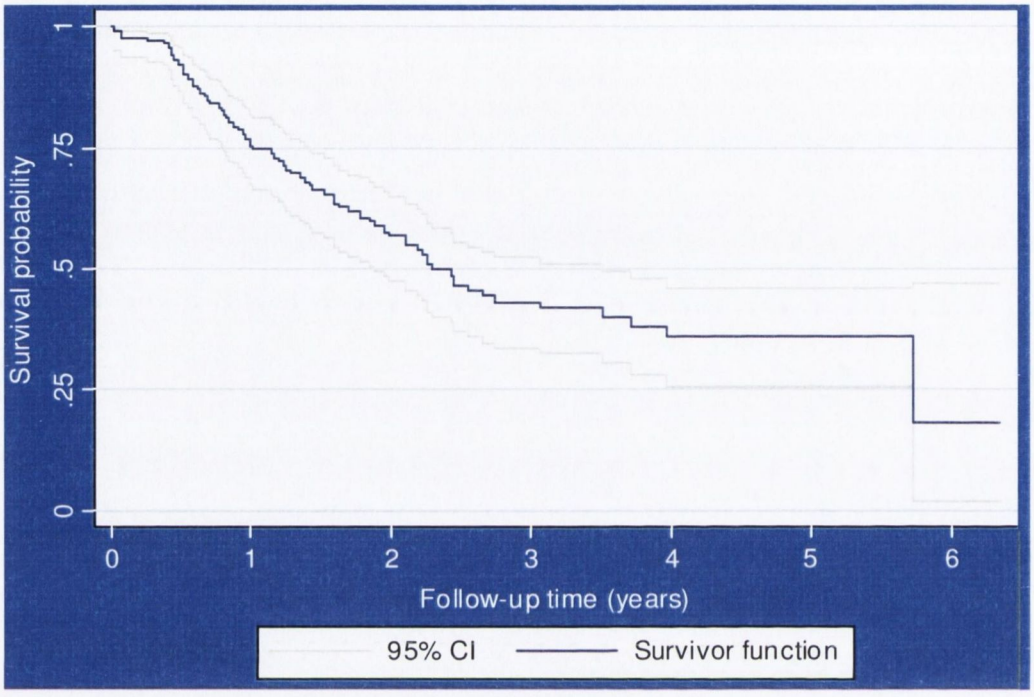


Table 4.8: Numbers At Risk: Overall survival for total population (adenocarcinoma and SCC) R0 resections, neo-adjuvant therapy and surgery together, n=148.

	Total	Deaths	No Follow up*	% Survival
Year 1	148	32	20	77%
Year 2	96	22	16	57.7%
Year 3	58	13	12	43%
Year 4	33	4	12	37%
Year 5	17	0	13	37%

*Patients were followed up at their local hospital and not at this unit

Figure 4.3: Kaplan Meier survival estimates per treatment approach for total population (adenocarcinoma and SCC), Neo-adjuvant versus Surgery (Log rank = 0.55).

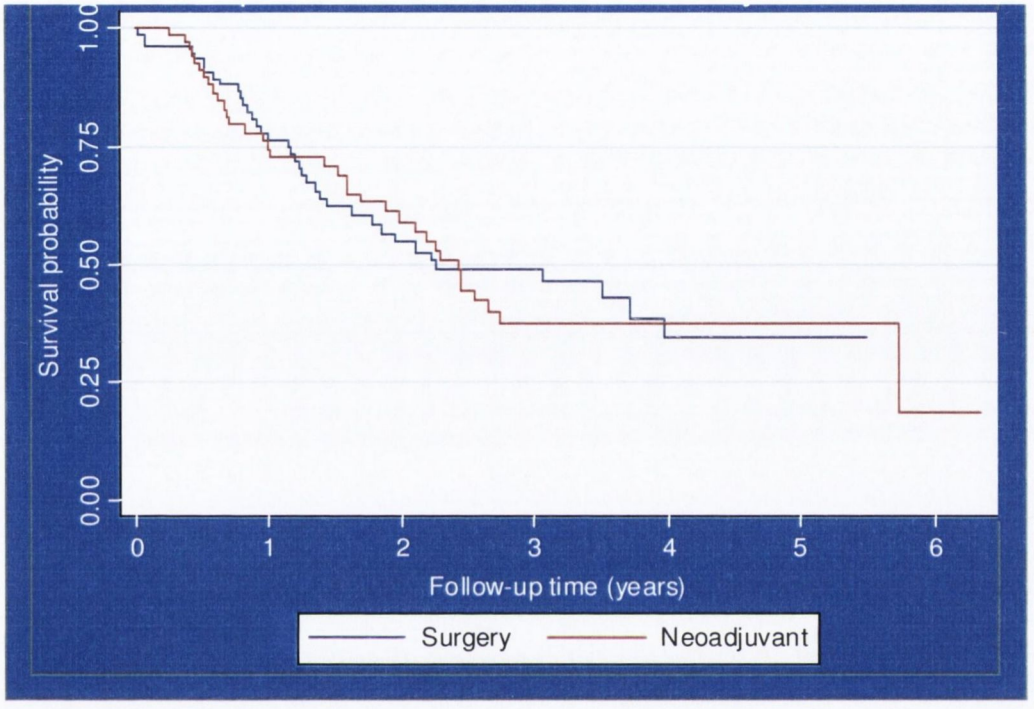


Table 4.9: Median Survival, and 1, 2 and 3 year survival in R0 adenocarcinoma and SCC, Neo-adjuvant versus Surgery, n=148.

	Neo-adjuvant	Surgery Only	P
Average Follow up among survivors	2.4 yrs	2.3 yrs	
Median Survival	2.43yrs	2.25 yrs	NS
1 year Survival	77%	77%	
2 Year Survival	60%	56%	
3 Year Survival	37%	49%	

Table 4.10: Numbers at Risk: Survival of R0 *Neo-adjuvant Only* (Adenocarcinoma and SCC), n=70.

	Total	Deaths	No Follow up*	% Survival
Year 1	70	17	11	77%
Year 2	44	9	5	60%
Year 3	30	10	7	37%
Year 4	13	0	4	37%
Year 5	9	0	7	37%

*Patients were followed up at their local hospital and not at this unit

Table 4.11: Numbers at Risk: Survival of R0 *Surgery only* (Adenocarcinoma and SCC), n=78.

	Total	Deaths	No Follow up*	% Survival
Year 1	78	18	12	77%
Year 2	52	13	11	56%
Year 3	28	3	5	49%
Year 4	20	4	8	37%
Year 5	8	0	6	37%
Year 6	2	0	2	37%

*Patients were followed up at their local hospital and not at this unit

Figure 4.4: Kaplan Meier survival estimates per morphology for total population Neo-adjuvant and Surgery, adenocarcinoma versus SCC

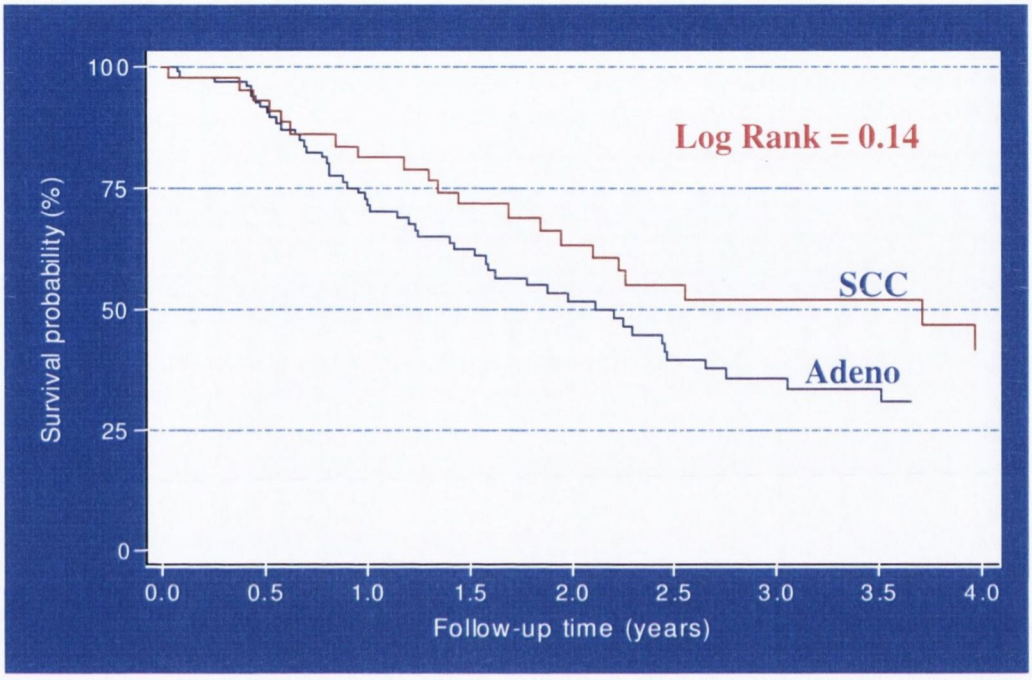


Table 4.12: 1, 2 and 3 year Survival in R0 neo-adjuvant and surgery only per morphology (adenocarcinoma versus SCC).

	Adenocarcinoma	SCC	P
1 Year Survival	73%	82%	
2 Year Survival	52%	64%	NS
3 Year Survival	36%	52%	

4.7 DISCUSSION

Oesophagectomy carries a very significant risk of postoperative morbidity and mortality. Earlam and Cunho-Melo (1980) reported an overall mortality rate of 29% from papers published between 1960 and 1979, and Muller (1990) reported a postoperative mortality rate of 13% from papers published between 1980- 1988. In a review by Jamieson et al (2004) of 70,756 patients covering the period 1990-2000, the reported mortality rate was 6.7 per cent. Bailey et al (2003) reviewed 1777 patients with oesophageal cancer who underwent resection at 109 Veterans Affairs hospitals between 1991 and 2000, and reported an approximate 50% major morbidity rate, and 10% mortality rate. In the United Kingdom, McCullogh et al (2003) reported a 14% in-hospital mortality rate from a multi-centre review of 365 patients. The compelling recent literature linking better outcomes with surgeon and hospital volume of > 100 cases per year (Migliore et al, 2007; Rodgers et al, 2007) has highlighted the unassailable fact that there is no common elective surgical procedure that carries the same operative risks (Van Lanschot et al, 2001; Begg et al 1998). It appears intuitive that factors that impact on immune well-being and organ function, in particular respiratory, may have great relevance to operative outcome after thoracic surgery, and in this regard rigorous assessment of the impact of neoadjuvant chemotherapy and radiation therapy is imperative.

This report represents the experience of a unit with a high-volume surgeon (>130 cases/year) and support team, with approximately 40 oesophageal resections a year, in a tertiary cancer centre with a long tradition of managing patients with oesophageal and thoracic malignancy. The groups compared are not randomised but are contemporaneous, and are equal in fulfilling strict criteria for surgery, including absence of T4 or M1 disease on CT imaging, and adequate performance status.

Neo-adjuvant chemo-radiotherapy followed by oesophagectomy has been the preferred treatment of choice over the past decade. The expected benefits of preoperative chemoradiotherapy are the preoperative elimination of potential systemic micro metastases in patients with both loco regional and locally advanced tumours, and the down staging of the primary tumour which may increase the R0 resection rate (i.e. curative resection/no residual disease) and thus may reduce the rate of local and distant recurrences, thereby increasing the chances of long-term survival (Lerut et al., 1999). Approximately 15-20% of patients experience a complete pathological response to

chemoradiation therapy and no tumour is found on final pathology (Lerut et al., 1999). Several studies have reported 3-year survival rates as high as 100% in this group (Ancona et al, 2001).

However, oesophageal resection is associated with high rates of perioperative morbidity and mortality (Bailey et al, 2003). Concerns have also been raised as to the increase in perioperative morbidity and mortality that is associated with neo-adjuvant therapy.

Neo-adjuvant therapy has been shown to be independently associated with perioperative mortality (Bailey et al., 2003). Bosset and co-workers in a study of 297 patients, demonstrated a statistically significant, threefold (12% versus 4%) increase in perioperative mortality in the neo-adjuvant chemo-radiotherapy group. This increase in mortality was thought to be due to an increased incidence of respiratory failure and mediastinal infection in the neo-adjuvant therapy group. Walsh and associates found a 2.8 fold increase in perioperative mortality (10.7 versus 3.7%) in the neo-adjuvant therapy group and Nygaard et al., observed a 1.8 fold (24% versus 13%) increase in the neo-adjuvant therapy group. However these differences did not reach statistical significance but it is likely that a type II error occurred and that had more patients been recruited the differences in perioperative mortality would have reached significance (Bailey et al, 2003).

In the present study it could be argued that neoadjuvant patients are fitter preoperatively-they were significantly younger, had significantly better Karnofsky performance scores and ECOG performance scores, better pulmonary function tests and had smaller tumours. There was no difference in smoking habits, BMI, degree of dysphagia or the incidence of concomitant co-morbid disease. The only major significant difference pre-operatively was the suppression of immune function in the neo-adjuvant arm as assessed by counts of neutrophils, lymphocytes and WCC. Perhaps this was a key factor in the finding that neoadjuvant patients had a significantly higher rate of sepsis, respiratory failure, ARDS and a trend towards increased in-hospital mortality when compared to surgery only patients.

Seven of the nine deaths in this series were in the neoadjuvant group. In fact each of these seven deaths was sepsis related and this may reflect host immuno-suppression by pre-operative CT/RT. Recently Heidecke et al (2002) showed that neo-adjuvant therapy was associated with significant immunosuppression in the host, specifically with defective proliferation of T cells after chemoradiotherapy, when compared to patients undergoing oesophagectomy alone. This deficiency has been hypothesised to impair the

host response to subsequent surgery and has been proposed to explain the higher risk of surgery after neo-adjuvant therapy. In this series we have shown significantly lower counts of WCC, neutrophils, lymphocytes and haemoglobin both pre-operatively and on post-operative days 1, 3 and 7 in the neo-adjuvant group versus the surgery only group. The standard requirement before surgery is for adequate neutrophil count recovery, but whether neutrophils, lymphocytes and other cells actually function normally in the blood, lungs and other tissues is unknown.

The overall operative mortality rate (in hospital) in this series was 6% which is comparable with other specialised units: Poon et al (7.2%), Gupta (6%), Orringer et al (5%), Lerut (3%) and much lower than rates reported by Moreno-Gonzales et al (16%), Junginger and Dutkowski (15%), Muller et al (13%) and Gurkan et al (12%), McCullach et al (12%). Our in-hospital mortality rate was twice as high in the neo-adjuvant group compared to the surgery alone group and this is similar to figures published in several phase II randomised trials (Urba 2001, Nygaard 2002, Walsh 1996). Bossets' trial in 1997 reported a mortality rate in neo-adjuvant group that was 3.4 times the mortality rate in the surgery alone group.

To date there have been six published prospective randomised controlled trials assessing the benefits of neo-adjuvant therapy in oesophageal cancer patients. A sole randomised trial from our institution showed a benefit of neo-adjuvant therapy in 113 patients with adenocarcinoma). However many of these trials focused on survival and failed to give much needed detail on in hospital morbidity associated with treatment. Two of these trials report on 30-day mortality only instead of in hospital mortality, which we have shown in this study.

The survival data reported in this study shows a median survival of between 2.2 and 2.4 years. There was no survival benefit for either treatment arm and no difference in survival between the two morphology groups.

This study is not randomised, and potential biases may exist. Nonetheless, this should not apply to the primary end-point, that of postoperative complications. The operative insult was similar in both groups, and all patients underwent a thoracotomy and one-lung anaesthesia as a component of their surgery. The multimodal group was younger, had significantly superior pre-operative pulmonary function tests, and a lower pathological

stage compared with the surgery only group, suggesting strongly that the negative impact of the only variable, chemoradiation, is a true effect.

The study is an observational study, a restricted cohort design, and this adapts principles of the randomised controlled trial design as follows (Horwitz et al, 1990): base-line criteria identified for patient eligibility; inclusion and exclusion criteria the same as in randomised trials; and statistical methods, including intention to treat analysis, is similar to that of randomised trials. The authors recognize that cancer outcomes in a non-randomised comparison should be extrapolated with caution, nevertheless, in cohorts with identical preoperative clinical stage no difference in overall outcomes has been observed, or any difference where outcomes for adenocarcinoma alone was analysed. These cancer outcome data at minimum support the conclusion of a recent trial (Burmeister et al, 2005) that a randomised trial would require the enrolment of many hundreds of patients to be adequately powered.

4.8 CONCLUSIONS

In this non-randomised comparison of neo-adjuvant therapy and surgery alone in patients undergoing curative R0 resection, neo-adjuvant therapy was associated with increased postoperative complications, particularly serious infectious complications, and no improvement in survival. Future efforts should aim to clearly define the status of neo-adjuvant therapy stage for stage, focus efforts on research into increasing the pCR rate, and limit detrimental effects of chemoradiation therapy on organ function.

CHAPTER 5

SERUM ALBUMIN LEVELS AS A PREDICTOR OF POST-OPERATIVE COMPLICATIONS IN PATIENTS UNDERGOING UPPER GASTROINTESTINAL SURGERY

Published in *The Journal of Gastrointestinal Surgery, 2007, Oct;11(10):1355-1360.*

"Hypoalbuminaemia on the first post operative day post Oesophagectomy may predict short-term adverse outcomes".

Aoife Ryan, Aine Hearty, Ruth S Prichard, Aileen Cunningham, Suzanne P Rowley, John V Reynolds.

5.1 SUMMARY

Objective: Changes in serum albumin may reflect systemic immuno-inflammation and hypermetabolism in response to insults such as trauma and sepsis. Oesophagectomy is associated with a major metabolic stress, and the aim of this study was to determine if the absolute albumin level on the first post-operative day was of value in predicting in-hospital complications.

Methods: A retrospective study of 200 patients undergoing oesophagectomy for malignant disease at St. James Hospital between 1998 and 2005 was performed. Patients who had pre and post-operative (days 1, 3 and 7) serum albumin levels measured were included in the study. Patients were sub-divided into three post-operative albumin categories <20 g/L, 20-25 g/L, >25g/L. Logistic regression analysis was performed to calculate the odds of morbidity and mortality according to the day 1 albumin level.

Results: Patients with an albumin of less than 20 g/L on the first post-operative day were twice as likely to develop post-operative complications than those with an albumin of greater than 20 g/L (60% versus 28% respectively, $p<0.011$). Correspondingly, these patients also had a significantly higher rate of Adult Respiratory Distress Syndrome (25% versus 5%, $p<0.001$), respiratory failure (30% versus 8%, $p<0.01$) and in-hospital mortality (30% versus 6% ($p<0.001$). On multivariate logistic regression analysis, day 1 albumin level was independently related to post operative complications (OR 0.89: 95% CI 0.83 – 0.96, $p<0.005$). In addition, albumin < 20 g/l on the first post operative day was associated with the need for further surgery and a return to ICU.

Conclusion: Serum albumin concentration on the first post-operative day is a better predictor of surgical outcome than many other pre-operative risk factors. It is a low cost test that may be used as a prognostic tool to detect the risk of adverse surgical outcomes.

5.2 INTRODUCTION

Oesophagectomy is associated with a high morbidity and mortality rate, the largest prospective outcome cohort in the literature reporting a morbidity rate of 50% and mortality rate of 10% (Bailey et al, 2003). Several pre-operative risk factors have been identified, including advancing age, co-morbid disease, preoperative chemo-radiotherapy, low body mass index (BMI) and decreased functional status (Bartels et al, 1998; Ferguson et al, 1997; Lund et al 1990). In addition to pre-operative factors, the early course postoperatively may help predict short-term outcomes. Numerous studies have looked at immune perturbations postoperatively as a predictor of Systemic inflammatory response syndrome (SIRS) and sepsis (Sweeney et al, 2005). Oesophagectomy induces profound changes in the endocrine, neuroendocrine and immune system as well as significant changes in organ function (Desborough, 2000). The release of these inflammatory mediators causes endothelial dysfunction with severe capillary leakage, massive loss of protein, and fluid shift from the intravascular space into the interstitium. In a subset of patients overwhelming stress response leads to systemic inflammatory response syndrome (SIRS), which may be associated with organ dysfunction and failure.

The acute phase response to surgery, measured by C-reactive protein (CRP) and IL-6, has been studied following major surgery and an exaggerated response may be associated with adverse outcomes (Karaylannakis et al, 1997; Fogler et al, 1998; Pepys & Hirschfield, 2003). These markers may inversely relate to serum albumin, yet to date no studies have addressed early post-operative hypo-albuminaemia as marker of morbidity risk. In this study, the value of early post-operative hypo-albuminaemia as a marker of outcome following oesophagectomy was studied, the hypothesis being that the post-operative day one serum albumin is an early marker of the systemic immunoinflammatory response to major trauma, and thus has prognosis implications for further clinical course. We report herein confirmatory evidence that a low albumin on the first postoperative day is by multivariate analysis a predictor of adverse outcomes.

5.3 PATIENTS AND METHODS

A retrospective study of 200 patients who underwent oesophagectomy for malignant disease in St. James Hospital, Dublin between 1999 and 2005 was performed. One hundred and ninety five patients had a thoracotomy as a component of their surgical management, either combined with an abdominal and neck exploration (3-stage) for mid and upper-oesophageal cancers, or cancer arising in long-segment Barrett's oesophagus; or with an abdominal exploration (2-stage) for most lower third and junctional tumours; or combined with a total gastrectomy for junctional tumours with significant gastric extension (Type III) (Benzoni et al, 2007; Cunningham et al, 2005). A 2-field lymphadenectomy (abdominal and thoracic) was performed in all cases. All patients were extubated immediately following surgery and managed in a high dependency unit (HDU). All patients with a gastric remnant had a pyloroplasty (refashioning of pylorus), and patients were fed enterally from 12 hours postoperatively via a needle catheter jejunostomy (8 French, Argyle, Tullamore, Ireland). Two senior anaesthesiologists look after oesophagectomy cases at this Institution, and four Intensivists run the HDU/ICU. All patients have a thoracic epidural, and the unit policy is to limit intravenous fluid administration intra-operatively and in the first 24hours.

Pre-operative medical co-morbidities were noted, as well as body mass index and percentage weight loss at presentation. The patients' age, pulmonary function, cigarette consumption was also noted. Performance status was assessed by American Society of Anaesthesiologists (ASA) physical status classification where ASA I= normal healthy patient, ASA II=mild systemic disease with no functional limitation, ASA III=moderate systemic disease with finctional limitations, ASA IV=severe systemic disease that is a constant threat to life, ASA V=moribund patient with life expectancy <24 hours without surgery (Lee et al, 1998).Intra-operative blood loss, length of operation, intent of surgery (palliative or curative), blood products given, and the type of operation was all recorded. Assessment of the length of stay in both the intensive care and the high dependency unit was noted, as was the length of inpatient stay post-operatively.

The blood results on these 200 cases, particularity serum albumin concentrations both preoperatively and on days 1, 3 and 7 post-operatively was recorded (Duly et al, 2003). Additionally results of full blood counts (with Haematocrit quantification) and renal profile analysis for urea and creatinine levels, which were performed daily were recorded

(Rey-Ferro et al, 1997). C-reactive protein and immune cell parameters were not routinely measured at that time.

5.3.1 Postoperative Complications

All complications from surgery to discharge from hospital were prospectively documented. Major post-operative complications, including, Adult Respiratory Distress Syndrome (ARDS), sepsis, multi-organ failure (MOF), renal failure, heart failure, respiratory failure, pneumonia/respiratory tract infection, empyema, major thromboembolic event, wound infection, anastamotic leak, pancreatic fistula, and in-hospital mortality were documented. Respiratory failure was defined as the requirement for mechanical ventilation beyond 24 hours after surgery. ARDS and multiple organ failure (MOF) were defined as per Bone et al (1992), sepsis required evidence of Systemic Inflammatory Response Syndrome (SIRS) with microbiological evidence of infection (Lever et al, 2007), and the diagnosis of pneumonia required either positive sputum cultures or clear clinical and radiographic evidence of consolidation (Drakopanagiotakis et al, 2008).

5.3 STATISTICAL ANALYSIS

Data manipulation and statistical analyses were conducted using SPSS® Version 11.0 for Windows™ (SPSS® Inc., Chicago, IL). Mean (± standard deviation) values for albumin taken at different stages pre-op and post-operatively were compared with each other using paired samples t-tests. The normal reference range for albumin was 35-50 g/l. Three albumin categories were also created based on day 1 albumin (<20g/l, 20-25/l, >25g/l). Cross-tabulation was used to compare albumin categories, post-operative complications and patient status with other categorical variables. Significant differences were tested using Pearson Chi-square analysis. Differences in mean laboratory data across categories of day 1 albumin status were evaluated using one-way analysis of variance (ANOVA). Where statistically significant effects were encountered ($p<0.05$), comparisons of means were made using Scheffe *post-hoc* multiple comparisons test. For values that did not comply with Levene's test for homogeneity of variance, the Tamhane *post-hoc* multiple comparisons test was used.

Binary logistic regression analysis was used to determine whether certain variables could predict post-operative complications (no/yes) and patient status (alive/dead). These

predictor variables included sex, age, smoking status, presence of co-morbid disease, post-operative complications, >10% weight loss, pre-operative and post-operative laboratory test results. Initially, all predictor variables were assessed independently. The models were used to generate odds ratios (OR) with their respective 95% confidence intervals (CI) to quantify the likelihood of having a post-operative complication or the likelihood of death.

Separate models were created for predicting post-operative complications and patient status. The set of variables remaining statistically significant at $p \leq 0.200$ in these initial models were incorporated to produce multiple logistic regression models. This approach identifies important variables for the final models that may not be identified using the traditional statistical cut-off point of $p<0.05$. In each of the final multiple regression models, significance was taken at $p<0.05$.

5.5 RESULTS

5.5.1 *Patient Characteristics*

Two hundred patients who underwent oesophagectomy for malignancy were studied – 143 males and 57 females. Patient demographics are described in *Table 5.1.* The median age at diagnosis was 61 years (range 29-77). One hundred and thirty five patients had a 2-stage oesophagectomy, 60 had a three stage and 5 patients had a transhiatial oesophagectomy. The median operative time was 5 hours (range 2 - 9.5 hours). The median length of stay in the HDU was 3 days (range 0-32 days) and the length of stay in hospital post surgery was 20 days (range 14-106).

5.5.2 *Post operative complications*

In total 118 patients (59%) developed a post-operative complication (*see table 5.2*). The most common post-operative complication following oesophagectomy was pneumonia (16%), sepsis (12.5%), respiratory failure (10.5%), mortality (8.5%), and ARDS (7.5%). The mean pre operative albumin level in oesophagectomy patients was 39 g/L. This fell significantly to 25.8 g/L (4.4) on the first post-operative day ($p<0.0001$).

5.5.3 *Post Operative Complications per Albumin level on day 1*

To examine the effect of post operative hypoalbuminaemia, patients were split into tertiles of serum albumin level on day 1. However the was no difference in

complications between the tertiles. Patients were then split into three albumin categories (<20g/L, 20-24.9 g/L and >25 g/L).

The demographics of each albumin group was analysed for association with sex, ASA grade, duration of surgery, type of regimen (multimodal or surgery alone), type of surgery, pathological stage (i.e. tumour stage on examination of resected specimen), and blood loss and blood transfusion within the first 24 hours (*see table 5.3*). There was a significant association between albumin < 20g/L and a three-stage oesophagectomy, squamous sub-type, and median blood loss and blood transfusions.

Table 5.1: Comparison of patient demographics, blood loss and operative time, average length of stay, post-operative complications and mortality post oesophagectomy - data shown as median (range), n=200.

Male: Female	143:57
Age	61(29-77)
Median BMI on Diagnosis	25.5
BMI < 20 kg/m2	9%
>10% wt loss in 6 months	33%
Co-Morbidities (yes: no)	125:75
ASA Grade	
One	81
Two	95
Three	19
Four	4
Surgery only: Multimodal	95:105
Blood transfusion in first 48hr	46%
Operative Time (hrs)	5 (2-9.5)
Stay in ICU (days)	0(0-39)
Stay in HDU (days)	3 (0-32)
Length of hospital stay post surgery (days)	20 (14-106)
Post operative Complication (yes: no)	118:82
In hospital mortality (yes: no)	17:183

ASA=American Society of Anaesthesiologists, BMI=Body Mass Index, HDU=High Dependency Unit, ICU=Intensive Care Unit

Table 5.2:

Post-operative complications in order of frequency post oesophagectomy (n=200)

Pneumonia/RTI	32 (16%)
Sepsis	25 (12%)
Respiratory Failure	21 (10%)
Mortality	17 (8%)
ARDS	15 (7%)
Renal failure	14 (7%)
Multi-organ failure	10 (5%)
Anastamotic leak	9 (4%)
Major thromboembolic event	7 (3%)
Pancreatic fistula	6 (3%)
Heart failure	3 (1.5%)
Empyema	2 (1%)
Wound Infection	2 (1%)

ARDS=Adult respiratory distress syndrome, RTI=respiratory tract infection

When the groups were analysed for post-operative morbidity significant associations were observed with postoperative complications in the < 20g/l group (*see table 5.4*). Patients with an albumin <20 g/L on the first post operative day were twice as likely to develop post operative complications than those with an albumin > 20 g/L (60% versus 28% respectively, p<0.01). When compared to patients with an albumin level > 20 g/L on post operative day 1, patients with an albumin < 20 g/L had significantly higher rate of ARDS, respiratory failure and were also 5 times more likely to die in hospital, see *table 5.4* and *figure 5.1.* Seventeen patients died in hospital post operatively, 6 of whom (30%) were in the albumin < 20 g/L group on post operative day 1. Cause of death was as follows: ARDS, Respiratory Failure, Sepsis (3); ARDS, Respiratory Failure, Sepsis, multiple organ failure (6); Sepsis, pneumonia (3); Sepsis, multiple organ failure (3); Heart Failure (1); Major thromboembolic event (1).

Patients with an albumin < 20g/L on the first post operative day were 3 times more likely to return to the Intensive care unit (30% versus 10%, p<0.03), and spent a significantly longer time there than patients who had an albumin > 20g/L (p=0.002), they also stayed significantly longer in the high dependency unit (p=0.02).

The data was then analysed according to the percentage drop from pre-operative levels to post operative day 1 levels. The mean (+SD) percentage decrease in serum albumin in the group with POD1 albumin < 20g/L was 53.6% (+10.37) compared with 30.7% (+9.45) in the group with an albumin > 20g/L (p=0.00001). However, the risk of mortality, ARDS, anastomotic leak, sepsis or pneumonia was not significantly associated with percentage change from preoperative levels to the first postoperative day.
On multivariate logistic regression analysis (see table 5.5) day 1 albumin level was independently related to post operative complications (OR 0.85: 95% CI 0.85 – 0.77, p<0.001), as was female sex, and current or previous history of tobacco use. This albumin assessment was controlled for urea and Haematocrit; median (range) urea level on the first postoperative day was 4.8 (3.3-113), 5 (2.5-11.7), and 5.8 (2.9-13.5) respectively in the < 20, 20-25, and > 25 subgroups. The median associated haematocrit was 0.3 (0.2-0.4) in all three albumin groups on the first postoperative day (see table 5.6).

Table 5.3: Patient demographics, surgical approach and outcome in three albumin Categories based on post operative day 1 level.

	Albumin <20 g/l	Albumin 20-24.9 g/l	Albumin >25 g/l	P
Age	62.5(44-70)	61(37-76)	60(29-77)	ns
Male/Female	14: 8	34: 19	94: 31	
Pre-operative albumin	40 (30-47)	38 (28-45)	40 (30-48)	ns
ASA Grade				
1	5 (23%)	24 (45%)	52 (42%)	
2	12 (54%)	21 (40%)	62 (50%)	
3	4 (18%)	7 (13%)	8 (6%)	ns
4	1 (4%)	1(2%)	2 (2%)	
Length of operation	6(3 – 9.5)	5.5 (3.75-8)	5.5(3.75-8)	ns
Surgery Only: Multimodal	11:11	22:30	62:62	ns
Operation Type				
Transhiatial	1(5%)	1(2%)	3(3%)	
2-Stage	10 (45%)	28(53%)	96(77%)	0.001
3-Stage	11 (50%)	24(45%)	26(20%)	
Surgical Intent				
Curative: Palliative	15:8	38:15	87:38	ns
Morphology				
Adenocarcinoma	10(45%)	30(57%)	93(73%)	
SCC	12(55%)	22(42%)	27(21%)	0.006
Other	0	1(2%)	5(5%)	
Pathologic Stage*				
0-2	10 (45%)	33 (62%)	74 (59%)	0.065
3-4	9 (41%)	19 (36%)	45 (36%)	
Unknown	3 (14%)	1 (2%)	8 (6%)	
Blood loss (mls)	2000(200-4000)	820(180-2875)	960(100 -3300)	0.005
Median units transfused	2	0.5	0	0.045

Pathological stage: T0=No evidence of primary tumour; T1=Tumour invades lamina propria and subucosa; T2=Tumour invades muscuularis propria; T3= Tumour Invades adventitia; T4 Tumour invades adjacent structures

Table 5.4: Post-operative complications according to albumin level on first post operative day post oesophagectomy.

	<20 g/l n=20	20-24.9 g/l n=53	>25 g/l n=126	P
Complication				
"All Complications"	12 (60%)	13 (24%)	37(29%)	0.01
ARDS	5 (25%)	0 (0%)	10 (8%)	0.001
Respiratory Failure	6 (30%)	5 (9%)	10 (8%)	0.01
Mortality	6 (30%)	1 (2%)	10 (8%)	0.001
Anastamotic Leak	1 (5%)	1 (2%)	7 (6%)	ns
Pneumonia/RTI	6 (30%)	8 (15%)	18 (14%)	ns
Sepsis	4 (20%)	4 (7.5%)	17 (13%)	ns
MOF	3 (15%)	1 (2%)	6 (5%)	ns
Renal Failure	3 (15%)	2 (4%)	9 (7%)	ns
Return to ICU				
No	14(70%)	48(90%)	113(90%)	
Yes	6 (30%)	5 (9%)	13 (10%)	0.03
Days in ICU				
0 days	10 (50%)	46 (87%)	101 (80%)	
1-3 days	3 (15%)	3 (6%)	15 (12%)	0.002
>3 days	7 (35%)	4 (8%)	10 (8%)	
Days in HDU				
0-1 Days	7 (35%)	6 (11%)	11 (9%)	
2-4 days	9 (45%)	32 (60%)	75 (60%)	0.01
>5 days	4 (20%)	15 (28%)	40 (32%)	
Length of Stay post op				
<24 days	12(60%)	37 (70%)	81 (64%)	
>25 days	8 (40%)	16 (30%)	45 (36%)	ns

ARDS = Adult respiratory distress syndrome, MOF= multiple organ failure, RTI = respiratory tract infection, ns = non-significant.

Figure 5.1:

Post Operative Complications following oesophagectomy according to serum albumin level on first post-operative day (n=200) *=p<0.01 (for trend)

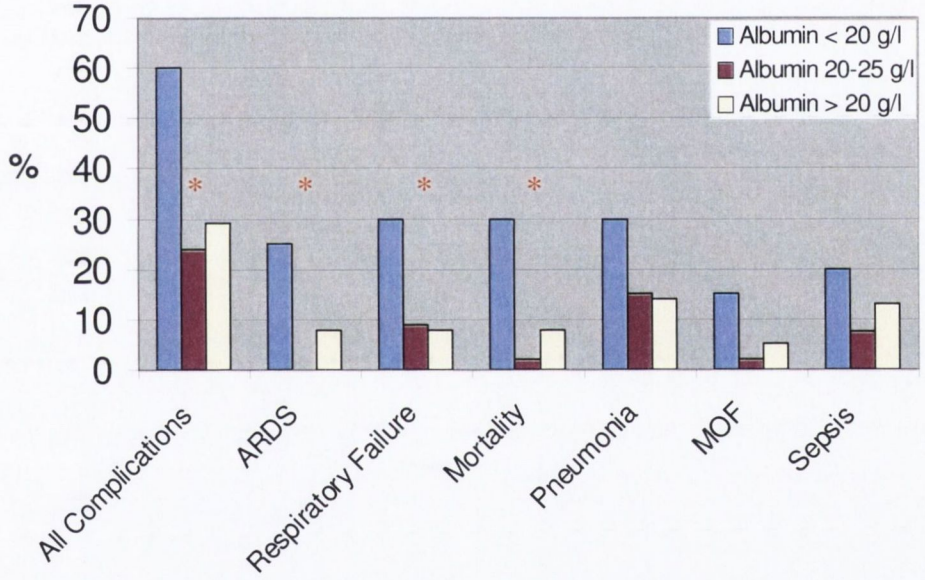


Table 5.5: Prediction of Post operative Complications after Oesophagectomy - Multivariate Logistic regression analysis controlled for day 1 haematocrit level, blood loss, length of operation, treatment intent (palliative or curative) and blood products given.

Factor	Odds Ratio	95% C.I.	P Value
Gender Female	3.3	1.34 – 8.1	0.009
Current Smoker	2.43	0.95 – 6.2	0.06
Ex Smoker	2.53	1.08 – 5.9	0.03
Day 1 Albumin	0.85	0.77 – 0.93	0.001

Table 5.6:

Post oesophagectomy complications versus age and laboratory data on post-operative day 1 (n=200).

	Post operative Complication						
	No			Yes			P
	N	Mean	SD	N	Mean	SD	
Age	138	59.1	10.3	62	61.2	10	ns
FEV1	124	2.9	0.9	54	2.7	0.8	ns
Day 1 Haemoglobin	137	11	1.7	61	10.8	0.8	ns
Day 1 Platelets	137	200	74	59	176	52.3	0.03
Day 1 WCC	136	10.4	3.5	62	9.6	3.2	ns
Day 1 Albumin	137	26.3	4.3	62	25	4.5	0.01
Day 1 Haematocrit	136	0.3	0.04	61	0.3	0.04	ns
Day 1 Urea	135	5.6	1.6	62	6.3	2.5	0.03

WCC=White Cell Count, SD=Standard Deviation, ns=non-significant, FEV=Forced expiratory volume

5.6 DISCUSSION

Upper gastrointestinal surgery for malignant disease imposes significant physiological stress on patients. Despite recent advances in the treatment of patients with oesophageal carcinoma, the overall morbidity following oesophageal resection remains high, with a hospital mortality rate of 5 – 10% (Jamieson & Mathew, 1999; Hulscher et al, 2001). Neo-adjuvant therapies such as chemotherapy, or chemo radiation, have been widely applied in recent years (Doty et al, 2002) but these treatment regimens have been associated with increased post-operative complication rates (Eguchi et al, 1999). Standardization of operative technique, improvement of pre-operative risk assessment and post-operative intensive care management have failed to impact significantly on the relatively high incidence of post-operative complications following oesophagectomy. The most frequently seen surgical complication contributing to substantial morbidity is anastamotic leak (Urschel, 1995; Gandhi & Naunheim 1997; Bailey et al, 2003). However, the most common and serious post-operative morbidity arises from pulmonary complications and most centres report a complication rate of 20% (Daly et al, 2000).

The literature has identified several pre-operative risk factors, amongst which is pre-operative hypo-albuminaemia, which has been shown to be an independent prognostic indicator of overall morbidity and mortality, and prolonged hospital stay, in surgical and critically ill patients (Gibbs et al, 1999). Hypo-albuminaemia is independently associated with the development of post-operative complications, especially the development of infective complications (Schwartz et al, 2004; Bone et al 1992; Dewar et al, 1992; Rey-Ferro et al, 1997; Mullen et al, 1979). In upper gastrointestinal cancer surgery low preoperative serum albumin levels are significantly correlated with anastamotic leak, general post-operative morbidity and post-operative mortality (Buzby et al, 1980; Detsky et al, 1987; Kudsk et al, 2003).

To-date, there are no studies addressing early postoperative hypoalbuminaemia in the literature concerning upper GI surgery. Because serum albumin levels decrease in acute illness and injury, as the liver reprioritizes protein synthesis from visceral proteins to acute phase reactant proteins, hypoalbuminaemia thus acts as a marker of underlying systemic immuno-inflammation and is referred to a 'negative acute phase protein (Soeters et al, 1990; Spanga et al, 1985; Dowd & Heatly, 1984). The decrease in albumin is a result of a combination of factors including haemodilution during fluid

resuscitation, and capillary leakage into the interstitial space. The degree of capillary hyper-permeability is proportional to the inflammatory response mounted by the patient, and therefore those with the greatest rate of vascular permeability are associated with the highest mortality. The development and degree of hypoalbuminaemia thus relates to the severity of the underlying traumatic insult and therefore to the ultimate outcome.

This hypothesis, that albumin may reflect immuno-inflammation and may be a marker of the magnitude of this response, was the primary focus of this study, which to our knowledge is the first report on early postoperative hypoalbuminaemia and short-term outcome after major upper gastrointestinal surgery. This study has demonstrated the positive association between a low serum albumin on the first post-operative day and the development of complications and overall in-hospital mortality. This was still significant when factors such as haematocrit and urea were taken into account, thus out-ruling the possibility of a dilutional effect on serum albumin concentrations. We have shown that a critical albumin level of < 20 g/l on the first post operative day was an independent predictor of complications - it was associated with a doubling of in-hospital complication rate, a 3.5 fold increase the rate of respiratory failure and a five fold increase in the incidence of ARDS and in-hospital mortality. It was also predictive of the need for longer HDU and ICU stays and the need to return to the operating theatre for further surgery. In fact on multivariate logistic regression only 3 factors could predict poor outcome: female gender, smoking and day 1 albumin. Importantly, the absolute level of albumin on the first postoperative day rather than a percentage change from preoperative levels was the significant measurement, suggesting that profound hypoalbuminaemia is a serum marker of a heightened systemic response with associated adverse risks. The equivalent outcomes in the 20-25g/L group and the 25-30g/L group are also consistent with this thesis.

A reduction in the serum albumin has also been positively associated with impaired immunological function and a reduction in the resistance to post-operative nosocomial infections. Hypoalbuminaemia results in a decrease in the production of the immune system proteins by the liver. Our study confirmed a significant association between low serum albumin and the development of infective complications, ARDS and pneumonia.

In this study there was no association of the low postoperative albumin level with preoperative neoadjuvant therapy or pathological stage, nor with preoperative serum

albumin levels, but it was significantly associated with the 3-stage resection, squamous pathology, and blood loss and requirement for blood transfusions. We acknowledge that exact details on intra-operative and early postoperative fluid balance would be helpful, and this is now automated, but this was not fully recorded during the study period. The albumin effect by multivariate analysis was significant when factors including haematocrit and urea were taken into account, thus the possibility that this represents a dilution effect on serum albumin concentrations from excessive fluid administration, although possible, is unlikely. Moreover, the consistency of anaesthesiology involved in these cases, and the integrated care pathway in the early postoperative period established in this Unit make it improbable that this represents solely an effect of fluid administration.

The association between a low serum albumin on the first post-operative day and the development of complications and overall mortality may be a sign of systemic immuno-inflammation and hypermetabolism, a marker of the host response to a severe operative insult. The operative insult may have been somewhat greater in this cohort, as reflected by blood loss and transfusion, and the higher number of 3-stage resections. The data at minimum suggests that an albumin less than 20g/L on the first postoperative day may identify a cohort postoperatively that should continue to be monitored closely in HDU or ICU. Further research on the relationship of hypoalbuminaemia to the early systemic immuno-inflammatory response following major surgery is required, as well as a better understanding of the therapeutic implications.

5.7 CONCLUSION

In conclusion this study demonstrates a significant association between a low serum albumin on the first post-operative day and the development of post-operative complications and overall mortality. It is a direct marker of the severity of the systemic inflammatory response and a low cost and simple laboratory test, with significant prognostic value.

CHAPTER 6:

NUTRITIONAL STUDIES IN UPPER GASTROINTESTINAL CANCER

CHAPTER 6A

POST-OESOPHAGECTOMY EARLY ENTERAL NUTRITION VIA A NEEDLE CATHETER JEJUNOSTOMY: 8-YEAR EXPERIENCE AT A SPECIALIST UNIT

Published in *Clinical Nutrition* (2006) Jun; 25(3):386-93

"Post Oesophagectomy Early Enteral Enteral Nutrition via a needle catheter jejunostomy: 8-year experience at a specialist unit".

Aoife Ryan, Suzanne P Rowley, Laura A Healy, Philomena M Flood, Narayanasami Ravi, John V Reynolds.

6A.1 SUMMARY

Background: The purpose of this study was to prospectively evaluate post-operative jejunostomy feeding in terms of nutritional, biochemical, gastrointestinal and mechanical complications in patients undergoing upper gastrointestinal surgery for oesophageal malignancy.

Methods: The study included 205 consecutive patients who underwent oesophagectomy for malignancy. All patients had a needle catheter jejunostomy inserted at the conclusion of laparotomy. Patients were followed prospectively to record nutritional intake, type of feed administered, rate progression, tolerance, weight changes and complications either mechanical, biochemical or gastrointestinal.

Results: Ninety-two per cent of patients were successfully fed exclusively by needle catheter jejunostomy post oesophagectomy, and 94% of patients were tolerating a maintenance regimen of 2000ml feed over 20 hours by day 2 post operatively. Patients spent a median of 15 days on jejunostomy feeding post surgery (range 2 -112 days), however 26% required prolonged jejunostomy feeding (>20 days). Minor gastrointestinal complications were effectively managed by slowing the rate of infusion, or administering medication. Three (1.4%) serious complications of jejunostomy feeding occurred, all requiring re-laparotomy, one resulting in death. Needle catheter jejunostomy feeding was extremely effective in preventing severe post-operative weight loss in the majority of oesophagectomy patients post op. However, oral intake was generally poor at discharge with only 65% of requirements being met orally. Sixteen patients (8%) patients required home jejunostomy feeding. By the first postoperative month a further 6% (12) patients were recommenced on jejunostomy feeding.

Conclusion: Needle catheter jejunostomy feeding is an effective method of providing nutritional support post oesophagectomy, and allows home support for the subset that fail to thrive. Serious complications, most usually intestinal ischaemia or intractable diarrhoea, are rare.

6A.2 INTRODUCTION

There is an emerging consensus that early postoperative nutritional support benefits the surgical patient at high risk of complications by decreasing septic morbidity, maintaining immunocompetence and improving wound healing (Baigrie et al, 1996). An increasing body of literature indicates functional advantages of early postoperative enteral feeding in ameliorating the stress response and in diminishing the risk of major postoperative infections (Myers et al, 1995; Beier-Holgerson & Boesby, 1996; Kudsk et al, 1992). Of all elective complex major operations, the procedure of oesophagectomy is associated with the highest risk of sepsis-related complications and mortality (Bailey et al, 2003), and this risk, as well as the large metabolic, endocrine and neuroendocrine response to this surgery makes it a particularly good model for studies of nutritional support or nutrient immunomodulation.

Needle catheter jejunostomy (NCJ) was first described in 1973 (Delaney et al, 1973). It is useful after oesophagectomy as normal food intake is delayed until any concerns about anastomotic healing and gastric emptying are abated, the average being approximately the 10^{th} postoperative day. NCJ allows provision of nutrition, fluid and electrolytes early after surgery and permits a safe means of administering many medications that might otherwise require central venous access or monitoring if given intravenously (Sarr et al, 1988). Once some oral feeding is permitted, patients almost uniformly experience early satiety and tend to eat smaller meals. Previous reports have estimated that the mean time required to achieve what the patients considered to be a socially acceptable diet was six months and that a significant amount of adjustment and experimentation with diet is necessary in the first three months following surgery (Ludwig et al, 2001). In this regard the presence of a NCJ provides a useful back up for patients who require supplementary enteral nutrition during this period of adjustment.

The use of the NCJ is not without risk, however, and as an adjunct to oesophageal resection serious complications, sometimes life-threatening, are well described (Han-Geurts et al, 2004; Biffi et al, 2000). The Oesophageal Surgical Unit at St. James's Hospital Dublin is a tertiary centre for oesophageal cancer, has a long experience with NCJ feeding post-oesophagectomy, and an academic interest in the immunologic benefits of enteral nutrition (Welsh et al, 1996; Reynolds et al, 1997), and this study reports the experience of this unit with jejunostomy feeding by evaluation of nutritional

and biochemical effects of early feeding, and detailing mechanical and other complications.

6A.3 PATIENTS AND METHODS

All patients who underwent Oesophagectomy from 1997 to 2004 and had a needle catheter jejunostomy placed at the time of surgery were selected for inclusion in this study. The medical details were obtained from the St. James's Hospital Upper Gastrointestinal Cancer database, which uses the Patient Analysis and Tracking System (PATS)^{TM}, Dendrite Clinical Systems, UK. Full nutritional details obtained from the dietetic record cards and the patient's medical notes were obtained and entered individually into PATS by a research dietitian. Data entered concerned nutritional status pre operatively (weight, height, Body Mass Index (BMI), pre illness weight, percentage weight loss, degree of dysphagia, oral intake and nutritional requirements. In addition Nutritional Risk Index was calculated (Veterans Affairs Total Parenteral Co-operative Study Group, 1991) by the equation: NRI = 1.519 x serum albumin (g/l) + 0.417 x (current weight/usual weight) x 100 with a score <83.5 indicating severe nutritional risk; 83.5-97.5 mild nutritional risk; 97.5 – 100 borderline risk; and a score >100 no nutritional risk. Subjective global assessment (SGA) was also calculated by assessment of weight loss in the previous six months, dietary intake in relation to usual intake, presence of gastrointestinal symptoms, functional capacity, and physical examination to assess loss of subcutaneous fat, muscle wasting and/or presence of peripheral oedema. SGA divided patients into 3 categories: well nourished, mild-moderately malnourished or severely malnourished (Detsky & Smalley, 1994). With regard to the jejunostomy: the type of feed administered, rate progression, tolerance, and complications either mechanical, biochemical or gastrointestinal, duration on feeding, and nutritional status on discharge from hospital and at out patient follow up were all documented.

Regarding the NCJ – it was inserted at conclusion of laparotomy by standard method (Sica et al, 2005) where a 8 Ch feeding catheter (Argyle, Tullamore, Ireland) is inserted through a cannula percutaneously in the left upper quadrant and inserted into the jejunum about 15 to 20cms from Duodenal-Jejunal flexure through a purse string suture. This spot is subsequently buried with seromuscular sutures continued proximally to create a 5 cm long subserosal tunnel. The exit point of the catheter is then sutured onto the pareites to protect against leakage. Prior to 2002 a 10 French catheter was used (Cook, USA) and since then an 8 French catheter was used (Argyle, Sherwood Medical, Tullamore,

Ireland). Feeding was commenced at 0800 am on the first postoperative day using a nutritionally complete whole protein isotonic feed (Fresubin Original, Fresenius Kabi, Stockholm). The initial rate of administration was 30ml/hr for eight hours, 50 mls/hour for 8 hours and 80 mls/hour for four hours. On the second postoperative day the infusion rate was increased to100mls/hour for 20 hours, with a four-hour rest period. Each patient was given sufficient calories and nitrogen to meet estimated nutritional requirements as calculated by the Schofield equation (Schofield, 1985). The feeding goal was 2000mls over 20 hours.

The NCJ was also used routinely to deliver additional water and electrolytes (Baxter Healthcare, Northampton, UK) to meet daily requirements or replace gastrointestinal losses such as nasogastric secretions. Medications absorbed in the small intestine were also given by the NCJ where possible.

Once oral intake was begun patients were given a 1.5kcal/ml feed over ten hours overnight to supplement oral intake until the time of discharge. Patients were followed prospectively to record nutritional requirements, type of feed administered, rate progression, tolerance, and complications either mechanical, biochemical or gastrointestinal. Patients were deemed to be fully tolerant of enteral feedings if they tolerated the full volume of feed prescribed without any GI symptoms. Patients were discharged with their NCJ in situ until follow up in out patients one month following discharge, at which stage nutritional status was reassessed. If the progress was considered satisfactory, the catheter was removed.

6A.4 RESULTS

6A.4.1 Nutritional Status at Diagnosis

205 patients had a needle catheter jejunostomy inserted at the time of oesophagectomy. The median age at diagnosis was 62 years (Range 29-83 years). The median BMI at diagnosis was 25.5kg/m^2 (Range 16 – 42 kg/m^2). Even though 57% of oesophagectomy patients were overweight or obese at diagnosis, 74% were actively losing weight and 34% had experienced clinically severe weight loss at diagnosis (defined as > 10% in 6 months or > 5% in one month (Blackburn, 1977) with 29% losing >10% of their body weight in less than 6 months. The median weight loss as a percentage of pre illness weight was 5.3%; the range was large, 0 to 40 per cent. Prior to surgery patients had a median duration of sub optimal (defined as oral intake less than 75% of estimated energy

and protein requirements) intake of 3 months (range = 0 – 18 months), and the median nutritional intake, as a percentage of requirements was only 69% for energy and 66% for nitrogen (see table 6A.1). When broken down per tumour morphology, patients with squamous cell cancer (SCC) exhibited a higher degree of malnutrition at diagnosis than patients with adenocarcinoma, with a lower BMI and 40% reporting clinically severe weight loss. In contrast even though 31% of adenocarcinoma cases had clinically severe weight loss at diagnosis 68% of patients remained overweight or obese at the time of surgery indicating a high prevalence of obesity prior to disease. Pre operative nutrition support was administered in 10 patients (5%) 4 were nasogastrically fed and 6 received pre operative Total Parenteral Nutrition (TPN).

6A.4.2 Nutrition Support post operatively

The majority of patients 74% (n= 151) underwent a 2-stage oesophagectomy, 20% (41) patients underwent a three stage Oesophagectomy, 2% (4) underwent a transhiatial oesophagectomy, 3% (6) underwent a 2-stage oesophagectomy and distal oesophagectomy, one patient an extended total gastrectomy with distal oesophagectomy. Feeding was commenced on the first postoperative day using a standard 1.0 kcal/ml feed. 92 patients (45%) remained on a standard 1.0kcal/ml feed throughout the feeding period, 41 (20%) received a fibre enriched 1.0 kcal/ml feed, 43 patients (21%) received a high energy 1.5 kcal/ml feed, 8 (4%) received a semi-elemental feed and 10 (5%) received a 2.0 kcal/ml renal feed. Patients were given a fibre enriched feed if they were complaining of constipation or required laxatives in the postoperative period. Semi-elemental feeds were only administered to patients who had intractable diarrhoea in an attempt to alleviate symptoms. The mean number of calories delivered per 24 hours was 2000kcal (range 1500-3000). Ninety four per cent (n=192) of patients achieved the target of a maintenance regimen of 2L enteral feed at 100ml/hour over 20 hours by the second post-operative day, 4% achieved maintenance rates by day three post op and the remaining 2% by day 4 post op. Patients spent a median of 0 days fasting (range 0-10 days).

Oesophagectomy patients spent a median of 15 days on nutrition support via needle catheter jejunostomy (range 2 -112 days). However 53 patients (26%) required prolonged nutrition support (>20 days) principally related to post operative complications, which delayed the implementation of oral feeding. The median length of time to first bowel motion was 5 days (see table 6A.2).

Table 6A.1: Nutritional status at Diagnosis per morphology in 205 Oesophagectomy cases

Median BMI at Diagnosis	25.5 (16.0 – 42.13) kg/m^2		
Median % Weight Loss	5.3 (0 – 40.3%)		
Clinically Severe Weight Loss*	34%		
Clinically Significant weight loss**	8%		
Non-significant weight loss	58%		
>10% Weight Loss	29%		
% Actively losing weight at Diagnosis	74%		
Subjective Global Assessment			
SGA Severe	6%		
SGA Mild-moderately malnourished	25%		
SGA Well nourished	47%		
Unavailable	22%		
Nutritional Risk Index			
Not Malnourished	47%(96)		
Mild Malnutrition	16%(33)		
Moderate Malnutrition	29%(59)		
Severe Malnutrition	4%(8)		
	Adenocarcinoma N=135	**SCC n=59**	**P Value**
Median BMI at Diagnosis (kg/m^2)	25.95 (16.8–42.1)	23.6 (16–30.1)	
Median % Weight Loss	5.1%(0 - 28%)	6 % (0-40.3%)	
Clinically Severe Weight Loss	31%(42)	40%(24)	0.0001
>10% Weight Loss	27%(36)	36%(21)	0.04
Underweight (BMI<18.5)	3% (4)	12% (7)	
Normal (BMI 18.5-24.9)	29% (39)	53% (31)	0.001
Overweight (BMI 25-29.9)	47% (63)	33% (19)	
Obese (BMI >30)	21% (28)	2% (1)	

*Clinically severe weight loss= >10% in 6 months or >7.5% in 3 months or >5% in one month.

**Clinically significant weight loss =10% in 6 months or 7.5% in 3 months or 5% in one month.

Table 6A.2: Nutrition Support Post operatively in 205 Oesophagectomy cases

Days on nutrition support	15(2 - 112)
Days on full NS	11(2 - 112)
Days on part NS	3 (0 - 48)
Days Fasting	0 (0 - 10)
Days to first Bowel Motion	5
Peri-op Weight loss (kg)	1.5 (0 - 25.6)
Peri-op weight Loss	2.3% (0-26)
Mean Weight on discharge (kg)	71 (39 - 125.7)
Mean BMI on Discharge	24.6 (16.3 – 40.6)
Weight Loss Classification	
Non-significant weight loss	65%(133)
Significant weight loss	8%(16)
Severe weight loss	24%(56)
Enteral Feeding	189 (92%)
Parenteral Feeding	16 (8%)
Intra Venous Fluids Only	0 (0%)

NS = Nutrition Support

6A.4.3. Biochemical, Gastrointestinal and mechanical complications of NCJ feeding

The biochemical, gastrointestinal and mechanical complications of jejunostomy feeding in this series are shown in Table 6A.3. A large percentage of patients required electrolyte supplementation post-operatively, phosphate requirements being the most common. Eighteen percent of patients reported constipation on jejunostomy feeding and 26% required laxative administration. Diarrhoea occurred in 22% of cases, most frequently in patients with a prolonged hospital stay, and this may have been antibiotic-related or in some cases due to *Clostridium difficile* infection. The incidence of mechanical complications was rare, however three (1.4%) serious complications of jejunostomy feeding occurred, all requiring re-laparotomy. One 54-year-old man developed an acute abdominal crisis on the fourth postoperative day; at laparotomy he was found to have patchy necrosis of dilated small and large bowel, with extensive infarction of the caecum and ascending colon. The necrotic bowel was resected but he died of a cardiac arrest a few hours after surgery. Another patient, a 53 year-old woman requiring prolonged feeding via a NCJ because of an anastomotic leak, developed a volvulus around her jejunostomy site leading to complete bowel obstruction a month postoperatively. At laparotomy her jejunostomy was taken down and she made a full recovery. A 56 year-old man developed *Escherichia coli* bacteraemia on the third postoperative day, and at laparotomy was found to have a volvulus and jejunal perforation. His jejunostomy was removed and he too made a full recovery.

Eighteen patients (9%), including the latter two above, required cessation of jejunostomy feeding and commencement of total parenteral nutrition (TPN). The reasons included prolonged ileus (1), actual or suspected chyle leak (8), severe clostridium difficile diarrhoea (1), severe diarrhoea (1), abdominal distension and abdominal pain (4) inadvertent tube removal/tube dislodgement (3). The infusion rate was adjusted or feeds withheld temporarily for the following reasons: nausea (8), abdominal distension (7); abdominal pain/cramps (2); raised PaCO2 (1); large positive fluid balance (3); severe diarrhoea (5); regurgitation of feed in gastric aspirates (1); leakage at jejunostomy site (2); dislodgement of catheter (3); and rigors (1).

The overall in-hospital morbidity and mortality is shown in Table 6.4. The overall morbidity rate was 45%, with the most common post operative complication being sepsis at 16%, organ failure 14% and pneumonia 12%. The mortality rate in this series was 4.3% (9 patients).

6A.4.4 Nutritional Course Postoperatively

NCJ feeding was extremely effective in preventing severe post-operative weight loss. Oesophagectomy patients lost a median of 1.5 kg (0 - 25.6kg). 38% of patients lost no weight at all post operatively. 65% of oesophagectomy patients had non-significant weight loss peri-operatively. The median BMI on discharge was 24.6 (Range 16.3 – 40.6 kg/m^2).

Oral intake as a percentage of requirements was inadequate in over 60% of patients on discharge. Oral intake as a percentage of nutritional requirements was only 70% for energy and 65% for nitrogen (IQ 50-74%). Eighty per cent (163) of oesophagectomy patients had a jejunostomy in situ on discharge from hospital, 13% (27) had their jejunostomy removed before discharge, 3% of jejunostomy tubes were dislodged (6) and the remaining 9 patients died in hospital post operatively (4%). Sixteen patients (8%) required home enteral feeding at discharge from hospital, 10 of these patients were discharged on night-time feeding only, and 6 on continuous enteral feeding.

Figure 6A.1 plots the median weight changes peri-operatively in 162 oesophagectomy cases from pre illness to follow up. By the first month out patient visit 82% of oesophagectomy patients were losing weight, and only 12% had managed to gain weight. Forty four percent of patients had lost clinically severe weight loss (>5% in one month) and 6% had clinically significant weight loss (5% in one month). Six percent (n=12) were sufficiently malnourished that they required re commencement of jejunostomy feeding at home to, prevent further weight loss.

Table 6A.3: Biochemical, Gastrointestinal and Mechanical complications of Jejunostomy Feeding in 205 cases

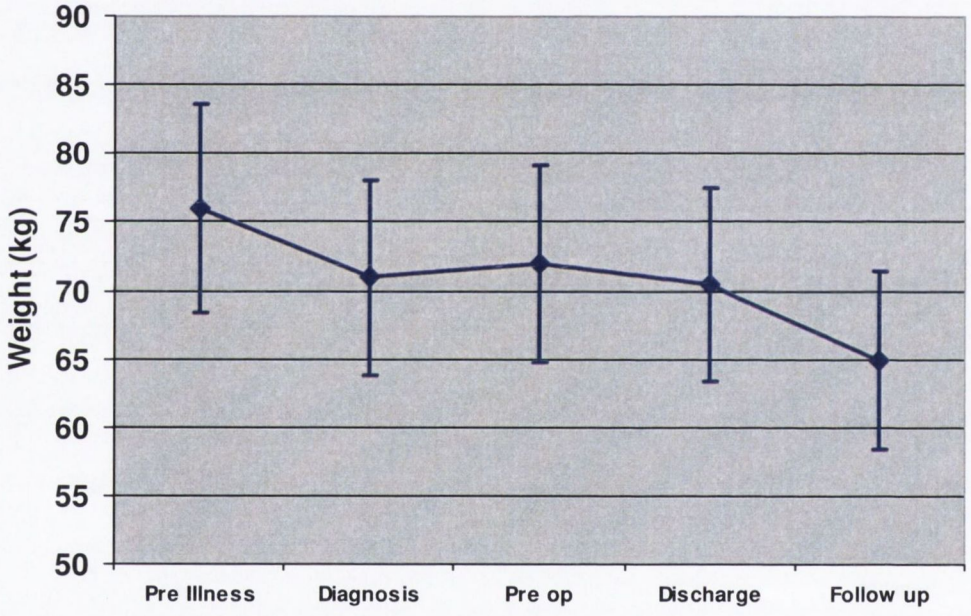
	Incidence
Electrolyte Supplementation	
Phosphate	37%(76)
Potassium	32%(66)
Sodium	8% (16)
Magnesium	20%(42)
Gastrointestinal Complications	
Constipation	18%(38)
Laxative requirement	26%(54)
Diarrhoea >3/day	11%(22)
Diarrhoea< 3/day	11%(22)
Nausea	16%(33)
Cramps	6%(13)
Abdominal Distension	4%(9)
Vomiting	3%(7)
Mechanical Complications	
Tube Dislodged	2.4%(5)
Tube Occlusion	3% (6)
Tube Split	0.5% (1)
Infection at entry site	1.4%(3)
Site Oozing	1.4%(3)
Bowel obstruction/volvulous	1.4%(3)

Table 6A.4: Post-operative morbidity and mortality

Median length of stay	20 days (4 - 126)
Median LnOS (excluding post op RIP pts)	20 days (11-126)
Median ICU stay	0 days (0-46)
Median HDU stay	4 days (0-32)
Median days ventilated	0 (0-33) days
Mortality rate	4.3% (9).
Post op morbidity	45%
No complication	55%
Organ failure	14%
ARDS	9%
Sepsis	16%
Pneumonia	12%
Wound infection/dehiscence	3%

ARDS= Adult respiratory distress syndrome, LnOS=Length of Stay, ICU=Intensive care Unit, HDU=High Dependency Unit, RIP=Rest in Peace

Figure 6A.1: Median Weight changes peri-operatively in 162 oesophagectomy cases from pre illness to follow up (Error Bars are 95% CI)



6A.5 DISCUSSION

The study herein confirms that early feeding via a needle-catheter jejunostomy following an oesophagectomy, although not without risks, is a safe procedure allowing early delivery of enteral nutrition and avoids the need for TPN with its attendant risks and expense. In addition to providing a safe, effective route for delivering enteral nutrition distal to the stomach and duodenum, the NCJ can be used to administer water, electrolytes, almost all types of medication, avoiding the need for parenteral preparations and thus reducing real costs and cost in terms of nursing time.

There is not uncommonly a long period of adjustment to the recommencement of oral intake, in part because of reduced satiety and in part from impaired gastric function (Lawlor et al, 2004), and the NCJ allows for this variation in the natural recovery of the uncomplicated patient. The principal benefit of the NCJ, however, is seen in patients with complications after oesophagectomy that markedly delay oral feeding. There is a paucity of published literature on nutritional outcomes after oesophagectomy and the majority of reports on jejunostomy feeding make no reference to nutritional outcomes. In this series 26% of patients undergoing oesophagectomy required enteral feeding for longer than 20 days, and this is in line with a recent report showing that 19% of patients require prolonged feeding after oesophagectomy (Sica et al, 2005). In addition to this, the routine discharging of these patients with their NCJ *in situ* allows for timely intervention in those that fail to thrive following discharge. As it is impossible to predict this outcome prior to surgery, the routine insertion of NCJ at the time of surgery avoids the need for later, often difficult invasive procedures, to achieve enteral access. In this series 14% of patients required home enteral feeding, as they were unable to meet nutritional needs orally or were not allowed oral intake. Since 60% of patients had sub-optimal oral intake on discharge, yet only 8% were discharged on supplemental feeds, and this was introduced in a further 6%, there is clear deficit in the delivery of nutritional treatment goals. This is acknowledged by the authors and this aspect of the audit may guide future policy.

The predominant complications of NCJ are diarrhoea and abdominal distension. These adverse gastrointestinal symptoms are not directly related to NCJ and are a common feature of early postoperative enteral feeding and they can be controlled in the vast majority of patients with appropriate reduction of the nutrient flow (Biffi et al, 2000).

Serious complications can occur, and the incidence of serious complications requiring surgical intervention was 1.4% in this series, with one death (0.6%). These figures are in line with previous reports (Sarr et al 1988; Han-Geurts et al 2004; Biffi et al, 2000; Wakefield et al, 1995; Pescovitz et al, 1995; Dent et al 1993; Chin et al, 2004). Two cases (1%) of intestinal volvulus around the fixed point of the jejunostomy site were reported, both from the early experience (1997 and 2000), and the surgeons make every attempt to site the catheter at most proximal suitable point in the jejunum to avoid redundant loops. No case of stricture was evident.

Functional intestinal complaints with jejunostomy feeding occur frequently but generally respond to alteration of the infusion rate or tube feeding formula. Occasionally, however, non-specific signs of intestinal disturbance progress to a syndrome of abdominal distension, hypotension, and hypovolemic shock resulting in extensive small bowel necrosis. The death in this series was from intestinal ischaemia, a rare but well-described complication (Myers et al, 1995; Han-Geurts et al, 2004; Chin et al, 2004; Schunn & Daky 1995; Yagi et al, 1999; Sica et al, 2005; Sarr & Mayo, 1988; McCarter et al, 1997). The mechanism is unclear, but hyperosmolarity of feed, bacterial overgrowth, decreased splanchnic blood flow, and adynamic ileus may all be contributory (Schunn & Daly 1995; Zetti et al, 2002). In this Unit, the feeding rate is advanced faster than in many Units that report their practice, and the low rate of ischaemic necrosis suggests that caveats with respect to this risk in the advancement of feeds may be incorrect. Notwithstanding this evidence from this study, it is clear that close daily monitoring of symptoms and feed tolerance is mandatory, and all who manage patients with NCJ feeds should know of the risks of bowel ischaemia and be aware of its appropriate management.

This study shows that early enteral feeding is effective in maintaining nutritional status and is associated with minimal weight loss in the perioperative period. A central mechanism observed in randomised trials is an approximate twofold increase in insulin levels in enterally compared with parenterally fed patients, resulting in a protein sparing effect with net positive nitrogen balance (Hockwald et al, 1997). By significantly impacting on protein loss, early post operative EN may potentially contribute to a decrease in post operative morbidity and mortality in upper GI cancer patients. Early enteral feeding impacts positively on whole body protein metabolism, and the hyperinsulinaemia induced by feeding decreases endogenous fat oxidation (Hockwald et

al, 1997). Nutritional fluid given enterally is completely absorbed even immediately following highly invasive oesophageal surgery. It has been suggested that this gut-directed therapy also modulates post surgical inflammatory responses, and encourages faster recovery of lymphocyte counts and attenuated levels of bilirubin and C-reactive protein compared with gut starvation after oesophagectomy (Aiko et al, 2001), although other studies fail to show a clear association between enteral nutrition and immune parameters (Reynolds et al, 1997). EN post oesophagectomy is associated with a significant increase in the levels of serum total protein and albumin (Yagi et al, 1999), and has also been shown to significantly attenuate gut permeability when compared to intravenous fluids only and is associated with significantly fewer post operative complications (Carr et al 1996). Postoperative starvation after oesophagectomy has been shown to be associated with poor nitrogen balance, poor gut mucosal integrity, a slower recovery in immune function and a more exaggerated inflammatory response (Aiko et al, 2001; Carr et al 1996).

There has been a real increase in the incidence of oesophageal adenocarcinoma in the western world in the last 20 years (Chow et al 1998; Lagergren et al 1999; Brown et al 1995), and overweight and obesity are clear risk factors, hence many patients now are overweight the time of surgery. In this series 43% were overweight and 15% were obese. Obese patients undergoing high risk surgery bring with them both technical difficulties for the surgeon as well as longer operative times (Blee et al, 2002), impairments in immune function, abnormal cardio respiratory function, metabolic derangements, abnormal haemostasis, higher incidence of post operative complications such as wound dehiscence, nosocomial infections, respiratory complications, delayed cardiac recuperation (Dickerson et al, 2002), and higher peri-operative weight loss (McWhirter & Pennington, 2002; Fettes et al, 2002). Obesity also induces significant changes in substrate metabolism. Contrary to the general belief that the abundant supply of adipose tissues will be the primary fuel, the injured obese patient experiences a relative block in both lipid metabolism and utilisation, resulting in significantly increased rates of protein mobilisation to provide substrates for the synthesis of glucose, resulting in increased nitrogen loss compared to equally injured non-obese patients (Jeevanandam et al, 1991). Starvation, or 'letting them live off their excess fat', is an inappropriate strategy, which places patients at risk for increased loss of lean body mass, and a standard nutritional regimen is prescribed for all our patients.

6A.6 CONCLUSION

In conclusion, this audit shows that NCJ feeding is a safe and effective method of providing nutritional support post oesophagectomy. It is well tolerated and is effective in preventing severe weight loss in the postoperative period. In a subset of patients who experience post operative surgical and medical complications NCJ allows for prolonged enteral access and avoids the need for TPN. For patients requiring home enteral feeding, insertion of a NCJ at the time of surgery avoids the need for invasive interventions at a later stage. Severe complications associated with this method of nutrition support are extremely rare. This audit has also highlighted for this Unit that many more patients than the 8% reported should be considered for home supplemental feeds via the jejunostomy, and this now informs current policy, as this nutritional deficit could negatively impact on complications and quality of life following hospital discharge.

CHAPTER 6B:
SHORT-TERM NUTRITIONAL IMPLICATIONS OF TOTAL GASTRECTOMY FOR MALIGNANCY, AND THE IMPACT OF PARENTERAL NUTRITIONAL SUPPORT

Published in *Clinical Nutrition, (2007)26; 718-727*

"Short-term Nutritional Implications of Total Gastrectomy for Malignancy, and the Impact of Parenteral Nutritional Support.

Aoife Ryan, Laura A Healy, Suzanne P Rowley, John V Reynolds.

6B.1 SUMMARY

Background: The purpose of this study was to report on nutritional outcomes in gastric cancer patients undergoing total-gastrectomy in terms of: nutritional status at diagnosis; the effectiveness of artificial nutrition support in the post operative period versus intravenous fluids only; long-term changes in nutritional status in the three months following surgery and finally, to examine if the provision of nutrition support post operatively attenuates changes in nutritional status post discharge.

Methods: This study included 90 consecutive patients who underwent total gastrectomy for malignancy. Each patient underwent an individual nutritional assessment at diagnosis, post operatively and at 3 month outpatient follow up. Patients were followed prospectively to record oral nutritional intake, artificial nutrition support administered, duration on nutrition support, post-operative complications. Nutritional status was reassessed at discharge from hospital and at out patient follow up three-months post discharge.

Results: There was a high prevalence of malnutrition at diagnosis with 46% of patients reporting clinically severe weight loss. Thirty one percent of patients lost >10% of their pre-illness weight at diagnosis. Dietary intake was inadequate in 72% of patients at diagnosis and 47% complained of dysphagia. Of the 90 gastrectomy cases, 42% were given TPN post-operatively and the remainder (53%) were maintained on intravenous fluids (IVF) alone. TPN patients spent a mean of 13.6 days on nutrition support versus IVF patients who spent a mean of 9.2 days fasting. IVF patients lost significantly more weight in hospital than TPN patients (5.2kg versus 3.1 kg, p=0.008). 69% of IVF patients lost severe amount of weight versus 34% in the TPN group (p=0.01). Post discharge IVF patients continued to lose significantly more weight than those given TPN post-operatively (7.5kg versus 2.9 kg, p=0.01) corresponding to 10.5% of their body weight from discharge to follow up versus 4.9% for TPN group (p=0.014). From pre illness to follow up, total gastrectomy patients lost an average of 15.5kgs however IVF patients lost significantly more - an average of 17.8 kg versus the TPN group who lost an average of 9.6 kg. There was no difference in post-operative complications between the two groups however, patients with >10% weight loss at diagnosis had a significantly higher mortality rate post-operatively than with less severe weight loss. On multivariate

logistic regression analysis >10% weight loss at diagnosis was the only predictive factor of post operative complications.

Conclusions: There is a high prevalence of malnutrition in gastric cancer patients undergoing surgery. Total Gastrectomy is associated with dramatic weight loss post-operatively, with patients losing an average of 15.5 kgs from diagnosis to 3-month follow up. Provision of nutrition support in the form of TPN post-operatively significantly reduces in-hospital weight loss and also helps to attenuate further weight loss post discharge.

6B.2 INTRODUCTION

Gastric cancer is the second leading cause of cancer deaths worldwide (Jemal et al, 2006; Greenlee et al, 2001). In Ireland approximately 440 new cases are diagnosed each year and 335 deaths from gastric cancer occur, making it the 7th most common cancer in men and the 8th most common cancer in women in Ireland (National cancer Registry 2006).

Approximately one-third of gastric cancer patients have stage I or II disease at the time of diagnosis, one quarter have stage III disease and the remaining 40% or so stage IV disease (Alberts et al, 2003). The only potentially curative treatment currently available for gastric cancer is surgery. The side effects of gastrectomy, in particular total gastrectomy, are however considerable, and include post-operative weight loss, anorexia, diarrhoea, and other metabolic and nutritional changes (Liedman, 1999). The recovery is very slow and often incomplete. Survival remains poor with a 5-year survival of approximately 25% in patients undergoing a curative resection.

Malnutrition is one of the major post-operative complications of radical subtotal or total gastrectomy for gastric cancer (Saito et al, 2001). Marked loss of adipose and lean tissue mass is common even years after surgery. Amongst the mechanisms involved in the aetiology of protein energy malnutrition include anorexia, iatrogenic starvation, inadequate efforts at oral feeding, postprandial symptoms, malabsorption, maldigestion, shortened intestinal transit time and bacterial overgrowth. This can manifest as severe weight loss compromising the quality of life, often leading to an unnecessary loss of muscle mass with impaired mobilisation and increased morbidity.

There is still considerable controversy in the literature regarding the optimal route of feeding post total gastrectomy. Many units do not routinely provide any form of nutrition support. In Chapter 6A we reported on the beneficial effects of immediate enteral feeding post oesophagectomy with a needle catheter jejunostomy. However, this has not been our practice after total gastrectomy, as the senior surgeon does not favour the insertion of a jejunostomy feeding tube in proximity to the anastomosis of proximal jejunum to the efferent jejunal limb of the Roux-en-Y reconstruction, and is also against naso-jejunal feeding across this anastomosis.

6B.3 AIMS AND OBJECTIVES

The aims of this study were to report on the experience of this tertiary unit in patients undergoing total gastrectomy, specifically recording the nutritional status at diagnosis, the effectiveness of artificial nutrition support in the post operative period, and to audit on long-term changes in nutritional status in the months following discharge, comparing patients who were nutritionally supported postoperatively and those that were not.

6B.4 PATIENTS AND METHODS

All patients who underwent a total gastrectomy from 1998 to 2006 were selected for inclusion in this study. The medical details were obtained from the St. James's Hospital Upper Gastrointestinal Cancer database, which uses the Patient Analysis and Tracking System (PATS)^{TM}, Dendrite Clinical Systems, UK. The nutritional details obtained from the dietetic record cards and the patients' medical notes, were entered into PATS. Where dietetic records were unavailable – patients were excluded form the study.

6b.4.1 Nutritional Assessment

Each patient underwent an individual nutritional assessment by a registered dietitian. Patients were weighed at their bedside in their bedclothes. Height was measured by a wall-mounted stadiometer. Body Mass Index was calculated using the standard formula weight/height^2. The patients were asked to report their usual or pre illness weight (at least one year prior to diagnosis) and this was confirmed by examination of past medical notes if available. Percentage weight loss was then calculated and graded according the Blackburn criteria (Blackburn et al, 1977). Individual energy requirements were calculated using the Schofield equation with adjustments for stress and activity level (Schofield, 1985). Protein requirements were estimated using the Elia table for nitrogen requirements (Elia, 1990). Additionally, nutritional status was classified by the Nutritional Risk Index (NRI) (Veterans Affairs total Parenteral nutrition group, 1991), and by Subjective Global Assessment (Detsky & Smalley 1994). NRI was calculated by the formula: NRI=1.519 x serum albumin (g/l) + 0.417 x (current weight/usual weight) x 100. The degree of dysphagia (if any) was recorded along with, oral intake of energy and nitrogen, and the type of artificial support administered pre-operatively (if any).

6b.4.2 Surgical Procedure

All patients underwent a total gastrectomy and had a laparotomy and abdominal lymphadenectomy. Gastrectomy patients were managed post operatively by extubation immediately following surgery and management in a high dependency unit (HDU) for the early post-operative days. Prior to 2002 it was standard practice to maintain gastrectomy patients solely on intravenous fluids (IVF) in the post-operative period until oral intake was allowed. Since 2002 patients were routinely given total parenteral nutrition (TPN) from 24 hours post operatively via a central venous catheter, inserted at the time of surgery. The rate progression of nutrition support, tolerance, and complications were documented, along with the duration on feeding and days spent fasting without any nutrition. Nutritional status was re-assessed on the day of discharge from hospital and at out patient follow up at one month and 3 months.

6B.5 STATISTICAL ANALYSIS

Data manipulation and statistical analyses were conducted using SPSS® Version 14.0 for Windows™ (SPSS® Inc., Chicago, IL). Mean (± standard deviation) for continuous variables were compared with each other using independent samples t-tests. Cross-tabulation was used to compare method of nutrition support and degree of weight loss with, post-operative complications and patient status with other categorical variables. Significant differences were tested using Pearson Chi-square analysis.

Binary logistic regression analysis was used to determine whether certain variables could predict post-operative complications (no/yes) and patient status (alive/dead). These predictor variables included sex, age, smoking status, presence of co-morbid disease, >10% weight loss, and route of nutrition support (if any). Initially, all predictor variables were assessed independently. The models were used to generate odds ratios (OR) with their respective 95% confidence intervals (CI) to quantify the likelihood of having a post-operative complication or the likelihood of death.

Separate models were created for predicting post-operative complications and patient status. The set of variables remaining statistically significant at $p \leq 0.200$ in these initial models were incorporated to produce multiple logistic regression models. This approach identifies important variables for the final models that may not be identified using the traditional statistical cut-off point of $p<0.05$. In each of the final multiple regression models, significance was taken at $p<0.05$.

6B.6 RESULTS

6B.6.1 Patient Characteristics

One hundred and ten patients underwent a gastrectomy for malignancy by a single consultant surgeon between February 1998 to October 2006. Of the 110 patients, 90 had full dietetic information available and were included in the study (*see table 6B.1*). The median age at diagnosis was 65 years (standard deviation 12 years, range 26-85 years). There were 58 males and 32 females. Seventy-five patients had adenocarcinoma (83%), 10 (11%) had gastrointestinal Stromal tumour (GIST) and 5 patients (6%) had gastric lymphoma. The majority of the tumours were located in the body of the stomach and the operative approach is described in table 6B.1.

At diagnosis 31% of patients were current smokers and 33% were ex smokers. There was also a high incidence of reported heavy alcohol consumption (>14 units/week for females or >21 units/week for males) prior to disease at 21%. Fifty-four percent of patients had co-morbid disease – the most common being cardiovascular disease (37%). Based on American Society of Anaesthesiology (ASA) grade (Saklad, 1941); 32% of patients were healthy, 38% had mild systemic disease, but 23% were ASA grade III i.e. severe systemic disease with definite functional limitation, and 7% had ASA grade IV – severe systemic disease that is a constant threat to life. Performance was assessed by Karnofsky score, a measure of quality of life (Grieco & Long 1984). Results above 80% indicate normal activity with few symptoms or signs of disease, results below indiated te need for help with adtivities of daily living and symptoms of disease requiring regular medical care. Our results showed that 19% of patients had a Karnofsky score of 80% or less. The majority of the tumours were located in the body of the stomach and in total 78% of patients underwent a total gastrectomy with a further 22% requiring total gastrectomy with either a distal oesophagectomy or a partial pancreatectomy.

6B.6.2 Nutritional Status at Diagnosis

The median weight at diagnosis was 72 kg (SD 17 kg, range 40 -116kg). The median BMI was 25 kg/m^2 (standard deviation 5 kg/m^2, range 15.1 – 48.3 kg/m^2). Based on BMI alone 11% of patients were underweight (BMI < 20 kg/m^2), 44% had a normal BMI (20-24.9 kg/m^2) and 45% were either overweight or obese (BMI > 25 kg/m^2). The median weight loss was 5.4 kg (standard deviation 5.3kg) representing 7.3% loss of usual weight (SD 7%). Seventy six percent of patients were losing weight at the time of diagnosis. When the weight loss was graded according to the Blackburn criteria (Blackburn, 1977), 46% of patients had clinically severe weight loss, 1% had clinically

significant weight loss, and the remainder (53%) had non- significant weight loss. Thirty one percent of patients lost > 10% of their usual pre illness weight (*see table 6B. 2*).Based on Subjective Global assessment (SGA) 58% of patients were classified as well-nourished, 31% had mild/moderate malnutrition and 11% were severely malnourished. On Nutritional Risk Index classification of malnutrition only 8% were severely malnourished but 37% had moderate malnutrition. Eighteen percent of patients had a serum albumin level below the normal reference range on admission. Dietary intake as a percentage of requirements was inadequate in 72% of patients at diagnosis. While 53% could swallow normal textured food the remaining patients could only tolerate soft foods (33%) or liquids only (3%), 4% of patients required nutritional support either as enteral or parenteral feeding.

6B.6.3 Post operative nutrition support

Of the 90 patients that underwent gastrectomy - 52 (58%) did not receive artificial nutrition support post operatively and were maintained on Intravenous Fluids (IVF) only. Thirty-eight patients (42%) were given Total Parenteral Nutrition (TPN) via a central venous catheter from the first post-operative day. Patients who were given TPN post operatively were significantly more malnourished pre-operatively than those who received IVF. Compared to patients who received IVF only, patients given TPN were significantly lighter as reflected by median weight (76.4 kg (SD 15.7) versus 66kg (SD 15.8), p=0.002 respectively) and BMI (26kg/m^2 (SD 5.2) versus 23.8 kg/m^2 (SD 4.7), p=0.049), and also had a greater degree of weight loss pre-operatively (4.7%(SD 5%) versus 10.8%(SD 8%), p=0.0001) (*see table 6B. 3*).

6B.6.4 Changes in Nutritional Status Post Operatively

There was no significant difference between the two groups in terms of the length of time to resumption of oral intake, which was 9 days in both groups (p=0.812). Patients given TPN spent a mean of 13.6 days on nutrition support. In contrast, IVF patients spent a mean of 9.2 days without any form of nutrition support, which was significantly longer than TPN patients who only spent a mean of 0.8 days without any nutrition (p=0.0001). As a result patients who received IVF only lost significantly more weight in hospital compared to TPN patients (5.2 kg versus 3.1 kg, p=0.008), which corresponded to a higher percentage weight loss in the IVF group (6.6% versus 4.6%, p=0.023). When graded according to the Blackburn criteria for weight loss (1977), 69% of patients in the IVF group had severe weight loss peri-operatively versus 34% in the TPN group (p=0.011) see table 6B.3.

TABLE 6B.1: Patient characteristics, tumour site, morphology and operation type

Age (years)	65(12)
Male/Female	58/32
Smoking status	
Current smoker	27 (31%)
Ex smoker	28 (33%)
Never smoked	31 (36%)
Alcohol Intake	
Heavy drinker	17 (21%)
Social drinker	36 (44%)
Non-Drinker	29 (35%)
ASA Grade	
Grade I	28 (32%)
Grade II	33 (38%)
Grade III	20(23%)
Grade IV	6 (7%)
Karnofsky score at Diagnosis	
100%	32 (37%)
90%	38 (44%)
80%	11 (13%)
70%	2 (2.5%)
60%	3 (3.5%)
Co-Morbid Disease	
None	40 (46%)
Cardiovascular disease	32 (37%)
Cardiovascular disease & Diabetes	2 (2.3%)
Cardiovascular disease & renal disease	2(2.3%)
Diabetes	3 (3.4%)
Renal disease	1 (1%)
Respiratory disease	7(8%)
Tumour Site	
Antrum Stomach	11 (12%)
Body of Stomach	33 (37%)
Fundus	10 (11%)
OG Junction	4 (4%)
Cardia	15 (17%)
Distal stomach	9 (10%)
Proximal	8 (9%)
Morphology	
Adenocarcinoma	75 (83%)
GIST	10 (11%)
Lymphoma	5 (6%)
Operation Type	
Total Gastrectomy & Distal Oesophagectomy	12 (13%)
Total gastrectomy & distal pancreatectomy	8 (9%)
Total Gastrectomy	70 (78%)

ASA Grade I =Healthy patient, Grade II =Mild Systemic Disease, Grade III =Severe systemic disease with definite functional limitation, Grade IV =Severe systemic disease that is a constant threat to life. GIST=Gastrointestinal Stromal tumour

TABLE 6B.2: Nutritional status at diagnosis prior to gastrectomy (n=90) values expressed as mean (standard deviation or %)

Weight at diagnosis (kg)	72 (±17)
Pre Illness Weight (kg)	77 (±15)
BMI at diagnosis (BMI kg/m^2)	25 (± 5)
Pre Illness (BMI kg/m^2)	27 (± 5)
Underweight (BMI<20 BMI kg/m^2)	10 (11%)
Normal (20-25 BMI kg/m^2)	38 (44%)
Overweight (25-30 BMI kg/m^2)	25 (29%)
Obese (>30 BMI kg/m^2))	14 (16%)
Weight loss (kg)	5.4 (± 5.3)
% Weight Loss	7.3 (±7.1)
Weight loss grading	
Non Significant	44 (53%)
Significant	1 (1%)
Severe	38 (46%)
>10% Weight loss	
Yes	28 (31%)
No	62 (69%)
Weight loss over previous 2 weeks	
Yes	67 (76%)
No	21 (24%)
Subjective Global Assessment	
SGA Severe	7 (11%)
SGA Mild/Moderate Malnutrition	19 (31%)
SGA Well nourished	36(58%)
Nutrition Risk Index	
NRI Severe	6 (8%)
NRI Moderate	29 (37%)
NRI Mild	12 (15%)
NRI non malnourished	32 (40.5%)
Serum albumin on admission	37 (± 3.7)
Serum albumin below normal on admission	16 (18%)
Dietary Intake at diagnosis	
Adequate	19 (28%)
Inadequate	48 (72%)
Dietary Change	
No Change/Optimal intake	22 (29%)
Sub-optimal/Normal Texture	18 (24%)
Soft	25 (33%)
Semisolid	5 (7%)
Hypocaloric liquid	2 (3%)
Enteral Feeding	1 (1%)
Total Parenteral Feeding	2 (3%)
% Energy requirements met at diagnosis	60% (29)
% Nitrogen requirements met at diagnosis	61% (29)

NRI=Nutrition Risk Index, SGA=Subjective Global Assessment, BMI=Body Mass Index

TABLE 6B.3 Changes in Nutritional Status post total gastrectomy for patients given Total Parenteral Nutrition (TPN) versus Intravenous Fluids (IVF), n=90.

	IVF only (n=52)	TPN (n=38)	P
	Mean (standard deviation)		
Pre operative weight (kg)	76.4 (15.7)	66kg (15.8)	0.002
BMI at diagnosis	26(5.2)	23.8(4.7)	0.049
Weight loss (kg)	3.7(4)	7.8(6)	0.0001
% weight loss at diagnosis	4.7(5)	10.8(8)	0.0001
Days in hospital post op	17.6(9)	21.8(20)	0.184
Days on Nutrition support	1(3.5)	13.6(17.7)	0.0001
Days on full feeding	0.6(2.1)	12.7(17)	0.0001
Days on night feeding	0.6(0.43)	0.7(2.7)	0.097
Days without any nutrition	9.2(3)	0.8(1.5)	0.0001
Days to oral intake	9 (1.8)	9 (2.6)	0.812
Weight on Discharge (kg)	71.2 (14.4)	63(14.6)	0.011
Peri-operative weight loss (kg)	5.2(2.9)	3.13(3.8)	0.008
% Weight loss as in-patient	6.6(3.6)	4.6(4.7)	0.023
Weight loss grading			
Non-significant	8 (18%)	16 (49%)	0.011
Significant	7 (13%)	5 (17%)	
Severe	30 (69%)	12 (34%)	
Weight at follow up (kg)	62.2(14)	61.7(14.6)	0.895
BMI at follow up	21.7(5.2)	22.1(4.4)	0.764
Weight loss post discharge (kg)	7.5(7.3)	2.9(3.9)	0.015
% weight loss at follow-up	10.5(8.8)	4.9(5.4)	0.014
Wt loss diagnosis to FUp (kg)	12(7.9)	6.3(6)	0.01
% wt loss diagnosis to FUp	16(9.5)	8.6(7.3)	0.008

FUp=Follow up, BMI=Body Mass Index

When the results were analysed including only patients with baseline pre operative malnutrition (i.e. >10% weight loss at diagnosis) patients given IVF only lost 3 times the amount of weight post operatively than those given TPN (6.5 kg versus 1.8 kg, p=0.001). When graded according to the Blackburn criteria of weight loss (Blackburn, 1977) 86% of IVF patients who had baseline malnutrition had severe weight loss versus 25% given TPN (p=0.01) see table 6B.4.

Despite this weight loss in hospital, the IVF group still weighed significantly more on discharge form hospital than the TPN group (71.2 kg versus 63 kg, p=0.011). However, by out patient follow up at 3 months, there was no difference in mean weight between the groups as IVF patients lost significantly more weight than the TPN group post discharge from hospital (7.5 kg versus 2.9 kg, p=0.015). In fact, the IVF group lost an average of 10.5 % of their body weight from discharge to follow up versus 4.9% in the TPN group (p=0.014). From pre illness to follow up, total gastrectomy patients lost an average of 15.5 kgs, however IVF patients lost significantly more - an average of 17.8 kg versus the TPN group who lost an average of 9.6 kg, p<0.01 (*see figure 6B. 1*).

6B.6.5 Post Operative Complications

In total 34% of patients developed a post-operative complication. The most common complication was sepsis (10%), followed by pneumonia (8%), respiratory failure (3.5%), and wound infection (3.5%). The in-hospital mortality rate was 4%. The mean length of stay was 19 days (SD 14.6 days).

When comparing patients given TPN to those given IVF only there was no significant difference in any post-operative complication or in the length of hospital stay (*see table 6B.5*). When the data was analysed by splitting patients with >10% weight loss pre operatively versus <10% weight loss, patients with >10% weight loss had a significantly higher rate of "any" post-operative complication, and a significantly higher mortality rate than patients who lost <10% body weight (26.2% versus 51.9%, p=0.036 and 11.1% versus 0%, p=0.027 respectively). There was no significant difference in any other complication or in the length of stay in ICU, HDU, or in-hospital days between the two groups. On multivariate logistic regression analysis controlling for age, gender, >10% weight loss pre operatively, co-morbid disease, and nutrition support (IVF or TPN), to examine predictors of post operative complications, 10% weight loss was the only significant predictor with an Odds Ratio of 3.1 (95% CI 1.0 – 9.6), p=0.04.

TABLE 6B.4: Changes in peri-operative nutritional status in patients with pre-operative malnutrition (>10% weight loss at diagnosis). Values shown as means (standard deviation).

	TPN	IVF	P Value	95%CI
Weight loss peri op (kg)	1.8 (2)	6.5(4)	0.001	2.2 – 7.1
% Weight loss peri op	3.3%(3.6)	10%(5.2)	0.001	3 – 10.5
% patients with >5% wt loss	100%	31%	0.005	
Non significant wt loss	0%	67%	0.01	
Significant Weight Loss	14%	6%		
Severe Weight Loss	86%	25%		

Figure 6B.1: Weight Changes from pre illness to 3-month follow up post gastrectomy for patients given Total Parenteral Nutrition versus Intravenous Fluids only (Values are mean and standard deviation)

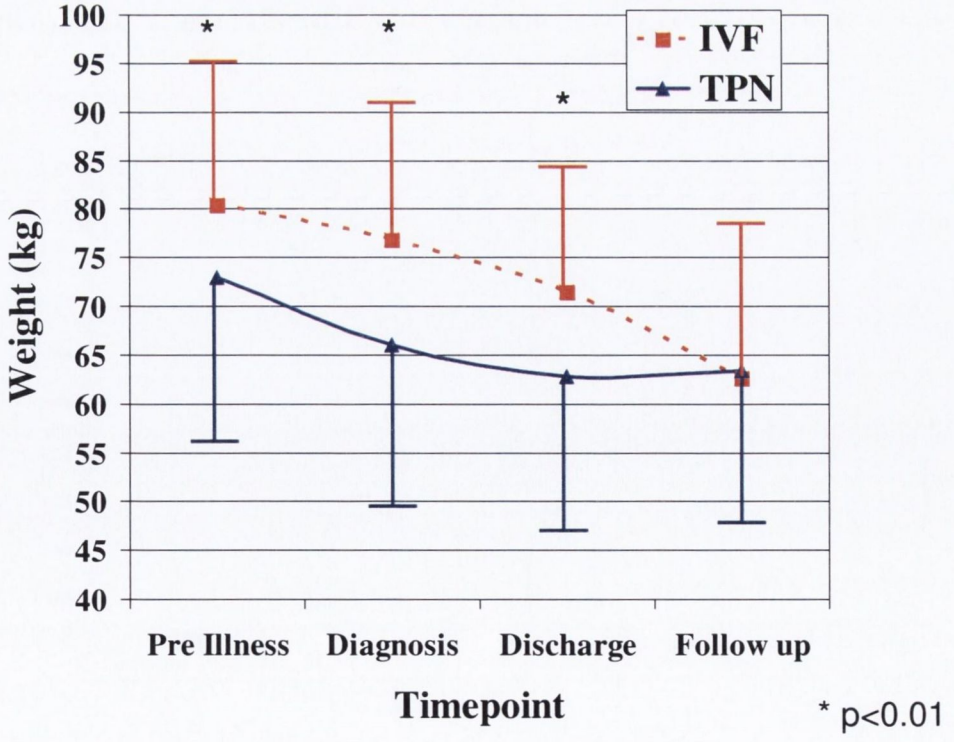


TABLE 6B.5: Post operative complications in patients given Total Parenteral Nutrition (TPN) versus Intravenous Fluids only (IVF), n=90

	IVF	TPN	P value
Any complication	27.5%	43.2%	0.189
Wound Infection	5.9	2.7	0.636
MOF	0	2.7	0.42
Respiratory Failure	2.0	8.1	0.305
Mortality	0	8.1	0.071
Pneumonia	7.8	8.1	1.000
Sepsis	5.9	16.2	0.158
Length of stay (days)	17.6(8.6)	21.8(20)	0.184

MOF=Multiple Organ Failure

TABLE 6B.5: Post operative complications in patients with or without 10% weight loss at diagnosis

	> 10% Weight loss	<10% Weight Loss	P Value
Any complication	51.9	26.2	0.036
Wound Infection	7.4	3.3	0.583
MOF	3.7	0	0.307
Respiratory Failure	7.4	3.3	0.583
Mortality	11.1	0	0.027
Pneumonia	11.1	6.6	0.671
Sepsis	18.5	6.6	0.126
Length of stay (days)	18.6 (10)	19.7(16.2)	0.728
Length of stay ICU (days)	2.9 (0.7)	0.8 (0.25)	0.218
Length of stay HDU (days)	2.0 (2.4)	2.4 (1.7)	0.176

6B.7 DISCUSSION

Radical surgery offers the only possibility for cure in patients with gastric cancer (Scutru et al, 2005; Liedman, 1999). The side effects of total gastrectomy are however considerable, and include post-operative weight loss, anorexia and other metabolic and nutritional changes (Liedman, 1999). Some patients can become nutritionally crippled with marked weight loss and poor tolerance to regular diets (Saito et al, 2001).

There are numerous mechanisms underlying malnutrition following total gastrectomy. Total Gastrectomy can dramatically reduce the reservoir into which patients can eat. Innervation of the stomach is also damaged leading to small stomach syndrome. Anorexia, absence of hunger sensations, and post-prandial abdominal discomfort occurs in many patients and can lead to a significant reduction in calorie intake. Approximately 85% of gastrectomy patients have eating-related symptoms (Bragelmann et al, 1996). Amongst the most frequently cited symptoms are: early satiety (48%), epigastric fullness (26%), epigastric pain (26%), reflux (43%), diarrhoea (22%) and nausea (17%). Diarrhoea commonly occurs within an hour or two of eating, and is probably partly caused by the vagotomy and possible lack of gastric hormones, but also partly from defective fat absorption due to pancreatic insufficiency, bacterial overgrowth, or short small-bowel transit time (Liedman, 1999; Ambrecht et al, 1988; Friess et al, 1996). Patients can become very selective in their choice of foods avoiding hyperosmolar, starchy and high volume foods. Maldigestion after total gastrectomy has been described as pancreaticocibal asynchronism – a condition where defective stimulation of biliary and pancreatic secretions by ingested food bypassing the duodenum, and inadequate mixing of biliary and pancreatic secretions with food occurs (Bragelmann et al, 1996). Loss of duodenal absorptive surface which is the principle site for absorption of iron, calcium, fat and carotene; stasis in the afferent loop can lead to bacterial overgrowth and abnormalities in bile salt metabolism. Bypass of the duodenum also results in decreased release of secretin and CCK and a decreased output of pancreatic enzymes leads to maldigestion and malabsorption. Malabsorption of dietary fat has been proposed as a major contributor to weight loss post total gastrectomy. Up to 50 g fat per day can be lost in stools – almost 7 times that of healthy controls (Sategna-Guidetti & Bianco, 1989; Cristallo et al, 1986). Malabsorption of amino acids has also been reported following gastrectomy resulting in a state of persistent proteolysis of lean tissue mass for long periods after surgery (Saito et al, 2001).

This study highlights the marked deterioration in nutritional status that occurs post total gastrectomy. We reported a mean weight loss of 15.5 kgs corresponding to a 13.3% percentage weight loss from diagnosis to follow up, similar to levels of weight loss reported by other authors (Sategna-Guidetti & Bianco 1989; Ludwig et al, 2001). Several studies have reported on severe weight loss following gastrectomy, which can be between 18-29 kgs and can span up to 4 years. Bozetti and colleagues (1990) reported on 44 disease-free patients after a mean of three years post op, and found that weight loss reaches its nadir at the 15th post op month. This study showed that cancer-free patients lose an average of 19.1 kgs (+/- 9.4kgs) after gastrectomy and report very inadequate oral intakes (24.8 +/- 13.7 kcals/kg ideal body weight/day). Bae et al (1998), reported an average pre-operative BMI of 22.2 +/- 0.45 kg/m^2 and post operative BMI of 18.9 +/- 0.38, that is an average of 15% less than pre operative weight ($p<0.01$). They found no significant correlation between the time since surgery and the magnitude of the weight loss. This group reported that weight loss increased until 4.2 years after total gastrectomy. In a study of 108 patients who underwent gastrectomy, Kiyama et al (2005) examined body compositional changes using bioelectrical impedance analysis. Results showed that body protein mass was lost preferentially in the first 14 days following surgery and continued until 6 months post op, but from 6 months to 1 year post op weight loss was from fat mass alone with no change in body protein. Total gastrectomy patients lost an average of 8.9 kg (SD 5) in the first 6 months and a further 4 kg (SD 3.4) in the second six months following surgery (Kiyama et al, 2005). These results are consistent with those of Liedman et al (1997) who showed that weight loss (10% of pre operative weight) occurs early after total gastrectomy and body fat decreased by 40% during the first 6 months post op. The selective wasting of body fat and sparing of lean body mass is probably an adequate adaptation to the new situation where eating is not as comfortable as before (Liedman et al, 1997).

Protein Energy Malnutrition has been shown to impact on quality of life in terms of functional status and psychosocial well-being (Crogan & Pasvogel, 2003; Peltz, 2002). In patients with advanced GI cancer a weight loss of just 2.5 kg or greater over a 2-6 week period can produce significant alterations in performance status (O'Gorman et al, 2000). Weight loss has also been shown to be a significant survival related factor post gastrectomy (Sanchez-Bueno et al, 1998). This study shows that while only 11% of patients were malnourished based on BMI alone, 76% were actively losing weight at diagnosis and 46% had clinically severe weight loss at diagnosis, with almost a third

losing greater than 10% of their body weight – a cut off point well known to be linked to post operative morbidity (Blackburn et al, 1977). The vast majority of gastric cancer patients have inadequate oral intakes at diagnosis and almost half have swallowing difficulties.

In the present study we report a 35% incidence of complications and a 4% mortality rate following total gastrectomy for malignancy. Patients who lost >10% of their body weight pre-operatively had a significantly higher rate of complications and a significantly higher mortality rate than patients who lost <10% body weight (26.2% versus 51.9%, p=0.019 and 11.1% versus 0%, p=0.027 respectively). On Multivariate logistic regression we report that > 10 % weight loss pre operatively was the only factor to significantly impact on post operative complications with an odds ratio of 3.1 (95% CI 1.0-9.6, p=0.04). These observations are similar to other published reports. Grossmann et al (2002), reported on 234 total gastrectomy cases for cancer with post-operative complication rate of 38% and a 30-day mortality rate of 7.7%. On multivariate logistic regression analysis a weight loss >10% in the six months prior to surgery was predictive of 30-day mortality. This increased mortality rate was also observed by Sitges-Serra et al (1988), who showed that patients who lost >20% of their body weight had a significantly higher mortality rate than those losing <20% of their body weight (23% versus 7%, p<0.05). Similar results were observed by Rey-Ferro et al (1997), who reported a 19% weight loss in patients who died post-operatively versus 9% weight loss in those who survived. Hill (1992) correlated weight loss with post operative morbidity and mortality rates and showed that weight loss of >20% and associated functional alterations presented a rate of complications 3-5 times greater, increasing the hospitalisation stay by 4-6 days (Hill, 1992; Windsor & Hill 1988).

Other means of assessing nutritional status such as the Nutritional Risk Index (NRI) and Prognostic Nutritional Index (PNI) have also been shown to be of value in terms of predicting post operative complications (Sitges-Serra et al, 1988; Rey-Ferro et al, 1997). By associating weight loss with albumin levels in the NRI as described by Buzby (1980), Rey-Ferro et al (1997) found a greater correlation between severe malnutrition (NRI<83.5) and post operative mortality and cellular immunosuppression. They reported 42.5% incidence of moderate malnutrition and 15% incidence of severe malnutrition in patients with gastric cancer according to the NRI - post-operative mortality rate of 33% was observed in the severely malnourished group and 6.5% in the moderately malnourished group. These groups also displayed cellular immunosuppression with poor CD4/CD8 ratios and this was related to post operative

mortality (Rey-Ferro et al, 1997). Sitges-Serra et al (1988) reported that patients with a PNI below 50% have a lower mortality rate than those with a PNI of at least 50% and it has been postulated that this difference is related to the lower resistance to infection in patients who are most malnourished – their sepsis related death rates have been reported to be five times higher than well-nourished patients.

The rationale of nil by mouth in the immediate post operative period following total gastrectomy, is to prevent postoperative nausea and vomiting and protect the anastamosis, allowing time for it to heal before being stressed by food. Nausea and vomiting occur more frequently after upper gastrointestinal surgery than after resection of the small intestine or colon (Silk & Gow, 2001). Patients with gastric cancer are frequently malnourished at diagnosis - weight loss often being the first alarm signal. The fact that no oral intake is allowed until the anastamosis has healed sufficiently (i.e. for up to a week after surgery) means that patients who are already malnourished at baseline can suffer further deterioration in nutritional status post operatively. These concerns, coupled with the catabolic response of the body to major surgery makes post-operative nutrition support logical in the early post-operative period for all patients undergoing upper gastrointestinal surgery.

Patients with gastric cancer who undergo total gastrectomy usually receive TPN for 7 to 10 days after surgery because of concern over the integrity of the oesophago-jejunal anastamosis (Kamei et al, 2005; Sand et al, 1997). There are very few reports published on early enteral feeding after total gastrectomy either by nasojejunal tube feeding or percutaneous catheter jejunostomy (Braga et al, 1996; Sand et al, 1997; Juhani et al, 1997). Some authors report there is no role for artificial feeding after total gastrectomy as it fails to impact on post-operative complications and length of stay. Few reports make any reference to weight loss, which continues beyond the surgeon's view, after discharge.

Failure to provide nutrition support results in prolonged periods fasting and in this study 69% of patients lost clinically significant amounts of weight in hospital, and continued to lose dramatic amounts of weight following discharge (an average of 10.5% of their body weight). In fact, IVF patients shed an average of 12 kgs corresponding to 16% of their body weight from diagnosis to follow up. While provision of TPN failed to impact on post operative complications in this study, the primary aim of providing nutrition support is to treat malnutrition and to prevent deterioration in nutritional status. From the present study it can be seen that the benefits of fighting weight loss during the hospital stay

continue in the post discharge time as well - provision of nutrition support in the form of TPN post operatively attenuated weight loss both in-hospital and post discharge. TPN patients, despite being more malnourished pre-operatively seemed to derive longer term benefits of nutrition support as they lost an average of 6 kgs from diagnosis to follow up, corresponding to 8.6% of body weight – half the weight loss experienced by the IVF group.

Providing patients with cancer-associated malnutrition with nutritional support has been associated with a better response to therapy and fewer treatment-related complications (Nayel et al, 1992; Heys et al, 1999), as well as improved immune function (den Broeder et al, 2000; Bozetti, 2001), performance status, outcome and quality of life (den Broeder et al, 2000; den Broeder et al, 1998; Barber et al, 1999a; McCarthy & Weihofen, 1999; Roberge et al, 2000; van Bokhorst et al, 2000). The interrelationship between nutrition and the immune system has become the focus of ever increasing attention with many substrates being recently identified as having an immune-modulating functions. These nutrients include glutamine, arginine, nucleotides and n-3 fatty acids, as well as selenium, vitamins E, C and β-carotene, at various concentrations (Windsor et al, 1998). Further studies are warranted to examine the role of immune-enhancing nutrients in the context of major upper gastrointestinal surgery.

6B.8 CONCLUSION

This study highlights the nutritional problems experienced by patients with gastric cancer undergoing surgery. There is a high prevalence of malnutrition at diagnosis, and total gastrectomy is associated with dramatic weight loss post-operatively, with patients losing an average of 15.5 kgs from diagnosis to 3-month follow up. Provision of nutrition support in the form of TPN post-operatively significantly reduces in-hospital weight loss and also helps to attenuate further weight loss post discharge. Although non-randomized, the experience of this Unit would support the provision of TPN to patients following total gastrectomy.

CHAPTER 7

A PROSPECTIVE RANDOMISED DOUBLE-BLINDED TRIAL TO INVESTIGATE THE EFFECTS OF AN ENTERAL NUTRITIONAL SUPPLEMENT ENRICHED WITH EICOSAPENTAENOIC ACID ON POST-OPERATIVE COMPLICATIONS, STRESS RESPONSE, IMMUNE FUNCTION, AND BODY COMPOSITION IN PATIENTS UNDERGOING SURGICAL TREATMENT FOR OESOPHAGEAL CANCER

7.1 SUMMARY

Background

The high morbidity rates observed following oesophagectomy reflect the profound changes in the endocrine, neuroendocrine and immune system as well as significant changes in organ function that occur following this type of operation. The model of multimodality management of oesophageal cancer lends itself well to studies with immunonutrition as it represents an ideal model of a major homogenous insult with predictable alterations of immune cell function and metabolism, with most patients developing a systemic inflammatory response syndrome (SIRS), and approximately 50% developing complications, with the risk of in-hospital mortality at approximately 6-10 percent, it also compromises nutritional status and quality of life. Although several studies have demonstrated the beneficial effects of immunonutrition on immune competence and patient outcome, controlled clinical trials focusing on the use of perioperative enteral omega-3 fatty acids alone are scarce. Eicosapentaenoic acid (EPA) is of interest in the context of major cancer surgery as it has potential to impact on both the inflammatory response to surgery, may reduce catabolism, and may also modulate immune function.

Objectives

The primary objectives were to examine the effects of perioperative EPA enriched Enteral Nutrition (EN) on post operative complications, nutritional status and the immuno-inflammatory response to major surgery.

Methods

In a double-blinded design, patients were randomised to receive a Standard EN formula or an isocaloric-isonitrogenous formula enriched with 2.2g EPA/day for 5 days pre-operatively (orally) and 21 days post-operatively via feeding jejunostomy tube. Body composition was assessed by segmental bioelectrical impedance analysis pre-operatively and on post-operative day 21. Post-op complications, systemic inflammatory response syndrome (SIRS), maximum body temperature and length of hospital stay were recorded. Several markers of coagulation, inflammation and immune function were determined pre-operatively and on post-operative days 1, 3 7, 14 and 21. Gas chromatography was performed to examine changes in the EPA content of serum and the phospholipid membrane

of peripheral blood mononuclear cells (PBMCs) at baseline and on post operative days 7 and 14. Non-parametric statistical analysis was performed. The study was adequately powered to detect a 10% difference in lean body mass.

Results

Fifty Three patients completed the study, (28 EPA enriched group, 25 standard enteral nutrition group). At baseline there was no significant difference in any pre-operative clinical characteristic including age, treatment modality, morphology, stage of disease, co-morbid disease, or operation type or nutritional status in terms of weight loss, serum albumin, Nutritional Risk Index, or dietary intake between the two groups. The majority of patients tolerated the enteral feed with no difference in the incidence of gastrointestinal symptoms such as diarrhoea between groups. The enteral product enriched with EPA was successful in significantly raising the serum EPA levels, and the EPA content of cell membranes of PBMCs, as determined by gas chromatography, while no change occurred in the standard EN arm.

Post operatively there was no significant difference in the incidence of any complication, days ventilated, days in ICU or HDU, or overall length of hospital stay (27 days versus 26 days, p=0.776). There was no significant difference in the incidence of SIRS on days 1-7 post op, however the mean maximal body temperature was significantly higher in the first week in the Standard EN arm (p=0.001).

Patients who received the EPA enriched feed maintained all aspects of their body composition peri-operatively. In contrast Standard EN patients lost a significant amount of lean mass, fat free mass fell by 1.9 kg (std dev 3.7), p=0.03. They lost significant amount of muscle from the leg (0.3kg (std dev 0.6, p=0.05; arm (0.17 kg (std dev 0.3), p=0.01) and trunk (1.44 kg (std dev 2.7), p=0.03). Eight percent of EPA patients (n=2) lost a 'severe' amount of weight (>5%) in hospital versus 39% (n=10) in the Standard EN arm (p=0.03).

There was no significant difference in the mean Prothrombin time or D-Dimer levels between the groups peri-operatively. There was also no difference in C-Reactive Protein, Serum Amyloid A, Erythrocyte Sedimentation Rate. Both groups showed a similar

perioperative response in terms of Interleukin (IL)-2, IL-4 and IL-6. For IL-8, levels were significantly higher in the standard EN arm compared to the EPA arm on day 7 and 14. On repeated measures analysis (Friedman test) there was no significant change in IL-8 levels over time for the EPA arm but there was a significant change for the Standard arm (chi squared=10.9, p=0.05). For IL-10 there was a more prolonged elevation in levels in the first post operative week in the standard arm than in the EPA arm. For TNF-α there was no significant change for the EPA arm but in the Standard arm there was a significant increase in levels on days 1, 7, and 14. For MCP-1 there was no change over time for the EPA enriched arm but the Standard arm had a significant change over time on repeated measures analysis (chi squared 13.2, p=0.022).

On lymphocyte subset analysis EPA fed patients had a better recovery of T-cell counts on post operative day 7 compared to Standard EN. CD4 cells fell in both groups post operatively but recovered by day 7. For CD8 Standard feed patients exhibited a greater decline in CD8 cytotoxic T cells in 1st post operative week compared to EPA fed patients where levels did not change. Thus EPA patients showed a significant increase in the CD4 to CD8 ratio in the first post operative week with no change in the standard EN arm. Natural Killer Cells increased significantly on day 1 in EPA patients whereas levels fell post operatively in standard arm. B cells increased significantly over time in both groups.

Conclusions

Enteral nutrition enriched with 2.2 g EPA/day for 5 days pre-op and 21 days post-oesophagectomy is associated with preservation of lean body mass, lower body temperature, improved immune function and an attenuated pro-inflammatory response to surgery compared with standard EN. The anabolic properties of EPA may have practical implications for patients not only with this cancer, but with other solid tumours such as lung and head/neck cancer. The anabolic properties of EPA may have practical implications for patients not only with this cancer, but with the increasing number of solid tumours where multimodality therapy may supplant surgery alone, including lung, head and neck, and rectal cancer.

7.2 INTRODUCTION

Oesophageal resection for cancer is associated with a significant risk of morbidity and mortality, approximately 50 and 10 per cent respectively in most series (Bailey et al, 2003). The complex of surgery involving both abdominal and thoracic dissection induces profound perturbations in the endocrine, neuroendocrine and immunological system, as well as significant changes in organ function (Senkal et al, 1999). The stress of surgery may be compounded by the increasing use of preoperative (neoadjuvant) therapy, either chemotherapy alone or combined with radiation therapy. Although the results of the neoadjuvant approach are encouraging, there is a concern that chemo-radiotherapy may increase operative risks further, particularity the risks of infectious complications (Bosset et al, 1997). In addition the prolonged treatment pathway has effects on nutritional status and quality of life. Endocrine, physiological and immune cell response to surgery, contribute to post-operative catabolism, and marked weight loss is normally observed.

There appears to be an emerging consensus that early postoperative nutritional support benefits the high-risk patient by decreasing septic morbidity, maintaining immunocompetence and improving wound healing (Baigrie et al, 1996). An increasing body of literature indicates functional advantages of early post operative enteral feeding in ameliorating stress response and in diminishing major postoperative infections (Myers, 1995; Beier – Holgerson & Boesby, 1996). Early postoperative enteral feeding after upper gastrointestinal cancer surgery has also been shown to impact positively on whole body protein metabolism (Hochwald et al, 1997) and has also been associated with a trend towards shorter length of stay (Biffi et al 2000).

Oesophagectomy lends itself well to studies on nutrition support as it represents an ideal model of a major homogenous insult with predictable alterations of immune cell function and metabolism, and a high risk of septic complications, weight loss and compromised quality of life. An improvement in nutrition support and immune function in the perioperative period could bring meaningful clinical benefits, and not surprisingly a new range of products, so called immunonutrition or nutrient immunomodulation is targeted on this premise. 'Immunonutrition' has been defined as 'modulation of the activities of the

immune system, and the consequences on the patient of immune activation, by nutrients fed in amounts above those normally encountered in the diet' (Grimble, 2001). Numerous clinical studies have been published examining the effects of immunonutrition using a variety of formulations and doses of immunonutrients as well as different types of operations/trauma. Several different commercial formulas are now available with varying concentrations of individual immunonutrients. The majority of trials on immunonutrition have used enteral formulations containing arginine, glutamine, omega-3 fatty acids, nucleotides (RNA) and branched chain amino acids (Xu et al, 2006; Senkal et al, 2005; Gianotti et al, 2002; Braga et a;, 2007; Beale et al, 1999)

Eicosapentaenoic acid (EPA), a long chain polyunsaturated fatty acid (PUFA) of the omega-3 (n-3) family, is of interest in the context of major cancer surgery as it has potential to impact on both the underlying metabolic abnormalities of tumour-induced weight loss, as well as modulation of immune function. When EPA is consumed at levels above that normally found in the diet, it replaces arachidonic acid (AA), an n-6 PUFA, in cell membrane phospholipids (Palombo et al, 1993; Morlion et al, 1996). It then acts as a substrate for the production of the 3 series prostaglandins and the 5 series leukotrienes that differ strikingly in potency from their respective 2- and 4- analogs normally synthesized from AA. Thus eicosanoids synthesized from the n-3 PUFAs (i.e. EPA) rather than the n-6 PUFAs (i.e. AA) have lower potential for promoting inflammation (Kudsk, 2006). Modulation of dietary fatty acids can therefore have an impact on many immune processes such as proliferation, phagocytosis, cytotoxicity and cytokine production (Fritshce, 2006). Increased intake of EPA also has a modulatory effect on the prevention and treatment of tumour related weight loss and cachexia. Through modification of eicosanoid production and subsequent reduction in proinflammatory cytokines, attenuation of tumour related weight loss occurs (Barber et al, 2001). Although several studies have demonstrated the beneficial effects of immunonutrition on immune competence and patient outcome, controlled clinical trials focusing on the use of peri-operative enteral omega-3 fatty acids alone are scarce. To date there is only one enteral EPA study in oesophageal surgery published with promising results (Aiko et al, 2005).

The immunomodulatory and anabolic properties of EPA merit evaluation in the setting of major upper GI cancer surgery. Moreover, the prolonged treatment has significant global and specific quality of life implications for the patient, and the anabolic properties of EPA may have practical implications for patients not only with this cancer, but with the increasing number of solid tumours where multimodality therapy may supplant surgery alone, including lung, head and neck, and rectal cancer.

7.3 PATIENTS AND METHODS

This study had ethical approval from the St. James's Hospital Ethics board and the Irish Medicines Board. All adult patients presenting to the Oesophageal Unit at St. James's Hospital from July 2005 to July 2007 with resectable oesophageal cancer were eligible for inclusion. The exclusion criteria included the following: patients with metastatic disease, non-operable cases, patients requiring chemotherapy/radiotherapy early following surgery, patients with known immunological disorder; emergency oesophagectomy cases; patients with cardiac, liver or renal failure; active small intestinal disease (e.g. Crohns disease); allergy to any of the ingredients; uncontrollable diabetes; use of medications known to affect eicosanoid metabolism in two weeks prior to trial; use of fish oil/n-3 fatty acids supplements; drug abuse; inadequate preoperative preparation; or pregnant women (see Appendices for consent forms, patient information leaflet and case report forms).

7.3.1 Work up and Staging

All patients had a clinical examination, oesophagoscopy, and computerized tomography of the neck, thorax and abdomen Cunningham et al, 2005). Endoscopic ultrasound (EUS) was not routinely utilised as access to EUS is limited at this centre. ^{18}-F-deoxyglucose PET scans was routine in all patients (Ott et al, 2006). Using CT-criteria, the mediastinal and left gastric nodes were classified as N1 (invaded) if the maximal transverse diameter of these nodes were larger than 1 cm. Resectable disease was defined as T_{1-3}, N_{0-1} (Cunningham et al, 2005). All tumours at the oesophago-gastric junction were assigned as Type I, II or III, as per Siewert & Stein (1998): Type I was adenocarcinoma of the distal oesophagus, usually arising in specialised intestinal metaplasia; Type II is a true adenocarcinoma of the cardia

arising immediately at the oesophago-gastric junction; and Type III is a subcardial gastric carcinoma infiltrating the oesophago-gastric junction and distal oesophagus from below.

All patients with localized disease (T_{2-3},N_{0-1}; predicted R0 resection) of the oesophagus or junction (Type I and II) were offered the option of either surgery alone or the multimodal regimen, patients with Type III OG junction tumours had surgery alone. Patients with more locally advanced disease were treated with radical radiation therapy and chemotherapy and excluded from this study.

7.3.2 Neoadjuvant Therapy

The majority of patients in the neoadjuvant treatment arm were given a standard protocol of chemoradiotherapy consisting of 40 Gy/15 fractions on days 1 to 5, 8-12 and 15-19, and concurrent chemotherapy of 5-Fluorouracil (15mg/kg) on days 1-5 and Cisplatin (75mg/m2) on day 7. Chemotherapy was repeated on week 6. Patients were restaged by CT and oesophagoscopy at week 8 and scheduled for surgery on week 9. Surgery took place if the neutrophil count was > $2x10^6/ml^{-1}$, if physical status and wellbeing had not significantly deteriorated, (as assessed by the consultant Surgeon) and if there was no evidence of local or systemic progression of disease on imaging.

7.3.3 Surgical Procedure

The vast majority of patients had a thoracotomy as a component of their surgical management, either combined with an abdominal and neck exploration (3-stage) for mid and upper-oesophageal cancers, or cancer arising in long-segment Barrett's oesophagus, or with an abdominal exploration (2-stage) for most lower third and junctional tumours, or combined with a total gastrectomy for junctional tumours with significant gastric extension (Type III) (Cunningham et al, 2005). A 2-field lymphadenectomy (abdominal and thoracic) was performed in all cases. All patients were extubated immediately following surgery and managed in a high dependency unit (HDU). All patients with a gastric remnant had a pyloroplasty, and patients were fed enterally from 12 hours postoperatively via a needle catheter jejunostomy. This was inserted at conclusion of laparotomy by standard method where a 8 Ch feeding catheter (Argyle, Sherwood Medical, Tullamore, Ireland) is inserted

through a cannula percutaneously in the left upper quadrant and inserted into the jejunum about 15 to 20cms from DJ flexure through a purse string suture. This spot was subsequently buried with seromuscular sutures continued proximally to create a 5 cm long subserosal tunnel. The exit point of the catheter was then sutured onto the pareites to protect against leakage (Sarr et al, 1988; Ryan et al, 2006). Feeding was commenced at 0800 am on the first postoperative day. The jejunostomy tube was also used routinely to deliver additional water and electrolytes (Baxter Healthcare, Northampton, UK) to meet daily requirements or replace gastrointestinal losses such as nasogastric secretions. Medications absorbed in the small intestine were also given through the jejunostomy where possible. A gastrograffin contrast study was routinely performed on postoperative day 7 or 8 before initiating oral fluids (Timaksiz et al, 2005).

7.3.4 Enteral Treatment Procedure

After fulfilling the inclusion criteria patients were randomized (both surgery only groups and patients post neoadjuvant chemoradiation) into two groups, one to receive 5 days of preoperative supplementation of an EPA enriched (2.2g EPA/day) supplement (treatment arm) and the other to receive an iso-caloric iso-nitrogenous standard nutritional supplement (control arm) without EPA (*See table 7.1 for enteral feeding protocol*). There was no significant difference in the micronutrient intakes between the 2 enteral feeds. Post operatively the enteral products were started using continuous infusion via an intra-operatively placed needle catheter jejunostomy and continued for 21 days. Both patients and investigators were blinded to the enteral nutrition administered, see Figure 7.1 for image of the enteral products used.

Enteral nutrition was started on the first post operative day at 30 mls/hour for 8 hours, 50 mls/hour for 8 hours and then 80 mls/hour for 4 hours, after a four hour feeding break the rate was increased to 100mls/hour on post operative day 2 infused over 20 hours will a four hour feeding break daily. This provided 2 litres of enteral product per day of which 500 mls was either the EPA enriched product or the standard enteral nutrition. The composition of the enteral feeds is given in *table* 7.2. Enteral feeding was the sole source of nutrition provided for 10 days until oral intake was clinically indicated, at which time the enteral feeding was reduced to 1000mls overnight of which 500mls was either the EPA enriched

feed or the standard comparator product. At day 14 post op, night-time jejunostomy feeding ceased and patients were required to take the study product or comparator orally for a period of 7 days until post operative day 21 at which point nutritional assessment was re-examined. For patients who were unable to take the study product orally it was administered via an enteral feeding pump at night-time to supplement intake.

Table 7.1: Schedule of Peri-operative Enteral Feeding

Pre op day 5 to day –1	2 x 200 ml orally as sip feed
POD 0	0
POD 1	1000ml Enteral Nutrition (500ml of which study product)
POD 2-10	2000ml Enteral Nutrition (500ml of which study product)
POD 11-14	1000ml Enteral Nutrition (500ml of which study product)
POD 15-21	2 x 240 ml orally as sip feed

Figure 7.1: Image of blinded study product

Table 7.2: Composition of the Diets per 100ml

Component		EPA enriched feed	Standard Feed
Energy (kJ)		526	526
(Kcal)		125	150
Protein (g)		6.65	6.3
Fat (g)		2.56	4.91
Linoleic Acid (g)		0.17	0.61
Linolenic Acid (g)		0.04	0.15
EPA (g)		0.45	0
DHA (g)		0.19	0
n-6:n-3 Ratio		1:4	4:1
Carbohydrate (g)		19.4	20
Water (g)		79.4	77
Vitamins & Minerals			
Vitamin A	mcg RE	205	160
Vitamin D	mcg	1.7	1.0
Vitamin E	mcg TE	20	3.02
Vitamin K	mcg	10	7.6
Vitamin C	mg	43	15
Folic Acid	mcg	169	40
Vitamin B1	mg	0.25	0.27
Vitamin B2	mg	0.29	0.31
Vitamin B6	mg	0.34	0.4
Vitamin B12	mcg	0.5	0.57
Niacin	mg NE	2.5	2.9
Sodium	mg	150	140
Potassium	mg	200	165
Iron	mg	1.7	2.2
Osmolarity (mOmol/l)		474	517

DHA=Docosahexaenoic acid, EPA= Eicosapentaenoic Acid

7.3.5 Assessment of Nutritional Status

All patients had a full nutritional assessment by a dietitian at baseline once informed consent was obtained. Weight was measured using a digital scales to within 0.1kg, without heavy outdoor clothing or shoes. Height was measured barefoot using a portable stadiometer (Seca) to within 0.5cm. Usual pre-illness weight at least one year prior to diagnosis was recorded and the degree of weight loss was calculated with the severity of that weight loss classified according to the Blackburn criteria for weight loss (1977). Weight loss was 'clinically significant' if it was 5% in one month, 7.5% in 3 months or 10% in six months or 'clinically severe' if greater than 5% at one month, > 7.5% in 3 months or >10% in six months. Body Mass Index (BMI) was computed as weight in kilograms divided by height in

meters squared (kg/m^2). BMI was defined using the World Health Organisation definitions, with a BMI of 20-25 kg/m^2 normal, overweight 25-29.9 kg/m^2, and obese >30 kg/m^2.

Segmental body composition was analysed using the Tanita BC 418 MA bioelectrical impedance analyzer (Tanita UK Ltd, Middlesex, UK) which gives precise information on the amount of lean and fat tissue in the trunk area and in each limb, as well as overall body composition and hydration status (see *Figure 7.2*). Patients were asked to stand bare-foot on the two silver foot pads and to make a fist around the arm pads while relaxing their arms by their sides. Measurements were taken in the morning after a light breakfast and were considered accurate if hydration status was between 40-50% for females and between 50-60% for males.

Figure7.2:
Bioelectrical impedance analyzer

A full diet history, documenting the degree of dysphagia (if any) was performed by a dietitian. From this the energy intake and protein intake was calculated. Individual energy requirements were calculated using the Schofield equation (Schofield, 1985) with adjustments for stress and activity level (Elia, 1990; see appendix for Nomogram). Protein requirements were estimated using the Elia table for nitrogen requirements (Elia, 1990). Intake was compared to requirements to determine if intake was adequate or not. Additionally, nutritional status was classified by the Nutritional Risk Index (NRI) (Veterans Affairs total Parenteral nutrition group, 1991). NRI was calculated by the formula: NRI=1.519 x serum albumin (g/l) + 0.417 x (current weight/usual weight) x 100. If the result was > 100 the patient was not malnourished; 97.5-100 indicated mild malnutrition; 83.5-97.5 indicated moderate malnutrition and results <83.5 were severe malnutrition.

7.3.6 Collection of Clinical Data and Postoperative Complications

The medical, dietetic and histopathology records of the cancer cases were recorded on a computerised upper Gastrointestinal Cancer Database (Patient Analysis and Tracking System ™ (PATS) Dendrite Clinical Systems, UK). Data recorded concerned age, sex,

tumour site, clinical and pathological staging, smoking and alcohol intakes, co-morbid disease, socio-economic status, reflux symptoms, medications, and the presence or absence of Barrett's oesophagus. Performance status was measured using the Eastern Co-operative Oncology Group (ECOG) grades of performance (Oken et al, 1982) where 0=fully active and able for all pre disease performance, 1= restricted I physically strenuous activities but ambulatory and able to carry out light work of a sedentary nature, 2= ambumatory and capable of self care but unable to carry out any work activities, 3- capable of only limited self-care, confined to bed or chair for >50% of waking hours, 4=completely disabled, cannot self care, bed bound. In addition the American Society of Anaesthesiologists (ASA) physical status classification was documented where ASA I=normal healthy patient, ASA II=mild systemic disease with no functional limitation, ASA III=moderate systemic disease with finctional limitations, ASA IV=severe systemic disease that is a constant threat to life, ASA V=moribund patient with life expectancy <24 hours without surgery (Lee et al, 1998). All complications from surgery to discharge from hospital were prospectively documented and recorded in the PATS system. Major post-operative complications, including, Adult Respiratory Distress Syndrome (ARDS), sepsis, organ failure: renal failure, heart failure, respiratory failure, pneumonia/respiratory tract infection, empyema, wound infection, anastomotic leak, and in-hospital mortality were documented. Respiratory failure was defined as the requirement for mechanical ventilation beyond 24 hours after surgery. ARDS and multiple organ failure (MOF) were defined as per Bone et al (1992), sepsis required evidence of Systemic Inflammatory Response Syndrome (SIRS) with microbiological evidence of infection, and the diagnosis of pneumonia required either positive sputum cultures or clear clinical and radiographic evidence of consolidation. SIRS was diagnosed by clinical manifestation of two or more of the following conditions: Temperature > 38°C or <36°C; Heart rate >90 beats per minute; Respiratory rate >20 breaths per minute or PaCO2 <32 mmHg; White blood cell count >12,000mm^3, <4,000mm^3 or >10% immature (band) forms (Bone et al, 1992).

7.3.7 Study End-points

The primary end points were clinical: to examine changes in nutritional status (specifically body composition), examine the incidence of post-operative complications, Systemic Inflammatory Response Syndrome, and length of hospital stay. The secondary endpoints

were laboratory markers such as inflammatory markers and cytokine production, immune cell counts, and finally gas chromatography to provide proof of concept.

7.4 LABORATORY MATERIALS AND METHODS

7.4.1 Routine Bloods

At baseline (recruitment) and on post operative days 1, 3, 7, 14 and 21 bloods were taken for the following: C-reactive protein, Serum Amyloid A, Erythrocyte Sedimentation rate, and coagulation including Prothrombin time, D-Dimers and platelet counts (Duly et al, 2003; Gurleyik et al, 1995). At these same time points serum was also frozen at -80 degrees for later analysis of cytokines and growth factors (Fitzgerald et al, 2005), as well as quantification of serum levels of EPA. On days pre op minus 5, and days 1, 3 and 7 post operatively, blood was taken for Lymphocyte Subset analysis by Flow Cytometry for T, B and Natural Killer cell quantification (Kalwak et al, 2003). Fresh samples of blood were also taken at day 7 in a subset of patients for analysis of membrane lipid content by Gas Chromotography.

7.4.2 Lymphocyte Subset quantification by Flow Cytometry

Fresh blood samples were obtained in an EDTA tube pre operatively and on post operative days 1, 3 and 7. Becton Dickson (BD) True Count tubes (cat. No.340334) were then labelled either with CD3 FITC/CD8 PE/CD45PerCP/CD4APC (cat. No.342447), or CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 APC (cat. No.342446) according to the table below (table 7.3). To the appropriate tube, 20ul of BD Multiset antibody was then added. To each tube, 50ul of either CD Chex control (CD Chex Plus (cat. No.340768-8) or CD Chex low (cat. No.340786-8)) or whole blood was added. Each tube was then mixed for 30 seconds, and allowed to incubate for 15 minutes in the dark. After incubation, 450ul of BD FACS Lysing Solution (cat. No.349202) was then added to each tube. All tubes were again mixed for 30 seconds and allowed to incubate at room temperature for fifteen minutes in the dark. After incubation, 50ul of the BD trucount control (cat. No.340335) was then added to

the control tube. All tubes were again mixed and each sample was then acquired and analysed using BD FACScanto software (version 2.1) (Owens et al, 2000)

Table 7.3: Four Colour Staining for lymphocyte subset analysis4 Colour Staining for lymphocyte subset analysis

Tube No.	Test	FITC	PE	PERCP	ABC
1	**CD Chex control Helper T Cell/ Cytotoxic T Cell**	CD3	CD8	CD45	CD4
2	**CD Chex control B cells**	CD3	CD16+CD56	CD45	CD19
3	**Trucount control**	CD3	CD8	CD45	CD4
4	**Sample Helper T Cell/Cytotoxic T Cell**	CD3	CD8	CD45	CD4
5	**Sample B cells**	CD3	CD16+CD56	CD45	CD19

7.4.4 Cytokine and Growth Factor Array

The Randox Evidence Investigator™ Cytokine and Growth Factors Array (see appendix) was used for the *in vitro* simultaneous quantitative detection of multiple related cytokine immunoassays (in parallel) from a single sample (Fitzgerald et al, 2005). The core technology is the Randox Biochip, a solid state device containing an array of discrete test regions of immobilized antibodies specific to different cytokines and growth factors. A sandwich chemiluminescent immunoassay is employed for the cytokine array. Increased levels of cytokine in a sample lead to increased binding of antibody labelled with horseradish peroxidase and thus an increase in chemiluminescence signal emitted. The light signal generated from each of the test regions on the biochip is detected using digital imaging technology and compared to that from a stored calibration curve. The concentration of analyte present in the sample is calculated from the calibration curve. The Evidence Investigator™ Cytokine array quantitatively tests for IL-2, IL-4, IL-6, IL-8, IL-10, MCP-1 and TNFα simultaneously.

7.4.5 Gas Chromatography Methodology

PBMC separation

Four 4ml lithium heparin (Vacuette Cat. No. 454084) samples of whole blood were collected. The contents of each tube, was then transferred to a 20ml sterilin (Sterilin Cat. No. 03001). 3ml of Hank's Balanced Salt Solution (HBSS) (Gibco Cat. No. 14170-088) was then added to each tube, which was then mixed on the vortex mixer and the washings were then added to the 20ml sterilin. The contents of each sterilin, was then mixed on the vortex mixer. The contents of each sterilin were then layered carefully onto 5ml of Lymphoprep (Axis Shield Cat. No. LYS 3773) in a new sterilin. Each sterilin was then centrifuged at 290G for 30 minutes at 4°C with no brake. The buffy coat layer at the interface between the lymphoprep and the plasma and medium layers was then collected using a plastic Pasteur pipette and transferred to a new 20ml sterilin. The sterilin was then filled with HBSS and mixed on the vortex mixer. Each sterilin was then centrifuged at 520G for 10 minutes. The supernatant was then poured off, and the pellet was re-suspended using the vortex mixer. 10ml of HBSS was then added to each sterilin, which was then mixed on the vortex mixer. Each sterilin was then centrifuged at 520G for 10 minutes. The supernatant was then poured off, and the pellet was re-suspended using the vortex mixer. 1ml of HBSS was then added to each sterilin, which was then mixed on the vortex mixer. A cell count was then carried out using ethidium bromide-acridine orange (EBAO) (4ml of 4mg/ml ethidium bromide stock was mixed with 10ml of 1% acridine orange stock and 1 litre of saline) and a Neubauer haemocytometer (for peripheral blood mononuclear cells (PBMC) only).

Monocyte separation

A hyper-osmotic Percol solution was prepared, by adding 48.5ml of Percoll (Sigma Cat. No. P1644) to 41.5ml of deionised water. A 1.6M solution of NaCl was then prepared. 10ml of the 1.6M NaCl solution was then added to the Percoll solution. The solution was then mixed vigorously on the vortex mixer. 3ml of PBMC cell suspension (adjusted to 20 x 10^6/ml) was carefully layered onto 10ml of the hyper-osmotic Percoll solution in a new sterilin. Each sterilin was then centrifuged at 580g for 15 minutes with the brake off. The buffy coat layer at the interface between the medium and Percoll layers was then collected using a plastic Pasteur pipette and transferred to a new 20ml sterilin. Each sterilin was then filled with

HBSS and then mixed on the vortex mixer. Each sterilin was then centrifuged at 350g for 7 minutes. The supernatant was then poured off, and the pellet was re-suspended using the vortex mixer. 10ml of HBSS was then added to each sterilin, which was then mixed on the vortex mixer. Each sterilin was then centrifuged at 350g for 7 minutes. The supernatant was then poured off, and the pellet was re-suspended using the vortex mixer. 1ml of HBSS was then added to each sterilin, which was then mixed on the vortex mixer. A cell count was then carried out using EBAO and a Neubauer haemocytometer. Cell numbers were adjusted to between 8-10 x 10^5/ml (Repnik et al, 2003).

Lipid Extraction (Bligh & Dyer method)

Serum samples were allowed to thaw over night at 4°C. 400ul of sample (serum or cell suspension) was then pipetted into a 16ml borosilicate glass (Lennox Cat. No. 400719), screw capped tube. 400ul of milliQ water was then added to each tube. 2ml of methanol (Sigma-Aldrich Cat. No. 34860), 1ml of chloroform (Sigma-Aldrich Cat. No. 52873-0), 40ul 5mM Butylated Hydroxyl Toluene in ethanol (Previously prepared in lab), and 100ul of heptadecanoic acid (2mg/ml) control were then added to each tube. Each tube was then mixed vigorously for 1 minute on the vortex mixer. 1 ml of milliQ water and 1ml of chloroform were then added to each tube. The tubes were then centrifuged at 3000rpm for 15 minutes at room temperature. The infranatants (organic phase) were then transferred to new 16ml borosilicate glass, screw capped tubes, using a glass Pasteur pipette. The supernatants (aqueous phase) were supplemented with 2ml of chloroform. The tubes were then mixed for 30 seconds on the vortex mixer and then centrifuged at 3000rpm for 15 minutes at room temperature. The infranatants (organic phase) were then also transferred to the corresponding 16ml borosilicate glass, screw capped tubes i.e the two extractions were pooled. The organic phase was then dried under nitrogen in a Meyer evaporator.

Transesterification (Ohta method)

To each tube of dried lipid extract, 1ml of methanol and 1ml of Boron Trifluoro Methanol 14% (Sigma-Aldrich Cat. No. B1252) were added. Each tube was then mixed for 30 seconds on the vortex mixer. Each tube is then placed in a water bath (100°C) for 40 minutes. During incubation, the tubes were repeatedly mixed to ensure complete dissolution. The caps were also checked to ensure there were no leaks. The tubes were then allowed to cool down to

room temperature. The resulting methyl esters were then extracted by adding 2ml of hexane (Sigma-Aldrich Cat. No.13938-6) and 2ml of milliQ water to each tube. Each tube was then mixed for 10 minutes on the vortex mixer. The tubes were then centrifuged at 2000rpm for 5 minutes at room temperature. The supernatants (hexane phase) were then transferred to new 16ml borosilicate glass, screw capped tubes. To optimize the methyl ester extraction, a further 2ml of hexane was added to each original tube. This tube was then mixed for 10 minutes on the vortex mixer. The tubes were then again centrifuged at 2000rpm for 5 minutes at room temperature. The supernatants (hexane phase) were then also transferred to the corresponding 16ml borosilicate glass, screw capped tubes. The methyl esters were then dried under nitrogen in a Meyer evaporator.

Preparation for GC analysis

A glass screw cap vial was then labelled. An 8mm silicon septum (AGB Cat. No. 08020563) was then inserted to each cap. A small glass insert was then inserted in to each screw cap vial. The PUFA 2 (Supelco Cat. No. 47015-U) and PUFA 3 (Supelco Cat. No. 47085-U) standards were prepared according to the manufacturers instructions. To each dried methyl ester, 200ul of hexane was added. The tubes were then mixed vigorously on the vortex mixer to ensure complete dissolution. The contents were then transferred to each corresponding glass vial/insert which was then capped. Each vial was then placed in the GC carousel rack and analysed on the GC (Shimadzu GC2010) using GC Solution software (version 2.21). (The column used was Omegawax^{TM} 250 (5^{o} to 280 ^{o}C) 30m X 0.25 nm ID, 0.25µm film (Cat No. 24136 Supelco). The carrier gas used was Helium, samples were heated to an oven temperature of 250^{o}C for 53 minutes per sample.

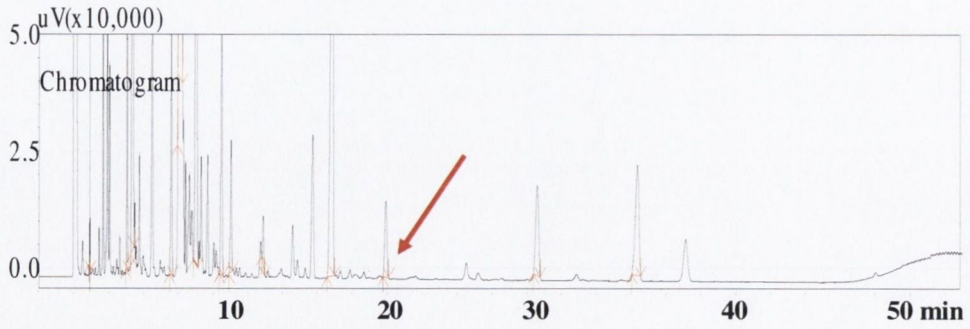


Figure 7.3a: PUFA 2 standard showing serum EPA at the 20 minute retention time (red arrow)

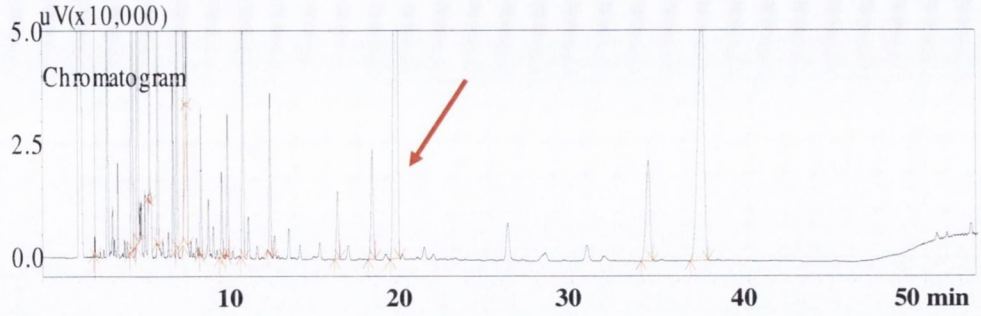


Figure 7.3b: PUFA 3 standard showing serum EPA at the 20 minute retention time (red arrow)

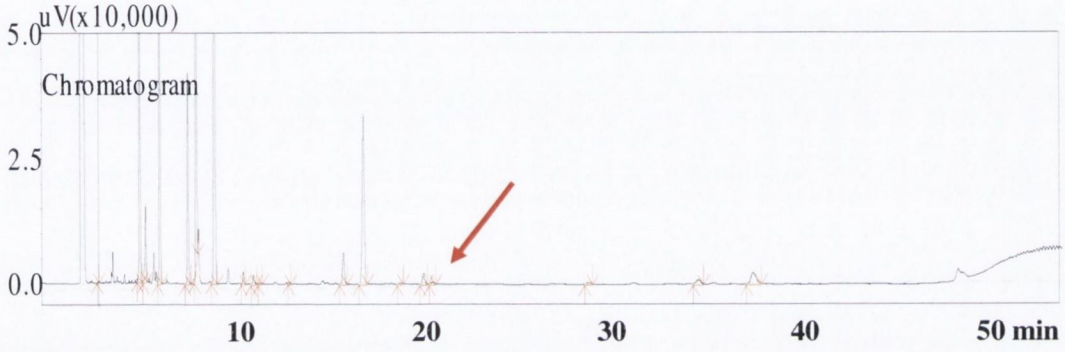


Figure 7.3c: Pronase treated patient at baseline showing low levels of serum EPA at the 20 minute retention time (red arrow)

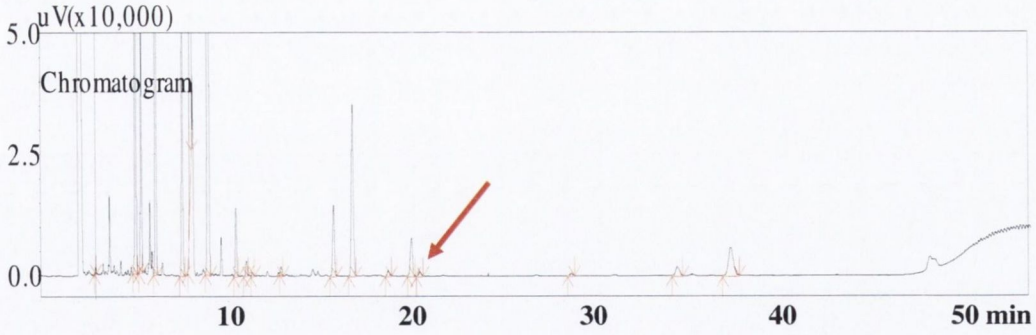


Figure 7.3d: Pronase treated patient at day 7 showing increased levels of serum EPA at the 20 minute retention time (red arrow)

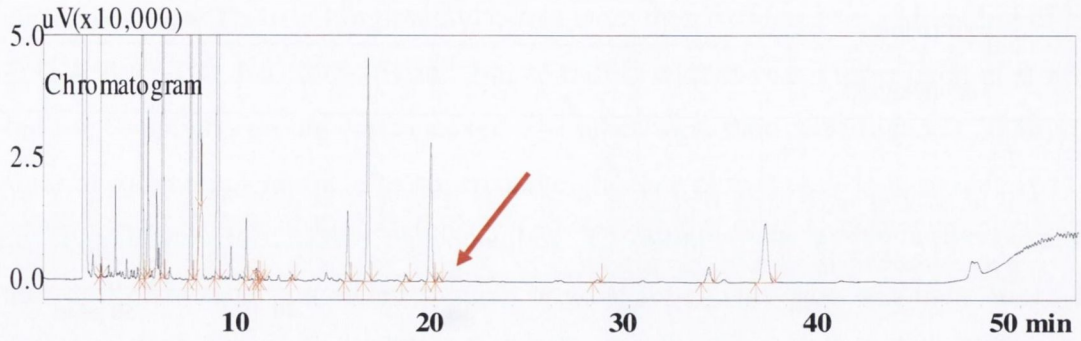


Figure 7.3e: Pronase treated patient at day 14 showing increased levels of EPA at the 20 minute retention time (red arrow)

7.5 STATISTICAL ANALYSIS

The randomisation procedure was performed by an independent statistician and the code was not unlocked until all analysis had been performed (randomisation procedure is included in Appendix). Statistical analysis was performed by SPSS (version 14) for Windows. For continuous normally distributed data Student's t-test either as independent or paired t-tests was performed and one way analysis of variance (ANOVA). Chi Squared test or Fishers exact tests were used to compare categorical variables. Mann Whitney U tests and Wilcoxon signed rank tests were used for data that was not normally distributed. For parametric continuous variables multiple analysis of variance using repeated measures ANOVA was used to compare the values pre operatively with the ones measured at several subsequent time points after surgery. Friedman Test (the non-parametric alternative to repeated measures ANOVA) was used to examine changes over time for data that was not normally distributed. "Mixed Between-within ANOVA" was used to examine the effects of the 2 enteral treatments on an independant variable over time and to see if the effect was related to the treatment administered (Pallant 2007). The most commonly reported statistic in this form of analysis is the Wilk's Lamda level with values <0.05 significcant. The Partial Eta Squared Values indicates the strength of the association – a value of 0.01 indicated a small effect, 0.06 a moderate effet and 0.14 a large effect (Cohen, 1988)

All results are presented as means (± Standard deviations) with 95% confidence intervals for normally distributed data or medians with 95% confidence intervals for non-parametric data. All statistical tests were two sided and two tailed, and a P value of <0.05 was taken to be significant.

7.6 RESULTS

In total 70 patients were recruited, gave signed consent and were randomised to either Standard EN or the EPA enriched treatment arm. However, 17 of these patients were withdrawn from the study by the lead investigator - 7 patients did not complete the full 5 day pre-operative feeding protocol due to unforeseen rescheduling of their operation dates, 1 lady was inoperable, 5 did not receive a feeding jejunostomy as they required a subtotal oesophago-gastrectomy, and 4 patients developed chylothorax within 3 days of surgery and required cessation of all enteral feeding and >10 days of Parenteral Feeding. Fifty three patients (28 EPA and 25 Standard enteral feed) were fully compliant with the pre and post operative feeding protocol and were therefore included in the final analysis.

7.6.1 Clinical Characteristics of Patients Pre operatively

Table 7.4 describes the clinical characteristics of patients pre operatively. There was a significant difference in the male to female ratio between the two enteral treatment groups (ratio 24:4 for EPA and 14:11 for Standard EN, p=0.036). There was no significant difference in the mean age (62 years EPA versus 65.7 years for Standard Feed, p=0.249, 95% CI[-9.1, 2.4], incidence of co-morbid disease, smoking status, alcohol intake per week, American Society of Anaesthesiologists (ASA) grade or Eastern Co-operative Oncology Group (ECOG) performance status (Oken et al, 1982). Fifty seven percent (n=16) of EPA patients had multimodal therapy versus 44% (n=11) of Standard EN (p=0.496). There was also no significant difference in the operation type, morphology, or TNM Clinical staging between the groups.

7.6.2 Pre operative nutritional status

The two enteral treatment groups were very similar at baseline nutritional assessment. There was no significant difference in the mean weight (73.6 kg for EPA arm and 77.2 for standard arm (p=0.38). There was no significant difference in the pre illness weight, the weight loss reported in kilograms or in the percentage weight loss (see table 7.5). There was a high prevalence of malnutrition at baseline with 18% of EPA enriched arm and 19% of the standard arm had >10% weight loss (p=1.000). Although there were no significant

difference between the two groups - 55% of EPA patients and 64% of standard EN patients had mild-moderate malnutrition on Nutritional Risk Index and 46% of EPA and 37% of standard EN patients had mild-severe malnutrition on Subjective Global Assessment. Seventy percent of EPA patients and 57% of Standard EN patients had to modify the consistency of their diet at baseline (p=0.194). Dietary intake of Energy was inadequate in 43% of EPA patients and 54% of Standard EN patients at baseline (p=0.443).

7.6.2.1 Tolerance of Enteral Feeding Regimen

All patients tolerated the enteral feeding regimen well and all were tolerating 2000mls/day at 100mls/hour by the second post operative day. There was no difference in the daily energy or protein intake between the two groups. Minor Gastrointestinal complaints such as constipation was reported in 1(4%) of EPA group and 4(16%) of Standard EN group (p=0.191) and 6(23%) of EPA group and 6(24%) of Standard group reported diarrhoea <3 times/day (p=0.938) which was often related to antibiotic use. The jejunostomy tube became occluded in 4(15%) of EPA patients and 1(4%) of Standard EN patients (p=0.350).

Table 7.4: Clinical Characteristics of patients pre operatively

		EPA enriched (n=28)	Standard feed (n=25)	P
Male:Female		24:4	14:11	0.036
Age (years)		62(11)	65.7(9)	0.249
Co-morbid Disease		14(56%)	15(60%)	1.000
Smoking:	Non smoker	7(27%)	7(28%)	
	Current smoker	10(39%)	8(32%)	0.880
	Ex smoker	9(35%)	10(40%)	
Alcohol:	Non Drinker	6(23%)	3(14%)	
	Social Drinker	14(54%)	15(68%)	0.574
	Heavy Drinker	6(23%)	4(18%)	
Treatment Pathway				
	Multimodal	16(57%)	11(44%)	
	Surgery only	12(43%)	14(56%)	0.496
ASA Grade				
	Healthy	7(32%)	2(11%)	
	Mild systemic disease	10(45%)	10(56%)	0.287
	Severe systemic disease	5(23%)	6(33%)	
Clinical Staging				
	T1	5(19%)	5(20%)	
	T2	3(8%)	2(8%)	0.986
	T3	20(74%)	18(72%)	
	N0	17(63%)	13(52%)	0.604
	N1	10(37%)	12(48%)	
	M0	27(100%)	25(100%)	1.000

Table 7.5: Nutritional status at baseline–values expressed as mean (std deviation)

	EPA enriched	Standard Feed	P
Weight (kg)	73.6(14)	77.2(13)	0.38
Pre illness weight (kg)	81(11)	82.6(15)	0.642
Wt loss (kg)	5.2(5.2)	5(5.7)	0.854
Wt loss (%)	6.6(6.6)	5.5(6)	0.554
> 10% weight loss (yes)	18%	19%	1.000
Severity of weight loss			
Non-significant	57%	67%	
Significant	19%	14%	0.816
Severe	24%	19%	
Albumin	38.7(3.5)	38.6(3)	0.939
Total Protein	69(5)	69.7(4.4)	0.558
Nutritional Risk Index			
Not malnourished	37%	36%	
Mild Malnutrition	13%	36%	0.138
Moderate Malnutrition	42%	28%	
Severe Malnutrition	8%	0%	
Subjective Global Assessment			
Well Nourished	54%	63%	
Mild Malnutrition	38%	33%	0.765
Severe Malnutrition	8%	4%	
Dietary Change			
No Change	30%	43%	
Soft foods only	40%	52%	
Semi-solids only	25%	5%	0.194
Liquids only	5%	0%	
Dietary Intake Adequate	57%	46%	0.443
Dietary Intake Inadequate	43%	54%	
% of energy requirements met	82(18)	83(17)	0.854
% of protein requirements met	81(15)	79(10)	0.893

7.6.3 Gas Chromotography and Cell membrane Fatty Acid Composition

Gas chromatography showed successful increase in the EPA levels in serum and in the cell membrane of peripheral blood mononuclear cells (PBMCs).

Table 7.6 shows the mean percentage of EPA in serum at baseline and on days 7 and 14 post operatively. In the EPA enriched arm the levels rose significantly from 1.2 % pre operatively to 3.2% on day 7 (p=0.04) and 2.8% (p=0.07) on day 14 post operatively. For the standard arm there was no significant change in EPA levels. Gas chromatography also illustrated the successful incorporation of EPA into the cell membrane of PBMCs in the EPA enriched arm with the percentage of EPA increasing from 0.3% at baseline to 1.7% on post operative day 7, in contrast the levels remained at 0.7% in the standard feed arm (p=0.005). Figures 7.4a and 7.4b illustrate the changes in the % EPA for both serum and PBMC membrane.

Table 7.6: Mean EPA % in Serum at baseline (pre supplementation) and on post operative days 7 and 14 in EPA Enriched patients Vs Standard Feed patients (n=20). Values are Mean (Standard Deviation)

	EPA Enriched	**Standard Feed**	**P**	**95% CI**
Serum % EPA				
Baseline (Day -5)	1.2 (1.0)	0.3(0.2)	0.10	-0.2, 1.98
Day 7	3.2(2.5)	0.3(0.05)	0.04	0.2, 5.5
Day 14	2.8(2.0)	0.3(0.07)	0.02	0.7, 4.3
PBMC membrane % EPA				
Baseline	0.3(0.3)	0.7(0.1)	0.174	-1, 0.3
Day 7	1.7(0.1)	0.7(0.1)	0.005	0.7, 1.2

Figure 7.4a: Percentage EPA in Serum on Gas Chromotography pre operatively (pre supplementation) and on post operative days 7 and 14. Error Bars: 95% Confidence intervals

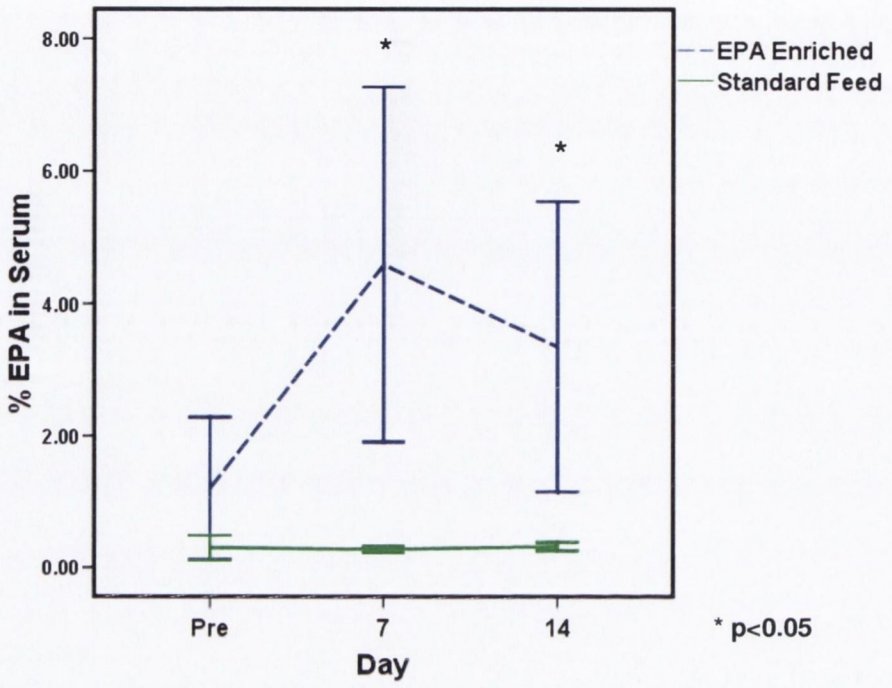
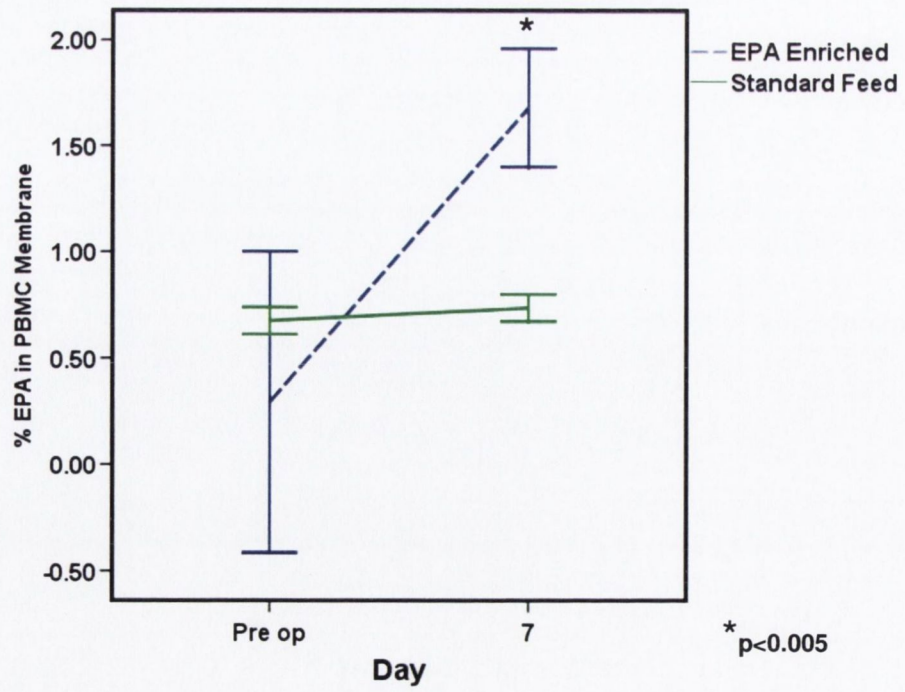


Figure 7.4b: Percentage EPA in membrane of PBMC on Gas Chromotography pre operatively (pre supplementation) and on post operative day. Error Bars: 95% Confidence intervals



7.6.3 Post operative complications

The most common post operative complication was pneumonia at 24%, sepsis at 14%, renal failure at 8%, and Anastomotic leak, Respiratory failure and wound infection all at 6%. The in-hospital mortality rate in this series was 0%. Table 7.7 describes the incidence of post operative complications per enteral feeding group. The overall morbidity rate (excluding pleural effusion and atelactasis) was 54% for the EPA arm and 40% for the standard enteral feeding arm (p=0.419). There was no statistically significant difference in any post operative complication between the two groups. The mean length of hospital stay was also not statistically different between the two enteral treatment arms (27.2 days for EPA arm and 26.2 days for the Standard enteral nutrition arm, p=0.776), nor was the duration of mechanical ventilation (0 days versus 3 days, p=0.211), days in the Intensive Care Unit (0.3 days versus 0.8 days, p=0.187), or days in High Dependency Unit (4.2 versus 3.7, p=0.390).

7.6.5 Systemic Inflammatory Response Syndrome (SIRS) and Maximum body temperature

The maximum body temperature was recorded on each patient from operative days 1-7 for both treatment arms. Figure 7.5 displays the results which found that the maximal body temperature was significantly greater in the Standard enteral treatment arm on post operative days 1, 3, 4, 5, 6 and 7 with a P value on independent T-Tests of <0.001. There was a trend towards a difference on post operative day 2 (p=0.093). Mixed between-within ANOVA was conducted to assess the impact of the 2 enteral products on body temperature. Both treatment arms had a significant change in body temperature over time: Wilk's Lamda=0.661, F (6. 27) =2.3, p=0.05, partial eta squared 0.339 indicating a large effect. The main effect comparing the 2 treatment arms was also significant suggesting that the differences were a result of the enteral treatment administered, Wilk's Lamda=0.576, F (6, 27) =3.3, p=0.01, partial eta squared=0.424 indicating a large effect.

Data on the incidence of SIRS was available for 34 patients. There was no significant difference in the percentage of patients with SIRS on days 1-7 post operatively between either treatment arm, or in the percentage of patients with SIRS for > 3 days or >5 days. (See Figure 7.6)

Table 7.7: Operative details and post operative complications per treatment approach.

(Values shown as mean (standard deviation) for continuous variables & P value on independent T-tests; for categorical data the number of cases (percentage) and P value on cross tabulation is given.)

	EPA enriched (n=28)	Standard Feed (n=25)	P
Length of operation (hours)	5.5(1.1)	5.1(1.5)	0.528
Operation Type			
2 stage oesophagectomy	16(57%)	18(72%)	
3 stage oesophagectomy	7(25%)	5(20%)	0.456
Transhiatial oesophagectomy	5(18%)	2(8%)	
Post op complication* (yes)	15(54%)	10(40%)	0.478
Sepsis	5(18%)	2(8%)	0.419
In hospital Mortality	0	0	1.000
Anastomotic Leak	1(4%)	2(8%)	0.610
Wound Infection	0(0%)	2(8%)	0.226
Empyema	1(4%)	0(0%)	1.000
Pneumonia	7(25%)	5(20%)	0.743
ARDS	1(4%)	0(0%)	1.000
Respiratory Failure	3(11%)	0(0%)	0.235
Heart Failure	1(4%)	0(0%)	1.000
Renal Failure	1(4%)	3(12%)	0.350
Vocal Cord Palsy	0(0%)	1(4%)	0.490
Re-ventilated (yes)	4(15%)	3(12%)	1.000
Blood Transfusion	6(23%)	6(24%)	0.938
SIRS > 3 days	3(18%)	7(41%)	0.259
SIRS > 5days	1(6%)	4(24%)	0.335
Return to HDU (yes)	4(15%)	3(12%)	1.000
Days in HDU	4.2(2)	3.7(2)	0.390
Length of Hospital stay (days)	27.2(15)	26.2(10)	0.776

*= post operative complication excluding pleural effusion & atelactasis
ARDS=Adult Respiratory Distress Syndrome, MRSA=Methacyllin resistant staphlococcus aureus, SIRS=Systemic Inflammatory Response Syndrome.

Figure 7.5: Mean maximum body temperature on post operative days 1-7 for EPA enriched versus Standard Enteral Feeding group.

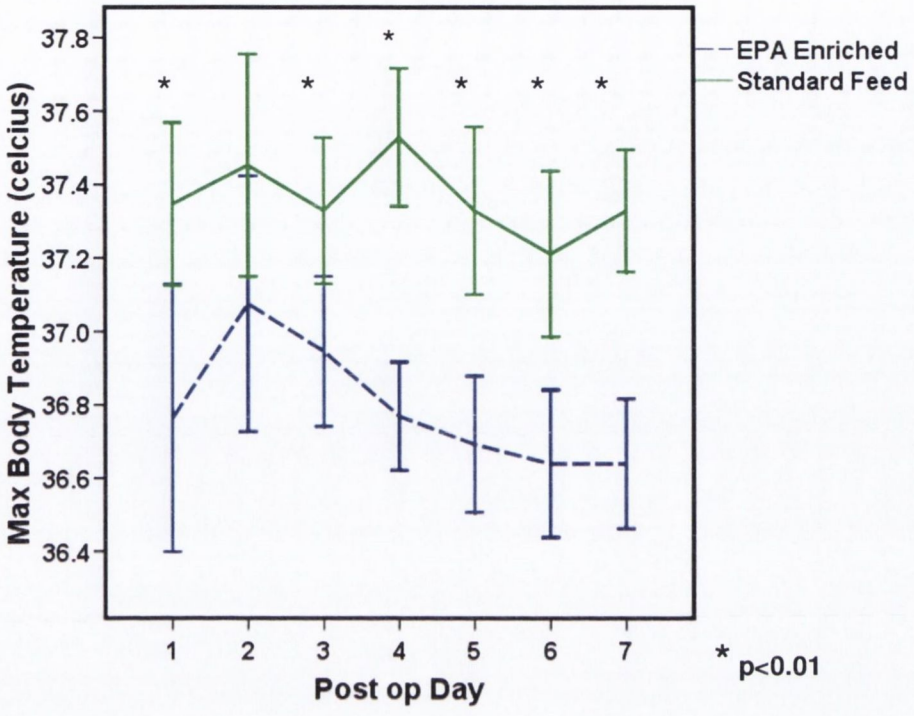
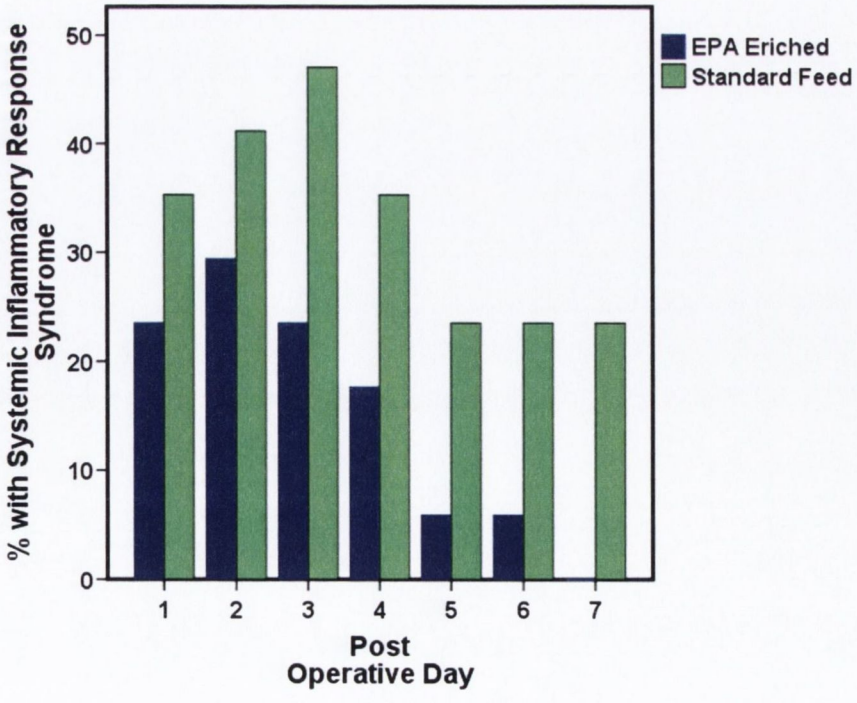


Figure 7.6: Percentage of patients with Systemic Inflammatory Response Syndrome on post operative days 1 – 7 for EPA enriched versus Standard Enteral Nutrition Patients.



7.6.6 Peri-operative changes in nutritional status - Body Composition Results

There was no significant difference in the energy or protein intake from enteral feeding between the two treatment groups at any post operative day. At discharge from hospital 82% of EPA patients and 83% of Standard EN patients were meeting their nutritional requirements (p=1.000).

Bioelectrical impedance analysis was performed at baseline and at day 21 post op to examine changes in segmental body composition in the peri-operative period. Table 7.8 describes the mean changes in body composition from pre operative values to post operative day 21. The results showed that the EPA enriched feeding group maintained all aspects of their body composition with no difference in any values from pre op to post operative day 21. EPA enriched patients lost an average of 1.2 kg of fat mass peri-operatively (p=ns) but maintained their fat free mass (55 kg pre op versus 55.3 kg post op, p=ns). In contrast, the Standard enteral feeding group lost significant amounts of weight, particularly of lean mass from pre operative values to post operative day 21. The Standard enteral nutrition group lost a mean of 1.8 kg in weight (std dev 3.3), p=0.03 [95%CI 0.17, 3.1], corresponding to a 0.56 (std dev 1.14) point drop in BMI, p=0.03 [95%CI: 0.06, 1.1]. Standard enteral nutrition patients lost a significant amounts of lean mass - fat free mass fell by 1.9 kg (std dev 3.7), p=0.03, [95%CI: 0.17, 3.6]. They lost significant amount of muscle from the legs, arms and trunk: leg (0.3kg (std dev 0.6, p=0.05 {95%CI -0.01, 0.57]); arm (0.17 kg (std dev 0.3), p=0.01 [95%CI: 0.04-0.3]) and trunk (1.44 kg (std dev 2.7), p=0.03, [95%CI: 0.12-2.76]), (see Figure 7.7).

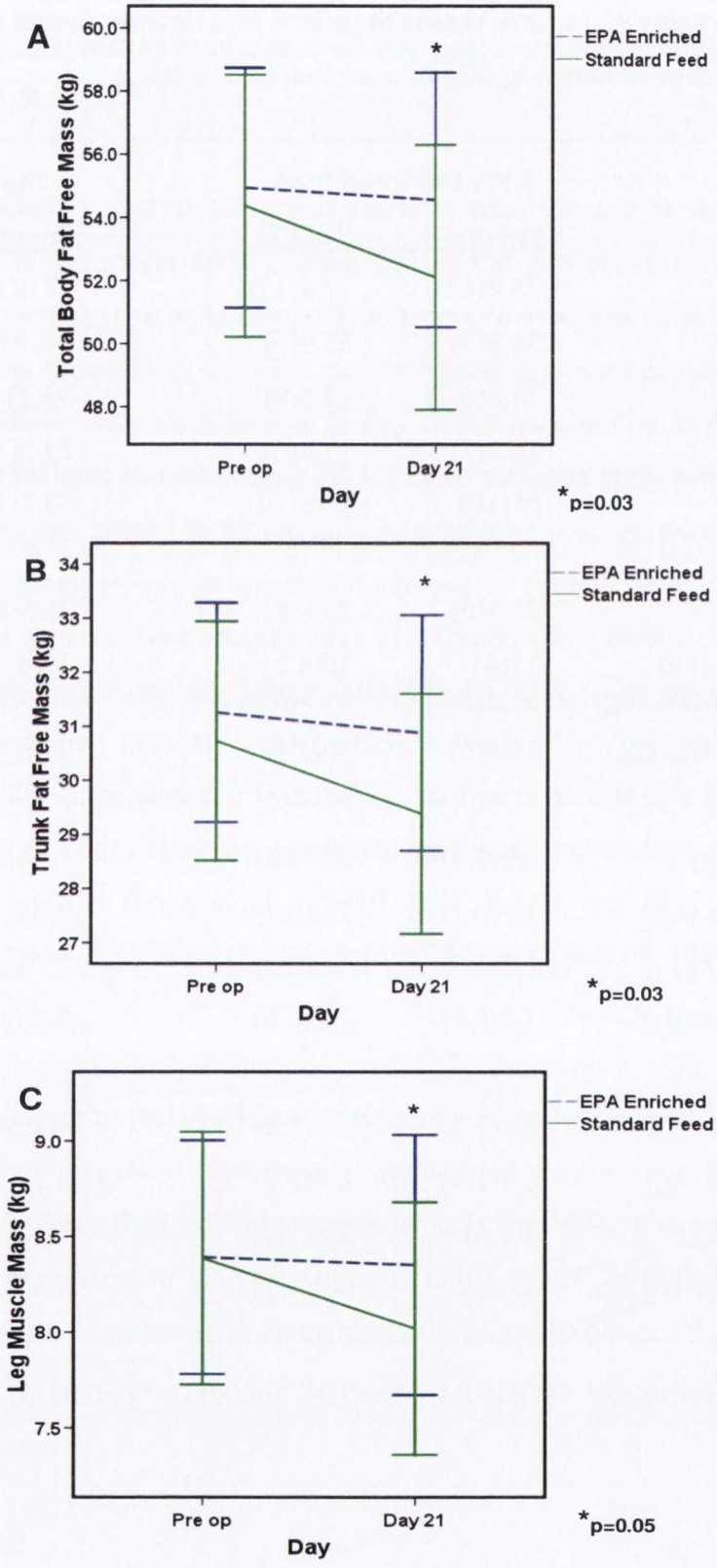
When graded according to the Blackburn criteria of weight loss (1977) 8% (n=2) of EPA enriched enteral feeding patients lost 'severe' amounts of weight (i.e. >5% in one month) versus 39% (n=10) of Standard Enteral nutrition patients (p=0.03). There was no significant difference in the percentage of patients requiring home enteral feeding at discharge from hospital (18% EPA (n=5) versus 24% standard EN (n=6), p=0.679).

Table 7.8: Changes in Segmental Body Composition from baseline pre operative values to post operative day 21 in EPA versus Standard EN. Values shown as mean (standard deviation). P Value denotes significance on Paired T-Tests.

	EPA Enriched feed		Standard feed	
	Pre op	POD 21	Pre op	POD 21
Weight (kg)	73.4(14)	72.4(13)	77.4(13)	75.6(13)*
% Hydration	54.8(5)	55.9(6)	51.4(5)	50.4(5.4)
% Fat	24.8(6.7)	23.5(8)	30(7)	31(7.2)
Fat Mass (kg)	18.5(7)	17.3(8)	23.3(7)	23.8(8)
Fat Free Mass	55(10)	55.3(10)	53.7(10)	51.8(9)*
Trunk Fat %	25.4(6)	23.8(8)	29.5(6)	31(6.6)
Trunk Fat Mass (kg)	11(4)	10(4.5)	13(4.2)	13.5(4.4)
Trunk Fat Free Mass (kg)	31.3(5.3)	31.3(5.5)	30.3(5)	29(5)*
Trunk Muscle Mass (kg)	29.8(5)	30(5.5)	29.5(5)	28(5)*
Left Arm Fat (kg)	0.9(0.4)	0.9(0.4)	1.2(0.5)	1.2(0.6)
Left arm Muscle (kg)	2.9(0.7)	2.8(0.7)	2.8(0.7)	2.7(0.6)**
Right Arm Fat (kg)	1.2(1.6)	0.8(0.3)	1.1(0.5)	1.1(0.5)
Right arm muscle (kg)	2.8(0.7)	2.8(0.6)	2.8(0.7)	2.6(0.7)***
Left Leg Fat (kg)	2.8(1.3)	2.7(1.4)	4(1.6)	4(1.6)
Left Leg Muscle (kg)	8.4(1.6)	8.4(1.7)	8.2(1.5)	8(1.5)
Right Leg Fat (kg)	3(1.4)	2.8(1.4)	4(1.5)	4.1(1.6)
Right Leg muscle (kg)	8.4(1.6)	8.5(1.7)	8.3(1.5)	7.9(1.5)$^{\Psi}$

POD = post operative day, ***p=0.04, **p=0.01, *p=0.03, $^{\Psi}$ p=0.05

Figure 7.7: Peri-operative changes in (A) Total body Fat Free Mass (kg), (B) Trunk Fat Free Mass (kg) and (C) Muscle mass in Leg (kg). P value denotes difference on Wilcoxon Signed Rank Test.



7.6.7 Blood Results

7.6.7.2 *Albumin and Total protein*

There was no significant difference in the mean albumin or total protein concentration at baseline or at any time point post operatively – with both groups displaying an almost identical response to surgery, see Figure 7.8 . On post operative day 7 there was a trend towards a higher total protein count in the EPA arm (62 g/L versus 59 g/L, p=0.07).

7.6.7.2 Coagulation: Prothrombin Time & D Dimers

There was no significant difference in the mean prothrombin time between the two groups at any time point peri-operatively (see Figure 7.9). There was no significant difference in the mean D-dimer level at baseline or on post operative days 1, 3, 7, 14 or day 21 between the two groups.

Figure 7.8: Mean albumin and total protein concentration in EPA enriched versus Standard feed at baseline and on post operative days 1, 3, 7, 14 and 21.

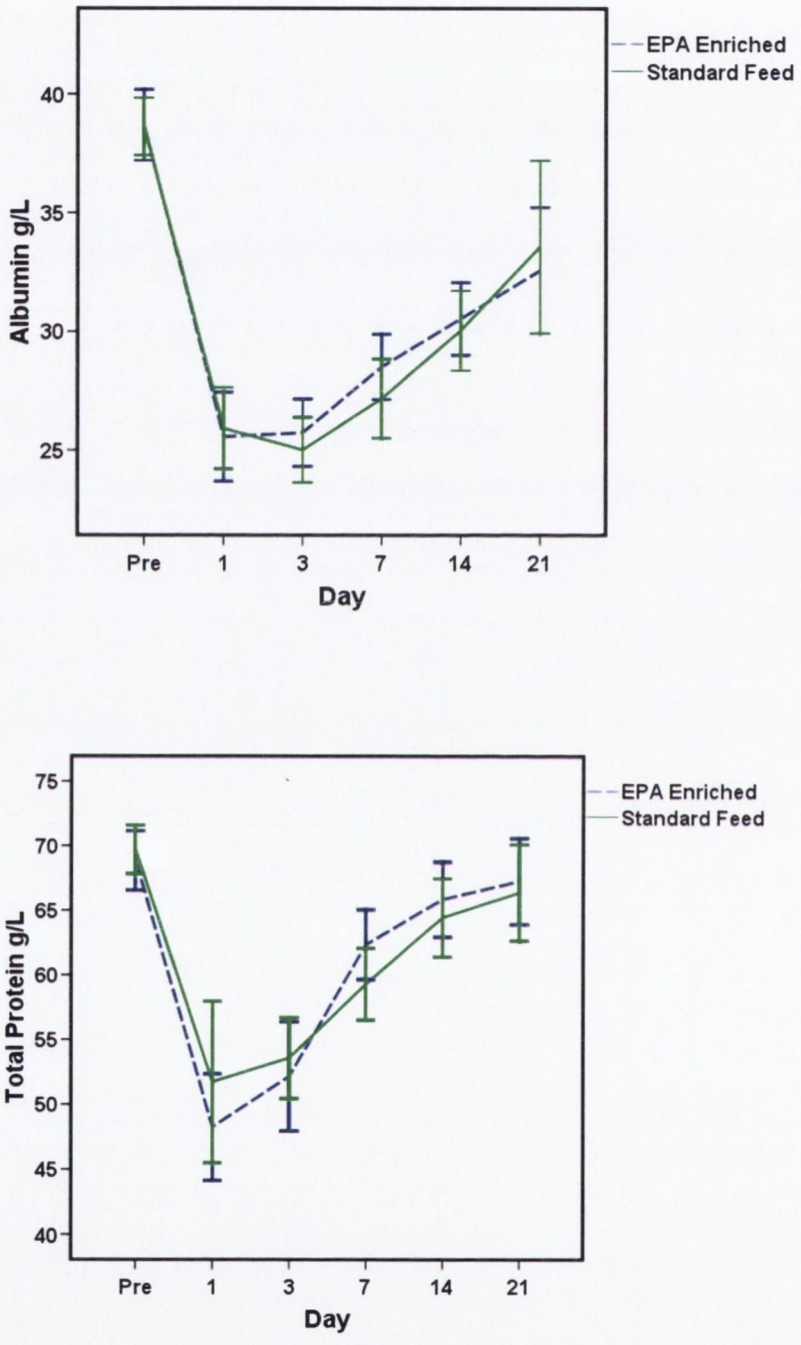
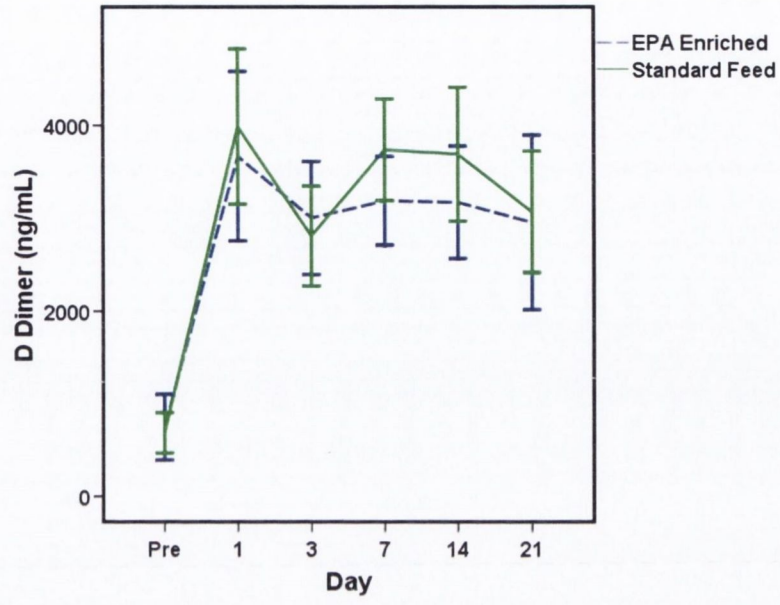
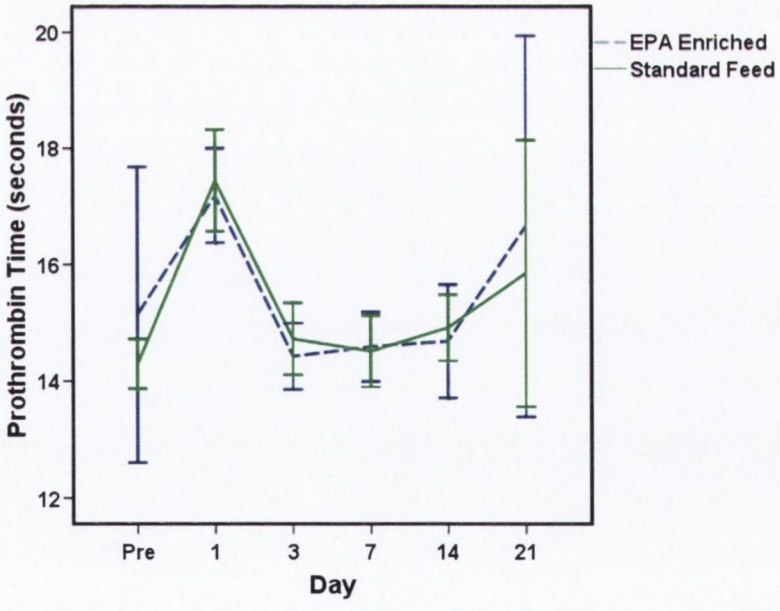


Figure 7.9: Mean Prothrombin Time and D-Dimer levels in EPA enriched versus Standard Enteral feeding group. Error Bars: 95% Confidence Intervals





7.6.8 Inflammatory Markers

7.6.8.1 C-Reactive Protein, Serum Amyloid A, Erythrocyte Sedimentation Rate

C-Reactive Protein

Figure 7.10 displays the mean CRP level peri-operatively for EPA enriched EN and the standard EN arm. On Mann Whitney U tests there was no significant difference in the CRP level between the 2 groups at any time point. The results of the Friedman tests indicated that there was a significant difference in the CRP levels across 6 time points (pre op, days 1, 3, 7, 14 and 21 post op); for EPA enriched: chi square=35.3, $p<0.005$, for Standard feed chi square=29.9, $p<0.005$. Inspection of the median values showed an increase in CRP until post operative day 3 and thereafter a decrease in both groups.

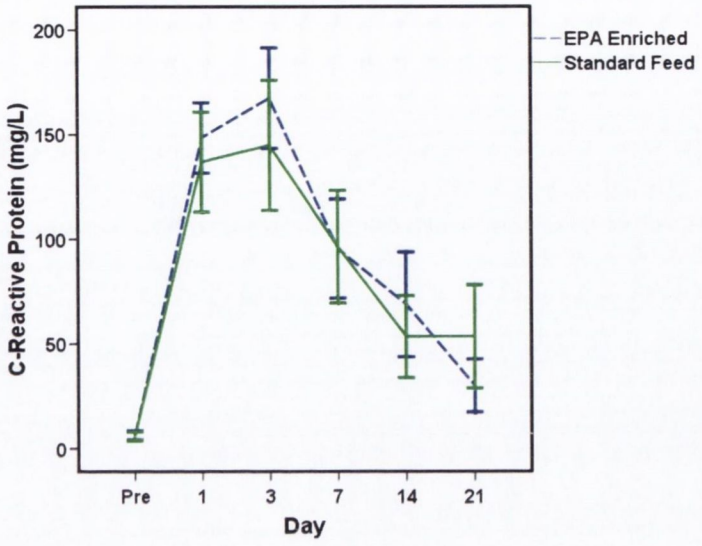
Serum Amyloid A

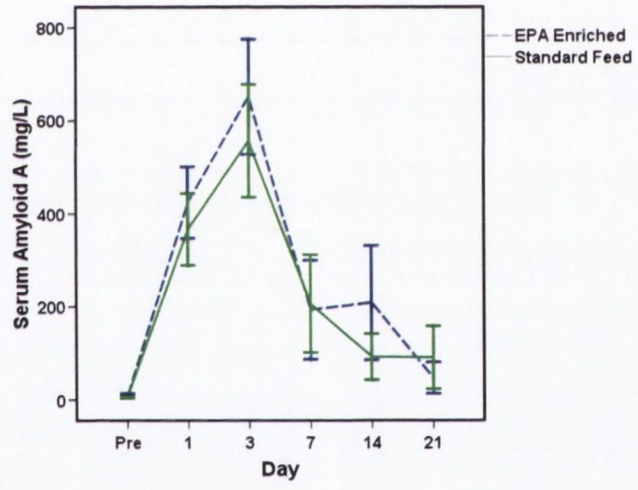
Figure 7.10 displays the mean SAA level peri-operatively for EPA enriched and standard EN arm. On Mann Whitney U tests there was no significant difference in the SAA level between the 2 groups at any time point. The results of the Friedman tests indicated that there was a significant difference in the SAA levels across 6 time points (pre op, days 1, 3, 7, 14 and 21 post op); for EPA enriched: chi square=23.3, $p<0.005$, for Standard feed chi square=19.8, $p=0.001$. Inspection of the median values showed an increase in SAA until post operative day 3 and thereafter a decrease in both groups.

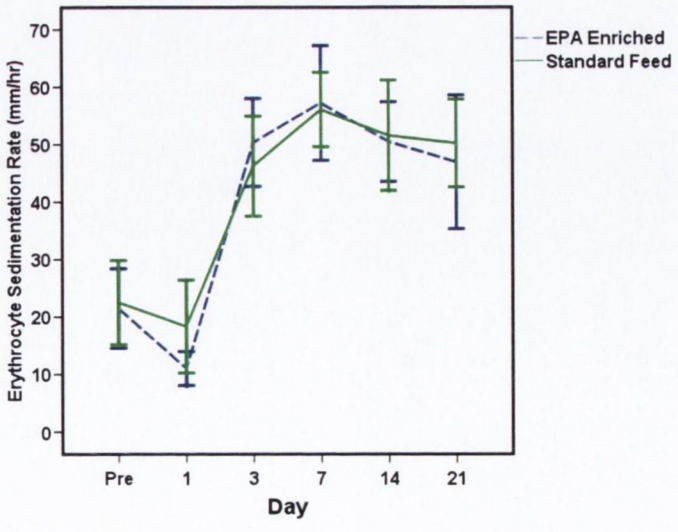
ESR

On Mann Whitney U tests comparing the two treatment arms there was no difference in the ESR level between the groups either pre or post operatively (see figure 7.10). Wilcoxon signed rank test revealed a statistically significant decrease in the EPA enriched arm on post operative day 1 compared to baseline ($p=0.028$) with no difference in the standard arm ($p=0.201$). The results of the Friedman tests indicated that there was a significant difference in the ESR levels across 6 time points (pre op, days 1, 3, 7, 14 and 21 post op); for EPA enriched: chi square=28, $p<0.005$, for Standard feed chi square=16.5, $p=0.006$. Inspection of the median values showed an initial drop in ESR on post operative day 1 and thereafter a significant increase in both groups.

Figure 7.10: C-Reactive Protein, Serum Amyloid A and Erythrocyte Sedimentation Rate levels at baseline and on days 1, 3, 7, 14 and 21 post oesophagectomy in EPA enriched EN patients versus Standard EN patients. Error bars are 95% CI.







7.6.8.2 Cytokine Results

Interleukin 4

On Mann Whitney U tests there was no significant difference between the groups at any time-point. On Wilcoxon Signed Rank testing comparing pre op levels to post operative levels there was no significant change in the IL-4 level in either treatment arm (pre op EPA Vs Standard: 2.7 Vs 2.5, p=0.43; Day 3: 1.7 Vs 1.6, p=0.37; Day 7: 3.1 Vs 2.5, p=0.12; Day 13: 3.1 Vs 2.8, p=0.34). On Friedman testing for non-parametric repeated measures analysis over 6 time-points (pre op, post op days 1, 3, 7, 14 and 21) there was no significant change over time in either group (EPA chi square=2.7, p=0.754, Standard: chi square 2.6, p=0.767).

Interleukin-6

On Mann Whitney U tests to compare differences between the two treatment arms there was no significant difference in the IL-6 level at any time point (see figure 7.11). On Wilcoxon Signed Rank testing comparing pre op levels to post op days 1, 3, 7, 14, and 21 there was a significant increase in the EPA arm on post operative days 1 (p=0.02), day 3 (p=0.007), day 7 (p=0.01), day 14 (p=0.01), and day 21(p=0.003). Similarly the Standard EN arm levels increased significantly on days 1 (p=0.003), day 3 (p=0.001), day 7 (p=0.003), day 14 (p=0.002), and day 21 (p=0.003). On Friedman testing for non-parametric repeated measures analysis over 6 time-points (pre op, post op days 1, 3, 7, 14 and 21) there was a significant change in levels over time for both groups: Standard group (chi squared=18.4, p=0.002), EPA arm (chi squared =21.6, p=0.001).

Interleukin 8

On Mann Whitney U tests to compare differences between the two treatment arms IL-8 levels were significantly higher on post operative days 7 and 14 in the Standard EN arm (p=0.05) (see figure 7.12A). On Wilcoxon Signed Rank testing comparing pre op levels to post op days 1, 3, 7, 14, and 21 there was a significant increase in the EPA arm on post operative days 1 (p=0.04), day 7 (p=0.022), day 14 (p=0.016), and day 21(p=0.009). For the Standard EN arm levels increased significantly on days 1 (p=0.008), day 7 (p=0.05) and day 14 (p=0.002). On Friedman testing for non-parametric repeated measures analysis over 6 time-points (pre op, post op days 1, 3, 7, 14 and 21) there was a significant change in levels

over time for the standard group only (chi squared=10.9, p=0.05) with no significant change over time for the EPA arm (chi squared =6.5, p=0.263).

Interleukin 10

There was no significant difference in the mean concentration of IL-10 between the two groups at any time-point on Mann Whitney U tests (see figure 7.12B). On Wilcoxon Signed Rank testing comparing pre op levels to post op days 1, 3, 7, 14, and 21 there was a significant increase in the EPA arm on post operative day 1 (p=0.058) and day 3 (p=0.028). For the Standard EN arm levels increased significantly on days 1 (p=0.008), day 3 (p=0.025) and day 7 (p=0.01). On Friedman testing for non-parametric repeated measures analysis over 6 time-points (pre op, post op days 1, 3, 7, 14 and 21) there was a significant change in levels in both groups over time, for EPA arm chi squared =11.3, p=0.045 and for the standard arm chi squared=12.9, p=0.025.

Tumour Necrosis Factor Alpha

There was no significant difference in the TNF alpha concentration between the two groups either at baseline or on post operative days 1, 3, 7, 14, or 21 on Mann Whitney U testing (see figure 7.13A). On Friedman testing for non-parametric repeated measures analysis over 6 time-points (pre op, post op days 1, 3, 7, 14 and 21) there was a significant change in TNF alpha levels over time for the Standard EN group (chi squared=13.5, p=0.019) but no change for the EP enriched arm (chi squared=2.9, p=0.714). On post hoc analysis by Wilcoxon signed rank tests there were no significant changes in the TNF alpha level on POD 1, 3, 7, 14, 21 or 28 in the EPA group compared with baseline, however in the Standard EN group there was a significant difference between the baseline TNF alpha level and the level on POD 1 (p=0.004), day 7 (p=0.033) and a trend on day 14 (p=0.07).

Monocyte Chemotactic Protein-1

There was no significant difference between the median concentration of MCP-1 between the two groups at any time point on Mann Whitney U tests (see figure 7.13B). Friedman Tests for repeated measures of non-parametric data showed that there was a statistically significant difference in the MCP-1 level across 6 time-points (pre op, day 1, 3, 7, 14 and 21 post op) for the standard EN arm only (chi square=13.2, p=0.022), with no change in the

EPA arm (chi square=1.83, p=0.872). Inspection of the median values showed an increase in MCP-1 levels in the EPA arm until post operative day 3 and thereafter a decrease. In the standard EN arm levels increased until day 7 and thereafter showed a decrease. Wilcoxon signed rank testing (for post hoc analysis) comparing the baseline MCP level to post operative days 1, 3, 7, 14 and 21 showed no significant differences for the EPA group but for the Standard EN group there was a significant increase from baselines to day 3 and day 7 the median level increased from 345 to 412on day 3, p=0.023, and from 345 to 440 on day 7 p=0.036.

Figure 7.11: Median Interleukin-6 levels at baseline and on days 1, 3, 7, 13 and 21 post oesophagectomy in EPA enriched EN patients versus Standard EN patients. Error bars: 95% CI.

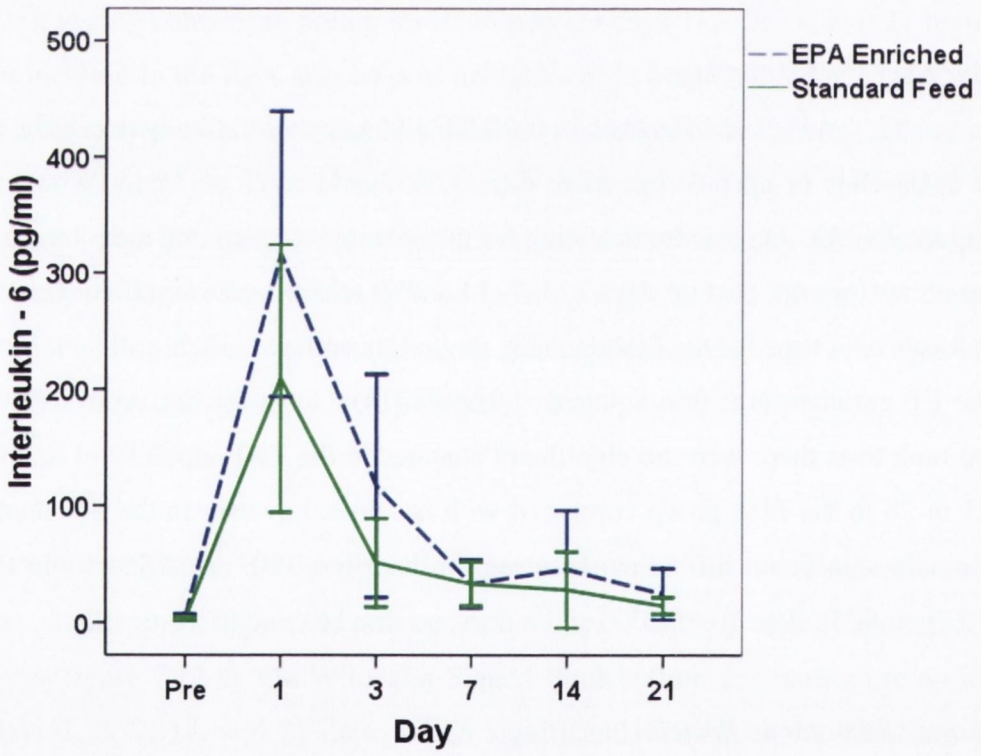


Figure 7.12: Median (A) Interleukin - 8, (B) Interleukin – 10 levels at baseline and on days 1, 3, 7, 13 and 21 post oesophagectomy in EPA enriched EN patients versus Standard EN patients. Error bars: 95% CI.

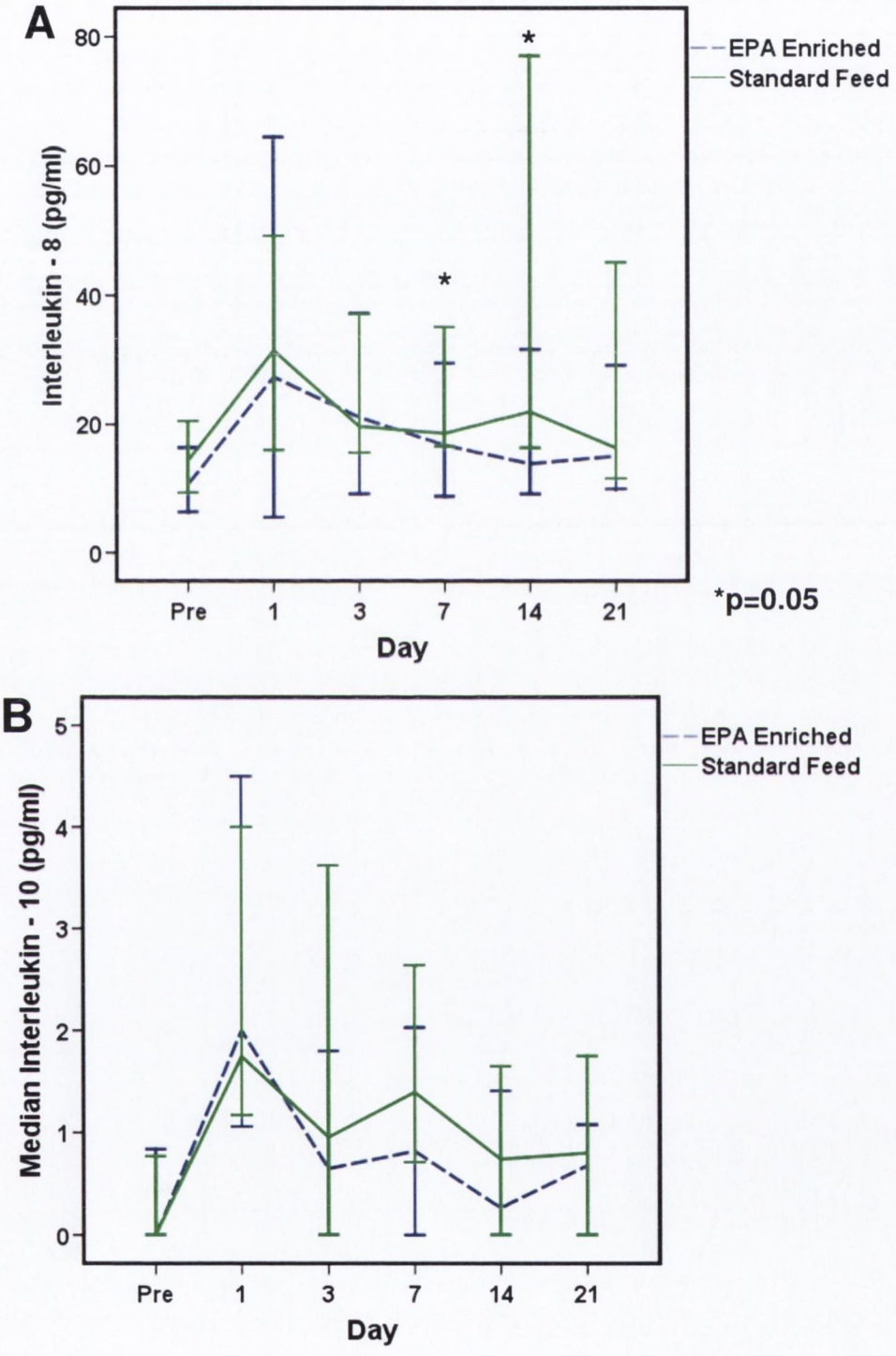
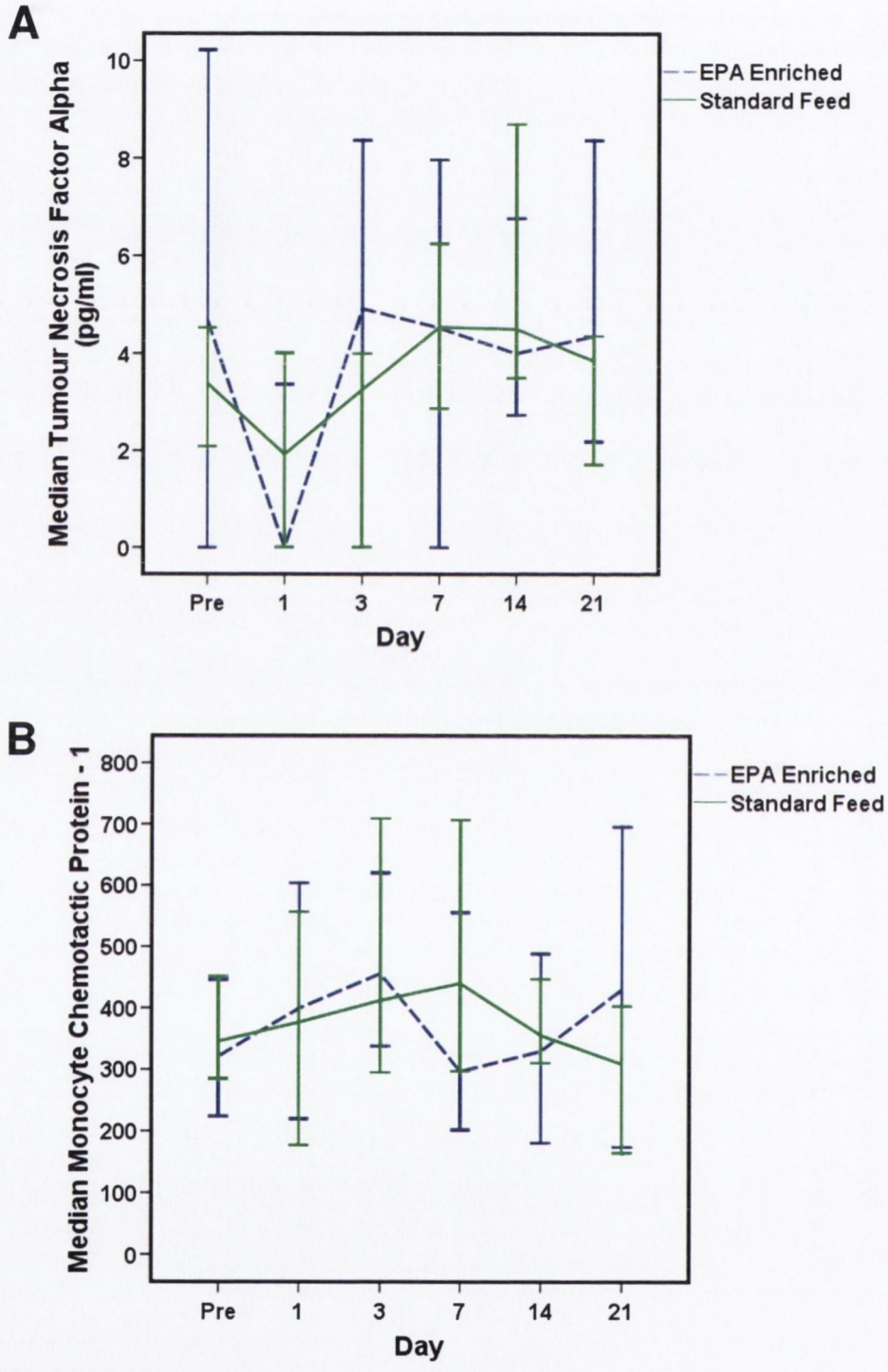


Figure 7.13: (A) Median Tumour Necrosis Factor alpha (B) Monocyte Chemotactic Protein -1 level at baseline and on days 1, 3, 7, 13 and 21 post oesophagectomy in EPA enriched EN patients versus Standard EN patients. Error bars: 95% CI.



7.6.8 Immune Function

7.6.9.1 Lymphocyte Subset Analysis

Lymphocyte subset analysis was performed pre operatively (prior to enteral supplements) and on post operative days 1, 3 and 7 by Flow Cytometry, the results are displayed in Figures 7.14, 7.15, 7.16 and 7.17 and Table 7.9. The analysis was also repeated excluding patients who received a blood transfusion (n=11) but the results did not change significantly.

T cells

There was no significant difference in the median T cell counts or the percentage of T-cells between the 2 groups at any time point. On repeated measures analysis (Friedman test) both groups had a significant change in the percentage of T cells over 4 time points (pre op, day 1, 3 and 7), for EPA the chi square was 13.5, p=0.003 and for Standard EN the chi square was 18.2, p=0.0001. Inspection of the median values by post hoc analysis showed that both groups had a significant decrease in the percentage of T cells on post operative days 1 and 3. By day 7 levels had recovered in the EPA arm (pre op 75% versus Day seven 71%, p=0.638) but remained significantly lower in the Standard EN arm (75% pre op versus 67% on day 7, p=0.01) (see figure 7.14(A) and table 7.9).

Helper T Cells (CD4)

There was no significant difference between the CD4 cell count or percentage of CD4 cells peri-operatively in either treatment arm with both groups showing a significant change over time on repeated measures analysis (Friedman Test) (EPA group: chi square=15.8, p=0.001; Standard EN: chi squared=13.2, p=0.004). Inspection of the median values on post hoc analysis showed that CD4 dropped on days 1 and 3 but recovered by post operative day 7 in both treatment arms (EPA group median pre op 46% versus 45% on day 7, p=0.221; Standard group median pre op 39 versus 38.4 on day 7, p=0.208) (figure 7.14(B), table 7.9).

Cytotoxic T cells (CD8)

There was no significant difference in the CD8 count or percentage CD8 cells between the two groups at any time-point on Mann Whitney Tests. On repeated measures analysis (Friedman test) there was no significant change over 4 time points (Pre op, days 1, 3, 7 post

op) in the EPA enriched arm (chi squared=5.2, p=0.159) but the Standard arm showed a significant change over time (chi squared=9.2, p=0.026). Inspection of the median values on post hoc analysis showed that CD4 cells decreased over time in the Standard arm but did not change in the EPA enriched arm (median pre op Standard EN 27% versus 21% on day 7, p=0.005; median pre op EPA enriched 20% versus 19% on day 7, p=0.06) (figure 7.15A, table 7.9).

CD4:CD8 Ratio

The median ratio of CD4:CD8 cells was calculated pre operatively and on days 1, 3, and 7 post operatively. There was no significant difference between the groups on Mann Whitney U tests at any time point. On repeated measures analysis (Friedman tests) over the peri-operative period the ratio increased over time for the EPA treatment arm from 1.8 pre op to 1.9, 2.1 and 2.3 on days 1, 3, and 7 respectively (chi square=11.8, p=0.008), for Standard EN the ratio went from 1.5 pre op to 1.4, 1.9 and 1.9 on days 1, 3 and 7 respectively (chi square=8, p=0.07). On post hoc analysis by Wilcoxon Signed rank tests the CD4:CD8 ratio had significantly increased in the EPA arm on post operative day 7 compared to baseline (1.8 pre op versus 2.3 day 7, p=0.004), with no significant change observed in the Standard arm (1.5 pre op versus 1.9 day 7, p=0.09) (see figure 7.15(b), table 7.9).

Natural Killer Cells

Pre operative NK cells were significantly higher in the Standard EN arm (median 19% Standard arm versus 11% in EPA arm, p=0.05). On repeated measures analysis (Friedman test) on 4 time points (pre op, day 1, 3 and 7) there was no significant change in the percentage of NK cells in the EPA arm (chi squared=5.6, p=0.132) but there was a significant change over time in the Standard arm (chi squared=8.6, p=0.035). Post hoc analysis showed that the % significantly increased in the EPA arm on day 1 (p=0.02) and there after decreased, by day 7 post op levels were not significantly different to baseline (pre op 11% versus day 7 16%, p=0.665). In the standard arm levels did not change at any time-point (figure 7.16(A), table 7.9).

B Cells

There was a significant difference in the B cell count and % B cells between the two groups on day 3 post op (EPA 10% versus Standard EN 16%, p=0.032). On repeated measures analysis (Friedman test) on 4 time points (pre op, day 1, 3 and 7) both groups showed a significant change over time, (EPA arm chi squared=12.2, p=0.006, Standard arm chi squared=8.7, p=0.03). On post hoc tests by Wilcoxon signed rank test, the percentage of B cells increased significantly in the EPA arm on days 1, 3 and 7 and in the Standard arm on days 1 and 7 (see figure 7.16(B), table 7.9).

Lymphocyte Count

There was no significant difference in the median lymphocyte count on any day peri-operatively between the two treatment arms, although there was a trend towards higher counts in the EPA arm at baseline (1032 versus 754, p=0.09). On repeated measures analysis (Friedman test) on 4 time points (pre op, day 1, 3 and 7) both groups showed a significant change in counts over time (EPA arm chi squared=22, p=0.001; Standard arm chi squared=17.1, p=0.001). On post hoc analysis the levels decreased significantly from baseline to day 7 in the EPA arm (1032 versus 567, p=0.003) but the levels were not significantly different from baseline in the Standard arm (754 versus 742, p=0.133), (figure 7.17, table 7.9)

Figure 7.14: Median Pre-operative and Days 1, 3, and 7 post operative (A) T-cell (B) CD4 Helper T-cells on Lymphocyte subset analysis for EPA enriched Feed versus Standard Feed. Error bars: 95% CI.

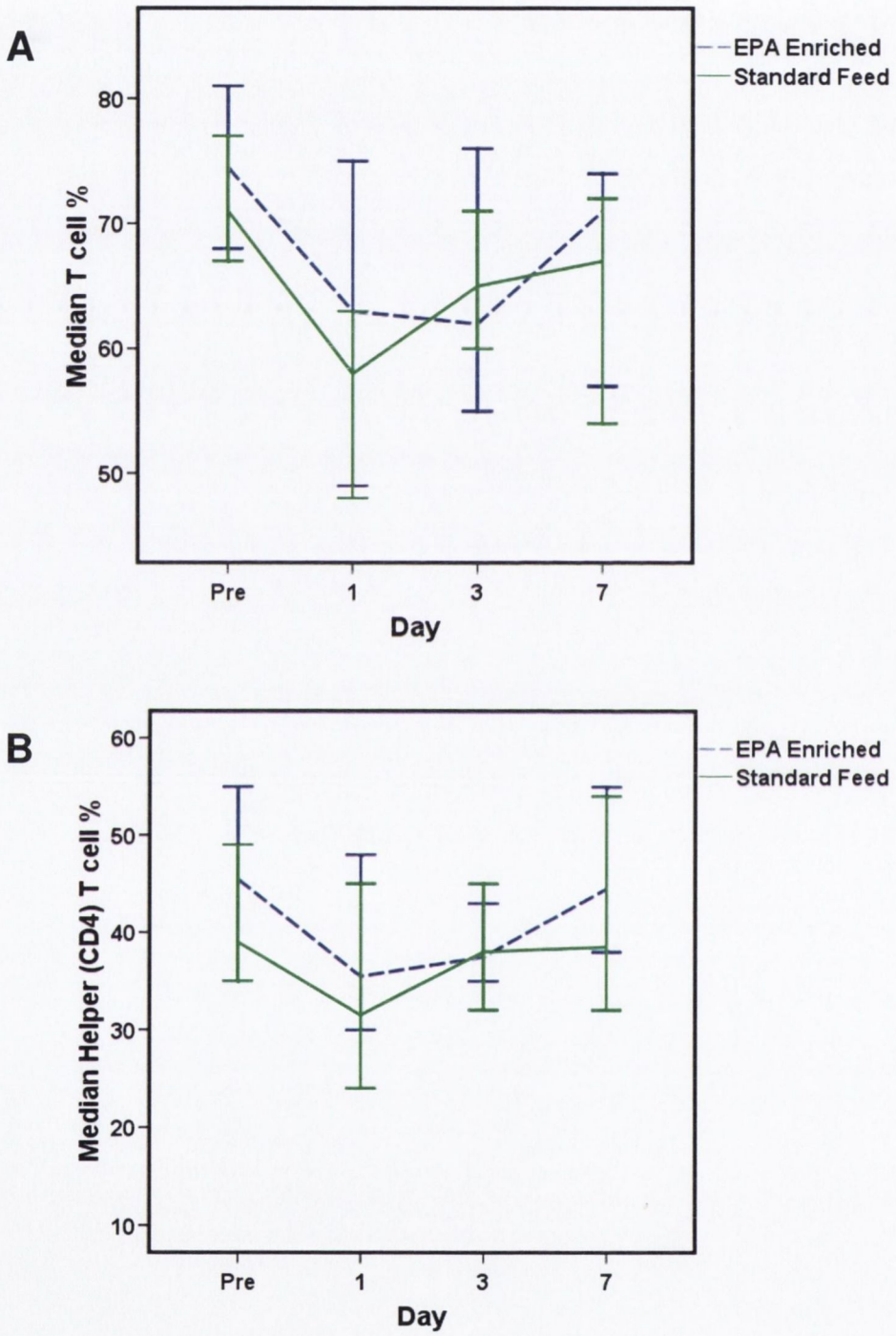


Figure 7.15: Median Pre-operative and Days 1, 3, and 7 post operative (A) CD8 Cytotoxic T-cells (B) CD4:CD8 Ratio on Lymphocyte subset analysis for EPA enriched Feed versus Standard Feed. Error bars: 95% CI.

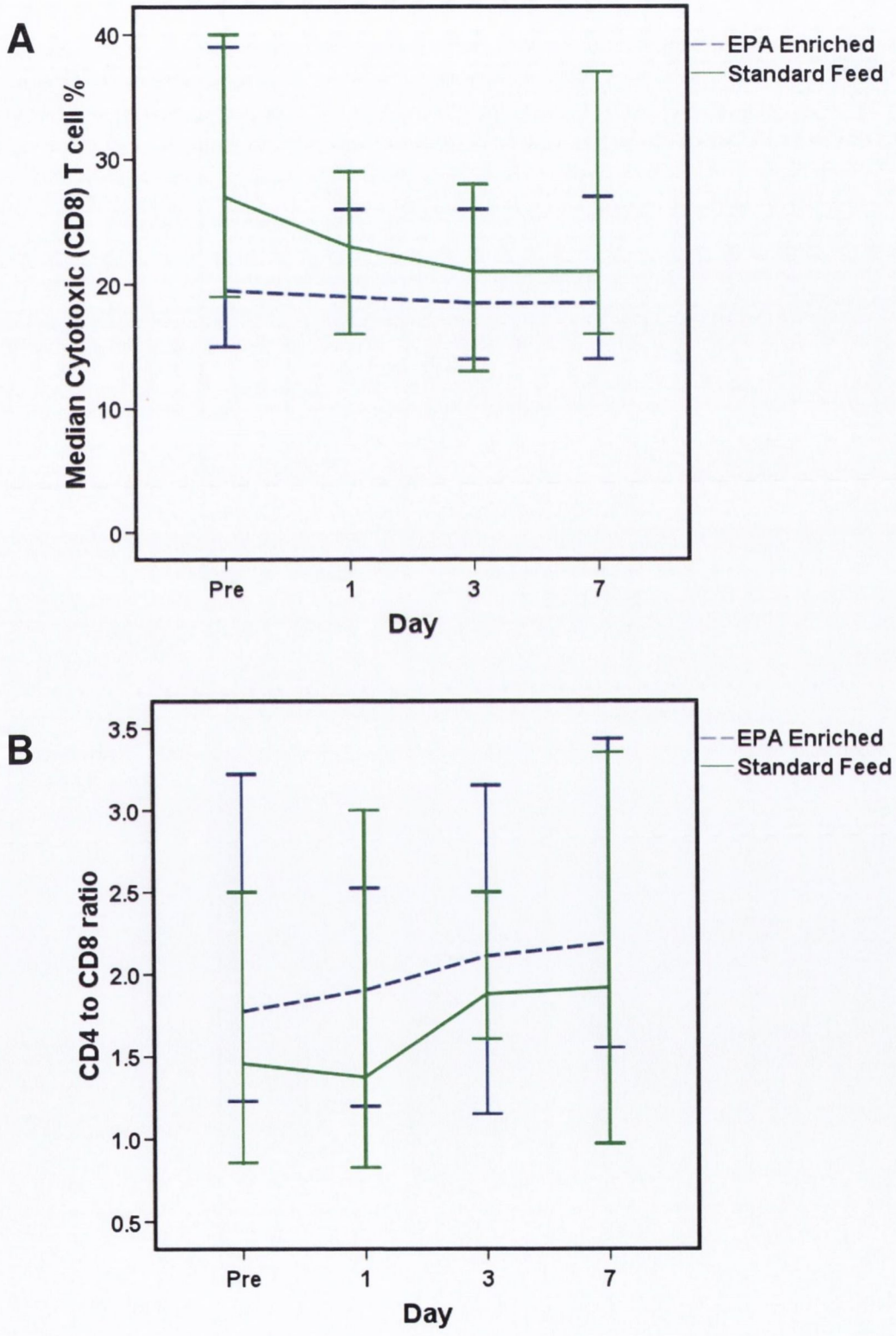


Figure 7.16: Median Pre-operative and Days 1, 3, and 7 post operative (A) Natural Killer Cells (B) B Cells on Lymphocyte subset analysis for EPA enriched Feed versus Standard Feed. Error bars: 95% CI.

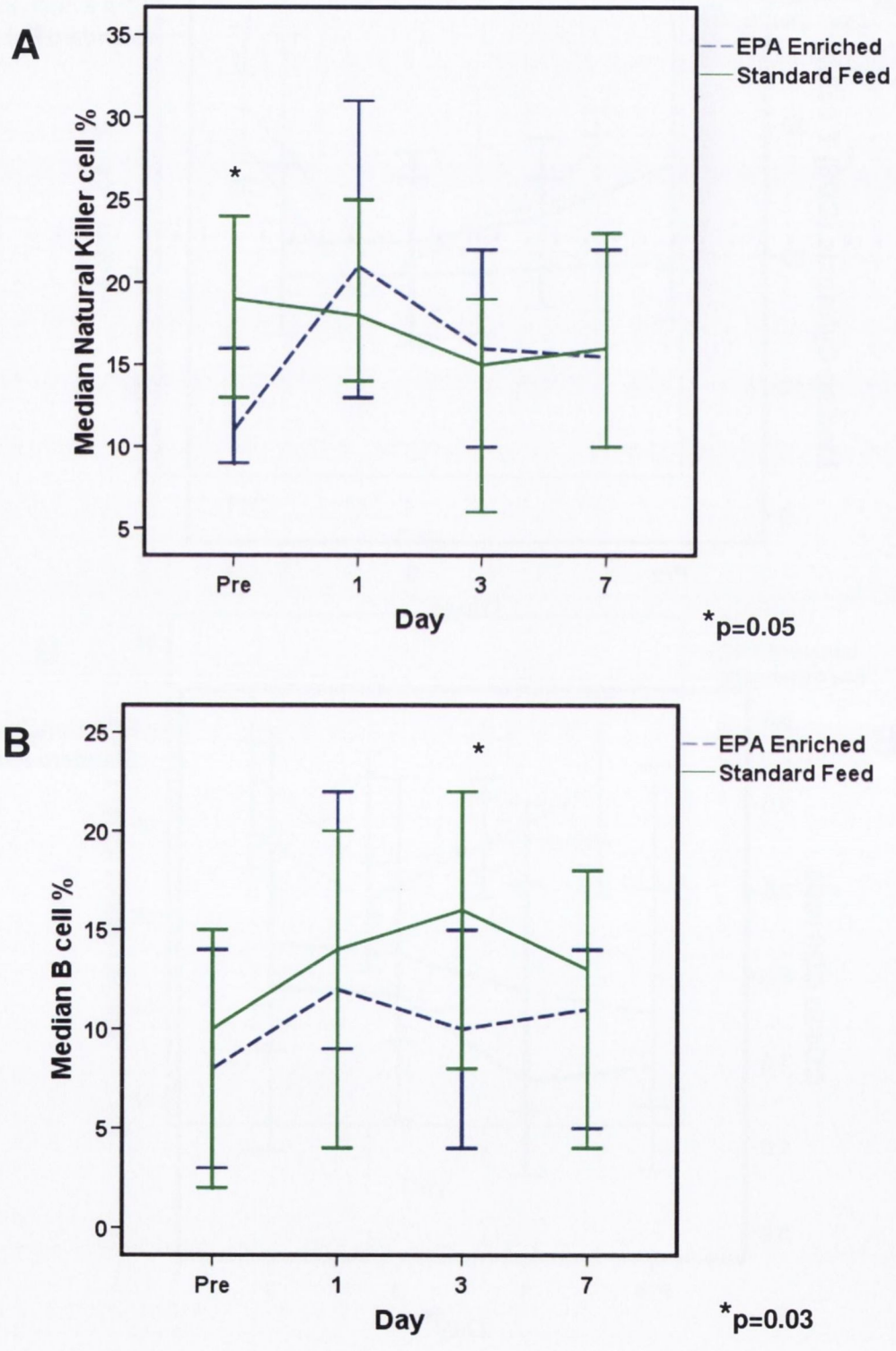


Figure 7.17: Median Pre-operative and Days 1, 3, and 7 post operative Lymphocyte Count (CD3) on Lymphocyte subset analysis for EPA enriched Feed versus Standard Feed. Error bars: 95% CI.

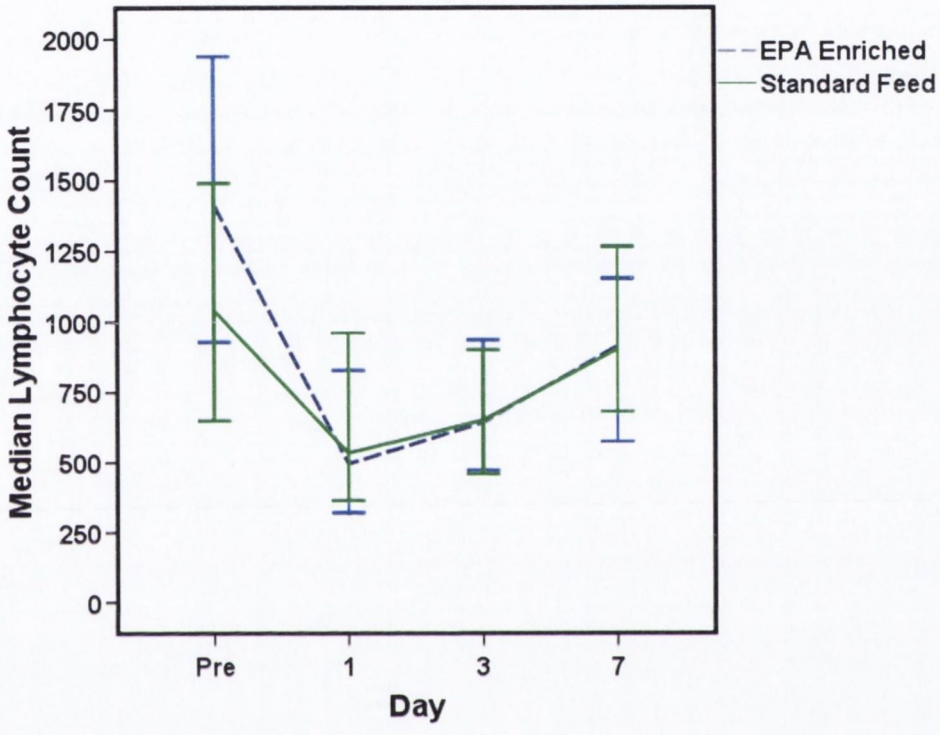


Table 7.9: Lymphocyte Subset results for EPA enriched versus Standard EN pre op and on days 1, 3, and 7 post op. Values expressed as medians.

	Standard EN				EPA Enriched			
	Pre	D1	D3	D7	Pre	Day1	Day 3	Day 7
T Cells %	71	58**	65**	67$^{**\Psi}$	75	63**	62**	71$^{\Psi}$
CD4 %	39	32**	38	39$^{\Psi}$	46	36**	38**	45$^{\Psi}$
CD8 %	27	23	21**	21$^{**\Psi}$	20	19	19**	19**
CD4:CD8 Ratio	1.5	1.4	1.9	1.9	1.8	1.9	2.1	2.3$^{**,\Psi}$
NK %	19*	18	15	16$^{\Psi}$	11	21**	16**	16
B Cell %	10	14**	16*,**	13$^{**,\Psi}$	8	12**	10**	11$^{**\Psi}$
Lymphocyte count	754	469**	574**	742$^{**,\Psi}$	1032	418**	616**	567$^{**,\Psi}$

*p<0.05 Between Groups (Mann Whitney U Test)

**p<0.05 from baseline (Wilcoxon Signed rank test)

$^{\Psi}$p<0.05 Friedman test repeated measures analysis over 4 time points

7.7 DISCUSSION

The high morbidity rates observed following oesophagectomy reflect the profound changes in the endocrine, neuroendocrine and immune system as well as significant changes in organ function that occur following this type of operation (Desborough, 2000). In fact, recent literature has highlighted the unassailable fact that there is no common elective surgical procedure that carries the same operative risks (Van Lanschot et al, 2001; Begg et al, 1998). The insult of oesophagectomy sets in motion a predictable immunologic response, with most patients developing a systemic inflammatory response syndrome (SIRS), and approximately 50% developing complications, with the risk of in-hospital mortality at approximately 6-10 percent (Bailey et al, 2003). It is also somewhat intuitive that the procedure of oesophagectomy results in significant nutritional consequences, although these are infrequently referred to in the literature. Endocrine, physiological and immune cell response to surgery contributes to post-operative catabolism after major oesophageal resections, and marked weight loss is normally observed. This is usually observed on a background of pre operative weight loss, with oesophageal cancer being reported as having the highest level of malnutrition compared with other digestive and extra-digestive cancers (Larrea, 1992).

The model of multimodality management of oesophageal cancer therefore lends itself well to studies with immunonutrition as it represents an ideal model of a major homogenous insult with predictable alterations of immune cell function and metabolism, and a high risk of septic complications, weight loss and compromised quality of life. Although several studies have demonstrated the beneficial effects of immunonutrition on immune competence and patient outcome, controlled clinical trials focusing on the use of perioperative enteral omega-3 fatty acids alone are scarce. Eicosapentaenoic acid (EPA), a long chain polyunsaturated fatty acid (PUFA) of the omega-3 (n-3) family, is of interest in the context of major cancer surgery as it has potential to impact on both the underlying metabolic abnormalities of tumour-induced weight loss, as well as modulation of immune function.

With increasing enteral or parenteral intake of n-3 PUFA, the n-3:n-6 PUFA value in the phospholipids spectrum of the cell membrane in various tissues changes in favour of n-3 PUFA (Palombo et l, 1993; Morlion et al, 1996). In fact dietary fatty acids of the omega 3 series are rapidly incorporated into cell membranes - incorporation of EPA into cell

membranes has been achieved in 5 days with oral administration whereas incorporation is much faster (hours) with IV administration (Carpentier et al, 1997; Senkal et al, 2005). As with any substance with supposed pharmacological action, immunonutrients should reach suitable tissue concentrations to be active (Gianotti et al, 2002). For this reason a key point is the anticipation of the provision of immunonutrients before surgery to obtain adequate levels at the time of surgical stress when the need for stimulation of the immune system is maximised. It was on this premise that we designed this peri-operative EPA study with 5 days of pre –operative 'loading of cells'. This change in cell membrane composition profoundly influences biologic responses, particularly during stress. These lipids influence membrane stability, membrane fluidity, cell mobility, the formation of receptors, binding of ligands to their receptors, activation of intracellular signalling pathways either directly or through the formation of eicosanoids, gene expression, and cell differentiation (Senkal et al, 2005).

Of most importance in terms of major surgical stress is the fact that alterations in membrane phospholipids directly influences the synthesis of lipid-derived mediators such as eicosanoids, phosphatidic acid, platelet-activating factor and the secondary messengers, diacylglycerol and ceramide (Ross, 1999; Suchner et al, 2000). By the action of the enzyme phospholipase A2, PUFA can be released from the membrane phospholipids and either act as a secondary messenger or alternatively serve as a precursor for the cyclo-oxygenase pathway (Suhner et al, 2000). The latter pathway metabolises arachidonic acid to the 2-series of prostaglandins, especially prostaglandins E2 and F2alpha and thromboxane A2. These products are vasoconstrictive and induce platelet aggregation (Kudsk, 2006). These immunosuppressive products impair cytotoxic T-cell function, cytokine secretion, leukocyte migration, and reticuloendothelial system function. In contrast, EPA derived thromboxane A3 is less active in platelet aggregation than thromboxane A2; EPA is converted to leukotriene B5 which results in decreased chemotactic migration and endothelial cell adherence, therefore EPA exerts major effects on the synthesis of leukotrienes by promoting an anti-inflammatory action. Modulation of dietary fatty acids can therefore have an impact on many immune processes such as proliferation, phagocytosis, cytotoxicity and cytokine production (Fritshce, 2006).

The mechanisms underlying the differential effects of omega 6 and omega 3 lipids on cytokine synthesis are relatively unknown (Aiko et al, 2005). Several studies have demonstrated that there is a close relationship between the release and metabolism of AA

from cell membranes and the generation of platelet activating factor (PAF). PAF is known to have a wide range of pro-inflammatory properties including increased chemotaxis, adherence, and aggregation of human neutrophils and monocytes. PAF also induces these cells to produce pro-inflammatory cytokines such as TNFα, IL-6 and IL-8 (Ruis et al, 1991). These cells in turn are capable of inducing cyclo-oxygenase 2 which metabolises AA and provides PGE2. Proinflammatory cytokines such as TNFα, IL-6 and IL-8, can act as pyrogens through increased production of PGE2 which can act directly on the brain causing an upward shift in thermo regulation (Coceani & Akarus, 1998). By substituting AA in cell membranes with EPA, PGE3 is preferentially formed and thus a more anti-inflammatory milieu arises with less risk of fever

Another potential benefit to this altered metabolic milieu is the observation that increased intake of EPA has a modulatory effect on the prevention and treatment of tumour related weight loss and cachexia. Through modification of eicosanoid production and subsequent reduction in proinflammatory cytokines, attenuation of tumour related weight loss has been observed in patients with advanced inoperable pancreatic cancer (Barber et al, 2001).

Although several studies have demonstrated the beneficial effects of immunonutrition on immune competence and patient outcome, controlled clinical trials focusing on the use of perioperative enteral EPA alone are scarce. Three trials on EPA following oesophageal cancer surgery have recently been published. Furkawa et al (1999) reported that the postoperative administration of EPA at a dose of 1.8 g/d either orally or enterally in combination with Total Parenteral Nutrition (TPN) reduces the stress response to oesophagectomy and stress-induced immune dysfunction compared to TPN alone. EPA supplementation significantly reduced the level of serum IL-6, and significantly improved the lymphocyte proliferation and Natural Killer cell activity on postoperative day 21 compared to TPN alone. Takagi et al, (2001) studied the effects of EPA on immune suppression induced by postoperative chemo-radiation therapy in a small cohort of 15 patients who underwent thoracic oesophagectomy and received post operative chemotherapy. For 1 week before surgery and 2 weeks after patients were fed by total parenteral nutrition – group 1 (n=5) were given TPN with 1.8 g/day of EPA and group 2 (n=10) were given TPN alone. The authors assessed Phytohemagglutin- and concanavalin-A stimulated lymphocyte proliferation, Natural Killer Cell activity and

total lymphocyte count 3 weeks after surgery and after post operative chemo-radiotherapy. The results revealed significantly less inhibition of cell mediated immunity in EPA treated patients undergoing thoracic oesophagectomy.

In the first and only study of enteral EPA after oesophagectomy for malignancy to date, Aiko et al (2005) performed a retrospective study of 27 patients and investigated whether supplementation of enteral nutrition with 2.25 g EPA/day affected platelet aggregation, coagulation activity and inflammatory response compared to standard enteral nutrition. Seventeen patients received an enteral formula for 7 days post operatively containing 2.25g EPA/day, the remaining 11 patients received a standard enteral formula for 7 days containing 0.7 g EPA/day. The results showed that administration of EPA in enteral nutrition significantly inhibited the post-operative decrease in platelet count, attenuated D-dimer levels and significantly decreased levels of the proinflammatory cytokine, IL-8, on days 1 and 3 post operatively. The anti-inflammatory effects of EPA were confirmed by the clinical findings of lower body temperature. There was also a decrease in the duration of fever in a number of patients. While these results seem promising it does seem surprising however that these authors were able to demonstrate decreased levels of pro-inflammatory cytokines and lower body temperature at day 3 post operatively as the patients had only received <1 g EPA on post operative day 1 and 1.1 gs and 2.2gs respectively on post operative days 2 and 3. Further studies with peri-operative administration of EPA are warranted to confirm these findings. There also is a need for long term studies addressing the issues of perioperative EPA supplementation on body composition and quality of life outcomes post major upper gastrointestinal surgery.

The present study aimed to address several of these issues. Firstly the results of the gas chromatography showed that peri-operative administration of an enteral formula enriched with 2.2 g of EPA was successful in significantly raising the serum levels of EPA on post operative day 7 and 14, and the EPA significantly incorporated itself into the fatty acid cell membrane of peripheral blood mononuclear cells by post operative day 7. Thus we were successful in preloading cell membranes with EPA with this regimen.

The post operative morbidity rates in the present study are similar to outcomes reported by some of the largest international centres in the World (Bailey et al, 2003). There were no deaths in the present study. While we failed to show any difference in the rate of post operative complications between the two treatment groups we did observe a significantly lower body temperature in the first post operative week in patients given peri-operative

EPA than in those given a standard enteral feed. The effect on body temperature was confirmed on mixed-between-within analysis of variance where the effect over time could be attributed to the enteral product. The mechanism may relate to reduced production of proinflammatory cytokines such as TNFα, IL-6 and IL-8, as these can act as pyrogens through increased production of PGE2 which can act directly on the brain causing an upward shift in thermo regulation (Coceani & Akarus, 1998). By substituting AA in cell membranes with EPA, PGE3 is preferentially formed and thus a more anti-inflammatory milieu arises with less risk of fever. While the standard group did have a higher incidence of SIRS on post operative days 1- 7, the result was not statistically significant possibly reflecting poor study numbers. Further larger studies are warranted to investigate whether immunomodulating nutrients have the potential to promote restoration of normal tissue function post operatively and prevent the occurrence of SIRS (O'Flaherty, 1999).

Perhaps the greatest success of this regimen was in terms of its anabolic effects. This study is the first to our knowledge to demonstrate that enteral EPA given before and after oesophagectomy is superior to standard enteral nutrition in terms of preservation of nutritional status. Only 8% of EPA enriched enteral feeding patients lost 'severe' amounts of weight (>5%) by post operative day 21 versus 39% of Standard Enteral nutrition patients (p=0.03). This finding was confirmed on segmental body composition by bioelectrical impedance analysis which was performed at baseline and at day 21 post op - the EPA enriched feeding group maintained all aspects of their body composition with no difference in any values from pre op to post operative day 21. In contrast, the Standard enteral feeding group lost significant amounts of weight, particularly of lean mass from pre operative values to post operative day 21. The Standard enteral nutrition group lost a mean of 1.62 kg in weight (std dev 3.3), p=0.03, corresponding to a 0.56 (std dev 1.14) point drop in BMI, p=0.03. Standard enteral nutrition patients lost a significant amounts of lean mass - fat free mass fell by 1.9 kg (std dev 3.7), p=0.03, they lost significant amount of muscle from the leg (0.3kg (std dev 0.6, p=0.05; arm (0.17 kg(std dev 0.3), p=0.01, and trunk (1.44 kg(std dev 2.7), p=0.03.

The mechanism by which EPA results in a more anabolic state is reported to be linked to alterations in hormone levels (Barber et al., 2001). In preclinical studies, EPA has been shown to attenuate the catabolic effects of Lipid Mobilising Factor and Proteolysis Inducing Factor (PIF) (Tisdale, 1996; Lorite et al., 1997; Islam-Ali et al., 2001).

Furthermore, EPA has been shown to reduce urinary PIF levels (Barber et al., 2001), attenuate cachexia (Wigmore et al., 1996; Barber et al., 1997) and has also been associated with the halting or reversal of weight loss in advanced malignancy (Barber et al, 2000; Barber et al, 1999b; Barber et al., 1999c; Fearon et al, 2001b; Wigmore et al, 2000), improvements in physical functioning and quality of life and a prolongation of survival (Gogos et al, 1998). Down regulation of systemic and tumour derived factors such as C-reactive protein, TNF α, and IL-6 leads to a more anabolic state. While inflammation is essential for healing, immune processes and successful recovery after injury (Chen et al, 2005), uncontrolled systemic inflammatory responses lead to organ dysfunction and adverse outcome (Bone, 1994). Rapid decrease in nitrogen balance, with loss of lean body mass and with catabolic response is associated with acute phase proteins through the release of pro-inflammatory cytokines such as TNF-a and IL-6 after injury (Wigmore et al, 1997).

In the present study, the remarkable increase of serum CRP detected in the 3 weeks post operatively indicates the occurrence of severe inflammation after an extensive en bloc oesophagectomy with oesophageal reconstructive surgery. CRP levels remained significantly elevated in both treatment arms on day 21 compared to baseline. This increase has been observed by other authors (Wang et al, 1998). There was a trend towards a lower CRP level in the EPA enriched arm on post operative day 21($p=0.09$). We also observed significant increases in Serum Amyloid A at day 21 post op in both groups.

We also examined the effect of peri-operative enteral administration of EPA on the production of IL-2, 4, 6, 8 10, TNFα and MCP-1. We found no differences between groups in the production of IL-2, IL-4, and IL-6. Interleukin-6 (IL-6) and interleukin-10 (IL-10), among other cytokines, play important roles in host responses under a stressed state. IL-6 is considered an integral mediator of the physiologic acute-phase response to injury. However, excessive and prolonged post-injury elevations of circulating IL-6 levels have been associated with higher rates of morbidity and mortality (Drost et al, 1993; Schluter et al, 1991). In the present study IL-6 levels increased 200 fold on post operative day 1 reflecting the severity of the surgical insult. Yamada et al (1998) reported that more marked increases in the serum IL-6 levels were observed after oesophagectomy when compared with those levels after lung lobectomy in patients receiving thoracotomy.

In the present study levels of IL-8, a proinflammatory cytokine increased significantly over time in the Standard EN arm only – levels remained significantly elevated on post operative day 7 and 14. IL-10 also remained significantly elevated on post operative day 7 in the Standard EN arm whereas levels had returned to baseline in the EPA enriched arm. IL-10 is a cytokine that counteracts inflammatory responses and is also able to down-regulate cellular immunity by inhibiting type-1 T-helper cells, thus causing an immunosuppressive state in surgically stressed individuals (Fiorentino et al, 1991; Klava et al, 1997). In the present study IL-10 levels remained significantly elevated on post operative day 7 in the standard EN arm but had returned to baseline in the EPA arm. IL-10 is capable of suppressing type-1 cytokine production, leading to a reduced ability to generate a delayed type of hypersensitivity response that is observed after surgery, burn, or trauma (Fiorentino et al, 1991). Klava et al. (1997) reported that IL-10 played a role in the development of postoperative immunosuppression in patients undergoing major abdominal surgery. Therefore, IL-10 has been considered a cytokine that inhibits cell-mediated immunity.

In the present study TNFα levels did not change significantly in the peri-operative period in the EPA treatment but did change significantly in the Standard EN arm with levels remaining significantly elevated on post operative days 1 and 7 compared to baseline. The higher ratio of omega 3 to omega 6 in membrane phospholipids is associated with a reduced production of proinflammatory cytokines like IL-6 and TNFα in response to an inflammatory stimulus (Caughey et al, 1996). The reason levels of TNFα differ between groups may relate to the ability of omega 3 PUFA to facilitate production of PGE3 in place of PGE2, the former being effective in relieving immunosuppression (Xu et al, 2006; Tsekos et al 2004). High levels of omega – 3 PUFA can decrease the synthesis of PGE2 and inhibit the formation of TNF-α. High PGE2 levels can also depress the cytotoxicity of macrophages, lymphocytes and Natural Killer cells (Kudsk et al, 1992).

Peri-operative EPA administration was also associated with lower production of Monocyte chemoattractant protein-1 (MCP-1), patients given standard EN showed significant increases on post operative days 3 and 7 compared to baseline with no change in the EPA arm. MCP-1 influences type II cytokine production and cell mediated immunity. MCP-1 levels are directly correlated with the severity of surgical stress and are also inversely correlated with cell mediated immunity (Shibasaki et al, 2006). .

Regarding immune function the percentage of T cells decreased significantly post operatively in both treatment groups. By day 7 levels had recovered in the EPA arm but remained significantly lower in the Standard EN arm. There was no significant difference between the percentages of CD4 cells peri-operatively in either treatment arm with both groups showing similar responses. For CD8 there was a significantly greater decline in levels on post operative days 1, 3 and 7 in the standard arm, in contrast the EPA arm maintained levels on days 1 and 3 and only had a significant decline form baseline on day 7. Thus EPA patients showed a significant increase in the CD4 to CD8 ratio in the first post operative week with no change in the standard EN arm. Natural Killer Cells increased significantly on day 1 in EPA patients whereas levels fell post operatively in standard arm. B cells increased significantly over time in both groups.
It has been reported that total lymphocyte count, CD4:CD8 ratio and natural killer cells activity decrease following surgical operation (Wang et al, 1998) and some workers have suggested that surgical operations induce a reversible depression of the cellular immune status that precedes plasma suppressive activity in its return to the preoperative level. In the present study the ratio of CD4 to CD8 cells increased significantly in the EPA arm from pre op to post op day 7. This change suggests that oesophageal resection results in a shift of the immunoregulatory system to helper cells when patients are given peri-operative EPA but not standard feeds. Activated CD4 cells secrete cytokines, which in turn activate various effector cell populations (Wang et al, 1998). For Natural Killer cells EPA administration seemed to prevent the reduction in NKC counts post operatively - patients demonstrated a significant increase in counts on post operative day 1 and a trend for post operative day 3. In contrast, in the standard EN arm counts fell significantly with no significant difference in the % of NK cells peri-operatively. Most patients with malignant tumours have low levels of NK cell activity, which is further depressed over 1-2 weeks following surgical stress (Hansbrough et al, 1984 Mafune & Tanaka, 2000). As with previous reports (Tsutsui et al, 1992) the lymphocyte counts fell significantly post oesophagectomy – with a similar drop in both treatment arms. While the immune cell results do not seem to impact on the clinical observation of infectious complications further studies with perioperative EPA are warranted to examine in greater detail the activities of these cells and their potential clinical relevance.

With regard to coagulation recent studies have shown that increased levels of D-Dimers predict the occurrence of deep-vein thrombosis after elective hip surgery (Eekhoff et al,

2000) and also the recurrence of venous thromboembolism after discontinuation of oral anti-coagulant therapy (Palareti et al, 2002). These high D-dimer levels indicate increased turnover of cross linked fibrin and signify a hypercoagulable state and are therefore a marker of a prothrombotic condition (Aiko et al, 2005). In the post operative period, inflammatory processes caused by surgery or underlying disease may lead to a marked increase in plasma D-dimer concentrations (Bounameux et al, 1992). Aiko et al (2005) in a study of oesophagectomy patients given 2 g of enteral EPA post operatively only showed a significant difference in the D-Dimer level on post operative day 2. This result is quite surprising as the patients only started the study product on day 1 post operatively and so it seems unlikely any differences on day 2 were related to EPA. This group recommended further studies that take serial measurements of a range of coagulation and fibrinolytic parameters post operatively. In the present study we found no difference in the D Dimer levels or Prothrombin time between EPA enriched feeding and standard enteral feeding even though we administered a similar amount of EPA peri-operatively to that in the study by Aiko et al (2005).

An important point in the interpretation of immunonutrition trials relates to the potential impact of genotype on immune responsiveness (Fritsche, 2006). This is illustrated by the findings of Grimble (2001) who investigated the impact of n-3 PUFA on ex vivo TNF-α biosynthesis. They found that their failure to observe an overall significant effect of n-3 PUFA on ex vivo TNFα biosynthesis was related to genetic polymorphism within the lymphotoxin gene in their subject population. Subjects in the lowest tertile of TNFα production responded to n-3 PUFA supplementation with a 43% reduction in production, in the other tertiles n-3 PUFA had no effect or increased production. Thus genetic variability in the human population may make it quite difficult to understand how biologically active nutrients, such as PUFA, affect the immune system and subsequently human health (Fritsche, 2006). Another important point is control of background diets before intervention. It is extremely difficult to reliably estimate intake of long chain PUFA and naturally occurring variations in AA intake may significantly impact responsiveness to n-3 PUFA supplementation (Fritsche, 2006). In the present study we have however successfully shown that the baseline n-3 PUFA intake was not difference between the two groups as confirmed by Gas Chromotography results of PBMC phospholipids membrane. In addition many of the published studies on n-3 PUFA are underpowered, with low subject numbers frequently cited as the explanation when

numerical differences between groups fail to reach statistical significance. While conducting the present study we learnt of the difficulties and challenges surrounding the conduction of randomised blinded trials and the length of time needed to achieve a respectable patient population that allows meaningful statistical analysis. Another problem in the interpretation of clinical trials with immunonutrients is varying degrees of PUFA supplementation, or use of too low a level of PUFA (Kew et al, 2003). In the present study we achieved a significant increase in serum and PBMC membrane levels with a dose of 2.2g/day of EPA which is available in commercially available formulas. We feel that future studies with EPA should aim for this level of supplementation to expand our understanding of EPA's role in immuno-inflammation and anabolism.

7.8 CONCLUSION

In conclusion this prospective randomised controlled trial provides first evidence that peri-operative enteral administration of 2.2g EPA/day improves nutritional status by modulating endogenous production of cytokines after major upper GI surgery. Our data further supports the hypothesis that a 'preloading' of cell membrane phospholipids with active precursors of desirable immune modulators is beneficial for patients undergoing major surgery for malignancy.

CHAPTER 8

GENERAL DISCUSSION & CONCLUSIONS

8.1 CONCLUSIONS

Oesophageal Cancer remains an important public health problem worldwide. Understanding and preventing the occurrence of this cancer are complicated by the fact that the two major histological subtypes, SCC and adenocarcinoma, differ substantially in their underlying patterns of incidence and key aetiologial factors. The main characteristic that they share is a high mortality rate.

The aetiology of oesophageal adenocarcinoma is only recently beginning to be understood. Although smoking still accounts for a large number of cases, it is not an overwhelming risk factor as it is for SCC. Alcohol appears to play very little role in the development of Adenocarcinoma. Instead chronic reflux and obesity appear to be the driving forces behind it's recent epidemic increase (Holmes & Vaughan 2006).

Evidence regarding the clear independent association between obesity and oesophageal adenocarcinoma is now accumulating. Data in this thesis provides the first Irish evidence supporting this observation. We have shown that obesity increases the risk of oesophageal cancer in a dose dependent manner – increasing the risk of lower oesophageal adenocarcinoma 11 fold and gastric cardia adenocarcinoma 3.5 fold. However, there is as yet no evidence that weight reduction, dietary improvement, and/or physical exercise will actually reduce the risk of adenocarcinoma, but this is a promising area of prevention research. Instead research needs to point to precursor lesions such as Barrett's oesophagus in an effort to understand the precise mechanisms linking obesity to cancer.

Future basic scientific research aimed at understanding how adipokines, insulin, and insulin–like growth factors stimulate cancer progression will aid in determining which factors are primary and which are secondary in the development of the metabolic syndrome and how we can potentially intervene in these pathways by use of pharmacological inhibitors, behavioral modification or gene therapy (Cowey & Hardy, 2006). The metabolic syndrome should be considered a high risk state for certain types of cancer and this relationship should be systematically explored across different cancer types as well as across cancerous precursors for other malignancies such as colorectal polyps. Individual components of the metabolic syndrome contribute to the development of several processes, including insulin resistance, aromatase activity, adipokine production, angiogenesis, glucose utilization, and oxidative stress/DNA damage, which can work together to increase cancer risk beyond that of the individual component alone and this too needs careful consideration in the context of large prospective case-control

studies. This thesis provides the first report on the incidence on metabolic syndrome in the precursor lesion for oesophageal adecnoarcinoma i.e. Barrett's oesophagus. This study should be expanded to incorporate better numbers to allow for age and sex matching. Data concerning the prevalence of metabolic syndrome amongst healthy controls is also urgently needed in Ireland.

Further studies evaluating cancer risk in patients diagnosed with the metabolic syndrome are essential for determining whether individual components of the metabolic syndrome act together or synergistically/additively to increase the risk of cancer development compared with individual risk factors. If the individual components of the metabolic syndrome are additive in predisposing to cancer, which may be the case, then controlling even just one or two of these components may significantly contribute to living a longer, healthier, cancer-free life.

Part II of this thesis adds to accumulating scientific knowledge on the risks of multimodality therapy for oesophageal cancer in terms of post operative infectious complications. Notwithstanding the controversy whether oncologic benefit accrues from multimodal regimens, informed decision-making requires better information on other end-points, including quality of life outcomes, toxicity of neoadjuvant regimens, and operative complications. Intuitively, the administration of chemotherapy and radiation therapy prior to major surgery presents an added challenge, both through treatment-related immunosuppression and direct tissue toxicity from radiation.

Better understanding of peri-operative risk factors aids in this decision making process. The literature has identified several pre-operative risk factors, amongst which is pre-operative hypo-albuminaemia, which has been shown to be an independent prognostic indicator of overall morbidity and mortality, and prolonged hospital stay, in surgical and critically ill patients (Gibbs et al, 1999). It is well known that Hypo-albuminaemia is independently associated with the development of post-operative complications, especially the development of infective complications (Schwartz et al, 2004; Bone et al 1992; Dewar et al, 1992; Rey-Ferro et al, 1997; Mullen et al, 1979). In upper gastrointestinal cancer surgery low pre-operative serum albumin levels are significantly correlated with anastamotic leak, general post-operative morbidity and post-operative mortality (Buzby et al, 1980; Detsky et al, 1987; Kudsk et al, 2003). Our hypothesis in chapter 5, that albumin may reflect immuno-inflammation and may be a marker of the magnitude of this response, was the primary focus of this study, which to our knowledge

is the first report on early postoperative hypoalbuminaemia and short-term outcome after major upper gastrointestinal surgery. This study has demonstrated the positive association between a low serum albumin on the first post-operative day and the development of complications and overall in-hospital mortality. This was still significant when factors such as haematocrit and urea were taken into account, thus out-ruling the possibility of a dilutional effect on serum albumin concentrations. We have shown that a critical albumin level of < 20 g/l on the first post operative day was an independent predictor of complications - it was associated with a doubling of in-hospital complication rate, a 3.5 fold increase the rate of respiratory failure and a five fold increase in the incidence of ARDS and in-hospital mortality. It was also was predictive of the need for longer HDU and ICU stays and the need to return to the operating theatre for further surgery. In fact on multivariate logistic regression only 3 factors could predict poor outcome: female gender, smoking and day 1 albumin. Importantly, the absolute level of albumin on the first postoperative day rather than a percentage change from preoperative levels was the significant measurement, suggesting that profound hypoalbuminemia is a serum marker of a heightened systemic response with associated adverse risks. The equivalent outcomes in the 20-25g/L group and the 25-30g/L group are also consistent with this thesis. Further research on the relationship of hypoalbuminemia to the early systemic immuno-inflammatory response following major surgery is required, as well as a better understanding of the therapeutic implications.

Chapter 6 of this thesis provides one of the first reports on needle catheter jejunostomy feeding post oesophagectomy that focuses not only on mechanical complications but also on nutritional outcomes. Many Irish Upper Gastrointestinal surgical units do not routinely provide any nutritional support post oesophagectomy, and those that do usually administer the jejunostomy feeds at very low infusion rates, inadequate to meet nutritional requirements. While the literature would agree that NCJ feeding is a safe means of providing nutrition support (Sarr & Mayo, 1988; Sica et al, 2005), nutritional outcomes are rarely reported. This audit shows that NCJ feeding is a safe and effective method of providing nutritional support post oesophagectomy. It is well tolerated and is effective in preventing severe weight loss in the postoperative period. In a subset of patients who experience post operative surgical and medical complications NCJ allows for prolonged enteral access and avoids the need for TPN. For patients requiring home enteral feeding, insertion of a NCJ at the time of surgery avoids the need for invasive

interventions at a later stage. Severe complications associated with this method of nutrition support are extremely rare. This audit has also highlighted the high incidence of rapid weight loss that occurs following discharge and that many more patients than the 8% reported in chapter 6 should be considered for home supplemental feeds via the jejunostomy - this now informs current Unit policy, as this nutritional deficit could negatively impact on complications and quality of life following hospital discharge.

Chapter 6B highlights a role for nutrition support post gastrectomy. There is again controversy in the literature regarding the optimum route or role for nutrition support post gastrectomy (Gabor et al, 2005) with very few long term nutritional outcome studies. Much of the published literature on the subject dates from the 1980's. Our research highlights the nutritional problems experienced by patients with gastric cancer undergoing surgery. There is a high prevalence of malnutrition at diagnosis, and total gastrectomy is associated with dramatic weight loss post-operatively, with patients losing an average of 15.5 kgs from diagnosis to 3-month follow up. Provision of nutrition support in the form of TPN post-operatively significantly reduces in-hospital weight loss and also helps to attenuate further weight loss post discharge. Although non-randomized, the experience of this Unit would support the provision of TPN to patients following total gastrectomy.

Finally in Chapter 7 we have conducted the first peri-operative double blinded randomised trial with Eicosapentaenoic acid in the setting of major cancer surgery. This study was designed as a direct result of the observations regarding nutritional deterioration in chapter 6A that occurs post oesophagectomy and also as a direct result of the high morbidity rates observed in chapter 4. We reported that enteral nutrition enriched with 2.2 g EPA/day for 5 days pre-op and 21 days post-oesophagectomy is associated with preservation of lean body mass, lower body temperature, improved immune function and an attenuated pro-inflammatory response to surgery compared with standard EN. The anabolic properties of EPA may have practical implications for patients not only with this cancer, but with the increasing number of solid tumours where multimodality therapy may supplant surgery alone, including lung, head and neck, and rectal cancer. Further longer term studies are needed to confirm our observations. Data is also needed on the potential impact of longer term supplementation of EPA enriched enteral nutrition on issues such as quality of life following discharge from hospital.

REFERENCES

Abou-Jawde RM, Mekhail T, Adelstein DJ, Rybiki LA, Mazzone PJ, Carroll MA, Rice TW. Impact of induction concurrent chemotherapy on pulmonary function and post-operative acute respiratory complications in esophageal cancer. *Chest*, 2005, 128(1):250-5.

Adelstein DJ, Rice TW, Becker M, Larto MA, Kirby TJ, Koka A, Tefft M, Zuccaro G. Use of concurrent chemotherapy, accelerated fractionation, and surgery for patients with esophageal carcinoma. *Cancer*, 1997, 80(6), 1011-1020.

Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends in Endocrinology and Metabolism* 2000, 11:327-332

Aiko S, Yoshizumi Y, Sugiura Y, Matsuyama T, Naito Y, Matsuzaki J, Maehara T. Beneficial effects of immediate enteral nutrition after esophageal cancer surgery. *Surgery Today,* 2001;31(11):971-8

Aiko S, Yoshizumi Y, Tsuwano S, Shimanouchi M, Sugiura Y, Machara T. The Effects of immediate enteral feeding with a formula containing high levels of omega-3 fatty acids in patients after surgery for esophageal cancer. *Journal of Parenteral and Enteral Nutrition,* 2005, 29(3): 141-147.

Alberti KG, Zimmet P, Shaw J. Metabolic syndrome-a new world-wide definition.A consensus statement from the international diabetes federation. *Diabetes Medicine,* 2006; 23:469-480.

Alberts SR, Cervantes A, van de Velde CJH. Gastric Cancer: epidemiology, pathology and treatment. *Annals of Oncology*, 2003:14 (supp 2), ii31-ii36.

Allison SP. The uses and limitations of nutrition support. *Clinical Nutrition*, 1992, 11:

319-330

Altorki N, Kent M, Ferrara RN, Port J. Three-field lymph node dissection for squamous cell and adenocarcinoma of the esophagus. *Annals of Surgery* 2002; 236: 177-183.

Altorki NK, Sunagawa M, Little AG, Skinner dB. High grade dysplasia in the columnar-lined esophagus. *American Journal of surgery* 1991, 161;97-99.

Ambrecht U, Lundell L, Lindstedt G, Stockbruegger RW. Causes of malabsorption after total gastrectomy with Rouz-en-Y reconstruction. *Acta Chirurgica Scandinavica.* 1988;154:37.

Ancona E, Ruol A, Santi S, Merigliano S, Sileni VC, Koussis H, Zaninotto G, Bonavina L, Peracchia A. Only pathologic complete response to neo-adjuvant chemotherapy improves significantly the long term survival of patients with resectable esophageal squamous cell carcinoma: final report of a randomised, controlled trial of preoperative chemotherapy versus surgery alone. *Cancer;* 2001;91:2165-74.

Andreyev HJ, Norman AJ, Oates J, Cunningham D, Why do patients with weight loss have a worse outcome when undergoing chemotherapy for gastrointestinal malignancies? *European Journal of Cancer* 1998 34 (4), pp. 503–509.

Apinop C, Puttisak P, Preecha N. A prospective of combined therapy in esophageal cancer. *Hepatogastroenterology* 1994; 41:391-3.

Argilés JM, Busquets S, Lopez-Soriano FJ, Cytokines in the pathogenesis of cancer cachexia. *Current Opinion in Clinical Nutrition and Metabolic Care*, 2003, 6(4);401–406.

Argilés JM, Moore-Carrasco R, Busquets Sand, Lopez-Soriano FJ. Catabolic mediators as targets for cancer cachexia. *Drug Discovery Today* 2003, 8(18);838–844.

Attwood SEA, Ball Cs, Barlow AP, Jenkinson L, Norris TL, Watson A. Role of intragastric and intraoesophageal alkalinisation in the genesis of complications in

Barrett's columnar lined lower oesophagus. *Gut.* 1993, 34:11-15.

Bae JM, Park JW, Yang HK, Kim JP. Nutritional status of gastric cancer patients after total gastrectomy. *World Journal of Surgery*, 1998 Mar;22(3):254-60;

Bahmanyar S, Ye W. Dietary patterns and risk of squamous-cell carcinoma and adenocarcinoma of the esophagus and adenocarcinoma of the gastric cardia: a population-based case-control study in Sweden. *Nutrition and Cancer*, 2006;54(2):171-8

Baigrie RJ, Devitt PG, Watkin DS. Enteral versus parenteral nutrition after oesophagogastric surgery: a prospective randomized comparison. *The Australian and New Zealand Journal of Surgery,* 1996, 66(10):668-70.

Bailey SH, Bull DA, Harpole DH, Rentz JJ, Neumayer LA, Pappas TN, Daley J, Henderson WG, Krasnicka B, Khuri SF. Outcomes after esophagectomy: a ten-year prospective cohort. *Annals of Thoracic Surgery*. 2003; 75(1):217-22.

Balart J, Balmana J, Rius X, Salazar R, Gallen M, Navarro S, Arcusa A, Gallardo E, Brunet J. Treatment of oesophageal cancer with preoperative chemoradiotherapy may increase operative mortality. Eur*opean Journal of Surgical Oncology*. 2003 Dec; 29(10): 884-9.

Barak N, Ehrenpreis ED, Harrison JR, Sitrin MD. Gastro-oesophageal reflux disease in obesity:pathophysiology and therapeutic considerations. *Obesity Reviews.* 2002, 3:9-15.

Barber MD, Fearon KCH, Tisdale MJ, McMillan DC,Ross JA. Effect of a fish oil-enriched nutritional supplement on metabolic mediators in patients with pancreatric cancer cachexia, *Nutrition and Cancer* 2001,40(2);118–124.

Barber MD, McMillan DC, Preston T, Ross JA, Fearon KC. Metabolic response to feeding in weight losing pancreatic cancer patients and it's modulation by a fish oil enriched nutritional supplement. *Clinical Science*, 2000;98:389-399.

Barber MD, Ross JA, and Preston T. Fish oil-enriched nutritional supplement attenuates

progression of the acute-phase response in weight losing patients with advanced pancreatic cancer. *Journal of Nutrition*, 1999a, 129:1120-1125.

Barber MD, Ross JA, Fearon KC, Disordered metabolic response with cancer and its management. *World Journal of Surgery* 2000, 24(6); 681–689.
Barber MD, Ross JA, Fearon KC. Changes in nutritional, functional, and inflammatory markers in advanced pancreatic cancer. *Nutrition and Cancer* 1999a, 35(2);106–110.

Barber MD, Ross JA, Fearon KCH. The anti-cachectic effects of fatty acids. *Proceedings of the Nutrition Society* 1998, 57:571-576.

Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KCH. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *British Journal of Cancer* 1999b, 81:80-86.

Barber MD, Wigmore SJ, Ross JA, Fearon KCH. Eicosapentaenoic acid attenuates cachexia associated with advanced pancreatic cancer. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 1997, 57;204.

Barr H, Shepard NA, Dix A, Roberts DJH, Tan WC, Krasner N. Eradication of high grade dysplasia in columnar-lined esophagus by photodynamic therapy with endogenously generated photoporphyrin-IX. *Lancet* 1996,348:584-585.

Bartels H, Stein HJ, Siewert JR. Preoperative risk analysis and post operative mortality of oesophagectomy for respectable oesophageal cancer. *British Journal of Surgery* 1998, 85, 840-844.

Bauer J, Capra S, Ferguson M. Use of the scored patient generated subjective global assessment tool in patients with cancer. *European Journal of Clinical Nutrition* 2002, 56:779-786.

Beale R, Bryg DJ, Bihari DJ. Immunonutrition in the critically ill: A systematic review of clinical outcome. *Critical Care Medicine*, 1999, 27, 2799-2805.

Beck AM, Ovesen L. At which body mass index and degree of weight loss should hospitalised elderly patients be considered at nutritional risk? *Clinical Nutrition* 1998:17(5):195-198.

Beck SA, Smith KL, Tisdale MJ. Anticachectic and antitumor effect of eicosapentaenoic acid and its effect on protein turnover, *Cancer Research* 1991, 51 (22);6089–6093.

Begg CB, Cramer LD, Hoskins WJ, Brennan MF. Impact of hospital volume on operative mortality for major cancer surgery. *JAMA Journal of the American Medical Association* 1998; 280: 1747-51

Beier-Holgerson R, Boesby S. Influence of postoperative enteral nutrition on postsurgical infections. *Gut,* 1996, 39(96):833-5

Benzoni E, Terrosu G, Bresadola V, Uzzau A, Intii S, Nocec L, Cedolini C, bresadola F, DeAnna D. A comparative study of the transhiatial laparosopic approach versus laparosopi gastric mobilisation and riht open transthoracic esophagectomy for esophageal cancer. *Journal of Gastrointestinal and liver disease,* 2007, 16(4):395-401.

Bergstrom A, Pisani P, Tenet V, Wolk A, Adami HO. Overweight as an avoidable cause of cancer in Europe. *International Journal of Cancer.* 2001, 91(3):421-30

Bianchini F, Kaaks R, Vainio H. Overweight, obesity, and cancer risk. *Lancet Oncology.* 2002, 3(9):565-74

Biffi R, Lotti M, Cenciarelli S, Luca F, Pozzi S, Zambelli M, Marzona L, Andreoni B. Complications and long-term outcome of 80 oncology patients undergoing needle catheter jejunostomy placement for early postoperative enteral feeding. *Clinical Nutrition.* 2000;19(4):277-9

Blackburn GL, Bistrian BR, Maini BS, Schlamm HT, Smith MF Nutritional and metabolic assessment of the hospitalized patient. *JPEN Journal of Parenteral and Enteral Nutrition.* 1977;1(1):11-22.

Blackstock AW. Esophageal Cancer, *Seminars in Radiation Oncology*, 2007, 17(1):1

Blay YJ, Negrier S, Combaret V, Attali S, Goillot E, Merrouche Y, Mercatello A, Ravault A, Tourani JM, Moskovtchenko JF. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma, *Cancer Research* 1992, 52(12); 3317–3322.

Blee TH, Belzer GE Lambert PJ. Obesity: is there an increase in peri-operative complications in those undergoing elective colon and rectal resection for carcinoma? *The American Surgeon* 2002, 68(2): 163-6

Blok WL, Katan MB, Van Den Meer JWM. Modulation of Inflammation and cytokine production by dietary (n-3) fatty acids. *The Journal of Nutrition* 1996, 126 (6):1515-1533.

Blot WJ, De Vesa SS, Kneller RW, Fraumeni JF Jr. Rising incidence of adenocarcinoma of the oesophagus and gastric cardia. *JAMA Journal of the American Medical Association*; 1991; 265: 1287-1289

Blot WJ, McLaughlin JK. The changing epidemiology of esophageal cancer. *Seminars in Oncology*, 1999, 26:2-8.

Bone RC, Sibbald WJ, Spring CL. The ACCP-SCCM consensus conference on sepsis and organ failure. *Chest* 1992; 101: 1481-3.

Bone RC. Sepsis and its complications: the clinical problem. *Critical Care Medicine*, 1994, 22:S8-11.

Bosset J-F, Gignoux M, Tiboulet J-P, et al. Chemoradiotherapy followed by surgery compared with surgery alone in squamous cell cancer of the esophagus. *New England Journal of Medicine* 1997; 337: 161-7.

Bounameaux H, Khabiri E, Huber O. Value of liquid crystal contact thermography and plasma level of D-dimer for screening for deep vein thrombosis following general abdominal surgery. *Thromb Haemost,* 1992, 67:603-606.

Boyle P & Ferlay J. Cancer incidence and mortality in Eurpoe, 2004. *Annals of Oncology,* 2005, 16:481-488.

Bozetti, ravera E, Cozzaglio L, Dossena G, Agradi E, Bonfanti G, Koukouras D, Gennari L. Comparison of nutritional status after total or subtotal gastrectomy. *Nutrition,* 1990;6(5):371-5.

Bozzetti F. Nutrition and gastrointestinal cancer, *Current Opinion in Clinical Nutrition and Metabolic Care* 2001, 4(6);541–546.

Braga M, Gianotti L, Radaelli G. Perioperative immunonutrition in patients undergoing cancer surgery: results of a randomized double-blind phase 3 trial. *Archives of Surgery,* 1999, 134:428-433.

Braga M, Vignali A, Gianotti L. Immune and nutritional effects of early enteral nutrition after major abdominal operations. *European Journal of Surgery* 1996;162:105-12.

Braga M. Immunonutrition: from laboratory to clinical practice. *Nutrition,* 2007, 23:368-370

Bragelmann R, Armbrecht U, Rosemeyer D, Schneider B, Zilly W, Stockbrugger RW. Nutrient malassimilation following total gastrectomy. *Scandinavian Journal of Gastroenterology,* Suppl, 1996;218:26-33

Braunschweig C, Gomez S, Sheean PM. Impact of declines in nutritional status on outcomes in adult patients hospitalised for more than 7 days. *Journal of the American Dietetic Association* 2000, 100:1316-1322.

Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the united states. *Surgical Oncology Clinics of North America*, 2002, 11:235-256.

Brown LM, Silverman DT, Pattern LM. Adenocarcinoma of the esophagus and esophagogastric junction in white men in the United States: alcohol, tobacco, and socio-economic factors. *Cancer Causes and Control*, 1994;5:333-40.

Brown, LM, Swanson CA, Gridley G, Swanson GM, Schoenberg JB, Greenberg RS, Silverman DT, Pottern LM, Hayes RB, Schwartz AG, Liff JM, Fraumeni JF, Hoover RN. Adenocarcinoma of the oesophagus: Role of obesity and diet. *Journal of the National Cancer Institute; 1995:* 87(2), 104-9.

Burmeister BH, Smithers BM, Gebski V , Fitzgerald L, et al. Surgery alone versus chemoradiotherapy followed by surgery for respectable cancer of the oesophagus: a randomised controlled phase III trial. *Lancet Oncology* 2005; 6: 659-68

Buzby GP, Mullen JL, Matthews DC. Prognostic nutritional index in gastrointestinal surgery. *American Journal of Surgery*. 1980; 139: 160 – 167

Cabal-Manzano R, Bhargava P, Torres-Duarte A, Marshall J, Bhargava P, Wainer IW. Proteolysis-inducing factor is expressed in tumours of patients with gastrointestinal cancers and correlates with weight loss, *British Journal of Cancer* 2001,84(12);1599–1601.

Calder PC. Dietary modification of inflammation with lipids. *Proceedings of the Nutrition Society*, 2002, 61:345-358.

Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids*, 2003, 38:343-352.

Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nature Reviews Cancer*. 2004; 4(8): 579-91

Calle EE, Rodriguez C, Walker-Thurmund K, Thun MJ. Overweight, Obesity and mortality from cancer in a prospectively studied cohort of U.S adults. *New England Journal of Medicine*. 2003; 348(17): 1625-1638.

Calle EE, Thun MJ, Petrelli J, Rodriguez C, Heath CW. Body Mass Index and mortality in a prospective cohort of US adults. *New England Journal of Medicine*, 1999;341:1097-105.

Cameron AJ, Ott BJ, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. *New England Journal of Medicine* 1985;313:857-9.

Cannon JG, Nerad JL, Poutsiaka DD. Measuring circulating cytokines. *Journal of Applied Physiology,* 1993; 75(4):1897-902

Cariati A, Casano A, Campagna A, Cariati E, Pescio G. Prognostic factors influencing morbidity and mortality in esophageal carcinoma. *Revista do Hospital das Clínicas.* 2002 Sep-Oct;57(5):201-4

CarpentierYA, Simoens C, Siderova V. Recent developments in lipid emulsions: relevance to intensive care. *Nutrition,* 1997, 13:73-78.

Carr CS, Ling KDE, Boulos P, Singer M. Randomised trial of safety and efficacy of immediate postoperative enteral feeding in patients undergoing gastrointestinal resection. *British Medical Journal*; 1996: 312:869-871

Caruso C, Lio D, Cavallone L, Franceschi C. Aging, longevity, inflammation, and cancer, *Annals of the New York Academy of Science* 2004,1028; 1–13

Caruso A. Licenziati S. Corulli M. Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation. *Cytometry,* 1997, 27(1):71-6.

Caughey GE, Mantzoris E, Gibson RA, Cleland LG. The effect on human tumour necrosis factor alpha and interleukin 1 beta production of diets enriched in n-3 fatty acids from vegetable and fish oil. *American Journal of Clinical Nutrition,* 1996, 63:116-122.

Caygill CPJ, Watson A, Lao-Sirieix P, Fitzgerald RC. Barrett's Oesophagus and adenocarcinoma. *World Journal of Surgical Oncology*. 2004. 2(1):12

Chak A , Lee T, Kinnard MF, *et al.* Familial aggregation of Barrett's oesophagus, oesophageal adenocarcinoma, and oesophagogastric junctional adenocarcinoma in Caucasian adults. *Gut* 2002;51:323–8.

Chalasani N, Wo JM, Hunter JG, Waring JP. Significance of intestinal metaplasia in different areas of esophagus including esophago-gastric junction. *Digestive Diseases Science* 1997, 42:603-607.

Chan A, Wong A. Is combined chemotherapy and radiation therapy equally effective as surgical resection in localized esophageal cancinoma? *International journal of radiation oncology, biology, physics*; 1999, 45(2), 265-270.

Chang CS, Poon SK, Lien HC. The incidence of reflux esophagitis among the Chinese. *American Journal of Gastroenterology* 1997, 92(4):668-71.

Chate A. A pilot audit of weight loss in upper gastrointestinal oncology outpatients. *Journal of Human Nutrition and Dietetics* 2006, 19(6):447-50.

Cheadle WG. The human leukocyte antigens and their relationship to infection. *American Journal of Surgery,* 1993, 165(2A Suppl):75S-81S.

Chen H, Tucker KL, Graubard BI. Nutrient intakes and adenocarcinoma of the esophagus and distal stomach. *Nutrition and Cancer*, 2002; 42:33-40.

Chen DW, Fei ZW, Zhang YC, Ou JM, Xu J. Role of enteral immunonutrition in patients with Gastric carcinoma undergoing major surgery. *Asian Journal of Surgery*, 2005, 28(2): 121-124.

Cheng KK, Sharp L, McKinney PA, et al. A case-control study of oesophageal adenocarcinoma in women: a preventable disease. *British Journal of Cancer.* 2000; 83(1): 127 – 32.

Chidel MA, Rice TW, Adelstein DJ, Kuplelian PA, Suh JH, Becker M. Resectable esophageal carcinoma:local control with neo-adjuvant chemotherapy and radiation therapy. *Radiology*, 1999;213:67-72

Chin KF, Townsend S, Wong W, Miller GV. A prospective cohort study of feeding needle catheter jejunostomy in an upper gastrointestinal surgical unit. *Clinical Nutrition.*

2004;23(4):691-6.

Choban PS, Flancbaum L. Nourishing the Obese Patient. *Clinical Nutrition* 2000 19(5): 305-311

Chow WH, Blaser MJ, Blot WJ, Gammon MD, et al. An inverse relationship between cagA+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Research* 1998; 58: 588-90.

Chow WH, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West B, Rotterdam H, Niwa S, Fraumeni JF. Body Mass Index and Risk of Adenocarcinoma of the oesophagus and gastric cardia. *Journal of the National Cancer Institute; 1998:* 90(2) 150-155.

Chow WH, Finkle WD, McLaughlin JK, *et al.* The relation of gastroesophageal reflux disease and its treatment to adenocarcinomas of the esophagus and gastric cardia. *JAMA: Journal of the American Medical Association* 1995;274:474–7

Coceani F, Akarus ES. Prostaglandin E2 in the pathogenesis of fever: an update. *Annals of the New York Academy of Science* 1998, 856:48-52.

Cohen JW. Statistcial Power analysis for the behavioural sciences, 1988, (2nd Edition). Hillsdate, NJ: Lawrence Erlbaum Associates.

Colangelo LA, Gapstur SM, Gann PH, Dyer AR, Liu K. Colorectal cancer mortality and factors related to the insulin resistance syndrome. *Cancer Epidemiology and Biomarkers Prevention,* 2002, 11:385-391.

Collard JM, Otte JB, Fiasse R. Skeletonizing en block esophagectomy for cancer. *Annals of Surgery*, 2001, 243:25-32.

Corish CA, Flood P, Mulligan S, Kennedy NP. Apparent low incidence of under nutrition in Dublin hospital in-patients: should we review the anthropometric thresholds for clinical practice? *British Journal of Nutrition*, 2000:84:325-335.

Cowey S, Hardy RW. The metabolic Syndrome: A high risk sate for cancer? *The American Journal of Pathology*, 2006, 169(5): 1505-1522.

Crew KD, Neugut AI. Epidemiology of upper gastrointestinal malignancies. *Seminars in Oncology*, 2004, 31:450-464.

Cristallo M, Braga M, Agape D, Primignani M, Zuliani W. Nutritional status, function of the small intestine and jejunal morphology after total gastrectomy for carcinoma of the stomach. *Surgery, Gynaecology and Obstetrics.* 1986, 163:225.

Crogan NL, Pasvogel A. The influence of protein-calorie malnutrition on quality of life in nursing homes. The *Journals of Gerontology. Biological Sciences and Medical Sciences*, 2003, 58:159-164.

Cunningham D, Jankowski J, Miles A. The effective Management of Upper Gastrointestinal Malignanices. UK Advances in Clinical Practice Series 2005. Aesculapius Medical Press (London) 2005.

Daly JM, Fry WA, Little AG. Esophageal cancer: results of an American College of Surgeons Patient Care Evaluation Study. *Journal of the American College of Surgeons*, 2000; 190:562-72.

Daly JM, Karnell L, Menck HR. National Cancer Data Base report on esophageal carcinoma. *Cancer.* 1996,78:1820-8.

Delaney HM, Carenevale NH, Garvey JW. Jejunostomy by a needle catheter technique. *Surgery.* 1973, 73:786-945.

Delgado-Aros S, Locke GR, Camilleri M. Obesity is associated with increased risk of gastrointestinal symptoms: A population based study. *American Journal of Gastroenterology* 2004;99:1801-6

Delves PJ, Roitt IM. The immune system: First of two parts. *New England Journal of Medicine* 2000; 343 (1): 37 – 49.

den Broeder E, Lippens RJ, van't Hof MA, Tolboom JJ, van Staveren WA, Hofman Z, Sengers RC. Effects of naso-gastric tube feeding on the nutritional status of children with cancer, *European Journal of Clinical Nutrition* 1998, 52(7); 494–500.

den Broeder E, Lippens RJJ, van 't Hof MA, Tolboom JJM, Sengers RCA, van den Berg AMJ, van Houdt NB, Hofman Z, van Staveren WA. Naso-gastric tube feeding in children with cancer: the effect of two different formulas on weight, body composition and serum protein concentrations, *Journal of Parenteral and Enteral Nutrition* 2000, 24(6); 351–360.

Dent D, Kudsk KA, Minard G, Fabian T, Nguyen T, Pritchard E, Pate L, Croce M. Risk of abdominal septic complications after feeding jejunostomy placement in patients undergoing splenectomy for trauma. *American Journal of Surgery*. 1993 Dec;166(6):686-9

Desborough JP. The stress response to Trauma and surgery. *British Journal of Anaesthesia*, 2000, 85(1):109-17.

Despres JP, Lemieu I. Abdominal obesity and Metabolic Syndrome. *Nature* 2006; 444(7121):881-7.

DeStefani E, Boffetta P, Deneo-Pellegrini H. The role of vegetable and fruit consumption in the aetiology of squamous cell carcinoma of the oesophagus: A case control study in Uruguay. *International Journal of Cancer*, 2005;116:130-5.

Detsky AS & Smalley PS. Is this patient malnourished? *Journal of the American Medical Association* (1994), 271, 54-58.

Detsky AS, Baker JP, O'Rourke K. Predicting nutrition-associated complications for patients undergoing gastrointestinal surgery. *Journal of Parenteral and Enteral Nutrition*. 1987; 11: 440 – 446

Devesa SS, Blot WJ, Fraumeni JF. Changing patterns in the incidence of oesophageal and gastric carcinoma in the United States. *Cancer*. 1998; 83(10): 2049-53.

Dewar L, Gelfand G, Finley RJ, Evans K, Inculet R, Nelems B. Factors affecting cervical anastomotic leak and stricture formation following esophagogastrectomy and gastric tube interposition. *American Journal of Surgery.* 1992; 163(5): 484-9

Dewar L, Gelfand G, Finley RJ, Evans K, Inculet R, Nelems B. Factors affecting cervical anastomotic leak and stricture formation following esophagogastrectomy and gastric tube interposition. *American Journal of Surgery*, 1992; 163(5): 484-9

Dhillon PK, Farrow DC, Vaughan TL, *et al.* Family history of cancer and risk of esophageal and gastric cancers in the United States. *International Journal of Cancer* 2001;93:148–52.

Diaz-Rubio M, Moreno-Elola-Olaso C, Rey E. Symptoms of gastro-oesophageal reflux: Prevalence, severity, duration and associated factors in a Spanish population. *Alimentary Pharmacology and Therapeutics* 2004;19(1):95-105.

Dickerson RN, Boschert KJ et al, Hypo caloric enteral tube feeding in critically ill obese patients, *Nutrition*, 2002, 18, 241-246

Dickson TM, Kusnierz-Glaz CR, Blume KG. Impact of the admission body weight and chemotherapy dose adjustment on the outcome of autologous bone marrow transplantation. *Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation.* 1999, 5:299-305.

Donington JS. Pre-operative preparation for esopageal surgery. *Thoracic Surgery Clinics*, 2005, 15(2):277-85.

Doty JR, Salazar JD, Forastiere AA et al. Post-esophagectomy morbidity, mortality and length of hospital stay after preoperative chemoradiation therapy. *Annals of Thoracic Surgery*, 2002; 74: 227 – 231

Dowd PS, Heatly RV. The influence of undernutrition on immunity. *Clinical Science* 1984; 66: 241 – 248.

Drakopanagiotakis F, Polychronopoulos V, Judson MA. Organising Pneumonia. *American Journal of Medical Science.* 2008, 335(1):34-9.

Drewitz DJ, Sampliner RE, Garewal HS. The incidence of adenocarcinoma in Barrett's esophagus: a prospective study of 170 patients followed 4.8 years. *American Journal of Gastroenterology* 1997;92:212-5.

Drost AC, Burleson DG, Cioffi WG, Jr. Plasma cytokines after thermal injury and their relationship on infection. *Annals of Surgery 1993,* 218, 74

Duly EB, Grimason S, Grimason P, Barnes G, Trinik TR. Measurement of serum albumin by capillary zone electrophoresis, bromocresol green, bromoresol purple and immunoassay methods. *Journal of Clinical Pathology,* 2003, 56(10):780-781.

Earlam R, Cunha-Melo JR. Oesophageal squamous cell carcinoma: A critical review of surgery. *British Journal of Surgery* 1980; 67: 381-390

Edington J et al. Prevalence of malnutrition on admission to four hospitals in England. *Clinical Nutrition* 2000; 19:191-195.

Eekhoff EMW, Rosendaal FR, Vandenbroucke JP. Minor events and the risk of deep vein thrombosis. *Thromb Haemost,* 2000, 83, 408-411.

Eguchi R, Ide H, Nakamura T et al. Analysis of postoperative complications after esophagectomy for esophageal cancer in patients receiving neoadjuvant therapy. *Japanese Journal of Thoracic and Cardiovascular Surgery.* 1999; 47: 552 – 558

Elia M, Van Bokhorst-De Van Der Schuren MAE, Garvey J, Goedhart A, Lundholm K, Nitenberg G, Stratton RJ. Enteral (oral or tube administration) nutrition support and eicosapentaenoic acid in patients with cancer: A systematic review. *International Journal of Oncology*, 2006, 28:5-23.

Elia M. Artificial nutrition support. *Medicine International*, 1990:82:3392-6.

El-Serag HB, Graham DY, Satia JA, Rabeneck L. Obesity is an independent risk factor for GERD symptoms and erosive esophagaitis. *American Journal of Gastroenterology,* 2005, 100:1243-1250.

Eng C, Spechler SJ, Ruben R, *et al.* Familial Barrett's esophagus and adenocarcinoma of the gastro-esophageal junction. *Cancer Epidemiology and Biomarkers Prevention* 1993;2:397–9.

Engel LS, Chow WH, Vaughan TL et al, Population attributable risks of oesophageal and gastric cancers. *Journal of the National Cancer Institute,* 2003; 17; 95(18): 1404 – 13.

Engeland A, Tretli S, Bjorge T. Height and Body Mass Index in Relation to Oesophageal Cancer; 23-year Follow-up of Two Million Norwegian Men and Women. *Cancer Causes Control.* 2004; 15(8): 837-43.

Enzinger PC, Mayer RJ. Oesophageal cancer. *New England Journal of Medicine*, 2003; 349:2241-52.

Espat NJ, Copeland EM, Moldawer LL. Tumor necrosis factor and cachexia: a current perspective, *Surgical Oncology*, 1994, 3(5); 255–262.

Espat NJ, Karpeh M. Reconstruction following total gastrectomy: a review and summary of randomised prospective controlled trials. *Surgical Oncology.* 1998, 7:65-69

Espat NJ, Moldawer LL Copeland EM. Cytokine-mediated alterations in host metabolism prevent nutritional repletion in cachectic cancer patients, *Journal of Surgical Oncology* 1995, 58(2);77–82.

Expert Panel on Detection, Evaluation and Treatment of high blood cholesterol in Adults, Executive summary of the third report of the National Cholesterol Education Programme (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Journal of the American Medical Association,* 2001, 285:2486-97.

Falconer JS, Fearon KC, Plester CE, Ross JA, Carter DC. Cytokines, the acute-phase response, and resting energy expenditure in cachectic patients with pancreatic cancer, *Annals of Surgery* 1994, 219(4);325–331.

Falconer JS, Fearon KC, Ross JA, Elton R, Wigmore SJ, Garden OJ, Carter DC. Acute-phase protein response and survival duration of patients with pancreatic cancer, *Cancer* 1995, 75(8); 2077–2082.

Fan YY, Ly LH, Barhoumi R, McMurray DN, Chapkin RS. Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production. *Journal of Immunology*, 2004, 173:6151-60.

Farrow DC, Vaughan TL, Sweeney C, *et al.* Gastroesophageal reflux disease, use of H2 receptor antagonists, and risk of esophageal and gastric cancer. *Cancer Causes and Control* 2000;11:231–8.

Fearon KCH. Nutrition support in cancer. *Clinical Nutrition*, 2001a, 20:187-190.

Fearon KCH, Von Meyenfeldt M, Moses AGW. An energy and protein dense, high n-3 fatty acid oral supplement promotes weight gain in cancer cachexia. *European Journal of Cancer* 2001b, 37:S27-28.

Ferguson MK, Martin TR, Reeder LB, Olak J. Mortality after esophagectomy:risk factor analysis. *World Journal of Surgery* 1997;21:599-604.

Fettes SA et al. Nutritional status of elective gastrointestinal surgery patients pre-and post-operatively. *Clinical Nutrition,* 2002, 21(3): 249-254.

Fink U, Stein HJ, Wilke H. Multimodal treatment for Squamous cell esophageal cancer. *World Journal of Surgery* 1995;19:198-204.

Fiorentino DF, Zlotnik A, Vieria P. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *Journal of Immunology 1991,* 146, 3444.

Fiorica F, DiBona D, Schepis F, Licata A, Shahied L, et al. Preoperative chemoradiotherapy for oesophageal cancer: a systematic review and meta-analysis. *Gut* 2004; 53: 925-930.

Fitzgerald RC, Farthing MJ. The pathogenesis of Barrett's oesophagus: a process in continuum or discontinuum. *Current Gastroenterology Reports,* 2000; 2: 421-4.

Fitzgerald SP, Lamont JV, McConnell RI, Benchikh EO. Development of a hig-throughput automated analyzer using biochip array technology. *Clinical Chemistry,* 2005, 51: 1165-76.

Flood V, Webb K, Lazarus R, Pang G. Use of self-report to monitor overweight and obesity in populations: some issues for consideration. *Australia New Zealand Journal of Public Health.* 2000; 24(1): 96-9.

Fogler C, Lindsey RW. C-Reactive Protein in orthopaedics. *Orthopaedics,* 1998;21:687-691.

Forastiere AA, Heitmiller RF, Kleinberg L. Multimodality therapy for esophageal cancer. *Chest.* 1997 Oct; 112(4 Suppl):195S-200S

Forastiere AA, Heitmiller RF, Lee DJ, Zahurak M, Abrams R, Kleinberg L, Watkins S, Yeo CJ, Lillemoe KD, Sitzmann JV, Sharfman W. Intensive chemoradiation followed by esophagectomy for squamous cell and adenocarcinoma of the esophagus. *Cancer Journal from Scientific American.* 1997 May-Jun;3 (3):144-52.

Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *Journal of the American Medical Association,* 2002; 16;287(3):356-9.

Fouladiun M, Korner U, Bosaeus I, Daneryd P, Hyltander A, Lundholm KG. Body composition and time course changes in regional distribution of fat and lean tissue in unselected cancer patients on palliative care—correlations with food intake, metabolism, exercise capacity, and hormones, *Cancer* 2005, 103(10);2189–2198.

Friedman J, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998, 395:763-770.

Friess H, Bohm I, Muller MV. Maldigestion after total gastrectomy is associated with pancreatic insufficiency. *American Journal of Gastroenterology.* 1996;91:341.

Fritsche K. Fatty Acids as Modulators of the Immune Response. *Annual Review of Nutrition*, 2006, 26:45-73.

Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model of integration of endocrine and metabolic signalling in energy metabolism regulation. *American Journal of Physiology and Endocrinology Metabolism, 2001,* 280: E827-E847.

Furukawa K, Tashiro T, Yamamoir H. Effects of soybean oil emulsion and eicosapentaenoic acid on stress response and immune function after a severely stressful operation. *Annals of Surgery,* 1999;229:255-261.

Gabor S, Renner H, Matzi V, Ratzenhofer B. Early enteral feeding compared with parenteral nutrition after oesophageal or oesophagogastric resetion and reonstruction. *British Journal of Nutrition*, 2005, 93(4):509-13.

Gaby C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* 1999; 340: 448 – 454.

Gammon MD, Schoenberg JB, Ahsan H. Tobacco, alcohol, and socio-economic status and adenocarcinoma of the oesophagus and gastric cardia. *Journal of the National Cancer Institute*, 1997, 89:1277-1284.

Gandhi SK, Naunheim KS. Complications of transhiatal esophagectomy. *Chest Surgery Clinics of North America* 1997; 7: 601 – 610

Ganem G, Dubray B, Raoul Y et al. Concomitant chemoradiotherapy followed, where feasible, by surgery for cancer of the esophagus. *Journal of Clinical Oncology* 1997 15:701-711.

Gao YT, McLaughlin JK, Blot WJ, *et al.* Risk factors for esophageal cancer in Shanghai, China. Role of cigarette smoking and alcohol drinking. *International Journal of Cancer* 1994;58:192–6.

Garavello W, Negri E, Talamini R. Family history of cancer, its combination with smoking and drinking, and risk of squamous cell carcinoma of the esophagus. *Cancer Epidemiology and Biomarkers Prevention,* 2005;14:1390-1393.

Gebski V, Burmeister B, Smithers BM, Foo K, Zalcberg J, Simes J, for the Australasian Gastro-intestinal Trial Group. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. *Lancet Oncology*. 2007, 8;226-34.

Geh JI, Crellin AM, Glynn-Jones R. Preoperative (neo-adjuvant) chemoradiotherapy in oesophageal cancer. *British Journal of Surgery;* 2001; 88-338.

Gerson LB, Ullah N, Fass R, Green C, Shetler K, Singh G. Does body mass index differ between patients with Barrett's oesophagus and patients with chronic gastro-oesophageal reflux disease. *Alimentary Pharmacology and Therapeutics* 2007; 25: 1079-86.

Gianotti L, Braga M, FOrtis C. A prospective, ranadomised clinical trial on perioperative feeding with arginine, omega-3 fatty acid, and RNA-enriched enteral diet. Effect on host response and nutritional status. *Journal of Parenteral and Enteral Nutrition* 1999, 23:314-20.

Gianotti L, Braga M, Landoni L, Mari G, Scaltrini F, Di Castelnuovo A, Di Carlo V Outcome of patients with cancer of the esophagogastric junction in relation to histology and surgical strategy. *Hepatogastroenterology;* 2003 Nov-Dec;50(54):1948-52

Gianotti L, Braga M, Nespoli L, Radaelli G, Beneduce A, Di Carlo V. A randomised controlled trial of preoperative oral supplementation with a specialized diet in patients with gastrointestinal cancer. *Gastroenterology,* 2002, 122:1763-1770.

Gibbs J, Cull W, Henderson W et al. Preoperative serum albumin level as a predictor of

operative mortality and morbidity. *Archives of Surgery* 1999; 134: 36 – 42

Gogos GA, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos NC, Kalfarentzos F. Dietary Ω-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy, *Cancer* 1998,82(2);395–402.

Gonzalez CA, Agudo A, Montes J. Tobacco and alcohol intake in relation to adenocarcinoma of the gastric cardia in Spain. *Cancer Causes and Control,* 1994, 5:88-89.

Goodman ER, Kleinstein E, Fusco AM. Role of interleukin 8 in the genesis of acute respiratory distress syndrome through an effect on neutrophils apoptosis. *Archives of Surgery* 1998, 133:1234-1239.

Graham DY, Yamaoka Y. Helicobacter Pylori and cagA: relationship with gastric cancer, and reflux oesophagitis and it's complications. *Helicobacter* 1998; 3 :145-51

Grant M & Kravits K. Symptoms and their impact on nutrition. *Seminars in oncology nursing,* 2000;16:112-121.

Greenlee RT, Hill-Harmon Mb, Murray T, Thun M. Cancer Statistics, 2001. *CA: A Cancer Journal for Clinicians.* 2001 Jan-Feb; 51(1):15-36.

Grieco A, Long C. Investigation of Karnofsky performance status is a measure of quality of life. *Health Psychology*, 1984;3:129-124.

Grimble RF. Nutritional modulation of cytokine biology. *Nutrition,* 1998, 14,634-640.

Grimble RF. Nutritional modulation of immune function. *Proceedings of the nutrition society,* 2001; 60: 389-397.

Grimble.RF, Nutritional therapy for cancer cachexia, *Gut* 2003, 52(10);1391–1392.

Grossmann EM, Longo WE, Virgo KS, Johnsn FE, Oprian CA, Henderson W, Daley J, Khuri SF. Morbidity and mortality of gastrectomy for cancer in Department of veterans affairs medical centers. *Surgery,* 2002, 131(5);484-490.

Groundwater P, Beck SA, Barton C, Adamson C, Ferrier IN, Tisdale MJ. Alteration of serum and urinary lipolytic activity with weight loss in cachectic cancer patients, *British Journal of Cancer*1990, 62(5);816–821.

Gupta NM. Oesophagectomy without thoracotomy: first 250 patients. *European Journal of Surgery* 1996;162:455-461.

Gurkan N, terzioglu T, Telzelman S et al. Transhiatial esophagectomy for oesophageal carcinoma. *British Journal of Surgery* 1991;78:1348-1351.

Gurleyik E, Gurleyik G, Unalmiser S. Accuracy of serum C-Reactive Protein measurements in the diagnosis of acute appendicitis compared with surgeons clinical impression. *Diseases of the Colon and Rectum,* 1995, 38(12):1270-74.

Guzik TJ, Mangalat D, Korbut R. Adipokines – novel link between inflammation and vascular function? *Journal of Physiology and pharmacology* 2006, 57(4):505-528.

Hampel H, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. *Annals of Internal Medicine*, 2005 Aug 2;143(3):199-211.

Han-Geurts IJ, Verhoef C, Tilanus HW Relaparotomy following complications of feeding jejunostomy in oesophageal surgery. *Digestive Surgery.* 2004;21(3):192-6.

Hansbrough JF, Bender EM, Zapata-Sirvent R, Andreson J. Altered helper and suppressor lymphocyte populations in surgical patients. *American Journal of Surgery*, 1984, 148:303-7.

Hansson LE, Sparen P, Nyren O. Increasing incidence in both histological types of esophageal carcinomas among men in Sweeden. *International Journal of Cancer*, 1993;

54: 402-7.

Harrison L. Nutritional status of patients admitted for elective surgery. *Proceedings of the Nutrition Society* 1997:56:265A

Heidecke CD, Weighardt H, Feith M, Fink U, Zimmerman F, Stein H, Siewert JR, Holzmann B. Neoadjuvant treatment of oesophageal cancer: Immunosuppression following combined radiochemotherapy. *Surgery* 2002; 132: 495-501.

Heitmiller RF, Forastiere AA, Kleinberg L, Zahurak M Neo-adjuvant chemoradiation followed by surgery for resectable esophageal cancer. *Recent Results of Cancer Research,* 2000;155:97-104.

Herskovic A, Matrz K, l-Sarraf M. Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with caner of the oesophagus. *New England Journal of Medicine.* 1992, 326;1593-98.

Heys SD, Walker LG, Smith I, Eremin O. Enteral nutritional supplementation with key nutrients in patients with critical illness and cancer: a meta-analysis of randomized controlled clinical trials, *Annals of Surgery* 1999, 229(4);467–477.

Hill GL. Body Composition research: implications for the practice of clinical nutrition. *Journal of Parenteral and Enteral Nutrition*, 1992, 16:179.

Hockwald SN, Harrison LE, Heslin MJ, Burt ME, Brennan MF. Early post operative enteral feeing improves whole body protein kinetics in upper gastrointestinal cancer patients. *American Journal of Surgery* 1997, 174(3), 324-330.

Holmes RS, Vaughan TL. Epidemiology and Pathogenesis of Esophageal Cancer. *Seminars in Radiation Oncology*, 2007; 17(1): 2-9.

Huang JQ, Subbaramiah S, Chen Y. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. *Gastroenterology*, 1998;114:1169-1179.

Huang Q, Zhang X, Jiang ZW, Liu BZ, Li N, Li JS. Hypoleptinemia in gastric cancer patients: relation to body fat mass, insulin, and growth hormone, *Journal of Parenteral and Enteral Nutrition* 2005, 29(4); 229–235.

Hulscher JB, Tijssen JG, Obertop H et al. Transthoracic versus transhiatal resection for carcinoma of the oesophagus: a meta-analysis. *Annals of Thoracic Surgery* 2001; 72: 306 – 313.

Iida K, Kadota J, Kawakami K. Immunological function and nutritional status in patients with hepatocellular carcinoma. *Hepatogastroenterology* 1999, 46:2476-2482.

Imdahl A, Schoffel U, Gunther R, Impact of neo-adjuvant therapy of perioperative morbidity in patients with esophageal cancer. *American Journal of Surgery* 2004; 187(1);64-68.

Ishikawa M, Kitayama J, Kazama S, Hiramatsu T, Hatano K, Nagawa H. Plasma adiponectin and gastric cancer. *Clinical Cancer Research* 2005, 11:466-472.

Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Camergo CA. Body Mass Index and Symptoms of Gastroesophageal reflux in women. *New England Journal of Medicine,* 2006, 354(22);2340-2348.

Jagoe RT, Goodship TH, Gibson GJ. The influence of nutritional status on complications after operations for lung cancer. *Annals of Thoracic Surgery*, 2003, 71:936-943.

Jamieson GG, Mathew G, Ludeman R, Wayman J, Myers JC, Devitt PG. Postoperative mortality following oesophagectomy and problems in reporting its rate. *British Journal of Surgery* 2004; 91: 943-97

Jamieson GG, Mathew G. Surgical management of oesophageal cancer: the western experience. In: Daly JM, Hennessy TPJ, Reynolds JV, eds. Management of Upper Gastrointestinal Cancer. London: WB Sanders; 1999: 183 – 199.

Jankowski JA, Wright NA, Meltzer SJ, Triadafilopoulous G, Geboes K, Casson AG, Kerr D, Young LS. Molecular evolution of the metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *American Journal of Pathology* 1999, 154(4): 965-973.

Jansson C, Johansson AL, Nyren O. Socioeconomic factors and risk of esophageal adenocarcinoma: A nationwide Swedish case-control study. *Cancer Epidemiology and Biomarkers Prevention*, 2005, 14:1754-1761.

Jeevanandam M, Young DH et al. Obesity and the metabolic response to severe multiple trauma in man. *Journal of Clinical Investigation*; 1991, 87: 262-269.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer Statistics 2006. *CA: Cancer Journal for Clinicians* 2006;56:106-130.

Jho DH, Babcock TA, Tevar R, Helton WS, Espat NJ. Eicosapentaenoic acid supplementation reduces tumor volume and attenuates cachexia in a rat model of progressive non-metastasizing malignancy, *Journal of Parenteral and Enteral Nutrition* 2002, 26(5);291–297.

Jochem VJ, Fuerst PA, Fromkes JJ. Familial Barrett's esophagus associated with adenocarcinoma. *Gastroenterology* 1992;102:1400–2.

Johannisson A. Thuvander A. Gadhasson IL Activation markers and cell proliferation as indicators of toxicity: a flow cytometric approach. *Cell Biology and Toxicology,* 1995, 11(6):355-66.

Johnston MH, Hammond AS, Laskin W, Kones DM. The prevalence and clinical characteristics of short segment s of specialised intestinal metaplasia in the distal esophagus on routine endoscopy. *American Journal of Gastroenterology* 1996, 91:1507-1511.

Juhani S, Markku L, Martti M. Enteral or parenteral feeding after total gastrectomy:prospective randomised pilot study. *European Journal of Surgery* 1997, 163:761-6.

Junginger T, Dutkowski P. Selective approach to the treatment of oesophageal cancer. *British Journal of Surgery* 1996;83:1473-1477.

Kabat GC, Ng SK, Wynder EL. Tobacco, alcohol intake, and diet in relation to adenocarcinoma of the esophagus and gastric cardia. *Cancer Causes and Control*, 1993;4:123-32.

Kamei H, Hachisuka T, Nakao M, Takagi K. Quick recovery of serum diamine oxidase activity in patients undergoing total gastrectomy by oral enteral nutrition. *American Journal of Surgery.* 2005, 189:38-43.

Karaylannakis AJ, Makri GG, Mantzioka A, Karousos D. Systemic stress response after laparoscopic or open cholecyctectomy: a randomized trial. *British Journal of Surgery*, 1997; 84: 467-471.

Karl RC, Schreiber R, Boulware D, Baker S, Coppola D Factors affecting morbidity, mortality, and survival in patients undergoing Ivor Lewis esophagogastrectomy. *Annals of Surgery.* 2000 May;231(5):635-43

Katz PO. Gastro-oesophageal reflux- state of the art. *Reviews in Gastroenterological Disorders*, 2001; 1:128-138.

Kauer WK, Peters JH, DeMeester TR, Ireland AP, Bremner CG, Hagen JA. Mixed reflux of gastric and duodenal juices more harmful to the oesophagus than gastric juice alone. The need for surgical therapy re-emphasised. *Annals of Surgery* 1995, 222:523-533.

Keller SM, Ryan LM, Coia LR, Dang P, Vaught DJ, Diggs C, Weiner LM, Benson AB. High dose chemoradiotherapy followed by esophagectomy for adenocarcinoma of the esophagus and gastroesophageal junction. *Cancer*, 1998, 83(1);9, 1908-1916.

Kelley ST, Coppola D, Karl RC Neo-adjuvant chemoradiotherapy is not associated with a higher complication rate vs. surgery alone in patients undergoing esophagectomy. *Journal of Gastrointestinal Surgery*. 2004 Mar-Apr;8(3):227-32.

Kelsen DP, Ginsberg R, Pajak TF et al. Chemotherapy followed by surgery compared with surgery alone for localised esophageal cancer. *New England Journal of Medicine* 1998; 339:1979-84.

Kemen M, Senkal M, Homann HH, Zumtobel V. Early post operative enteral nutrition with arginine, omega-3 fatty acids and ribonucleic acid-supplemented diet versus placebo in cancer patients: an immunologic evaluation of Impact. *Critical Care Medicine,* 1995, 23:652-659.

Kenler AS, Swails WS, Driscoll DF, DeMichele SJ, Daley B, Babineau TJ, Peterson MB Bistrian BR. Early enteral feeding in postsurgical cancer patients. Fish oil structured lipid-based polymeric formula versus a standard polymeric formula. *Annals of Surgery* 1996, 223(3);316–333.

Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *Journal of Clinical Endocrinology and Metabolism.* 2004;89(6):2548-56.

Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R. Lack of effect of foods enriched with plant or marine-derived n-3 fatty acids on human immune function. *American Journal of Clinical Nutrition* 2003, 77:1287-95.

Kim R, Weissfeld JL, Reynolds JC, Kuller LH. Etiology of Barrett's Metaplasia and esophageal adenocarcinoma. *Cancer Epidemiology and Biomarkers Prevention* 1997, 6:369-377.

Kinney JM. The influence of calorie and nitrogen balance on weight loss. *British Journal of Clinical Practice* 1988, 42:Suppl 63, 114-120.

Kiyama T, Mizutani T, Okuda T, Fujita I, Tokunaga A, Tajiri T, Barbul A. Postoperative changes in body composition after gastrectomy. *Journal of Gastrointestinal Surgery,* 2005, 9(3):313-319.

Klava A, Windsor ACJ, Farmery SM. Interleukin-10. A role in the development of postoperative immunosuppression. *Archives of Surgery,* 132 (1997), p. 425.

Knapp ML, al-Sheibani S, Riches PG, Hanham IW, Phillips RH. Hormonal factors associated with weight loss in patients with advanced breast cancer, *Annals of Clinical Biochemistry* 1991, 28(5);480–486.

Kopelman P. Obesity as a medical problem. *Nature*, 2000;404:635-43.

Kubo A, Corley D. Body Mass Index and Adenocarcinomas of the Esophagus and Gastric cardia: a systematic review and meta-analysis. *Cancer Epidemiology and Biomarkers Prevention.* 2006;15(5):872-878.

Kuczmarski MF, Kuczmarski RJ, Najjar M. Effects of age on validity of self-reported height, weight, and body mass index: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *Journal of the American Dietetic Association.* 2001; 101(1): 28-34.

Kudsk KA, Croce MA, Fabian TC, Minard G, Tolley EA, Poret HA, Kuhl MR, Brown RO. Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma. *Annals of Surgery.* 1992;215(5):503-11

Kudsk KA, Tolley EA, DeWitt C et al. Preoperative albumin and surgical site identify surgical risk for major postoperative complications. *Journal of Parenteral and Enteral Nutrition*, 2003; 27: 1 – 9.

Kudsk KA. Immunonutrition in Surgery and Critical care. *Annual Review of Nutrition* 2006, 26:463-79.

Kudsk KA. Visceral protein response to enteral vs parenteral nutrition and sepsis in trauma patients *Surgery* 1994;116(3):516-523

Kurk SJ, Barbul A. Role of arginine in trauma, sepsis, and immunity. *Journal of Parenteral and Enteral Nutrition*, 1990, 14(s5):226-229S.

Kuzu MA, Terzioğlu H, Genç V, Erkek AB, Ozban M, Sonyürek P, Elhan AH, Torun N. Preoperative nutritional risk assessment in predicting postoperative outcome in patients

undergoing major surgery. *World Journal of Surgery,* 2006, 30(3):378-90.

La Vecchia C, Negri E, Lagiou P, Trichopoulos D. Oesophageal adenocarcinoma: a paradigm of mechanical carcinoma? *International Journal of Cancer,* 2002;102(3):268-70.

Lagergren J, Bergström R, Lindgren A. The role of tobacco, snuff and alcohol use in the aetiology of cancer of the oesophagus and gastric cardia. *International Journal of Cancer,* 2000; 85:340–6.

Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastro-esophageal reflux as a risk factor for esophageal adenocarcinoma. *New England Journal of Medicine,* 1999;340:825-31.

Lagergren J, Bergström R, Nyrén O. Association between body mass index and adenocarcinoma of the oesophagus and gastric cardia. *Annals of Internal Medicine.* 1999;130: 883-890.

Lagergren J, Bergstrom R, Nyren O. No relation between body mass and gastro-oesophageal reflux symptoms in a Swedish population based study. *Gut* 2000;47:26-29.

Lagergren J, Ye W, Bergström R, *et al.* Utility of endoscopic screening for upper gastrointestinal adenocarcinoma. *JAMA: Journal of the American Medical Association* 2000;284:961–2

Lagergren J, Ye W, Lindgren A, *et al.* Heredity and risk of cancer of the esophagus and gastric cardia. *Cancer Epidemiology and Biomarkers Prevention* 2000;9:757–60.

Lagergren J. Adenocarcinoma of oesophagus: what exactly is the size of the problem and who is at risk? *Gut*, 2005, 54, i1-i5.

Lai PB, Ross JA, Fearon KC, Anderson JD, Carter DC. Cell cycle arrest and induction of apoptosis in pancreatic cancer cells exposed to eicosapentaenoic acid in vitro. *British Journal of Cancer* 1996, 74(9);1375–1383.

Langer CJ, Hoffman JP, Ottery FD, Clinical significance of weight loss in cancer patients: rationale for the use of anabolic agents in the treatment of cancer-related cachexia, *Nutrition* 2001, 17 (Suppl 1); S1–S20.

Lankford KV. Mosunjac M. Hillyer CD. Effects of UVB radiation on cytokine generation, cell adhesion molecules, and cell activation markers in T-lymphocytes and peripheral blood HPCs. *Transfusion,* 2000, 40(3):361-7.

Law S, Fok M, Chow S, Chu KM, Wong J. Preoperative chemotherapy versus surgical therapy alone for squamous cell carcinoma of the esophagus: a prospective randomized trial. *Journal of Thoracic and Cardiovascular Surgery*. 1997 Aug; 114(2):210-7.

Law S, Kwong D, Tung H, et al. Preoperative chemoradiation for squamous cell oesophageal cancer:a prospective randomised trial. *Canadian Journal of Gastroenterology* 1998; 12: Suppl B: 56B, abstract.

Lawlor P, McCullough JA, Byrne PJ, Reynolds JV. Gastric myoelectrical activity post-chemoradiotherapy & oesophagectomy: A prospective study using subscapular surface recording' *Diseases of the Oesophagus, 17* (2004) 77-81

Layke JC, Lopez PP. Esophageal Cancer: A review and Update. *American Family Physician*, 2006:73(12):2187-2191.

Le Prise E, Etienne PL, Meunier P, et al. A randomised study of chemotherapy, radiation therapy, and surgery versus surgery for localized squamous cell carcinoma of the esophagus. *Cancer* 1994; 73: 1179-84

Lee CH, Lee JM, Wu DC. Independent and combined effects of alcohol intake, tobacco smoking and betel quid chewing on the risk of oesophageal cancer in Taiwan. *International Journal of Cancer,* 2005, 113:475-482.

Lee HK, Vaporciyan AA, Cox JD, Tucker SL, et al. Postoperative pulmonary complications after preoperative chemoradiation therapy for oesophageal carcinoma: correlation with dose-volume histogram parameters. *International Journal of Radiation*

Oncology, Biology and Physics 2003; 57: 1317-22

Lee A, Lum ME, O'Regan WJ, Hillmann KM. Early post-operative emergencies requiring an intensive care team intervention. The role of ASA physial status and after jours surgery. *Anaesthesia,* 1998, 53(6):529-35.

Lehnert T. Multimodal therapy for Squamous carcinoma of the oesophagus. *British Journal of Surgery* 1999:86:727-39.

Leichman L, Steiger Z, Seydel HG, Vaitkevicius VK Combined preoperative chemotherapy and radiation therapy for cancer of the esophagus: the Wayne State University, Southwest Oncology group and Radiation Therapy Oncology Group experience. *Seminars in Oncology.* 1984 Jun;11(2):178-85.

Lerut T, Coosemans W, De Leyn P, et al. Reflections on 3-field lymphadenectomy in carcinoma of the esophagus and gastrooesophageal junction. *Hepato-Gastroenterology* 1999; 46: 717-725.

Lerut T, Coosemans W, Van Raemdonck D, Decker G. Treatment of esophageal carcinoma. *Chest;* 1999;116:463S-465S.

Lerut T. Oesophageal carcinoma:past and present studies. *European Journal of Surgical Oncology;* 1996;22:317-323

Lever A, Mackenzie I. Sepsis: definition, epidemiology and diagnosis. *British Medical Journal,* 2007, 335(7625): 879-83

Levi F, Ollyo JB, Vecchia C. The consumption of tobacco, alcohol and the risk of adenocarcinoma in Barrett's esophagus. *International Journal of Cancer* 1990;45:852–4.

Levine DS, Haggitt RC, Blount PL, Weinstein WM, Reid BR. An endoscopic biopsy protocol can differentiate high grade dysplasia from early adenocarcinoma in Barrett's oesophagus. *Gastroenterology* 1995, 105:40-50.

Lewis SJ, Egger M, Sylvester PA, Thomas S. Early enteral feeding versus "nil by mouth" after gastrointestinal surgery: systematic review and meta-analysis of controlled trials. *British Medical Journal,* 2001, 323(7316):773-6.

Li JY, Ershaw AG, Chen ZL. A case control study of cancer of the esophagus and gastric cardia in Linxian. *International Journal of Cancer*, 1989;43:755-61.

Liedman B, Andersson H, Bosaeus I, Hugosson I, Lundell L. Changes in body composition after gastrectomy:results of a controlled, prospective clinical trial. *World Journal of Surgery*, 1997; 21(4):416-20.

Liedman B. Symptoms after total gastrectomy on food intake, body composition, bone metabolism, and quality of life in gastric cancer patients – is reconstruction with a reservoir worthwhile? Nutrition. 1999, 15(9):677-682.

Linblad M, Rodriguez LA, Lagergren J. Body Mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. *Cancer Causes and Control*, 2005,16:285-294.

Lindsted KD, Singh PN. Body Mass and 26 y risk of mortality among men who never smoked: a re-analysis of men from he Adventist Mortality study. *International Journal of Obesity and Related Metabolic Disorders,* 1998;22:544-8.

Liparoto SF. Myszka DG. Wu Z. Analysis of the Role of the Interleukin-2 Receptor γ Chain in Ligand Binding. *Biochemistry,* 41 (8), 2543 -2551, 2002.

Liu JF, Wang QZ, Hou J. Surgical treatment for cancer of the oesophagus and gastric cardia in Hebei, China. *British Journal of Surgery* 2004, 91(1): 90-8.

Liu JF, Watson DI, Devitt PG, Mathew G, Myburgh J, Jamieson GG. Risk factor analysis of post-operative mortality in oesophagectomy. *Diseases of the Esophagus.* 2000;13 (2):130-5.

Locke GR, Talley NJ, Fett SL. Risk factors associated with symptoms of

gastroesophageal reflux. *American Journal of Medicine* 1999;106(6):642-9.

Lord GM, MAtarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998,394:897-901.

Lorite MJ, Cariuk P, Tisdale PJ. Induction of muscle protein degradation by a tumour factor, *British Journal of Cancer* 1997, 76(8);1035–1040.

Ludwig DJ, Thirlby RC, Low DE. A prospective evaluation of dietary status and symptoms after near-total oesophagectomy without gastric emptying procedure. *American Journal of Surgery* 2001, 181, 454-458.

Lund O, Kimose HH, Aagaard MT et al. Risk stratification and long-term results after surgical treatment of carcinomas of the thoracic esophagus and cardia. A 25-year retrospective study. *Journal of Thoracic and Cardiovascular Surgery* 1990; 99:200-9.

MacDonald JS, Smalley SR, Benedetti J et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *New England Journal of Medicine* 2001; 345:725-30.

Mafune K, Tanaka Y. Influence of multimodality therapy on the cellular immunity of patients with esophageal cancer. *Annals of Surgical Oncology,* 2000, 7(8):609-616.

Manson JE, Willett WC, Stampfer MJ. Body Weight and mortality among women. *New England Journal of Medicine*, 1995;333:677-85.

Martin L, Lagergren J, Jia C, Linblad M, Rouvelas I, Viklund P. The influence of needle catheter jejunostomy on weight development after oesophageal cancer surgery in a population based study. *European Journal of Surgical Oncology* 2007, 33(6):713-7.

Marzio R, Mauël J, Betz-Corradin S. CD69 and regulation of the immune function. *Immunopharmacol Immunotoxicol.* 1999, 21(3):565-82.

Mathys-Vliegen EM and Tygat GNJ. Twenty-four hour pH measurements in morbid obesity: effects of massive overweight, weight loss and gastric distension. *European Journal of Gastroenterology and Hepatology.* 1996;8 :635-640.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta call function from fasting plasma glucose and insulin concentrations in man. *Diabetologia,* 1985; 28:412-419.

Mayne ST, Navarro SA. Diet, obesity and reflux in the etiology of adenocarcinoma of the esophagus and gastric cardia in humans. *Journal of Nutrition*, 2002, 132:3467S-3470S.

Mayne ST, Risch a, Dubrow R. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiology and Biomarkers Prevention*, 2001;10:1055-62.

McAleer D, McPhillips FM, Fitzgerald SP, McConnell RI, rodriguez ML. Application of Evidence Investigator for the simultaneous measurement of soluble adhesion molecules: L-, P-, E- selectins, VCAM-1, ICAM-1 in a biochip platform. *Journal of Immunoassay and Immunochemistry*. 2006;27(4):363-78.

McCarter MD, Gomez ME, Daly JM. Early post operative enteral feeding following major upper gastrointestinal surgery. *Journal of Gastrointestinal Surgery*, 1997, 1:278-285.

McCarthy D, Weihofen D. The effect of nutritional supplements on food intake in patients undergoing radiotherapy, *Oncology Nursing Forum* 1999,26(5);897–900.

McCarthy DO. Rethinking nutritional support for persons with cancer cachexia, *Biological Research for Nursing* 2003,5(1);3–17.

McCarthy SN, Gibney MJ, Flynn A; Irish Universities Nutrition Alliance. Overweight, obesity and physical activity levels in Irish adults: evidence from the North/South Ireland food consumption survey. *Proceedings of the Nutrition Society.* 2002 Feb; 61(1): 3-7.

McCulloch P, Ward J, Tekkis PP; ASCOT group of surgeons; British Oesophago-Gastric Cancer Group. Mortality and morbidity in gastro-oesophageal cancer surgery: initial results of ASCOT multicentre prospective cohort study. *British Medical Journal.* 2003, 22;327(7425):1192-7

McMillan DC, Elahi MM, Sattar N, Angerson WJ, Johnstone J, McArdle CS. Measurement of the systemic inflammatory response predicts cancer-specific and non-cancer survival in patients with cancer, *Nutrition and Cancer* 2001, 41(1–2); 64–69.

McWhirter JP, Pennington CR. Incidence and recognition of malnutrition in hospital. *British Medical Journal* 1994; 308:945-948

Medical Research Council Oesophageal Cancer Working party. Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. *The Lancet*; 2002;359:1727-33.

Meneu-Diaz JC, Blazquez LA, Vicente E, Nuno J, Quijano Y, Lopez-Hervas P, Devesa M, Fresneda V. The role of multimodality therapy for resectable esophageal cancer. *American Journal of Surgery.* 2000 Jun;179(6):508-13.

Menke-Pluymers MBE, Hop WC, Dees J. Risk factors for the development of an adenocarcinoma in columnar-lined (Barrett's) esophagus. *Cancer*, 1993;72:1155-1158.

Migliore M, Choong CK, Lim E, Goldsmith KA, Ritchie A, Wells FE. A surgeons case volume of oesophagectomy for cancer strongly influences operative mortality rate. *European Journal of Cardiothoracic Surgery.* 2007, 32(2):375-80.

Misra A, Vikram NK. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue deposits. *Nutrition.* 2003; 19:457-466

Moayyedi P, Axon ATR. Gastro-oesophageal reflux disease: the extent of the problem. *Alimentary pharmacology & therapeutics*, 2005;22 Suppl 1:11-9.

Moayyedi P, Talley NJ. Gastro-oesophageal reflux disease. *The Lancet*, 2006, 367:

2086-100.

Moller DE, Kaufman KD. Metabolic syndrome: a clinical and molecular perspective. *Annual Reviews in Medicine*, 2005, 56:45-62.

Morales TG, Bhattacharyya A, Johnson C, Sampliner RE. Is Barrett's Oesophagus associated with intestinal metaplasia of the gastric cardia? *American Journal of Gastroenterology* 1997, 92:1818-1822.

Moreno-Gonzalez E, Garcia-Garcia I, Gonzalez-Pintol et al. Results of transhiatial esophagectomy for esophageal cancer and other diseases. *Hepato-Gastroenterology*. 1992;39:439-442.

Morlion BJ, Torwesten E, Lessire A, Sturm G, Peskar BM, Furst P, Puchstein C. The effects of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in postoperative trauma. *Metabolism* 1996, 45:1208-1213.

Mueller JM, Erasmi H, Stelzner M et al. Surgical therapy of oesophageal carcinoma. *British Journal of Surgery* 1990;77:845-857.

Mullen JL, Gertner MH, Buzby GP. Implications of malnutrition in the surgical patient. *Archives of Surgery,* 1979; 114: 121 – 125

Murray L, Johnston B, Lane A. Relationship between body mass and gastro-oesophageal reflux symptoms: The Bristol Helicobacter Project. *International Journal of Epidemiology* 2003;32(4):645-50.

Mutlu EA. Nutrition in the care of the cancer patient. *Nutrition in Clinical Care,* 2000;3:3-23.

Myers JG, Page CP, Stewart RM, Schwesinger WH, Sirinek KR, Aust JB . Complications of needle catheter jejunostomy in 2,022 consecutive applications. *American Journal of Surgery,* 1995, 170(6):547-50; discussion 550-1.

Nakamura K, Mriyama Y, Kariyazono , Hamada N, Toyohira H, Taira A, Yamada K. Influence of the pre-operative nutritional state on inflammatory response after surgery. *Nutrition,* 1999, 15(11/12):834-841.

Nandurkar S, Locke GR, Fett S. Relationship between body mass index, diet, exercise and gastro-oesophageal reflux symptoms in a community. *Alimentary Pharmacology and Therapeutics* 2004;20(5):497-505.

National Cancer Registry 2006. Trends in Irish Cancer Incidence 1994-2002, with projections to 2020. (http://www.ncri.ie/pubs/pubfiles/proj_2020.pdf).

Nayel H, el-Ghoneimy E, el-Haddad S. Impact of nutritional supplementation on treatment delay and morbidity in patients with head and neck tumors treated with irradiation, *Nutrition* 1992, 8(1);13–18.

Nevarro SA, Mayne ST, Risch HA. Dietary and lifestyle patterns and the risk of subtypes of esophageal and gastric cancer: factor analysis. *Journal of Nutrition*, 2004;134:3521S-3547S.

Nilsson M, Johnsen R, Ye W, Hveen K, Lagergren J. Obesity and estrogen as risk factors for gastroesophageal reflux symptoms. *Journal of the American Medical Association.* 2003, 290(1): 66-72.

Nilsson M, Lundegardh G, Carling L. Body Mass and reflux esophagitis: an estrogen dependent association? *Scandinavian Journal of Gastroenterology* 2002;37:626-30.

Nishi M, Hiramatsu Y, Hioki K, Kojima Y, Sanada T, Yamanaka H, Yamamoto M. Risk factors in relation to postoperative complications in patients undergoing esophagectomy or gastrectomy for cancer. *Annals of Surgery*, 1988, 207 (2) 148-154.

Nitenberg G & Raynard B. Nutrition support of the cancer patient: issues an dilemmas. *Critical Reviews of Oncology and Haematology*, 2000;34:137-168.

Nozoe T, Kimura Y, Ishida M, Saeki H, Korenaga D, Sugimachi K. Correlation of pre-

operative nutritional condition with post-operative complications in surgical treatment for oesophageal carcinoma. *European Journal of Surgical Oncology* 2002 Jun;28(4):396-400.

Nygaard K, Hagen S, Hansen HS, Hatlevoll R, Hultborn R, Jakobsen A, Mantyle M, Modig H, Munck-Wikland E, Rosengren B et al., Pre-operative radiotherapy prolongs survival in operable esophageal carcinoma: a randomised multi-centre study of pre-operative radiotherapy and chemotherapy. The second Scandinavian trial in esophageal cancer. *World Journal of Surgery* 1992;16 (6): 1104-9.

O Brien TF. Lower Oesophageal sphincter pressure (LESP) and esophageal function in obese humans. *Journal of Clinical Gastroenterology.* 1980, 2:145-8.

O'Flaherty, Immunonutrition in surgical practice. *Proceeding of the Nutrition Society* 1999, 58(4): 831-7.

O'Gorman P, McMillan DC, Mc Ardle CS. Prognostic factors in advanced gastrointestinal cancer patients with weight loss. *Nutrition Cancer; 2000;37(1):36-40.*

Ogunwobi O, Mutungi G, Beales ILP. Leptin Stimulates Proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun Nh2-Terminal Kinase Activation. *Endocrinology*, 2006, 147(9);4505-4516.

Oken MM, Creech RH, Tormey DC, Horton K, Davis TE, McFadden ET, Carbone PP. Toxicity and Response riteria of the Eastern Co-operative Oncology Group. *American Journal of Clinical Oncology*, 1982, 5:649-655.

Okuya S, Tanabe K, Taniawa Y, Oka Y. Leptin increases the viability of isolated rat pancreatic islets by suppressing apoptosis. *Endocrinology* 2001;142:4827-30.

Orlando RC. Overview of the mechanisms of gastroesophageal reflux. *American Journal of Medicine.* 2001. 111 Suppl 8A: 174S-177S.

Orringer MB, Marshall B, Stirling Mc et al., Transhiatial esophagectomy for benign and malignant disease. *Journal of Cardiovascular Surgery* 1993;105:265-277.

Ott K, Weber WA, Lordick F. Metabolic imaging predicts response, survival, and recurrence in adenocarcinoma of the esophagogastric junction. *Journal of Clinical Oncology*, 2006; 24:4692-98.

Owens MA, Vall HG, Hurley AA, Wormsley SB. Validation and Quality Control of immunophenotyping in clinical flow Cytometry. *Journal of Immunological Methods,* 2000, 243(1-2):33-50.

Palareti G, Legnani C, Cosmi B, Guazzaloca G, Pancani C, Coccheri S. Risk of venous thromboembolism recurrence: high negative predictive value of D-dimer performed after oral anticoagulation is stopped. *Thromb Haemost,* 2002, 87:7-12.

Pallant J. SPSS Survival Manual. 2007 (3rd Edition) Mc Graw Hill, London.

Palmblad J, Gyllenhammar H. Effect of dietary lipids on immunity and inflammation. Review article. *Acta Pathologica, microbiologica et immunologica Scandinavica*

Palombo JD, Bistrain BR, Fechner KD, Blackburn GL, Fose RA. Rapid incorporation of fish or olive oil fatty acids into rat hepatic sinusoidal cell phospholipids after continuous enteral feeding during endotoxaemia. *American Journal of Clinical Nutrition*, 1993; 57:643-649.

Peltz G. Nutrition support in cancer patients: A Brief review and suggestion for standard indications criteria. *Nutrition Journal* 2002; 1:1-5.

Pepys MB, Hirschfield GM. C-Reactive Protein: a critical update. *Journal of Clinical Investigation*, 2003; 111(12):1805-1812.

Pera M, Cameron A, Trastek V, Carpenter H, Zermeister A. Increasing incidence of adenocarcinoma of the esophagus and eosphagogastric junction. *Gastroenterology* 1993; 104: 510-513.

Pera M, Manterola C, Vidal O. Epidemiology of esophageal adenocarcinoma. *Journal of Surgical Oncology*, 2005, 92:151-159.

Pera M. Trends in the Incidence and Prevalence of Specialised intestinal metaplasia, barrett's esophagus, and adenocarcinoma of the gastroesophageal junction. *World Journal of Surgery* 2003,27:999-1008.

Persson and B. Glimelius, The relevance of weight loss for survival and quality of life in patients with advanced gastrointestinal cancer treated with palliative chemotherapy. *Anticancer Research* 2002, 22 (6B); 3661–3668.

Pescovitz MD, Mehta PL, Leapman SB, Milgrom ML, Jindal RM, Filo RS. Tube jejunostomy in liver transplant recipients. *Surgery.* 1995 Jun;117(6):642-7

Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *International Journal of Cancer*, 1999;83:18-29.

Plata-Salaman CR. Central nervous system mechanisms contributing to the cachexia-anorexia syndrome, *Nutrition* 2000, 16(10);1009–1012.

Poon RTP, Law SYK, Chu KM et al. Esophagectomy for carcinoma of the esophagus in the elderly. Results of current surgical management. *World Journal of Surgery*, 1998:227:357-364.

Poplin E, Fleming T, Leichman L, Seydel HG, Steiger Z, Taylor S, Vance R, Stuckey WJ, Rivkin SE Combined therapies for squamous-cell carcinoma of the esophagus, a Southwest Oncology Group Study (SWOG-8037). *Journal of Clinical Oncology.* 1987 Apr;5(4):622-8.

Powell J, McConkey CC. The rising trend in oesophageal adenocarcinoma and gastric cardia. *European Journal of Cancer Prevention*, 1992; 1:265-9.

Powell-Tuck J. Nutritional consequences of gastrointestinal disease. *Bailliere's Clinical Gastroenterology.* 1988, 2(4):715-27

Poynton AR, Walsh TN, O'Sullivan G, *et al.* Carcinoma arising in familial Barrett's esophagus. *American Journal of Gastroenterology* 1996;91:1855–6.

Prach AT, MacDonald TA, Hopwood DA, Johnston DA. Increasing incidence of Barrett's oesophagus: education, enthusiasm or epidemiology. *The Lancet* 1997, 350:933.

Quigley EM. Gastro-ooesophageal reflux disease – spectrum or continuum? *Quarterly Journal of Medicine,* 1997; 90: 75-8.

Schluter B, Konig B, Bergmann U. Interleukin-6—a potential mediator of lethal sepsis after major thermal trauma: evidence for increased IL-6 production by peripheral blood mononuclear cells. *Journal of Trauma 1991,* 31: 1663

Stratton RJ, Green CJ, Elia M, Disease-related Malnutrition: an Evidence-based Approach to Treatment, CABI Publishing, Wallingford (2003).

Ravasoc P, Moneiro-Grillo I, Vidal PM, Camilo Me. Nutritional deterioration in cancer: the role of disease and diet *Clinical Oncology*, 2003;15:443-450.

Reid GL, Barrett MT, Galipeau PC, Sanchez CA, Neshat K, Cowan DS, Levine DS. Barrett's Oesophagus: ordering the events that lead to cancer. *European Journal of Cancer Prevention* 1996, 5(suppl2):57-65.

Repnik U, Knezevic M, Jeras M. Simple and cost effectcive isolation of monocytes from buffy coats. *Journal of Immunological Methods,* 2003, 278(1-2), 283-292.

Rey-Ferro M, Castano R, Orozco O, Serna A, Moreno A. Nutritional and immunologic evaluation of patients with gastric cancer before and after surgery. *Nutrition.* 1997 Oct;13(10):878-81

Reynolds JV, Kanwar S, Welsh FK, Windsor AC, Murchan P, Barclay GR, Guillou PJ. 1997 Harry M. Vars Research Award. Does the route of feeding modify gut barrier function and clinical outcome in patients after major upper gastrointestinal surgery?

Journal of Parenteral and Enteral Nutrition, 1997 Jul-Aug;21(4):196-201.

Reynolds JV, Ravi N, Hollywood D, Kennedy MJ, Rowley SP, Ryan AM, Hughes N, Carey M, Byrne P. Neoadjuvant chemoradiation may increase the risk of respiratory complications and sepsis after transthoracic esophagectomy. *Journal of Thoracic and Cardiothoracic Surgery.* 2006, 132(3);549-55.

Richter JE, Falk GW, Vaezi MF. Helicobacter Pylori and gastroesophageal reflux disease: the bug may not be all that bad. *American Journal of Gastroenterology*, 1998; 93-1800-2.

Rigaud D, Merrouche M, Le Moel G, Vatier J, Paycha F, Cadiot G, Naoui N, Mignon M. Factors of gastroesophageal acid reflux in severe obesity. *Gastroentérologie clinique et biologique*. 1995; 19(10): 818-25.

Rivadeneira DE, Evoy D & Fahey T Jr. Nutrition support of the cancer patient. *CA: Cancer Journal for Clinicians*, 1998;48:69-80.

Rizk NP, Bach PB, Schrag D, Bains MS, Turnbull AD, Karpeh M, Brennan MF, Rusch VW. The impact of complications on outcomes after resection for esophageal and gastroesophageal junction carcinoma. *Journal of the American College Surgery*; 2004, 198(1):42-50.

Roberge C, Tran M, Massoud C, Poirée B, Duval N, Daemcour E, Frout D, Malvy D, Joly F, Lebailly P, Henry-Amar M. Quality of life and home enteral tube feeding: a French prospective study in patients with head and neck or oesophageal cancer. *British Journal of Cancer* 2000, 82(2);263–269.

Robinson SC, Coussens LM, Soluble mediators of inflammation during tumor development, *Advances in Cancer Research* 2005,93;159–187.

Rodgers M, Jobe BA, O'Rourke RW, Sheppard B, Diggs B, Hunter JG. Case volume as a predictor of inpatient mortality after oesophagectomy. *Archives of Surgery.* 2007, 142(9):829-39.

Romero Y , Cameron AJ, Locke GR 3rd, *et al.* Familial aggregation of gastroesophageal reflux in patients with Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology* 1997;113:1449–56.

Ross JA, Moses AGW, Fearon KCH. The anti-catabolic effects o n-3 fatty acids. Current opinion in Clinical Nutrition and Metabolic Care. 1999, 2, 219-226.

Ruhl CE, Everhart JE. Overweight, but not dietary fat intake, increases risk of gastro-oesophageal reflux disease hospitalisation. The NHANES I epidemiologic follow-up study. First National Health and Nutrition Examination Survey. *Annals of Epidemiology* 1999, 9(7):424-35.

Ruis NM, Rose JK, Valone FH. Tumour necrosis fctor release by human monocytes stimulated with platelet-ctivating factor. Lipids, 1991, 26:1060-1604.

Ryan AM, Rowley SP, Fitzgerald AP, Ravi N, Reynolds JV. Adenocarcinoma of the oesophagus and gastric cardia: Male preponderance in association with obesity. *European Journal of Cancer* 2006; 42: 1151-8.

Ryan A, Rowley SP, Healy LA, Flood P, Ravi R, Reynolds JV.
Post Oesophagectomy Early Enteral Enteral Nutrition via a needle catheter jejunostomy: 8-year experience at a specialist unit. *Clinical Nutrition* 2006, 25(3):386-93

Sagar PM, Ackroyd R, Hosie KB, Patterson JE, Stoddard CJ, Kingsnorth AN. Regression and progression of Barrett's esophagus after anti-reflux surgery. *British Journal of Surgery* 1995, 82:806-810.

Saito A, Noguchi Y, Yoshikawa T, Doi C, Fukuzawa K, Matsumoto A, Ito Y, tsuburaya A, Nagahara N. Gastrectomized patients are in a state of chronic protein malnutiriton: analyses of 23 amino acids. *Hepato-gastroenterology.* 2001, 48:585-589.

Saklad M. Grading of patients for surgical procedures. *Anesthesiology*, 1941;2:281-4.

Sampliner RE. Practice guidelines on the diagnosis, surveillance and therapy of Barrett's

oesophagus. *American Journal of Gastroesterology* 1998; 93: 1028-31.

Sanchez-Bueno F, Garcia-Marcilla JA, Perez-Flores D, Perez-Abad JM, Vicente R, Aranda F, Ramirez P, Parrilla P. Prognostic factors in a series of 297 patients with gastric adenocarcinoma undergoing surgical resection. *British Journal of Surgery*. 1998, 85(2):255-60

Sand J, Luostarinen M, Matikainen M. enteral or parenteral feeding after total gastrectomy:prospective rndomised pilot study. *European Journal of Surgery,* 1997, 163:761-766.

Sarr MG, Mayo S. Needle catheter jejunostomy: an unappreciated and misunderstood advance in the care of patients after major abdominal operations. *Mayo Clinic Proceedings.* 1988; 63(6):565-72

Sategna-Guidetti C, Bianco L. Malnutition and malabsorption after total gastrectomy. A pathophysiologic approach. *Journal of Clinical Gastroenterology*, 1989;11(5):518-24.

Scarpulla G, Camilleri S, Galante P, Manganaro M, Fox M. The Impact of prolonged pH measurements on diagnosis of Gastroesophageal reflux disease: 4 day wireless pH studies. *American Journal of Gastroenterology,* 2007, 102(12):1642-7.

Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition Clinical Nutrition*, 1985;82: 3392-6

Schunn CD, Daly JM. Small bowel necrosis associated with postoperative jejunal tube feeding. *Journal of the American College of Surgeons.* 1995 Apr;180(4):410-6

Schwartz SR, Yueh B, Maynard C et al. Predictors of wound complications after laryngectomy: A study of over 2000 patients. *Otolaryngology and Head and Neck Surgery*, 2004; 131(1): 61-8.

Scurtu R, Groza N, Otel O, Goia A, Funariu G. Quality of Life in patients with esophagjejunal anastamosis after total gastrectomy for cancer. *Romanian Journal of*

Gastroenterology. 2005, 14(4):367-372.

Senkal M, Haker R, Linseisin J, Wolfram G, Homann HH, Stehle P. Preoperative oral supplementation with long chain omega 3 fatty acids beneficially alters phospholipids fatty acid patterns in liver, gut mucosa and tumour tissue. *Journal of Parenteral and Enteral Nutrition*, 2005, 29 (4): 236-240.

Senkal M, Mumme A, Eickhoff U, Geier B, Spath A, Kemen M. Early post operative immunonutrition: clinical outcome and cost comparison analysis in surgical patients. *Critical Care Medicine*, 1997, 25:1489-1496.

Seydel HG, Leichman L, Byhardt R, Cooper J, Herskovic A, Libnock J, Pazdur R, Speyer J, Tschan J. Preoperative radiation and chemotherapy for localized squamous cell carcinoma of the esophagus: a RTOG Study. *International Journal of Radiation Oncology, Biology, Physics.* 1988 Jan; 14(1):33-5.

Shaheen N, Provenzole D. The epidemiology of gastroesophageal reflux disease. *American Journal of Medical Science* 2003;326:264-73.

Sheeran P, Hall GM. Cytokines in anaesthesia. *British Journal of Anaesthesia,* 1997;78:201-19.

Shibasaki H, Furukawa K, Yamamori H, Kimura F, Tashiro T, Miyazaki M. The post-operative level of serum monocyte chemoattractant protein-1 and its correlation with the severity of surgical stress. *Journal of Surgical Research,* 2006, 136(2):314-9.

Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *Journal of Clinical Investigation* 2006, 116:1793-1801.

Sica GS, Sujendran V, Wheeler J, Soin B, Maynard N. Needle catheter jejunostomy at oesophagectomy for cancer. *Journal of Surgical Oncology;* 2005, 15;91(4):276-9.

Sierra-HonigmannMR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulous A. Biological actions of leptin as an angiogenic factor. *Science* 1998, 81:1683-86.

Siewert JR, Stein HJ, Feith M, Bruecher BL, Bartels H, Fink U. Histologic tumour type is an independent prognostic parameter in esophageal cancer: lessons from more than 1000 consecutive resections at a single centre in the Western World. *Annals of Surgery*, 2001; 234:360-7.

Siewert JR, Stein HJ. Classification of adenocarcinoma of the oesophago-gastric junction. *British Journal of Surgery*; 1998; 85: 1457-9.

Silk DBA, Gow NM. Post-operative starvation after gastrointestinal surgery. *British Medical Journal, 2001;* 323:761-2.

Siman JH, Forsgren A, Berglund G. Helicobacter Pylori infection is associated with a decreased risk of developing oesophageal neoplasms. *Helicobacter,* 2001 ;4 :310-6.

Sitges-Serra A, Rafecas A, Jaurrieta E. Malnutrition, resectability and post-operative outcome in gastric cancer patients. *British Journal of Clinical Practice,* 1988, Suppl 63:15-19.

Smith KJ, O Brien SM, Smithers BM, Gotley DC, Webb PM, Green AC, Whiteman DC. Interactions among smoking, obesity, and symptoms of acid reflux in Barrett's Oesophagus. *Cancer Epidemiology and Biomarkers Prevention.* 2005; 14(11):2481-2486.

Soerjomataram I , De Vries E, Pukkala E, Coebergh JW. Excess of cancers in Europe: A study of eleven major cancers amenable to lifestyle change. *International Journal of Cancer.* 2007 Mar 15;120(6):1336-43._

Soeters PB, Von Meyenfeldt MF, Meijerink WJHJ et al. Serum albumin and mortality (Letter). *The Lancet* 1990; 335: 348.

Somasundar P, Yu AK, Vona-Davis L, McFadden DW. Differential effects of leptin on cancer in vitro. *Journal of Surgical Research.* 2003(b), 113(1): 50-5.

Spanga G, Siegel JH, Brown G et al. Reprioritization of hepatic plasma protein release in trauma and sepsis. *Archives of Surgery*, 1985; 120: 187 – 198.

Spechler SJ, Robbins AH, Rubins HB, et al. Adenocarcinoma and Barrett's esophagus: an overrated risk? *Gastroenterology* 1984; 87:927-33.

Spechler SJ. Barrett's esophagus. *Seminars in Oncology,* 1994,21:431-437.

Staal-van den Brekel AJ, Dentener MA, Schols AM, Buurman WA, Wouters EF. Increased resting energy expenditure and weight loss are related to a systemic inflammatory response in lung cancer patients, *Journal of Clinical Oncology* 1995,13(10); 2600–2605.

Stahl M, Wilke H, Fink U et al. Combined preoperative chemotherapy and radiotherapy in patients with locally advanced esophageal cancer: Interim analysis of a phase II trial. *Journal of Clinical Oncology*; 1996; 14: 829-837

Stein DJ, El-Serag HB, Kuczynski J, Kramer JR, Sampliner RE. The association of body mass index with barrett's oesophagus. *Alimentary Pharmacology and Therapeutics,* 2005; 22:1005-1010.

Stein HJ, Hoeft S, DeMeester TR. Reflux and motility pattern in Barrett's Oesophagus. *Diseases of the Oesophagus*, 1992, 5: 21-28.

Stevens J Plankey MW, Williamson DF. The body mass index-mortality relationship in White and African American women. *Obesity Research*, 1998;6:268-77.

Strassmann G, Kambayashi T. Inhibition of experimental cancer cachexia by anti-cytokine and anti-cytokine-receptor therapy, *Cytokines and Molecular Therapy* 1995, 1(2); 107–113.

Stulnig TM. Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. *International Archives of Allergy and Immunology*, 2003, 132:310-21.

Suchner U, Kuhn KS, Furst P. The scientific basis of immunonutrition. *Proceedings of the Nutrition Society*, 2000, 59:553-563.

Sungurtekin H, Sungurtekin U, Balci C, Zencir M, Erdem E. The influence of nutritional status on complications after major intraabdominal surgery. *Journal of the American College of Nutrition,* 2004,23(3):227-32

Sutton P, Clarke P. Neo-adjuvant treatment for oesophageal cnacer. *Gastrointestinal Cancer*; 2000;3:231-38.

Swails WS, Kenler AS, Driscoll DF, DeMichele SJ, Babineau TJ, Utsunamiya T, Chavali S, Forse RA, Bistrian BR. Effect of a fish oil structured lipid-based diet on prostaglandin release from mononuclear cells in cancer patients after surgery, *Journal of Parenteral and Enteral Nutrition* 1997, 21(5); 266–274.

Sweeney, KJ, Kell MR, Ravi N, Reynolds JV. Major upper gastrointestinal surgery is associated with an antigen-dependent proinflammatory T cell response. *British Journal of Surgery* 2005, 92(8):989-95.

Tabira Y, Okuma T, Kondo K, Yoshioka M, Mori T, Tanaka M, Nakano K, Kitamura N Does neo-adjuvant chemotherapy for carcinoma in the thoracic esophagus increase postoperative morbidity? *Japanese Journal of Thoracic and Cardiovascular Surgery.* 1999;47(8):361-7.

Takagi K, Yamamori H, Furukawa K, Miyazaki M, Tashiro T. Perioperative supplementation of EPA reduces immunosuppression induced by postoperative chemoradiation therapy in patients with esophageal cancer, *Nutrition* 2001, 17(6); 478–479.

Takagi K, Yamamori H, Toyoda Y, Nakajima N, Tashiro T. Modulating effects of the feeding route on stress response and endotoxin translocation in severely stressed patients receiving thoracic esophagectomy. *Nutrition,* 2000 May;16(5):355-60

Takatsuka H, Takemoto Y, Iwata N, Suehiro A, Hamano T, Okamoto T, Kanamaru A, Kakishita E. Oral eicosapentaenoic acid for complications of bone marrow transplantation, *Bone Marrow Transplantation* 2001, 28(8); 769–774.

Takesaki T, Gao CM, Wu JZ. Dietary protective and risk factors for esophageal and stomach cancers in a low-epidemic area for stomach cancers in Jiangsu province, China: Comparison with those in a high-epidemic area. *Japanese Journal of Cancer Research*, 2001:92:1157-1165.

Terrosu G, Cedolini C, Bresadola V, Baccarani U, Uzzau A, Signor M, Fongione S, Buffoli A, Iop A, Vigevani E, Sacco C, Cartei G, Bresadola F. Preoperative chemoradiotherapy in cancer of the thoracic esophagus. *Diseases of the Esophagus.* 2003; 16(1):9-16.

Terry P, Lagergren J, Wolk A. Reflux-inducing dietary factors and the risk of adenocarcinoma of the esophagus and gastric cardia. *Nutrition and Cancer*, 2000;38:186-191.

Terry P, Lagergren J, Ye W, Nygren O, Wolk A. Antioxidants and cancers of the esophagus and gastric cardia. *International Journal of Cancer*, 2000;87:750-4.

Terry P, Lagergren J, Ye W. Inverse association between intake of cereal fibre and risk of gastric cardia caner. *Gastroenterology*, 2001;120:387-391.

Testi R, Phillips JH, Lanier LL. T cell activation via Leu-23 (CD69). *Journal of Immunology,* 1989,15;143(4):1123-8.

Tevar R, Jho DH, Babcock T, Helton WS, Espat NJ. Omega-3 fatty acid supplementation reduces tumor growth and vascular endothelial growth factor expression in a model of progressive non-metastasizing malignancy, *Journal of Parenteral and Enteral Nutrition* 2002, 26(5);285–289.

Tillet WS, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *Journal of Experimental Medicine* 1930; 52: 561 – 571.

Timaksiz MB, Deschamps C, Allen MS, Johnson DC, Pairolero PC. Effectiveness of screening aqueous contrast swallow in detecting clinically significant anastomotic leaks after esophagectomy. *European Surgical Research*, 2005, 37(2):123-8.

Tisdale MJ. Inhibition of lipolysis and muscle protein degradation by EPA in cancer cachexia, *Nutrition* 1996, 12(Suppl 1);S31–S33.

Todorov P, Cariuk P, McDevitt T, Coles B, Fearon K, Tisdale M. Characterization of a cancer cachectic factor, *Nature* 1996, 379(6567);739–742.

Todorov PT, Field WN, Tisdale MJ. Role of a proteolysis-inducing factor (PIF) in cachexia induced by a human melanoma (G361). *British Journal of Cancer* 1999, 80(11);1734–1737.

Tran GD, Sun XD, Abnet CC. Prospective study of risk factors for esophageal and gastric cancers in the Linxian population trial in China. *International Journal of Cancer*, 2005; 113:456-463.

Trevisan M, Liu J, Muti P, Misciagna g, Menotti A, Fucci F. Markers of insulin resistance and colorectal cancer mortality. *Cancer Epidemiology and Biomarkers Prevention,* 2001, 10:937-941.

Tsekos E, Reuter C, Stehle P, Doeden G. Peri-operative administration of parenteral fish oil supplements in a routine clinical setting improves patient outcome after major abdominal surgery. *Clinical Nutrition*, 2004, 23:325-330.

Tsuchiya T, Shimizu H, Horie T, Mori M. Expression of leptin receptor in lung : leptin as a growth factor. *European Journal of Pharmacology* 1999, 365:273-279.

Tsutsui S, Morita M, Kuwano H. Influence of pre operative treatment and surgical operation on immune function of patients with esophageal carcinoma. *Journal of Surgical Oncology*, 1992, 49:176-181.

Urba SG, Orriner MB, Turrisi A, Iannettoni M, Forastiere A, Strawderman M. Randomised trial of preoperative chemoradiotherapy versus surgery alone in patients with locoregional oesophageal carcinoma. *Journal of Clinical Oncology* 2001; 19: 305-13.

Urschel JD, VasanH. A meta-analysis of randomised controlled trials that compared neo-adjuvant chemoradiation to surgery alone for respectable esophageal cancer. *American Journal of Surgery* 2003 Jun;185(6):538-43.

Urschel JD. Esophagogastrostomy anastomotic leak rates complicating esophagectomy: A review. *American Journal of Surgery*, 19

van Bokhorst-de van der Schueren MAE, Langendoen SI, Vondeling H, Kuik DJ, Quak JJ, van Leeuwen P. Perioperative enteral nutrition and quality of life of severely malnourished head and neck cancer patients: a randomized clinical trial, *Clinical Nutrition,* 2000, 19(6);437–444.

Van Cutsem E, Arends J. The causes and consequences of cancer-associated malnutrition. *European Journal of Oncology Nursing* 2005, 9(Suppl 2);S51-63.

Van der Veen AH, Dees J, Blankensteijn JD, Van Blankenstein M. Adenocarcinoma in Barrett's oesophagus: an overrated risk. *Gut* 1989;30:148.

Van Lanschot JJ, Hulscher JB, Buskens CJ, Tilanus HW, ten Kate FJ, Obertop H. Hospital volume and hospital mortality for esophagectomy. *Cancer;* 2001: 15;91(8):1574-8

Vaughan TL, Davis S, Kristal E. Obesity, alcohol, and tobacco as risk factors for cancers of the gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiology and Biomarkers Prevention*, 1995;4:85-92.

Vaughan TL, Kristal AR, Blount PL, et al. Nonsteroidal anti-inflammatory drug use, body mass index, and anthropometry in relation to genetic and flow cytometric abnormalities in Barrett's oesophagus. *Cancer Epidemiology and Biomarkers*

Prevention. 2002;11(8): 745-52.

Veith M, Masoud B, Meining A. Helicobacter Pylori infection :protection against Barrett's mucosa and neoplasia? *Digestion* 2000;62:225-31.

Veterans Affairs Total Parenteral Nutrition Cooperative Study Group, 1991. Perioperative total Parenteral nutrition in surgical patients. *New England Journal of Medicine* 325; 525-532.

Villegas R, Creagh D, Hinchion R, O Halloran D, Perry IJ. Prevalence and Lifestyle determinants of the metabolic syndrome. *Irish Medical Journal*, 2006, 97(10); 300-3.

Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-Reactive Protein Levels in Overweight and obese adults. *Journal of the American Medical Association,* 1999, 282(22):2131-2135.

Von Meyenfeldt M. Cancer-associated malnutrition: An introduction. *European Journal of Oncology Nursing* 2005, 9(Suppl 2); S35-S38

Wakefield SE, Mansell NJ, Baigrie RJ, Dowling BL. Use of a feeding jejunostomy after esophagogastric surgery. *British Journal of Surgery*. 1995 Jun; 82(6):811-3

Walsh T, Grannell M, Mansoor S. Predictive factors for success of neo-adjuvant therapy in upper gastrointestinal cancer. *Journal of Gastroenterology and Hepatology* 2002;17:S172-S175.

Walsh TN, Noonan N, Hollywood D, Kelly A, Keeling N, Hennessy TP. A comparison of multimodality therapy and surgery for esophageal adenocarcinoma. *New England Journal of Medicine;* 1996 Aug 15;335(7):462-7

Wang LS, Lin HY, Chang CJ, Fahn HJ, Huang MH, Lin CFJ. Effects of enbloc oesophagectomy on nutritional and immune status in patients with esophageal carcinoma. *Journal of Surgical Oncology,* 1998, 76:90-98.

Weinsier RL, Hunter GR, Gower BA. Body fat distribution in white and black women: Different patterns of intra-abdominal and subcutaneous abdominal adipose tissue utilization with weight loss. *American Journal of Clinical Nutrition*2001; 74(5):631-6.

Welsh FK, Farmery SM, Ramsden C, Guillou PJ, Reynolds JV. Reversible impairment in monocyte major histocompatibility complex class II expression in malnourished surgical patients. *JPEN Journal of Parenteral and Enteral Nutrition.* 1996 Sep-Oct; 20(5):344-8.

Wenzel J. Henze S. Brähler S. The expression of human leukocyte antigen-DR and CD25 on circulating T cells in cutaneous lupus erythematosus and correlation with disease activity. *Experimental Dermatology*, 2005 14(6): 454-459.

Weston AP, Badr As, Topalovski M. Prospective evaluation of the prevalence of gastric Helicobacter Pylori infection in patients with GERD, Barrett's esophagus, Barrett's dysplasia, and Barrett's adenocarcinoma. *American Journal of Gastroenterology,* 2000;95:387-94.

Whooley BP, Law SC, Murthy SC et al. Analysis of reduced death and complication rates after esophageal resection. *Annals of Surgery* 2001; 233:338-4

Wigmore SJ, Barber MD, Ross JA, Tisdale MJ, Fearon KC. Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutrition and Cancer,* 2000;36:177-184.

Wigmore SJ, Fearon KC, Ross JA. Modulation of human hepatocyte acute phase protein production in vitro by n-3 and n-6 poluunsaturated fatty acids. *Annals of Surgery,* 1997, 225:103-11.

Wigmore SJ, Fearon KC, Maingay JP, Ross JA. Down-regulation of the acute-phase response in patients with pancreatic cancer cachexia receiving oral eicosapentaenoic acid is mediated via suppression of interleukin-6, *Clinical Science* 1997, 92(2); 215–221.

Willett WC, Manson JE, Stampfer MJ. Weight, weight change, and coronary heart disease in women: risk within the normal weight range. *Journal of the American Medical Association,* 1995;273:461-5.

Wilson LJ, Ma W, Hirshowitz BI. Association of obesity with hiatus hernia and esophagitis. *American Journal of Gastroenterology,* 1999; 94:2840-2844.
Windsor AC, Kanwar S, Li AG, Barnes E, Guthrie JA, Spark JI, Welsh F, Guillou PJ,

Reynolds JV.Compared with parenteral nutrition, enteral feeding attenuates the acute phase response and improves disease severity in acute pancreatitis.*Gut* 1998,42(3):431-5.

Windsor JA, Hill GL. Weight loss with physiologic impairment – a basic indicator of surgical risk. *Annals of Surgery.* 1988, 207:290.

World Health Organisation Consultation on Obesity. Preventing and managing the global epidemic: Report of a WHO consultation on Obesity, Geneva, 3-5 June 1997, 1-276 Geneva, Switzerland, WHO. 1998.

Wright TA. High grade dysplasia in Barrett's metaplasia. *British Journal of Surgery* 1997, 84:760-766.

Wu AH, Crabtree JE, Bernstein LA. Role of Helicobacter Pylori cagA+ strains and the risk of adenocarcinoma of the stomach and esophagus. *International Journal of Cancer*, 2003;103: 815-821.

Wu AH, Wan P, Bernstein L. A multiethnic population-based study of smoking, alcohol and body size and risk of adenocarcinomas of the stomach and esophagus (United States). *Cancer Causes Control* 2001;12:721–32.

Wu-Williams AH, Yu Mc, Mack TM. Life-style, workplace, and stomach cancer by subsite in young men of Los Angeles county. *Cancer Research*, 1990, 50:2569-76.

Xu H, Barnes GT, Yang Q. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*

2003;112(12):1821-30.

Xu J, Zhong Y, Jing D, Wu Z. Pre operative enteral immunonutrition improves post operative outcomes in patients with gastrointestinal cancer. *World Journal of Surgery*, 2006, 30:1284-89.

Yagi M, Hashimoto T, Nezuka H, Ito H, Tani T, Shimizu K, Miwa K. Complications associated with enteral nutrition using catheter jejunostomy after esophagectomy. *Surgery Today,* 1999;29(3):214-8

Yang PC, Davis S. Incidence of cancer of the esophagus in the US by histologic subtype. *Cancer,* 1988;61:612-617.

Yamada T, Hisanaga M, Nakajima Y. Serum interleukin 6, interleukin 8, hepatocytes growth factor and nitric oxide changes during thoracic surgery. *World Journal of Surgery*, 1998, 22:783.

Ye W, Chow WH, Lagergren J, *et al.* Risk of adenocarcinomas of the oesophagus and gastric cardia in patients with gastroesophageal reflux diseases and after antireflux surgery. *Gastroenterology* 2001;121:1286–93

Ye W, Held M, Lagergren J. Helicobacter Pylori infection and gastric atrophy: Risk of adenocarcinoma and squamous cell carcinoma of the esophagus and gastric cardia. *Journal of the National Cancer Institute*, 2004;96:388-396.

Zetti G, Tagiabue F, Barabino M, Fontana S, Ceppi M, Samori G. Small bowel necrosis associated with postoperative enteral feeding. *Chirurgia Italiana*; 2002, 54(4):555-8.

Zhang ZF, Kurtz RC, Sun M, *et al.* Adenocarcinomas of the esophagus and gastric cardia: Medical conditions, tobacco, alcohol, and socioeconomic factors. *Cancer Epidemiology and Biomarkers Prevention* 1996;5:761–8.

Zhang ZF, Kurtz RC, Yu GP. Adenocarcinomas of the esophagus and gastric cardia: The role of diet. *Nutrition and Cancer*, 1997;27:298-309.

Zheng Y, Li F, Qi B, Luo B, Sun H, Liu S, Wu X. Application of perioperative immunonutrition for gastrointestinal surgery:a meta-analysis of randomised controlled trials. *Asia Pacific Journal of Clinical Nutrition,* 2007, 16 Suppl 1:253-7.

Ziegler SF, Ramsdell F, Alderson MR. The activation antigen CD69. *Stem Cells.* 1994;12(5):456-60.

APPENDIX 1

Ethical approval for the following studies was sought and approval given, subject to informed consent. Copies of the patient information leaflets and the consent forms for the two studies listed below are included in the Appendix. Also attached are copies of letters sent to patients or care providers, datasheets of Products used, label text from the products used, Hospital DRA forms, procedures for reporting adverse events, and randomisation procedures.

Prospective investigation of the incidence of central adiposity, metabolic syndrome, insulin resistance and adipo-cytokine secretion amongst patients with Gastro-oesophageal reflux disease and Barrett's Oesophagus **(Chapter 3).**

A randomised double blinded trial to investigate the effects of an enteral nutritional supplement enriched with eicosapentaenoic acid on post operative complications, stress response, immune function, body composition and quality of life in patients undergoing surgical treatment of oesophageal cancer **(Chapter 7)**

APPENDICES FOR CHAPTER 3

Professor Reynolds' Acid Reflux and Barrett's Oesophagus Clinic
Department of Surgery

Secretaries: ph. 4103595; fax. 4546534

e-mail reynoldsec@stjames.ie
Oesophageal Nurse Specialist: Sr Jenny Moore 4162650; bleep 296

Ref. JR/AL/o
__/08/2006

Mr

Dear Mr_____,

This is to inform you that we have established a Clinic at St James's Hospital for patients with severe acid reflux and/or the condition of Barrett's oesophagus. The second Clinic will be on Thursday 14th September. If you can attend I would be grateful if you could contact my secretary at the above number for an appointment time.

When I meet with you at the Clinic I would also like to discuss the possibility of your participation in some studies relating to acid reflux and how we may improve your condition.

With very best wishes,

Yours sincerely,

Prof John Reynolds, M.Ch., FRCSI
Consultant Surgeon

Patient Information Leaflet

PROSPECTIVE INVESTIGATION OF THE INCIDENCE OF CENTRAL ADIPOSITY, METABOLIC SYNDROME, INSULIN RESISTANCE AND ADIPOKINE SECRETION AMONGST PATIENTS WITH GASTRO-OESOPHAGEAL REFLUX DISEASE/ BARRETT'S OESOPHAGUS UNDERGOING UPPER GI PH MANOMETRY STUDIES.

Dear Patient

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives or your doctor if you wish. Ask us if there is anything that is not clear or if you would like more information.

Please take time to decide whether or not you wish to take part in this study.
Thank you for reading this.

Why have I been chosen?

You have been chosen to take part in this study as you are undergoing tests for acid reflux called pH Manometry. On the morning of this test we would like to screen you for diabetes, high cholesterol, and check the levels of some hormones produced by fat tissue in your blood. We would also like to check your body composition, height, weight and blood pressure. These tests will give us very helpful information about how body weight is related to acid reflux.

What will happen to me if I take part?

If you agree to take part we will take some blood samples approximately 20mls (4 teaspoons) to check your cholesterol, blood sugar, insulin levels and we will measure your blood pressure. We will measure your weight, height, waist circumference and using a special body composition analyser we will measure the fat and muscle composition of your body by asking you to stand on a special weighing scales. These tests should last no longer than 20 minutes.

What will happen if I chose not to take part?

If you choose not to take part you are free to go home after your pH Manometry tests.

What do I have to do?

If you agree to take part you will have blood tests taken and your body composition checked and then you are free to go home. This is a once off measurement and so you will not be called back to the hospital or have to take any additional medications as a result of this study.

What are the side effects/risks of taking part?

The various tests and measurements in this study are not harmful in any way. The blood samples will be taken using a needle and syringe and are expected to cause only minor discomfort such as bruising or a build up of blood under the skin.

What are the possible benefits of taking part?

There are many potential benefits to taking part – you will be able to have free

screening for diabetes, high cholesterol, and high blood pressure as well as having your body composition checked. Should any of your blood tests reveal abnormal results we will inform you GP. This may mean that if you have any of these conditions you will be treated by your GP at an earlier stage.

What about confidentiality?

All of your study records will remain strictly confidential. The researchers including your GP, the ethics committee and regulatory authorities will have access to your original medical records for the purpose of collecting data, verifying that the data is correct and checking that the study is conducted properly. By signing this form you are allowing your doctor, the researchers and the study staff to permit these people to see your medical records.

Confidentiality is promised in all cases and your identity will not be disclosed to the public. Any information that may leave the hospital, apart from that which we send to your GP will have your name and address removed and you will only be identified by your initials and study number. Under the Access to Health Records Act (1990), you may ask to see your study records.

What do you do with my information?

The information collected in this study will be processed to meet the purpose of the clinical study. It may also be used in reports of the study or for scientific presentations. You will not be identified in any such publication. The information obtained from this study, which relates to you may be used for future medical research, either in this field, or in a new area (but only with further ethics committee approval).

Compensation

Participation in this study is covered by an approved policy of insurance in the name of St. James's Hospital. In addition the medical practitioners involved in this study have current medical malpractice insurance cover. St. James's Hospital will comply with the ABPI guidelines and Irish Law (statutory and otherwise) in the unlikely event of your becoming ill or injured as a result of participation in this clinical study.

Payment for the study

There is no payment for patricipation in this study

Who do I call if I have questions or problems?

Aoife Ryan, Research Dieititan St. James's Hospital (01) 4162180	**OR**	Dr Patrick Byrne Head of the GI function unit St. James's Hospital (01) 4162845.

Patient Consent Form

PROSPECTIVE INVESTIGATION OF THE INCIDENCE OF CENTRAL ADIPOSITY, METABOLIC SYNDROME, INSULIN RESISTANCE AND ADIPOKINE SECRETION AMONGST PATIENTS WITH GASTRO-OESOPHAGEAL REFLUX DISEASE/ BARRETT'S OESOPHAGUS UNDERGOING UPPER GI PH MANOMETRY STUDIES.

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.
I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.
Name of sponsor:

PARTICIPANT'S NAME: _____

PARTICIPANT'S SIGNATURE: _____

Date:_____

Date on which the participant was first furnished with this form:_____

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained:-

NAME OF CONSENTOR, PARENT or GUARDIAN:_____

SIGNATURE:_____

RELATION TO PARTICIPANT:_____

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness.

NAME OF FIRST WITNESS:_____SIGNATURE:_____
NAME OF SECOND WITNESS: _____SIGNATURE:_____
Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's signature:_____Date:_____

CASE REPORT FORM

Prospective investigation of the incidence of nutritional status, central adiposity, metabolic syndrome, insulin resistance and adipokine secretion amongst patients with Barrett's oesophagus.

Aoife Ryan
Research Dietitian
University Department of Clinical Surgery
St. James's Hospital
4162180

Patient Number: _____

Name:_____

MRN:_____

DOB:___/_____/____

- ☐ **Gender: Male**
- ☐ **Female**

Diagnosis:_____

Medication:_____

Smoking:_____

Alcohol:_____

Date Patient assessed: ____/____/_____

Date Samples Taken: ____/____/_____

Informed Consent

Please ensure that the subject has signed and dated his/her informed consent before any trial specific assessment. The patient has signed the consent form for his/her inclusion in this study and for usage of the patient's (protected) medical data.

- Is the patient capable of giving consent — Yes ☐ No ☐

If no, please explain:

Date of Consent ☐☐☐☐☐☐ (d d m m y y)

Inclusion/Exclusion Criteria

Inclusion criteria

*If any of the criteria is checked **no**, do not include the patient in the study.*

	YES	NO
1. Is patient >18 years and with confirmed Barrett's oesophagus?	☐	☐

Exclusion criteria

If any of the criteria are checked yes, do not include the patient in the Study

Does the patient meet each of the following criteria?	YES	NO
1. Does the patient have a cardiac pacemaker in situ?	☐	☐
2. Does the patient have histologically confirmed cancer of the oesophagus, gastric cardia or stomach?	☐	☐
3. Has the patient ever undergone Laparoscopic Nissans Fundoplication or any other form of anti-reflux surgery?	☐	☐
4. Is the patient Pregnant?	☐	☐

Date Samples Taken

d	d	m	m	y	y

BIOCHEMISTRY

	Value	Units	Normal Range
Glucose Control			
Fasting Glucose		mmol/l	2.8 – 8.3
HbA1c		%	4.8-6.9
Fasting Insulin		mU/L	0-12
HOMA-IR **Fasting glucose X fasting insulin** 22.5		Low=high insulin sensitivity High= insulin resistance	
Lipid Profile			
Total Cholesterol		mmol/l	3.0 – 5.2
HDL Cholesterol		mmol/l	1.0 – 2.1
LDL		mmol/l	2 – 3.36
Total: HDL Cholesterol		mmol/l	Ratio
TAG		mmol/l	0.5 – 2.0
Liver Profile			
Albumin		g/l	35-50
Total Protein		g/l	60-80
Total Bilirubin		umol/L	0-17
Alkaline Phosphatase		IU/L	40-120
Gamma-GT		IU/L	10-55
LDH		IU/L	230-450
AST		IU/L	7-40
Calcium		mmol/L	2.2-2.7
Inorganic Phosphate		mmol/L	0.8-1.4
Inflammatory Markers			
Cortisol		ng/ml	
CRP		mg/l	
Metabolic Syndrome – 3 or more of following			

Impaired Glucose Tolerance Fasting glucose > 6.1mmol/l or on therapy	Yes No		
Dyslipidaemia Fasting TAG >1.7mmol/l Low HDL <1.04mmol/l men <1.29mmol/l women	Yes No Yes No		
Abdominal Obesity Waist >102 cm men >88cm women	Yes No		
Arterial Hypertension **Blood Pressure** >130/85mmHg	Yes No		
Adipokines			
DATE SERUM FROZEN: _____/_____/_____			
DATE SERUM ANALYSED: _____/_____/_____			
Leptin		ng/ml	
Resistin		ng/ml	
Adiponectin		ng/ul	
TNF-α		ng/ml	
IL-6			

Date measurement taken

d	d	m	m	y	y

PHYSICAL ACTIVITY

Physical Activity	
Leisure Time 1 = no exercise at all; **2 = regular physical activity up to 2 h per week (jogging, biking, swimming, playing tennis, heavy gardening, etc.);** 3 = regular physical activity for >2 h per week.	
Work 1 = sedentary; 2 = moderate; 3 = heavy.	
Overall Physical Activity Score	

Date measurement taken

d	d	m	m	y	y

BLOOD PRESSURE

	Value	Units	Normal Range
Blood Pressure			
BP		mm/Hg	
Pulse Rate		Beats/min	

Date assessment performed

d	d	m	m	y	y

NUTRITIONAL ASSESSMENT

	Value	Units	Normal Range
Anthropometry			
Weight		kg	
Height		m	
BMI		kg/m^2	

Ideal Weight for Height			
Waist Circumference		cm	
Bio-Electrical Impedance			
Overall Body Composition			
Fat		%	
Fat		kg	
Fat Free Mass		kg	
Total Body Water		kg	
Hydration		%	
Trunk			
Fat		%	
Fat Mass		kg	
Fat Free Mass		kg	
Predicted Muscle Mass		kg	
Right Leg			
Fat		%	
Fat Mass		kg	
Fat Free Mass		kg	
Predicted Muscle Mass		kg	
Left Leg			
Fat		%	
Fat Mass		kg	
Fat Free Mass		kg	
Predicted Muscle Mass		kg	
Body Composition continued			
Right Arm			
Fat		%	
Fat Mass		kg	
Fat Free Mass		kg	
Predicted Muscle Mass		kg	

Left Arm Fat Fat Mass Fat Free Mass Predicted Muscle Mass		 % kg kg kg	
Nutritional intake			
Diary completed		Yes No	
Energy Intake		_____kcals/24 hours	
Composition of Diet - Macronutrients			
 Carbohydrate Protein Fat		**% Energy**	
Composition of Diet - Micronutrients			

APPENDICES FOR CHAPTER 7

Patient Information and Consent

A RANDOMISED DOUBLE BLINDED TRIAL TO INVESTIGATE THE EFFECTS OF AN ENTERAL NUTRITIONAL SUPPLEMENT ENRICHED WITH EICOSAPENTAENOIC ACID ON POST OPERATIVE COMPLICATIONS, STRESS RESPONSE, GENE EXPRESSION, IMMUNE FUNCTION, BODY COMPOSITION AND QUALITY OF LIFE IN PATIENTS UNDERGOING SURGICAL TREATMENT OF OESOPHAGEAL CANCER.

Patient Information Sheet

Dear Patient
You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives or your doctor if you wish. Ask us if there is anything that is not clear or if you would like more information.

Please take time to decide whether or not you wish to take part in this study. Thank you for reading this.

Purpose of Study

It is normal after oesophagectomy (the operation you are about to undergo) not to be allowed to eat food orally for up to 11 days. During this time it is normal to be given a nutritional liquid feed to patients through a feeding tube that is inserted into your small bowel at the time of your operation. This nutritional feed contains energy, protein and all the vitamins and minerals your body needs while you recover from your operation.
This study aims to investigate the benefits of a new nutritional supplement that is fortified with omega-3 fats (the fats found naturally in fish oils) on the immune system, your weight and quality of life after your surgery. Recent research suggests that this nutritional supplement may significantly improve immune function, nutritional status and quality of life. If you agree to take part in this study you will randomly be assigned to take a nutritional feed *with* omega 3 fat or *without* omega 3 fats (the normal feeds we usually give patients). Both nutritional feeds will look the same and you and the doctors conducting this study will not know which supplement you are on until after the trial is over. The omega feed tastes and looks the same as the standard nutritional feed and does not taste or smell of fish!

If you agree to take part in this study you will be asked to take a nutritional drink (400 mls, approximately two cups) two times a day orally for the 7 days before your surgery. After you surgery it is routine to feed patients through a feeding tube and we will use this tube to give you either the standard nutritional feed or the omega 3 feed 10 days post surgery. Once your surgeon feels it is safe for you to eat normal food again we will supplement your oral intake with one liter of feed at night time while you sleep until you are ready to be discharged from hospital. You will be given a feeding tube whether or not you agree to partake in this study, and you will also be fed at night time whether or not you agree to take part in this study. On discharge (approximately three weeks

after you operation we will ask you to take two drinks a day either orally or you can administer them easily through you feeding tube using a plastic syringe, for one month. When you return for your first out patient visit your doctor will stop the supplements and remove your feeding tube. The study will not involve any additional medical procedures other than what is routinely carried out after this type of surgery.

Why have I been chosen?

If you are willing to join the study, you will be one of about 160 patients taking part in the study. In order to be eligible for this study, you need to meet certain criteria. If your doctor decides that you meet the criteria, you will begin the study procedures after you sign this consent form.

What will happen to me if I take part?

If you take part in this study we will perform initial blood tests and a detailed nutritional assessment. The nutritional intervention will be the current standard protocol for the treatment of this condition. The study will involve blood tests (usually 2-5ml) at the beginning and on days 1, 7 14 and 21 days after your surgery, and on your one month, months and 6 months follow up visits in out patients. This will be to assess the effects of the supplement on your immune system. Genetic testing of your blood samples will also be performed. You will also be reviewed by a Dietitian for a full nutritional assessment at regular intervals throughout the observation. A nurse specialist will also assess your quality of life using a questionnaire. All of these procedures are normal apart from extra blood tests is you agree to take part.

What will happen if I chose not to take part?

Standard nutritional feeds, which are protocol in this hospital will be given to you should you not wish to participate. You will also be seen by a hospital dietitian regularly and also by the specialist cancer nurse

What do I have to do?

- It will involve taking a nutritional drink two times a day for seven days before your surgery. This will be provided free of charge by the hospital.
- After your surgery instead of giving you a standard nutritional product through your feeding tube we will give you one of the two nutritional products we are investigating.
- The study will not involve any additional medical procedures other than what is routinely carried out.
- You can eat and drink as normal and there is no restrictions on activity levels, medications etc.

What is the drug or procedure being tested?

Prosure (the omega 3 supplement) and Ensure Plus (the standard supplement without omega 3) are nutritional drinks that provides an additional source of energy, protein,

vitamins and minerals in a pleasant milkshake style drink. These drinks fill the gap between your body's' needs and what you can manage to take in the form of food. Therefore, these drinks are boosting your nutritional intake in order to improve your nutritional status.

What are the alternative treatment(s)?

You do not have to participate in this study to receive treatment for your condition. A well balanced diet, which may or may not include nutritional supplements, is appropriate for your condition. Your study doctor will discuss the risks and benefits of these alternative treatments with you.

What are the side effects/risks of taking part?

The various tests and measurements in this study are not harmful in any way. The blood samples will be taken using a needle and syringe and are expected to cause only minor discomfort such as bruising or a build up of blood under the skin. As the safety of omega 3 oils are not understood in pregnancy we may need to perform a pregnancy test in women of child bearing age before you may take part in this trial. If this test is negative you will need to be informed regarding contraceptive methods while taking the supplements in the trial.
Like any nutritional product, this may occasionally cause unwanted effects, mainly upset stomach, nausea or diarrhoea though they are usually mild and do not last long. None of these effects are likely to damage your health, and your doctor will always take precautions to remove or reduce these effects. As in any research study, unforeseeable risk may cause unforeseen problems or complications to occur.

What are the possible benefits of taking part?

Partaking in this study may reduce your risks of infections after surgery by benefiting your immune system Your quality of life may also improve and taking part will almost certainly benefit your nutritional status as it will involve more intensive input from a Dietitian. It may also benefit subsequent patients with your condition.

We hope that both (all) treatments will help you. However this cannot be guaranteed. The information we get from this study may help us treat future patients undergoing oesophagectomy better.

What if new information becomes available?

If we find out any more information on the treatment that is being studied this will be given to you in writing. This may change the way you feel about taking part in the study and you are free to withdraw at any time. If you decide to continue in the study you will be asked to sign a new (updated) consent form to confirm that this new information has been explained to you.

What happens when the research study stops?

When the study finishes further treatment will be continued as deemed appropriate by the patients doctor.

What about confidentiality?

All of your study records will remain strictly confidential

Representatives of the supporting company for this study (Abbott Laboratories), independent companies monitoring the study and auditing the results, the researchers including your GP, the ethics committee and regulatory authorities will have access to your original medical records for the purpose of collecting data, verifying that the data is correct and checking that the study is conducted properly. By signing this form you are allowing your doctor, the researchers and the study staff to permit these people to see your medical records.

Confidentiality is promised in all cases and your identity will not be disclosed to the public. Any information that may leave the hospital, apart from that which we send to your GP and to allow product to be sent to your home, will have your name and address removed and you will only be identified by your initials and study number. Under the Access to Health Records Act (1990), you may ask to see your study records.
This study is being conducted according to the requirements of the Irish Data Protection Act. Study data may be sent to organizations outside of the European Union (EU), and this may be by computer. Countries outside of Ireland may not have laws that protect your privacy to the same extent as UK laws do, but we will take all reasonable steps to protect your privacy. By signing the consent form you are agreeing that your anonymous medical information from the study may be sent outside the EU for analysis.

What do you do with my information?
The information collected in this study will be processed to meet the purpose of the clinical study. It may also be used in reports of the study or for scientific presentations. You will not be identified in any such publication. The information obtained from this study, which relates to you may be used for future medical research, either in this field, or in a new area (but only with further ethics committee approval).

What if I want to stop taking part in this study?

Your participation is completely voluntary and you can decide not to take part in the study at any time. This will not affect your care in any way, either now or in the future. If your personal circumstances change and you no longer wish to take part you may leave at any time. If you choose to stop taking part in the trial, your study doctor will make arrangements for your care to continue. You do not have to give a reason for leaving the study and this will not affect how your doctor cares for you. If at any time you decide to stop taking part in the study, you should talk to the study doctor so that you can stop safely.

Your study doctor may stop the study at any time with or without your permission. Your study doctor may choose to take you out of the study if it is in your best interest or because you may not be following the instructions properly. If you withdraw from the study you can also ask in writing to stop further access to your personal information. However any request does not apply to data already collected as part of the study.

Compensation

Participation in this study is covered by an approved policy of insurance in the name of St. James's Hospital. In addition the medical practitioners involved in this study have current medical malpractice insurance cover. St. James's Hospital will comply with the ABPI guidelines and Irish Law (statutory and otherwise) in the unlikely event of your becoming ill or injured as a result of participation in this clinical study.

Payment for the study

The nutritional product and all study procedures are provided to you at no charge. There are no anticipated personal expenses to you. You will be offered reasonable travel expenses, but you will not be paid for taking part in the study.

Who do I call if I have questions or problems?

'Please contact the study doctor/dietician below at any time, if you would like more information about any part of this study, your rights as a study subject or if you would like more information about what to do in the case of a study related injury, or if you would like to see the ABPI guidelines.

Doctor	*Professor John Reynolds*
	Dept of Clinical Surgery
Address:	*St James Hospital,*
	Dublin 8.
Telephone:	*01 4162212*
Dietitian:	*Aoife Ryan*
	Dept of Clinical Nutrition,
Address:	*St James Hospital,*
	Dublin 8.
Telephone:	*01 4162251*

Patient Consent Form

Title of research study:
A RANDOMISED DOUBLE BLINDED TRIAL TO INVESTIGATE THE EFFECTS OF AN ENTERAL NUTRITIONAL SUPPLEMENT ENRICHED WITH EICOSAPENTAENOIC ACID ON POST OPERATIVE COMPLICATIONS, STRESS RESPONSE, GENE EXPRESSION, IMMUNE FUNCTION, BODY COMPOSITION AND QUALITY OF LIFE IN PATIENTS UNDERGOING SURGICAL TREATMENT OF OESOPHAGEAL CANCER.

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.
I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I understand that my blood samples may be used for genetic research. I also understand that I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a supporting company, a signed copy will be sent to that sponsor.

Name of sponsor:

Participants Name:_____

Participants Signature: _____

Date:_____

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained:-

Name of Consentor/Parent /Guardian:_____

Signature:_____

Relation to Participant:_____

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him/her for the illness.
Name of 1st witness: _____Signature:_____
Name of 2nd witness: _____ Signature:_____
Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's signature:_____
Date:_____

FINAL LABEL TEXT FOR ENTERAL FEEDS FOR CLINICAL TRIAL

ACA-IREL-04-03 study: **EPA**

1. Tetra pack carton label:

Abbott Laboratories, 4051 Kingswood Drive, Citywest Business Campus, Dublin 24, Eire

PROTOCOL NUMBER: ACA-IREL-04-03
CONTENTS: Ensure PLUS (Vanilla flavour) / ProSure (Vanilla flavour)

_____ carton(s) to be taken _____ times per day

DATE OF SUPPLY: __/___/___

PATIENT No. / INITIALS: _____/_____

DIRECTIONS FOR USE: This product is ready for use. SHAKE WELL. Open immediately before use.

STORAGE INSTRUCTIONS:
This product may be stored unopened at room temperature. Once opened, it should be covered and stored in a refrigerator and any contents discarded after 24 hours. The ingredients and nutrient content for this formula are given in the research protocol.

CAUTION: NUTRITIONAL PRODUCT FOR INVESTIGATIONAL USE ONLY.
KEEP OUT OF REACH OF CHILDREN. FOR ENTERAL USE ONLY.

INVESTIGATOR: Prof. J. Reynolds, St. James' Hospital, Dublin 8, Eire.

Batch Number: (BN issued by Brecon Pharmaceuticals)

Expiry Date (issued by Abbott)

Manufactured by: ABBOTT LABORATORIES B.V., Ross Product Manufacturer,
Postbus 626, 8000 AP, Zwolle, Netherlands.

2. Ready to Hang Bottle label:

Abbott Laboratories, 4051 Kingswood Drive, Citywest Business Campus, Dublin 24, Eire

PROTOCOL NUMBER: ACA-IREL-04-03
CONTENTS: Ensure PLUS (Vanilla flavour) / ProSure (Vanilla flavour)

_____ bottle(s) to be taken _____ times per day

DATE OF SUPPLY: __/___/___

PATIENT No. / INITIALS: _____/_____

DIRECTIONS FOR USE: This product is ready for use. SHAKE WELL. Open immediately before use.

STORAGE INSTRUCTIONS:
This product may be stored unopened at room temperature. Once opened, it should be covered and stored in a refrigerator and any contents discarded after 24 hours. The ingredients and nutrient content for this formula are given in the research protocol.

CAUTION: NUTRITIONAL PRODUCT FOR INVESTIGATIONAL USE ONLY.

KEEP OUT OF REACH OF CHILDREN. FOR ENTERAL USE ONLY.

INVESTIGATOR: Prof. J. Reynolds, St. James' Hospital, Dublin 8, Eire.

Batch Number: (BN issued by Brecon Pharmaceuticals)

Expiry Date (issued by Abbott)

Manufactured by: ABBOTT LABORATORIES B.V., Ross Product Manufacturer, Postbus 626, 8000 AP, Zwolle, Netherlands.

3. Outer carton label text : TBD

PROCEDURES FOR MONITORING AND RECORDING ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS (CHAPTER 7)

ADVERSE EVENT DATA COLLECTION FORM

NAME:.....DATE:.....TIME:.....

Reporter Details **Name:**..... ☐ Patient/Consumer ☐ Doctor ☐ Other, please state: ..... Address:..... .... Telephone Number:..... Fax Number:.....	**Patient Details** **Initials:**..... **Sex:** ☐ Male ☐ Female **Age/D.O.B:**..... **Weight (Kg):**..... **Height (cm)** .....

Description of event(s) and any treatment given:

Date of Onset:.....

Description:.....

.....

Outcome:

☐ Recovered ☐ Recovering ☐ Others, please state below

.....

Medication Details (including self-medication & herbal remedies)

Name of medication(s)	Is this a suspected drug? Y/N	Dose (with units)	Frequency eg BD	Route eg iv	Length of time on medication		Indication
					Start Date / Duration	Stop Date	

Other Information:
Patient Initials ____ Patient Number _____

Description of the Event (one per row)	**Date and Time of onset**	**Type**	**Date and Time of resolution**	**seriousness**	**Severity**	**Relation to study product**	**Outcome**

Seriousness
1=not serious
2=SERIOUS
Severity
1=mild*
2=moderate*
3=severe*

Relation to study product
1=unrelated*
2=unlikely*
3=possible*
4=probable*
5=definite*

Action Taken
1=none
2=study product dosage reduced
3=study product interrupted from ___ to ____
4=study product suspended permanently
5=specific therapy, please specify_____
6=hospitalisation

Outcome
1=recovered
2=recovering
3=not recovering
4=recovered with sequelae
5=death
6=unknown
definitions are given in protocol

Definitions of Adverse Events

Toxicity	0	1	2	3	4
Bruising	none	Localised at site of blood sample	generalised	-	-
Wound infection at jejunostomy site	none	cellulitis	Superficial infection	Infection requiring IV antibiotics	Necrotizing fascitis
Anorexia	none	Loss of appetite	Oral intake significantly decreased	Requiring IV fluids	Requiring full enteral or parenteral feeding
Constipation	none	Requiring stool softener or dietary modification	Requiring laxatives	Requiring manual evacuation or enema	Obstruction or toxic megacolon
Diarrhoea	none	Increase of < 4 stools /day over pre treatment	Increase 4-6 stools/day or nocturnal stools	Increase > 7 stools/day or incontinence or need for IV fluids for hydration	Physiologic consequences Requiring Intensive care
Dyspepsia/heartburn	none	mild	moderate	severe	-
Intestinal fistula	none	-	-	present	Requiring surgery

Nausea	none	Able to eat	Oral intake significantly decreased	No significant intake requiring IV fluids or enteral nutrition	-
Vomiting	none	1 episode in 24 hours over pre treatment	2-5 episodes in 24 hours over pre treatment	>6 episodes in 24 hours over pre treatment or need for IV fluids	Requiring parenteral nutrition, or physiologic consequences requiring intensive care
Peritonitis	none	-	-	Present requiring IV antibiotics	Present requiring IV antibiotics, physiologic consequences requiring intensive care
Abdominal Pain/cramping	none	Mild pain not interfering with function	Moderate pain; pain on analgesics interfering with function, but not interfering with activities of daily living	Severe pain; pain on analgesics severely interfering with activities of living	disabling
Adult respiratory distress syndrome	absent	-	-	-	present
Fistula	none			Requiring intervention	Requiring surgery
Hypercalcemia	WNL	> ULN - 11.5 mg/dl > ULN - 2.9 mmol/L	>11.5 - 12.5 mg/dl > 2.9 - 3.1 mmol/L	>12.5 - 13.5 mg/dl > 3.1 - 3.4 mmol/L	> 13.5 mg/dl > 3.4 mmol/L
Hypercholesterolemia	WNL	> ULN - 300 mg/dl > ULN - 7.75 mmol/L	> 300 - 400 mg/dl > 7.75 - 10.34 mmol/L	> 400 - 500 mg/dl >10.34 - 12.92 mmol/L	> 500 mg/dl > 12.92 mmol/L
Hyperglycemia	WNL	> ULN - 160 mg/dl > ULN - 8.9 mmol/L	> 160 - 250 mg/dl > 8.9 - 13.9 mmol/L	> 250 - 500 mg/dl > 13.9 - 27.8 mmol/L	> 500 mg/dl > 27.8 mmol/L or ketoacidosis
Hyperkalemia	WNL	> ULN - 5.5 mmol/L	> 5.5 - 6.0 mmol/L	> 6.0 - 7.0 mmol/L	> 7.0 mmol/L
Hypermagnesemia	WNL	> ULN - 3.0 mg/dl > ULN - 1.23 mmol/L	-	> 3.0 - 8.0 mg/dl > 1.23 - 3.30 mmol/L	> 8.0 mg/dl > 3.30 mmol/L
Hypernatremia	WNL	> ULN - 150 mmol/L	>150 - 155 mmol/L	>155 - 160 mmol/L	>160 mmol/L
Hypertriglyceridemia	WNL	> ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 10 x ULN	> 10 x ULN

Hyperuricemia	WNL	> ULN - ≤ 10 mg/dl ≤ 0.59 mmol/L without physiologic consequences	-	> ULN - ≤ 10 mg/dl ≤ 0.59 mmol/L with physiologic consequences	> 10 mg/dl > 0.59 mmol/L
Hypocalcemia	WNL	<LLN - 8.0 mg/dl <LLN - 2.0 mmol/L	7.0 - < 8.0 mg/dl 1.75 - < 2.0 mmol/L	6.0 - < 7.0 mg/dl 1.5 - < 1.75 mmol/L	<6.0 mg/dl < 1.5 mmol/L
Hypoglycemia	WNL	<LLN - 55 mg/dl <LLN - 3.0 mmol/L	40 - < 55 mg/dl 2.2 - < 3.0 mmol/L	30 - < 40 mg/dl 1.7 - < 2.2 mmol/L	< 30 mg/dl < 1.7 mmol/L
Hypokalemia	WNL	<LLN - 3.0 mmol/L	-	2.5 - <3.0 mmol/L	<2.5 mmol/L
Hypomagnesemia	WNL	<LLN - 1.2 mg/dl <LLN - 0.5 mmol/L	0.9 - <1.2 mg/dl 0.4 - < 0.5 mmol/L	0.7 - < 0.9 mg/dl 0.3 - < 0.4 mmol/L	< 0.7 mg/dl < 0.3 mmol/L
Hyponatremia	WNL	<LLN - 130 mmol/L	-	120 - <130 mmol/L	<120 mmol/L
Hypophosphatemia	WNL	<LLN -2.5 mg/dl <LLN - 0.8 mmol/L	≥2.0 - <2.5 mg/dl ≥0.6 - <0.8 mmol/L	≥1.0 - <2.0 mg/dl ≥0.3 - <0.6 mmol/L	< 1.0 mg/dl <0.3 mmol/L
Metabolic/Laboratory-Other (Specify, _____)	none	mild	moderate	severe	life-threatening or disabling

ST. JAMES'S HOSPITAL

DESIGNATED RESEARCH ACTIVITY PROPOSAL

HOSPITAL APPROVAL FORM

PREAMBLE

The Hospital Board has adopted guidelines governing approval procedures to apply for certain designated research activity (DRA) to be undertaken at St. James's Hospital. The guidelines are concerned primarily with resource and financial control issues. In essence, formal hospital approval is required for all DRAs before they can proceed at or involve St. James's Hospital. DRAs comprise all research that is:

- **Sponsored (wholly or partially); and**
- **Non-sponsored but requiring use of hospital resources which are not currently budgeted.**

This form must be completed in respect of all proposed research of these types. Copies of the relevant guidelines are available from the CEO's Office on request.

---

1. TYPE OF RESEARCH ACTIVITY [please tick]

SPONSERED [LEVEL 5 DRA]

- **Sponsored Medicines Trial** ☐
- **Other Sponsored Clinical Based Research Project/Programme** X
- **Sponsored Non-Clinical Based Research Project/Programme** ☐

NON-SPONSERED [LEVEL 4 DRA]

- **Non-Sponsored Clinical Based Research Project/Programme** ☐
- **Non-Sponsored Non-Clinical Based Research Project/Programme** ☐

2. PROJECT DETAILS

- **Title of Project** (*code number where applicable or other reference identifier)*

A RANDOMISED DOUBLE BLINDED TRIAL TO INVESTIGATE THE EFFECTS OF AN ENTERAL NUTRITIONAL SUPPLEMENT ENRICHED WITH EICOSAPENTAENOIC ACID ON POST OPERATIVE COMPLICATIONS, STRESS RESPONSE, GENE EXPRESSION, IMMUNE FUNCTION, BODY COMPOSITION AND QUALITY OF LIFE IN PATIENTS UNDERGOING SURGICAL TREATMENT OF OESOPHAGEAL CANCER.

- **Please provide a very brief description of the proposed research activity.**

Title
A randomized double blinded trial to investigate the effects of an enteral nutritional supplement enriched with Eicosapentaenoic acid on post operative complications, stress response, gene expression, immune function, body composition and quality of life in patients undergoing surgical treatment of oesophageal cancer.

Investigational medicinal Product(s):
Prosure (Abbott Laboratories)
Comparator:
Ensure Plus (Abbott Laboratories)

Study Objectives
To examine whether prolonged supplementation with a nutritional supplement enriched with Eicosapentaenoic acid is superior to standard nutritional products in terms of reducing post operative complications, ameliorating the stress response to surgery and enhancing immune function, promoting anabolism and improving quality of life in patients undergoing surgical treatment of oesophageal cancer.

Study Design
Prospective randomized double blinded controlled trial.

Inclusion Criteria
Adult (male & female) patients >18 years with resectable oesophageal cancer:
Exclusion Criteria

- Patients with metastatic disease,
- Non-operable cases,
- Patients requiring chemotherapy/radiotherapy early following surgery
- Patients with known immunological disorder
- Emergency oesophagectomy cases
- Patients with cardiac, liver or renal failure
- Active small intestinal disease eg Crohns disease
- Allergy to any of the ingredients
- Uncontrollable Diabetes

- Use of medications known to affect eicosanoid metabolism in two weeks prior to trial
- Use of fish oil/n-3 fatty acids
- Drug Abuse
- Inadequate preoperative preparation
- Pregnant women

Primary Endpoints:

- post operative SIRS, sepsis and organ failure
- quality of life and
- nutritional status post oesophagectomy

Secondary Endpoints:

- Effects on the immuno-inflammatory response,
- Effect on gene expression profiling and cytokines post oesophagectomy

3. **PLEASE LIST KEY PARTIPANTS IN THE PROPOSED RESEARCH ACTIVITY AS FOLLOWS:** ***(Sponsors Details are not to be included here)***

- **Principle Investigator(s):**

Professor John Reynolds

- **Other Researchers:***(please specify proposed role, name, qualifications and position)*

Ms Aoife Ryan – Clinical Trial Co-ordinator. Aoife will be responsible for all aspects of the study and will be conducting it on behalf of Professor Reynolds.
BSc Human Nutrition and Dietetics
Current Role: Senior Clinical Nutritionist : research (reading for PhD)

Ms Nicola Miller – Research Scientist
BSc Biotechnology
MSc Industrial Microbiology
PhD Human Molecular Genetics
DipRCPath Molecular Genetics
Current Role: Senior Clinical Scientist, Cancer Molecular Diagnostics

- **Contact Details for this Study:**

Ms Aoife Ryan on extension 2180.

4 **RESOURCE USAGE**

- **Will the proposed research activity involve use of Hospital Resources?**

YES [X] **NO** [ ]

- **If Yes, please indicate extent of such resource use in the following format:**

A research dietician has been appointed to run this study

- **Facilities**
  - **Accommodation**
  - **Equipment**
  - **Procedures**
  - **Diagnostic/Physiological tests**
  - **Other**

- **Staff** ***(where not included in above)***

Pharmacy will dispense study medication

Standard Bloods (Biochem and Haem)

- **Consumables** ***(where not included in above)***

- **Where resource use involves combined service/research mix please estimate related approximate distribution.**

SERVICE: _____10_____% **RESEARCH: ____90_____%**

[SCHEDULE SHOULD BE ATTACHED IF NOT POSSIBLE TO SUMMARISE ABOVE]

5. FUNDING ARRANGEMENTS

Is the proposed research activity to be supported or sponsored by grant/funding assistance from extern individual(s), organisation(s) or companies?

YES [X] **NO** [ ]

If YES: please complete the following:

- **Please list/name sponsors:**

Abbott Laboratories (Ireland) Ltd, 4051 Kingswood Drive,

Citywest Business Campus,

Dublin 24

- **Please provide details of grant/funding arrangements to apply** ***(to contain details of total grant/funding to be made available and basis for calculation)***

Please see enclosed document

- **In calculating grant/funding levels to apply, are sponsors making provision for envisaged use of Hospital resources?**

YES [ ] **NO** [X]

No, we are just changing the enteral feed a patient will be given therefore there is no additional use of hospital resources. In fact the hospital will save on the nutritional products that would have otherwise been consumed if the trial was not running (as the product is being supplied to hospital free of charge)

- **If Yes - please state clearly outline related provisions if not evident in information already furnished above.**

- **Please outline how grants/funds allocated are to be utilised in the proposed research project:**

See enclosed budget

- **Where grants/funding are to be utilised to employ additional personnel, please specify the extent to which such staff will contribute to hospital services.**

NA

- **Please outline in general terms the benefits likely to accrue to the hospital arising from this research activity.**

  International representation/publication of results
  Possibility of shorter hospital stay for patients if results are favourable

6. FINANCIAL ACCOUNTING AND CONTROL ARRANGEMENTS
(Applies to Level 5 Research Only)

Please outline financial and accounting control provisions to be effected with respect to the proposed research activity as follows:

- **Name/Address of Financial Accounting Agency:**
  - **St. James's Hospital Finance Department** [ ]
  - **Trinity College** (Haughton Insitute) [X]
  - **Other** [ ]

If Other, please furnish details as above:

Where the accounting agency is not St. James's Hospital, please confirm production and availability to the Hospital of the following accounting documentation:

- **Income & Expenditure Accounts** [X]
- **Audited Accounts** [X]
- **Control provisions in positions for Research Fund Accounts** [X]
- **Transaction details of Research Fund Accounts** [X]

Do you wish the Hospital's Finance Department to undertake these Financial Accounting and Control provisions for the proposed research activity?

YES ☐ **NO** ☒ X

(Note: A nominal administrative charge will apply to this service).

7. INDEMNIFICATION

In general where a sponsoring agent is involved, it is necessary for that agent to provide standard indemnification cover to the hospital. You should submit the relevant indemnification documentation in the standard format with this approval form. The relevant research activity may not proceed in the absence of this provision.

8. RESEARCH ETHICS COMMITTEE APPROVAL

Is evidence of formal approval from the Research Ethics Committee for the proposed Research Activity attached to this DRA Form?

YES ☒ X **NO** ☐ **N/A** ☐

If No, please submit evidence of Ethics Committee Approval as soon as it has been obtained.

9. DECLARATION

I confirm that provided herein and attached are accurate and disclose the complete resource implications and grants/funding provisions applicable for the specified proposed research activity.

_____ _____

Applicant and Principle Investigator **Date**

10. APPROVAL

SIGNED: _____

Deputy CEO/Operations Manager

DATE: _____

Date

Clinical Trial: Prosure CT No: 900/429/1

Name: _____
MRN: _____
Randomisation Code: _____

Systemic Inflammatory Response Syndrome

Date of Surgery: ___/___/___	POD 1 ___/___/___	POD 2 ___/___/___	POD 3 ___/___/___	POD 4 ___/___/___	POD 5 ___/___/___	POD 6 ___/___/___	POD 7 ___/___/___
Temperature >38^{0}C ***or*** < 36^{0}C							
Heart Rate > 90 beats/minute							
Respiratory Rate > 20 breaths/minute ***or*** $PaCO_2$<32 mmHg							
White blood count >12,000mm3, <4,000 mm^3 ***or*** >10% immature (band) forms							
SIRS? YES/NO							

Signed by Lead Investigator:_____
Professor John V Reynolds

Clinical Trial: Prosure CT No: 900/429/1

Name: _____

MRN: _____

Randomisation Code: _____

Enteral Product Received

POD –5	POD –4	POD-3	POD-2	POD-1	POD1	POD2	POD3	POD4	POD5	POD6	POD7	POD8	POD9
___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___
POD 10	POD 11	POD 12	POD 13	POD 14	POD 15	POD 16	POD 17	POD 18	POD 19	POD 20	POD 21	POD 22	POD 23
___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___	___/___

Signed by Sub Investigator: _____
Aoife Ryan
Research Dietitian

Randomisation Procedure for Prospective Randomised Double Blinded Trial with EPA (Chapter 7)

1 product B
2 product A
3 product A
4 product A
5 product A
6 product B
7 product B
8 product A
9 product A
10 product B
11 product A
12 product A
13 product A
14 product B
15 product B
16 product B
17 product A
18 product B
19 product A
20 product A
21 product A
22 product A
23 product B
24 product A
25 product A
26 product B
27 product B
28 product B
29 product B
30 product A
31 product B
32 product A
33 product B
34 product A
35 product A
36 product B
37 product B
38 product B
39 product A
40 product B
41 product B
42 product A
43 product B
44 product B
45 product B
56 product A

57 product A
58 product A
59 product B
50 product B
51 product A
52 product B
53 product A
54 product B
55 product A
56 product B
57 product A
58 product B
59 product A
60 product A
61 product A
62 product A
63 product B
64 product B
65 product A
66 product A
67 product A
68 product B
69 product B
70 product B
71 product A
72 product A
73 product B
74 product A
75 product A
76 product A
77 product B
78 product B
79 product B
80 product B
81 product A
82 product B
83 product A
84 product A
85 product A
86 product B
87 product A
88 product A
89 product B
90 product B

Standard Operating Procedure for the Randox Evidence Investigator™ Cytokine and Growth Factors Array

The Sandwich Assay

The biochip carriers were placed in the carrier handling tray, before addition of samples and reagents, to facilitate handling during manual assays.

The addition of reagents was performed by pipetting towards the front edge of the biochip, with the tip of the pipette pointed towards the back of the well, as this was deemed the optimal position, and the addition time did not exceed 10 minutes.

a) 200µls of assay diluent was pipetted into each well, followed by 100µls of calibrator, to the first 9 wells, Tri-level control to the following 3 wells and of patient sample to the remaining 42 wells.

b) The side of the carrier was gently tapped to facilitate reagent mixing, and the holding tray secured to the base plate of the thermoshaker using the central screw.

c) The plates were then incubated for 1 hour at 37^0 C at 370rpm, after which time an alarm sounded and the handling tray with carriers was removed. The reagents were subsequently discarded to waste using a sharp flicking action of the handling tray, to minimise cross-contamination between wells.

d) The wells were then washed using the concentrated wash buffer with approximately 350µls added to each cell in 2 quick wash cycles. Care was taken not to overfill the wells in order to reduce potential for well-to-well contamination and the side was again gently tapped to release any reagents trapped below the biochip. The buffer was then decanted with a sharp flicking action. The wash procedure was repeated for another 4 cycles, each time leaving the biochips to soak in wash buffer for 1 minute at each cycle. After a final wash the carriers were tapped on lint free tissue to remove residual fluid.

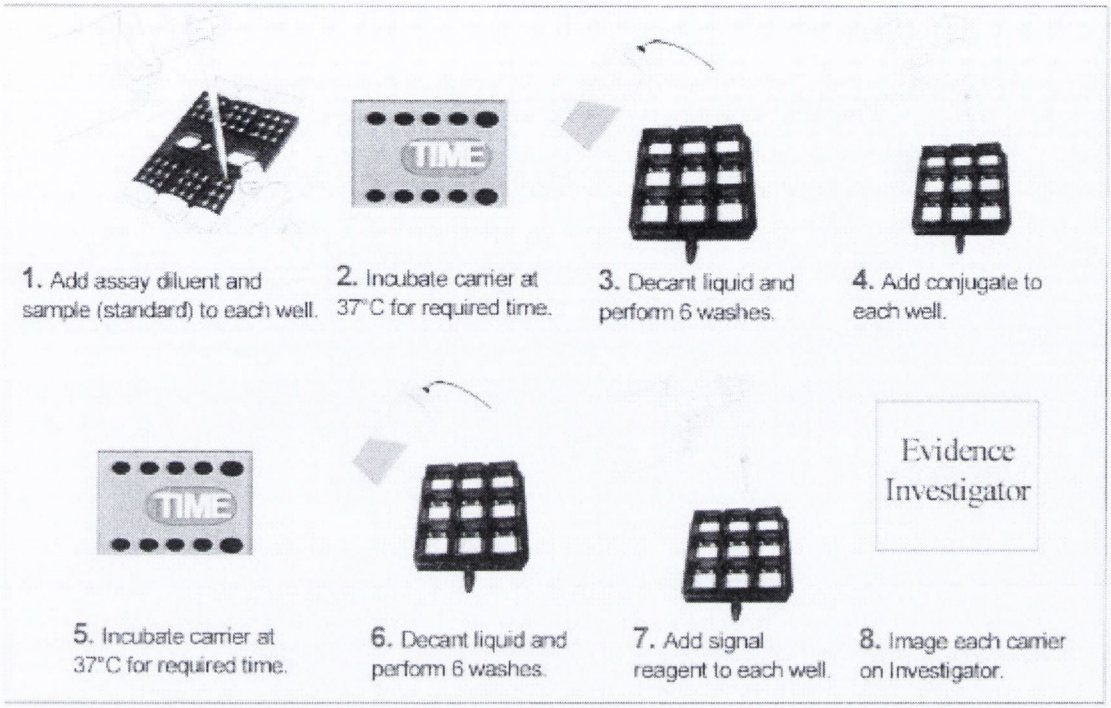
e) 300 µls of conjugate was then immediately pipetted to each well, gently tapped to ensure mixing, and secured once again to the base tray plate of the thermoshaker for incubation at the same time, frequency and temperature as previously outlined.

f) When the one hour incubation period was complete, the conjugate solution was decanted with the sharp flicking action and 2 wash cycles were immediately carried out. 350 µls of wash buffer was instilled to each biochip tapped, and decanted into the waste, followed by a further 4 wash cycles, with a 2 minute soaking time at each cycle. After the final wash, the wells were filled with wash buffer and left to soak until directly prior to imaging, and no rack was left longer than 30 minutes as recommended.

g) Imaging of each rack occurred individually. Those that awaited imaging were protected by foil from light, and immediately prior to imaging were taken from the rack and tapped on lint free tissue to remove residual wash buffer.

250 µls of working signal reagent was added to each well and covered for incubation at room temperature for 2 minutes, after which time the carrier was placed in the Evidence

Investigator for imaging of the biochips. All the remaining carriers were left in buffer until they too were imaged.



h) The carrier was taken inside the instrument and the door automatically closed to create a light-tight environment. The thermoelectrically-cooled CCD camera simultaneously captured an image of all nine biochips in the carrier and dedicated software quantified the light signal output from each discreet test region on the surface of each biochip. The system captures a Coupling device image and quantifies the Relative Light Units (RLUs). The system then reports an assay result based on the calibration values. When the images were captured, the door opened and prompted the user to remove the carrier, and load the next. This process continued until all carriers in the work list were processed.

On completion of the assay, the carriers were disposed of in an appropriate biohazard waste disposal bin. The system was then shut down allowing the camera to warm up to room temperature and the software likewise when the camera reaches a temperature of 15.

Elia Nomogram Providing guide to the adjustment in Basal Metabolic Rate for a level of metabolic stress (From Elia, 1990)