Mechanisms underlying obesity-related insulin resistance

Nilanjana Tewari

BScMedSci (Hons), MBChB, MRCS, MSc

Submitted to the University of Nottingham

for the degree of Doctor of Philosophy

July 2016

Table of Contents

Table of Contents……ii

Abstract……viii

Declaration……ix

Acknowledgements……x

Presentations arising from this thesis……xii

Publications arising from this thesis……xiv

List of abbreviations……xv

Chapter 1 Introduction……15

1.1 Background ……16

1.2 Methods……18

1.3 Definitions……18

1.4 Surgery and insulin resistance ……20

1.5 Obesity and insulin resistance ……22

1.6 Mechanisms underlying obesity-related Insulin Resistance – Adipokines 32

1.7 Adipose tissue: an endocrine organ……43

1.8 Fatty Acids and insulin resistance……48

1.9 Body composition, sarcopenia, myosteatosis and insulin resistance……50

1.10 Preoperative carbohydrate loading ……52

Chapter 2 Hypotheses……56

Chapter 3 Common methods……58

3.1 Approvals from regulatory bodies ……59

3.2 Hyperinsulinaemic-euglycaemic clamp……59

3.3 Anthropometrics……60

3.4 Dual energy x-ray absorptiometry (DXA) 61
3.5 Body composition analysis of CT scans 62
3.6 Bioelectrical impedance analysis 63
3.7 SF-36 and M-SGA questionnaires 64
3.8 Skeletal muscle and adipose tissue biopsies 65
3.9 RNA extraction 66
3.10 cDNA preparation 67
3.11 TaqMan low density gene array analysis 68
3.12 Collection of blood samples 70
3.13 Analysis of blood samples 71
3.14 Composition of study drinks 73
3.14 Statistical analyses 74
3.15 Funding for studies 74

Chapter 4 Investigating the relationship between the distribution and physiology of adipose tissue and skeletal muscle and postoperative inflammation and insulin resistance 76

4.1 Background 77
4.2 Study hypothesis 79
4.3 Methods 79
4.4 Results 83
4.5 Discussion 105

Chapter 5 A comparison of surrogate indices of insulin sensitivity against the hyperinsulinaemic-euglycaemic clamp in two study populations 115

5.1 Background 116
5.2 Study hypothesis 116
5.3 Methods 116
5.4 Results 117
5.4 Discussion 122

Chapter 6 The effects of an intensive nutritional support programme on body composition, insulin resistance and outcomes during neoadjuvant chemotherapy for oesophagogastric cancer: A before and after pilot study 126

6.1 Background 127
6.2 Study Hypothesis 128
6.3 Methods 129
6.4 Results 135

6.5 Discussion.....152
Chapter 7 A comparison of three methods of assessing body composition…158
7.1 Background 159
7.2 Study hypothesis.....160
7.3 Methods.....160
7.4 Results.....161
7.5 Discussion.....164
Chapter 8 Critique of thesis……..166
Chapter 9 Future Directions……..174
Bibliography……176
Appendix 1. Adipose and skeletal muscle gene card template…….……199

List of Figures

Figure 1.1. Mechanisms underlying obesity-associated insulin resistance 24
Figure 1.2. Mechanism of free fatty acids (FFAs) induced IR and enhanced inflammatory responses in adipose tissue 49
Figure 4.1. Consort diagram..... 83
Figure 4.2. Change in blood glucose concentration during HEC 88
Figure 4.3. Change in insulin sensitivity with surgery..... 90
Figure 5.1. Comparison of methods of determining insulin sensitivity.....120
Figure 5.2. Correlation between methods in obese and non-obese subgroups in Study 1 121
Figure 5.3. Correlation between methods in obese and non-obese subgroups in Study 2 122
Figure 6.1. Study regimen.....130
Figure 6.2. SF-36 individual scores 141
Figure 6.3. Lean mass and fat mass (FM) on DXA before (DXA1) and after (DXA2) NAC..144
Figure 6.4. Fat mass (FM) and fat free mass (FFM) on CT before (CT1) and after (CT2) NAC 145
Figure 6.5. Insulin sensitivity before and after NAC 146
Figure 6.6. Carbohydrate and fat oxidation during clamps pre and post NAC 147
Figure 6.7. Non-oxidative glucose disposal pre and post NAC 148
Figure 6.8. Calorimetry in control subjects 149
Figure 6.9. Non-oxidative glucose disposal in cancer patients and controls 150
Figure 6.10. Metabolite concentrations 151
Figure 6.11. Metabolite concentrations during NAC. 152
Figure 7.1. Correlation between CT and DXA.....161
Figure 7.2. Correlation between BIA and DXA/CT 162
Figure 7.3. Bland Altman analysis of DXA, CT and BIA.....163

List of Tables

Table 1.1. Definitions of terminology ..... Error! Bookmark not defined.
Table 1.2. Mechanistic studies of obesity and insulin resistance (adipose tissue inflammation/ macrophage infiltration) **27**
Table 1.3. Mechanistic studies of obesity and insulin resistance (adipokines). **34**
Table 3.1. Composition of study drinks **73**
Table 4.1. Exclusion criteria **79**
Table 4.2. Study outline **81**
Table 4.3. Demographic details of study patients **86**
Table 4.4. Results of different methods of body composition analysis **87**
Table 4.5. Time points for blood sampling **92**
Table 4.6. Gene changes in obese compared with non-obese **99**
Table 4.7. Postoperative gene expression differences in omental fat placebo obese vs non-obese subjects **104**
Table 5.1. Results of indices of insulin sensitivity **118**
Table 5.2. Proportion of subjects with IR estimated with each technique **118**
Table 5.3. Correlations of HEC and QUICKI/HOMA-IR at each timepoint **120**
Table 6.1. Exclusion criteria **129**
Table 6.2. INSP vs standard care **131**
Table 6.3. Demographic data **136**
Table 6.4. Anthropometrics **138**
Table 6.5. SF-36 domain scores **142**
Table 6.6. Daily energy and protein deficits prior to commencing NAC **143**
Table 6.7. Nutritional supplementation during NAC **144**
Table 6.8. Insulin resistance per cohort and per visit **146**

Abstract

This thesis investigates the effect of body composition on insulin resistance and the impact of supplementation with nutritional support or carbohydrate treatment. Insulin resistance occurs as a response to a number of stressors, including surgery. However, the mechanism underlying the development of insulin resistance is as yet unclear. Adipose tissue distribution appears to play a role in the development of insulin resistance and obesity-related complications.

In obese and non-obese patients undergoing open abdominal surgery who received preoperative carbohydrate or placebo, there was a significant fall in perioperative insulin sensitivity and changes in the expression of genes relating to carbohydrate and fat oxidation. There was no influence of perioperative carbohydrate or obesity on change in insulin sensitivity. Patients undergoing neoadjuvant chemotherapy for oesophageal cancer underwent pre and post chemotherapy assessment of insulin sensitivity and body composition. There was a significant reduction in insulin sensitivity despite minimal change in body composition and adequate nutritional intake.

These studies have provided further information about the optimal methods for assessment of insulin sensitivity and body composition as well as an insight into mechanisms underlying the association between body composition and insulin sensitivity.

Declaration

Except where acknowledged in the acknowledgements and text, I declare that this dissertation is my own work and is based on research that was undertaken by me in the Division of Surgery, School of Clinical Sciences, University of Nottingham Medical School from 3rd August 2011 to 5th August 2014.

Nilanjana Tewari

Acknowledgements

The work undertaken in this thesis would not have been possible without the support of a number of individuals to whom I am indebted.

- I would like to thank my supervisors Professor Dileep N Lobo and Professor Ian A Macdonald for their constant encouragement, provision of timely and useful feedback.
- Dr. Andrew Bennett and the staff of the FRAME laboratory were hugely supportive during the laboratory phase of the studies.
- Dr. Elizabeth Simpson and the staff of the David Greenfield Human Physiology Laboratory made all their resources and knowledge available to me and I am grateful for their patience in training me in the methods used to measure insulin sensitivity for the clinical studies included.
- Mr. Sherif Awad provided invaluable help in devising study protocols and initiating the clinical trials in Chapters 4 and 6.
- Mr. Julian Williams, Mr. Ayan Banerjea and Professor John Scholefield kindly allowed me to recruit patients from their operating lists and provided intraoperative samples for the studies detailed in Chapter 4.
- Mr. James Catton, Mr. Simon Parsons and Mr. Neil Welch allowed me to recruit patients with oesophagogastric cancer and provided intraoperative samples.
- Dr. Carolyn Chee and Dr. Francis Stephens very kindly allowed me to use the calorimetry data they had obtained from a healthy volunteer study as a comparator against cancer patients in the study in Chapter 6.

- Kim Ward, Pete Johnson, Sarah Farnan, Lauren Tarr and all staff of the surgical CLRN provided vital assistance with clamp studies, muscle biopsies and processing of samples.
- Tracey Shepherd, Sam Warburton and the staff of the NDDC BRU were generous with their time and resources and provided support during the clamp studies and muscle biopsies.
- Sally Cordon and Karen Swift and their staff assisted with analysis of serum metabolites and have made themselves always available to me to answer any questions.
- Kate Shepherd and Val Heath provided practical assistance with setting up all the studies and also helped with blinded preparation of study drinks.

- CORE, Nutritional Research Foundation, NUH Charities and ESPEN provided funding for these studies and without their generous support, the work would not have been possible. I would particularly like to mention Alice Kington at CORE who has been a valuable source of information and support.

- Finally, to my family and friends. Thank you for your patience, endurance, tolerance and support.

Presentations arising from this thesis

1. A comparison of three methods to assess body composition
   N Tewari, S Awad, IA Macdonald, DN Lobo
   Accepted for presentation to the Society for Academic and Research Surgery (SARS) annual meeting, London, January 2016, Burnand Prize session.

2. Cellular mechanisms underlying obesity-associated insulin resistance
   N Tewari, S Awad, A Bennett, IA Macdonald, DN Lobo.
   Accepted for presentation to SARS annual meeting, London, January 2016, Patey Prize session.

3. A Randomised Study of Factors Mediating Alterations in Inflammatory Responses and Insulin Sensitivity Following Major Abdominal Surgery (NCT01470534)
   S Awad, N Tewari, F Duška, IA Macdonald, DN Lobo
   European Society for Clinical Nutrition and Metabolism (ESPEN) 2014 Geneva

4. Determining Changes in Insulin Sensitivity Following Major Abdominal Surgery: Homa-IR VS The Hyperinsulinaemic-Euglycaemic Clamp–Data from a Randomised Study (NCT01470534)
   N Tewari, S Awad, F Duška, IA Macdonald, DN Lobo.
   ESPEN 2014 Geneva

5. The Impact of an Intensive Nutritional Support Programme During Neoadjuvant Chemotherapy for Upper Gastrointestinal Cancer.
   N. Tewari, S. Awad, J.A. Catton, S. Madhusudan, I.A. Macdonald, D.N. Lobo.
   ESPEN 2014 Geneva

Submitted abstracts

1. A comparison of three methods to assess body composition
N Tewari, S Awad, IA Macdonald, DN Lobo
Clin Nutr 2016

2. Cellular mechanisms underlying obesity-associated insulin resistance
N Tewari, S Awad, A Bennett, IA Macdonald, DN Lobo.
Clin Nutr 2016

Publications arising from this thesis

Published abstracts

1. A Randomised Study of Factors Mediating Alterations in Inflammatory Responses and Insulin Sensitivity Following Major Abdominal Surgery (NCT01470534)
   S. Awad, N. Tewari, F. Duška, I.A. Macdonald, D.N. Lobo
   Clinical Nutrition Supplements 2014; 33 (Supplement 1): S2–S3

2. Determining Changes in Insulin Sensitivity Following Major Abdominal Surgery: Homa-IR VS The Hyperinsulinaemic-Euglycaemic Clamp–Data from a Randomised Study (NCT01470534)
   N. Tewari, S. Awad, F. Duška, I.A. Macdonald, D.N. Lobo
   Clinical Nutrition Supplements 2014; 33 (Supplement 1): S21

3. The Impact of an Intensive Nutritional Support Programme During Neoadjuvant Chemotherapy for Upper Gastrointestinal Cancer.
   N. Tewari, S. Awad, J.A. Catton, S. Madhusudan, I.A. Macdonald, D.N. Lobo
   Clinical Nutrition Supplements 2014; 33 (Supplement 1): S51-S52

Published papers

1. Obesity-related insulin resistance: Implications for the surgical patient.
   N. Tewari, S. Awad, IA Macdonald, DN Lobo.
   International Journal of Obesity 2015 Jun 1. doi: 10.1038/ijo.2015.10 [Epub ahead of print]

2. Submitted for publication
   A comparison of three methods to assess body composition
   N Tewari, S Awad, IA Macdonald, DN Lobo
   Submitted to Clinical Nutrition, September 2015

List of abbreviations

ATGL	Adipose Triglyceride Lipase
BMI	Body Mass Index
CLS	Crown Like Structure
DXA	Dual Energy X-Ray Absorptiometry
ER	Endoplasmic Reticulum
FABP	Fatty Acid Binding Protein
FASN	Fatty Acid Synthase gene
FFA	Free Fatty Acid
FOXC2	Forkhead Box Protein C2
HEC	Hyperinsulinaemic-Euglycaemic Clamp
HOMA (IR)	Homeostatic Model Assessment (Insulin Resistance)
HSL	Hormone Sensitive Lipase
IKK	I κB kinase
IL	Interleukin
IMCL	Intramyocellular Lipid
IR	Insulin Resistance
IS	Insulin Sensitivity
JNK	C-jun NH2 Terminal Kinase
MAPK	Mitogen Activated Protein Kinase
MK	Midkine
MRI	Magnetic Resonance Imaging
NFKB1	Nuclear factor kappa-B p105 subunit
OGTT	Oral Glucose Tolerance Test
PBEF	Pre B Cell Colony Enhancing Factor
PERK	Protein Kinase RNA-like Endoplasmic Reticulum Kinase
POAF	Postoperative Atrial Fibrillation
SAT	Subcutaneous Adipose Tissue
SOCS	Suppressor of Cytokine Signalling
STAT	Signal Transducer and Activation of Transcription
TAG	Triacylglycerol
TNF-α	Tumour Necrosis Factor Alpha
UPR	Unfolded Protein Response
VAT	Visceral Adipose Tissue

Chapter 1 Introduction

1.1 Background

Insulin resistance (IR) may be caused by decreased sensitivity to insulin, a decrease in maximal response to insulin, or a combination of both. The molecular mechanisms underlying these distinct forms of IR may be different (Kahn, 1978). In fact, IR has been found to affect various insulin signalling pathways differentially, even in the same tissue (Cusi et al., 2000).

IR is associated with reduced cellular glucose uptake and oxidation, despite the existence of high concentrations of glucose and insulin in the blood (Hollenbeck and Reaven, 1987). The metabolic response to starvation, injury and trauma encompasses the termination of anabolic processes, release of fat, carbohydrate and protein, and the acute phase response (Thorell et al., 1999, Cuthbertson, 1980a, Cuthbertson, 1980b, Cuthbertson, 1929, Cuthbertson, 1930). Short periods of fasting (less than 24 hours) are adequate to deplete liver glycogen stores (Nilsson and Hultman, 1973, Awad et al., 2010b), and this leads to increased gluconeogenesis and the detrimental effects of the catabolic state (Cahill et al., 1966). In pathological states, such as diabetic nephropathy, there may also be deposition of glycogen in renal tubules (Gatica et al., 2015).

After a meal or glucose load, hyperglycaemia and hyperinsulinaemia work together to suppress hepatic glucose production and stimulate glucose uptake by visceral and peripheral tissues (Ferrannini et al., 1985, Katz et al., 1983). Glucose is transported to the cell and oxidised subsequently to carbon dioxide and water or converted to glycogen, which is stored in the liver or skeletal muscle (DeFronzo

and Tripathy, 2009). Some glucose is also metabolised to glycerol phosphate for re-esterification of ingested fat for lipid storage and some is used in fatty acid synthesis (Wolfe, 1998). The rate of glucose oxidation is decreased after fasting and most studies agree that non-oxidative glucose disposal is unchanged (Mansell and Macdonald, 1990, Webber et al., 1994). However, in one study on lean and obese subjects with and without diabetes, both the oxidative and non-oxidative components of glucose disposal decreased after fasting (Duska et al., 2005). These effects were seen even in healthy subjects after fasting (Can et al., 2009, Svanfeldt et al., 2005) but may be exaggerated in obese subjects (Damsbo et al., 1991). During periods of fasting a greater proportion of energy is derived from protein oxidation in lean compared with obese subjects (Gallagher and DeLegge, 2011).

Inflammation is also implicated in the aetiology of IR. Obesity-associated inflammation starts in adipose tissue and the liver, with elevated macrophage infiltration and expression of pro-inflammatory cytokines which enter the bloodstream to cause systemic inflammation (Ye, 2013). Several inflammatory markers have been associated with an increased risk of IR (Thorand et al., 2003, Hu et al., 2004, Liu et al., 2007). However, data on the association between inflammatory markers and IR are not consistent (Marques-Vidal et al., 2013).

IR can be present in up to 41% of obese patients without diabetes (Liu et al., 2007). This can rise in the postoperative period and be associated with an increased risk of postoperative complications.

1.2 Methods

Search strategy

Searches of the Medline (Ovid, PubMed, Embase), Science Citation Index and Google™ Scholar databases were performed using the key words surgery, insulin sensitivity, insulin resistance, metabolic, nutrition, carbohydrate, obese, obesity, inflammation, perioperative, pre-operative, post-operative, surgical outcomes in various combinations with the Boolean operators AND, OR and NOT. Animal and human studies published from 1985 to 2014 and key earlier articles were included. Articles published in languages other than English, those published only in abstract form and case reports were excluded. Key journals, textbooks on nutrition and metabolism, and the reference lists of key articles, were also hand searched.

1.3 Definitions

According to the World Health Organisation, "IR is a state in which, under hyperinsulinaemic-euglycaemic conditions, glucose uptake is below the lowest quartile for the population under investigation (Alberti and Zimmet, 1998)." Definitions and methods to measure IR/insulin sensitivity are summarised in **Error! Reference source not found.**.

Table 1.1. Definitions of terminology

Insulin sensitivity	Defined in terms of the concentration of insulin required to cause 50% of its maximal effect on glucose transport (Hollenbeck and Reaven, 1987).
Insulin resistance (IR)	State in which, under hyperinsulinaemic-euglycaemic conditions, glucose uptake is below the lowest quartile for the population under investigation (Alberti and Zimmet, 1998).
Hyperinsulinaemic-euglycaemic clamp	Considered the 'gold-standard' method for quantifying insulin secretion and resistance, used to measure either how well an individual metabolises glucose or how sensitive an individual is to insulin (DeFronzo et al., 1979). The plasma insulin concentration is acutely raised and maintained by a continuous infusion of insulin. Meanwhile, the plasma glucose concentration is held constant at basal levels by a variable glucose infusion. When the steady-state is achieved, the glucose infusion rate equals glucose uptake by all the tissues in the body and is, therefore, a measure of tissue insulin sensitivity (DeFronzo et al., 1979).
Homeostasis Model Assessment (HOMA-IR)	A method which derives an estimate of insulin sensitivity from mathematical modelling of fasting plasma glucose and insulin concentrations (Matthews et al., 1985).
Insulin Sensitivity Index (ISI)	A method which evaluates whole body insulin sensitivity from the oral glucose tolerance test (OGTT) (Matsuda and DeFronzo, 1999).
Quantitative Insulin Sensitivity Check Index (QUICKI)	A method which obtains an index of insulin sensitivity from a fasting blood sample (Katz et al., 2000).

1.4 Surgery and insulin resistance

The stress response following elective surgery has been characterised (Clarke, 1970, O'Keefe et al., 1974, Desborough, 2000). Major abdominal surgery is associated with up to 50% reduction in insulin sensitivity postoperatively (Thorell et al., 1994). Additionally, elective surgery leads to transient impairment in glucose tolerance (Nygren et al., 1997). Glucose uptake in peripheral tissues is reduced and production of glucose via gluconeogenesis is enhanced with resultant hyperglycaemia (Bisgaard et al., 2004). The exact mechanisms underlying postoperative IR have not been fully elucidated yet, but it is known that surgery leads to the release of stress hormones and inflammatory mediators (e.g. cytokines, cortisol, catecholamines, glucagon) (Thorell et al., 1996). In addition, surgery is a stimulus for the acute phase response mediated by cytokines such as interleukin (IL)-6 (Akintola et al., 1997). The magnitude of the IR response to surgery is proportionate to the magnitude of surgical trauma (Thorell et al., 1993, Faria et al., 2009). Severe acute pain has also been associated with a decrease in insulin sensitivity (Greisen et al., 2001) and postoperative IR is partially attenuated by epidural analgesia (Uchida et al., 1988).

Postoperative IR is most pronounced on the day after surgery and persists for up to three weeks after uncomplicated elective open abdominal operations (Thorell et al., 1994, Wang et al., 2010). Development of IR is associated with reduced insulin-stimulated glucose uptake in skeletal muscle and adipose tissue, increased hepatic glucose production and hyperglycaemia (Nygren et al., 1997).

Development of postoperative IR has been shown to be associated independently with increasing duration of hospital stay (Thorell et al., 1999). A study that utilised intraoperative hyperinsulinaemic-euglycaemic clamps (HECs) examined the effects of changes in insulin sensitivity on surgical outcomes following cardiac surgery (Sato et al., 2010). Amongst 130 patients with diabetes and 143 without diabetes studied, those with diabetes with poor glycaemic control (HbA1c >6.5%) had a greater incidence of major complications (p=0.010) and minor infections (p=0.006). Independent of the presence of diabetes mellitus, for each 1 mg/kg/min decrease in insulin sensitivity (M value as obtained from the HEC), the incidence of major complications increased (odds ratio [OR]=2.23, p=0.004) as did the incidence of severe infections (OR=4.98, p=0.010) and minor infections (OR=1.97, p=0.003) (Sato et al., 2010).

In a study of 925 critically ill surgical patients (Mowery et al., 2009), an intensive glucose control protocol was studied which used a surrogate marker of IR (M - not to be confused with the M value obtained from HEC) as a multiplier to adjust the rate of insulin infusion. The study demonstrated that as the magnitude of IR increased, mortality increased (M=0.061 in non-survivors *vs*. M=0.058 in survivors, p=0.02). However, a weakness of the aforementioned study was that there was significant heterogeneity in the study population, which included patients with and without diabetes. In addition, glucose concentration was sampled every 2 hours and 'M' in this study was used to calculate insulin dose based on the previous 2 hour period. Nevertheless, such data from a large surgical patient

cohort lend support to the relationship between development of IR and increased surgical mortality (Mowery et al., 2009).

1.5 Obesity and insulin resistance

It has long been postulated that obesity is a risk factor for development of complications after elective surgery, yet the underlying reasons/mechanisms have not been identified. Identifying these mechanisms may be of clinical relevance, especially given the rising prevalence of obesity globally. Presently, over 30% of the adult population in the USA is obese (Ogden et al., 2014). Obesity has been shown to be associated with increased risk (0.05% in non-obese *vs*. 2.8% in obese and 4% in severely obese, $p<0.01$) of developing postoperative nosocomial infections (Choban et al., 1995), ileus and wound infections after laparoscopic colorectal surgery (Pikarsky et al., 2002) and an increased risk of complications following laparoscopic adrenalectomy (Dancea et al., 2012). Obesity has also been proposed to be a risk factor for wound and respiratory complications after intestinal surgery (patients with BMI 30-40 kg/m^2 were 1.44 times more likely to develop a wound complication compared with normal-weight patients, and those with BMI >40 kg/m^2 were 1.84 times more likely to develop a wound complication and 1.55 times more likely to develop a respiratory complication compared with normal-weight patients) (Wakefield et al., 2012) and increased length of stay (9.5 *vs*. 6.9 days, $p=0.02$) (Pikarsky et al., 2002).

Obesity is associated with the 'metabolic syndrome' which encompasses IR. In a retrospective analysis of 5,304 consecutive patients who underwent an isolated

coronary artery bypass grafting (CABG) procedure, operative mortality was 2.4% in patients with the metabolic syndrome and 0.9% in patients without it ($p < 0.0001$). Presence of the metabolic syndrome was a strong independent predictor of operative mortality (relative risk 3.04 [95% confidence interval (CI) 1.73 to 5.32], $p = 0.0001$) (Echahidi et al., 2007b). In 5085 patients who underwent isolated CABG surgery, of whom 1468 (29%) were obese (body mass index ≥30 kg/m^2) and 2320 (46%) had metabolic syndrome, postoperative atrial fibrillation (POAF) occurred in 1374 (27%) of the patients. Obesity was associated (p<0.001) with increased incidence of POAF in the whole cohort. In these patients, metabolic syndrome was the only metabolic factor to be significantly associated with higher incidence of POAF (12% versus 6%, p=0.01) (Echahidi et al., 2007a).

The association between obesity and IR has been studied in animals and humans (Karakelides et al., 2010, Weisberg et al., 2003, Wang et al., 2011). Studies on obese male C57BL/6 mice suggested that adipose tissue inflammation, focused on macrophages recruited to and activated by expanding adipose tissue mass, was the mechanistic link between obesity and IR (Strissel et al., 2007). In humans, insulin-resistant obesity was associated with increased visceral fat area, increased macrophage infiltration into omental adipose tissue, enlarged adipocyte size in both omental and subcutaneous fat depots, and omental adipocyte IR (Figure 1.1).

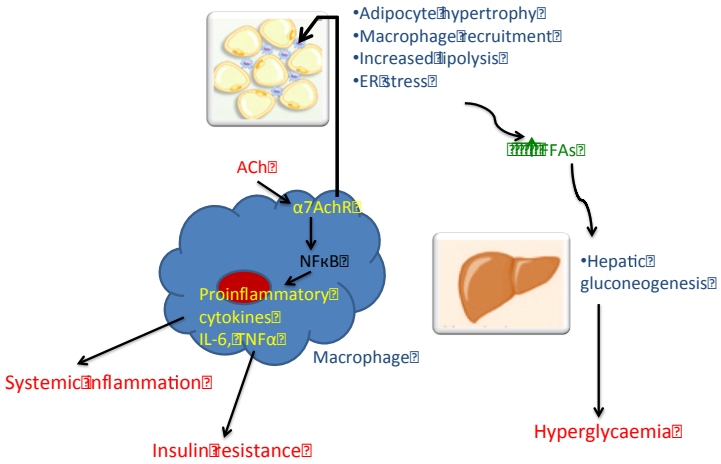


Figure 1.1. Mechanisms underlying obesity-associated insulin resistance

Obesity results in expansion of adipose tissue mass with adipocyte hypertrophy. This leads to an inflammatory response driven by macrophage infiltration and there is evidence of endoplasmic reticulum (ER) stress. Within the macrophage, release of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF) is triggered by the binding of acetylcholine (Ach) to the 7α subunit of its receptor via the NFκB pathway. This leads to systemic inflammation and insulin resistance. There is an increase in lipolysis which leads to an increase in free fatty acids (FFAs) which trigger hepatic gluconeogenesis and hyperglycaemia(Tateya et al., 2013).

Obesity was associated with elevated serum concentrations and dysregulation of adipokines and cytokines produced by visceral fat. These include resistin, adiponectin, tumour necrosis factor alpha (TNF-α) and several interleukins (Kloting et al., 2010, Borst et al., 2005, Mitterberger et al., 2010, Boden, 2009, Donohoe et al., 2011, Indulekha et al., 2011). Studies suggest that a defect in insulin signalling, i.e. a post-receptor defect, may be present in insulin-resistant obesity (Black et al., 1982, Sandqvist et al., 2011).

Excess adipose tissue results in elevated concentrations of pro-inflammatory cytokines, leading to an imbalance between inflammatory and anti-inflammatory stimuli, causing persistent low-grade inflammation (Gletsu et al., 2006). Adipose tissue from insulin-resistant obese subjects had an increased number of CD4+ T cells (which produce IL-22 and IL-17) compared with insulin sensitive obese and lean subjects (Apovian et al., 2008). A study of non-obese and obese patients undergoing abdominal surgery demonstrated an increase in postoperative plasma IL-6 concentrations and adipose tissue content of IL-6 compared with baseline. The increase in IL-6 in plasma and in both visceral (VAT) and subcutaneous adipose tissue (SAT) was exaggerated in severely obese (BMI 48.4±0.7 kg/m^2) patients, compared with non-obese (BMI 24.1±0.9 kg/m^2) patients (Gletsu et al., 2006).

Macrophage infiltration of adipose tissue (Table 1.1) has also been seen in obese humans compared with lean controls, as demonstrated in a study of 29 severely obese women and 60 controls (Harman-Boehm et al., 2007). A recent study of baseline fat biopsies from participants in a weight loss programme confirmed the finding of elevated numbers of macrophages in the adipose tissue of obese individuals (Fjeldborg et al., 2014). In another study, paired omental and subcutaneous fat biopsies revealed elevated macrophage infiltration into omental rather than subcutaneous fat and this was exaggerated by obesity, particularly if predominantly intra-abdominal. The number of omental fat macrophages ($r=0.636$, $p=0.001$), but not of subcutaneous fat macrophages ($r=0.170$, $p=0.427$), correlated with waist circumference (Harman-Boehm et al., 2007, Gauthier et al., 2011).

Table 1.1. Mechanistic studies of obesity and insulin resistance (adipose tissue inflammation/ macrophage infiltration)

qPCR quantitative polymerase chain reaction, **AChR** acetylcholine receptor, **RT-PCR** reverse transcriptase polymerase chain reaction, **BMI** body mass index, **SAT** subcutaneous adipose tissue, **VAT** visceral adipose tissue, **HOMA-IR** homeostatic model assessment, **IL-6** interleukin-6, **eAT** epididymal subcutaneous adipose tissue, **iAT** inguinal subcutaneous adipose tissue, **CSF1** colony stimulating factor, **MCP1** monocyte chemoattractant protein, **IR** insulin resistance, **AMPK** 5' adenosine monophosphate-activated protein kinase

Authors	Subjects/study groups	Intervention	Analysis	Results
Animal Studies				
(Weisberg et al., 2003)	Lean C57BL/6J male mice, lean C57BL/6J female mice fed high-fat diet, moderately obese C57BL/6J male mice, B6.CgAy/$^{+}$ female mice, B6.VLepob/ob severely obese male mice, B6.Vlepob/ob severely obese female mice	Fed liquid formula diet of corn oil, carbohydrate and protein	Total RNA extracted from perigonadal tissue, cDNA hybridised to microarrays, qPCR	Expression of 1304 mRNA transcripts correlated significantly with body mass. Of most significantly correlated, 30% encoded proteins characteristic of macrophages
(Strissel et al., 2007)	Male C57BL/6 mice	Fed high-fat diet for 20 weeks	Intraperitoneal insulin tolerance tests, eAT and iAT and liver harvested for histological, immunohistochemical and gene expression analyses, blood samples for measurement of adipokines	Frequency of adipocyte death in eAT increased from <0.1% at week 1 to 16% at week 12 ($p<0.001$). Serum concentrations of leptin and resistin but not adiponectin elevated after one week of high-fat feeding ($p<0.001$)
(Wang et al., 2011)	Male C57BL/6J mice, male α7nAChR-/- (knockout) mice, male obese diabetic db/db mice	High fat and nicotine or low fat diet for 11-24	Cell culture of peritoneal macrophages, measurement of blood glucose, glucose tolerance	Adipose tissue possesses a functional cholinergic signalling pathway, activation of cholinergic anti-inflammatory pathway by nicotine suppressed inflammation in adipose tissue, independent of changes in body weight and adiposity

		weeks	tests, hyperinsulinaemic-euglycaemic clamp, tissue homogenising and immunoblotting to detect 28phosphor- and total protein levels, RNA extraction and RT-PCR, adipocyte isolation	
Human Studies				
(Gletsu et al., 2006)	13 non-obese (BMI≤30 kg/m^2) and 33 severely obese patients (BMI≥40 kg/m^2)	Undergoing elective laparoscopic surgery	Biopsies taken while under general anaesthesia at start and end of surgery: Abdominal SAT, VAT. Blood samples taken at start and end of surgery for cytokines and HOMA-IR	Severely obese patients had higher preoperative IL-6 concentrations compared with non-obese ($p<0.01$). Postoperatively IL-6 increased in both groups, but was exaggerated in severely obese ($p<0.001$). VAT and SAT at start of operation contained comparable amounts of IL-6 in non-obese, but VAT contained less IL-6 than SAT in severely obese ($p<0.05$). At end of surgery, IL-6 concentrations in VAT and SAT increased but magnitude of increase greater in severely obese ($p<0.005$).
(Harman-Boehm et al., 2007)	60 controls undergoing elective open abdominal surgery and 29 severely obese (BMI ≥30kg/m^2) women undergoing elective laparoscopic abdominal surgery	Nil (samples taken preoperatively)	Hyperinsulinaemic-euglycaemic clamp to estimate IR in cohort 1, HOMA-IR used in cohort 2, paired omental and subcutaneous fat biopsies examined histologically and mRNA, protein levels quantified	Elevated macrophage infiltration into omental vs subcutaneous fat seen in lean subjects and exaggerated by obesity. Concentrations CSF1 mRNA and MCP1 correlated with number of omental macrophages ($r=0.258$, $p<0.051$ and $r=0.521$, $p<0.0001$)
(Gauthier et al., 2011)	8 obese patients (BMI 42-60kg/m^2) undergoing elective surgery	Nil (samples taken preoperatively)	HOMA-IR to estimate IS, omental and SAT analysed for mRNA levels of genes involved in inflammation	AMPK activity 2 fold lower in omental than subcutaneous fat, and associated with increased expression of multiple inflammatory genes

(Bigornia et al., 2012)	92 obese participants (BMI ≥30 kg/m^2) undergoing elective bariatric surgery	Nil (samples taken preoperatively)	HOMA-IR to estimate IS, omental, mesenteric and subcutaneous fat biopsies subjected to immunohistochemistry to determine presence of macrophage crown-like structures (CLS)	CLS status in subcutaneous fat highly sensitive and modestly specific for CLS status (marker of inflammation) in visceral fat. HOMA-IR and plasma insulin concentrations positively associated with CLS status in all adipose tissue depots

Recent studies have suggested that macrophage accumulation in subcutaneous fat in a histopathological pattern of crown-like structures (CLS) is associated with overproduction of cytokines, altered adipose tissue gene expression and systemic IR (estimated using homeostasis model assessment [HOMA]) in obese individuals (Wentworth et al., 2010, Apovian et al., 2008, Bremer et al., 2011, van Beek et al., 2014). CLS status has also been studied as a marker of inflammation in visceral fat depots in 92 obese participants during bariatric surgery (Bigornia et al., 2012). In this study, fasting IR as measured by HOMA-IR, was associated with CLS positive status in all adipose tissue depots (Bigornia et al., 2012).

An association between abdominal adiposity and IR has previously been demonstrated (Abate et al., 1995, Goodpaster et al., 1997, Kelley et al., 2000, Raji et al., 2001, Wagenknecht et al., 2003, Sites et al., 2000, Ross et al., 2002a, Ross et al., 2002b). Studies, utilising the gold-standard HEC (DeFronzo et al., 1979) and dual absorptiometry X-ray (DXA) scans/CT/hydrodensitometry to evaluate body composition, have been performed in healthy middle-aged men (Abate et al., 1995), sedentary men and women (Goodpaster et al., 1997), and lean and obese glucose tolerant men and women (Kelley et al., 2000). These studies demonstrated that subcutaneous fat mass correlated with IR.

The same effect was seen in Asian Indians, who had physiological markers of IR, dyslipidaemia and increased cardiovascular risk (Raji et al., 2001), and in pre- and post-menopausal women (Sites et al., 2000, Ross et al., 2002b). This correlation was also demonstrated in a large study of 999 Hispanic and 458 African-American men

and women as part of the Insulin Resistance Atherosclerosis Study (IRAS) Family Study (Wagenknecht et al., 2003).

Data from the European Group for the Study of Insulin Resistance suggested that only around 25% of obese individuals [body mass index (BMI) ≥35 kg/m^2] were insulin sensitive, as determined by the HEC (Ferrannini et al., 1997). Although the authors found no significant differences in body composition between those who were insulin sensitive and insulin resistant, they hypothesised that in some individuals weight gain may occur in ways or at sites that do not interfere with insulin action or glucose uptake (Ferrannini et al., 1997). IR has been associated with a lower rate of weight gain in Pima Indians (Swinburn et al., 1991) and in the San Antonio heart study, a higher concentration of fasting insulin was associated with lower likelihood of gaining weight (Valdez et al., 1994). However, in the more recent RISC study, no association was found between insulin sensitivity and weight gain (Rebelos et al., 2011). Therefore, the association between weight gain, body composition and IR has not yet been clearly established.

Whole-body insulin mediated glucose uptake is decreased in obese individuals (Laakso et al., 1990). IR is related to the degree of obesity and decreases after weight loss (Ferrannini et al., 1997, Drapeau et al., 2011). In a study of patients undergoing cardiac surgery, negative correlations were observed between insulin sensitivity and body weight (Sato et al., 2010), and in healthy volunteers insulin concentrations and IR, assessed using the HOMA-IR formula (Matthews et al., 1985), were increased significantly in obese subjects compared with lean controls (Silha et al., 2003). Insulin stimulation of glycogen formation, glucose oxidation and

non-oxidative glycolysis were all depressed in the muscle of severely obese patients (Friedman et al., 1994). This has been demonstrated on analysis of skeletal muscle cell cultures derived from biopsies from severely obese individuals (Dohm et al., 1988).

1.6 Mechanisms underlying obesity-related Insulin Resistance – Adipokines

Adiponectin

Dysregulation of adipokines (Table 1.2) contributes to the pro-inflammatory environment associated with obesity (Simon et al., 2009). Adiponectin has been identified as an insulin-sensitising, adipocyte-derived protein which is decreased in obesity (Weisberg et al., 2003, Mitterberger et al., 2010, Horakova et al., 2010, Stepien et al., 2011, Stepien et al., 2014). Adiponectin-deficient mice were prone to diet-induced obesity and IR (Kubota et al., 2002). In mouse hepatocytes, adiponectin improved suppression of gluconeogenesis by insulin (Miller et al., 2011). A study of 77 obese (BMI ≥30) and 23 non-obese (BMI ≤30) women showed that adiponectin secretion from subcutaneous adipose tissue (SAT) was reduced in obesity and insulin resistance (Hoffstedt et al., 2004). In a study of patients undergoing elective abdominal surgery, total adiponectin concentrations were lower in obese than in non-obese subjects ($p<0.05$) (Kovacova et al., 2012). Secretion of total adiponectin was lower in obese than in non-obese subjects in VAT but not in SAT (Kovacova et al., 2012). This finding was mirrored in another study of obese subjects undergoing gastric bypass surgery and non-obese subjects undergoing elective abdominal surgery (Hammes et al., 2012). However, in a study of 117 women and 59 men, adiponectin concentration and waist circumference

were predictors of IR, measured by HOMA-IR, in women but not in men (Bonneau et al., 2014). In a study of Danish patients undergoing elective cardiac surgery, high concentrations of adiponectin were associated with increased 365-day mortality (Mikkelsen et al., 2010). This study was not designed to detect causality. The incongruent findings from studies of adiponectin may be explained by differences in study design, methods used to study insulin sensitivity and heterogeneity of subjects. Therefore, this requires further evaluation in clinical studies.

Table 1.2. Mechanistic studies of obesity and insulin resistance (adipokines). **TNF α tumour necrosis factor alpha, PKC Protein Kinase C, IgG Immunoglobulin G, rt-qPCR** reverse transcriptase quantitative polymerase chain reaction, **IL-6** interleukin 6, **MCP-1** monocyte chemoattractant protein 1, **GLUT-4** glucose transporter type 4, **HepG2** hepatocellular carcinoma cell line, **Foxa2** forkhead box protein A2, **VF** visceral fat, **LKB1** liver kinase B1**, AMPK** 5' adenosine monophosphate-activated protein kinase, **VAT** visceral adipose tissue, **SAT** subcutaneous adipose tissue, **HOMA** homeostatic model assessment, **IR** insulin resistance, **ERK** extracellular signal-related kinase, **JNK** c-JUN N-terminal kinase, **CRP** C-reactive protein, **BMI** body mass index, **HSL** hormone sensitive lipase, **ATGL** HSL lipase adipose triglyceride lipase, **DXA** dual energy X-ray absorptiometry, **MRI** magnetic resonance imaging, **QUICKI** quantitative insulin sensitivity check index, **A-FABP** adipocyte fatty acid binding protein, **T2DM** type 2 diabetes mellitus, **IGT** impaired glucose tolerance, **CURES** Chennai Urban Rural Epidemiological Study, **CT** computed tomography, **STAT3** signal transducer and activator of transcription3, SOSC3 suppressor of cytokine signalling3, **NAMPT** Nicotinamide phosphoribosyltransferase, **NFκB1** nuclear factor of kappa light polypeptide gene enhancer in B cells 1, **ADIPOQ** adiponectin coding gene, **LEP** leptin gene **IRS1** insulin receptor substrate1**, P1K3R1 , SLC2A4** solute carrier family2**, IκBκB** inhibitor of kappa light polypeptide gene enhancer in B cells**, cAMP** cyclic adenosine monophosphate**, GLP-1R** glucagon-like peptide receptor

Author, year	Subjects/study groups	Intervention	Analysis	Results
Cell/ tissue culture				
(Hotamisligil et al., 1994)	Murine 3T3L1 or F442A cells	Adipocytes treated with cytokines	Analysis of Insulin Receptor Tyrosine Kinase activity in cells	Low TNF-α concentrations induce significant resistance to the effects of insulin on glucose uptake
(Rosenzweig et al., 2002)	Murine skeletal muscle cell cultures	Cells subjected to treatment with TNF-α	Immunoprecipitation, glucose uptake analysis, Western blot, PKC activity	Two distinct mechanisms of TNF-α opposing upstream elements in insulin signalling pathway
(Steppan et al., 2001)	Mouse 3t3-L1 fibroblast cells, C57/BL/6J mice and female FVB/N mice	Adipocytes treated for 48h with dimethyl sulphoxide or rosiglitazone, mice subjected to fasting for 48 h, chow diet or fasting for 48 h followed by unrestricted chow. rosiglitazone treatment	Immunoblot assays, immunohistochemistry of adipocytes. Epididymal fat dissected from mice for RNA and protein analysis, serum resistin, plasma glucose, glucose tolerance tests, glucose transport assays.	Resistin gene expression down regulated by rosiglitazone treatment. Mouse serum resistin concentrations decreased after 48h fast and reversed by refeeding. Serum resistin concentrations elevated in mice fed on high fat diet. Anti-resistin IgG potentiated insulin-stimulated glucose uptake by 42% ($p<0.04$).
(Fu et al., 2006)	Mouse 3t3-L1 fibroblast cells	Resistin gene expressed using lentiviral vector	Cell culture, glucose transport assays, Western blots, resistin assays, rt-QPCR	30% decrease in maximal insulin-stimulated glucose transport rate in cells over-expressing resistin.

				Overexpression of resistin increased concentrations of TNF-α, IL-6 and MCP-1. GLUT4 mRNA levels reduced 51% in cells overexpressing resistin.
(Pandey et al., 2009)	Hep g2 cells	Cells incubated with TNF-α or insulin or TNF-α followed by insulin treatment	Western blotting, immunofluorescence microscopy, chromatin immunoprecipitation assay, RNA isolation, rt-PCR, glucose production assay	Pre-incubation of cells with TNF-α, followed by insulin, prevented insulin-mediated nuclear exclusion of Foxa2. TNF-α pre-incubation attenuated insulin-induced inhibition of expression of gluconeogenic enzymes and hepatic glucose production.
Animal studies				
(Kubota et al., 2002)	Adiponectin deficient mice, heterozygous adiponectin deficient mice and wild type controls	Fed standard diet or high-fat diet	Blood sampling, RNA preparation, Northern blot analysis, Vascular cuff injury modelling	Glucose-lowering effect of insulin was significantly impaired in adiponectin deficient compared with heterozygous and controls (p=0.03).
(Borst et al., 2005)	Male Sprague-Dawley rats	Retroperitoneal and epididymal fat deposits removed in 8 rats, sham surgery performed in 8 rats	Muscle strips analysed to assess glucose transport, glucose tolerance testing, TNFα measured in muscle extracts, resistin and adiponectin measured in plasma	VF removal resulted in an improvement in skeletal muscle insulin responsiveness. Serum adiponectin was reduced 30% in VF removed animals, significant correlation between VF and serum adiponectin (p<0.001). Serum resistin reduced 26% in VF removed animals.
(Miller et al., 2011)	Mice with deletion of LKB1, an upstream regulator of AMPK	Nil	Hyperinsulinaemic-euglycaemic clamp, gene expression analysis from mouse hepatocytes	Loss of LKB1 in the liver partially impaired the ability of adiponectin to lower serum glucose.
Studies in healthy subjects				
(Johnson et al., 2001)	44 black and white healthy premenopausal women	Nil	Anthropometrics, body composition analysis using hydrodensitometry, measurement of VAT and SAT using MRI, oral glucose tolerance test, frequently	SAT adipocytes were less sensitive to antilipolytic effect of insulin compared with gluteal adipocytes (p<0.01)

			sampled intravenous glucose tolerance test, SAT biopsy, adipocyte isolation, determination of adipocyte size, in vitro lipolysis measurement, determination of glucose transport	
(Papadia et al., 2003)	85 severely obese women	Nil	Venous blood, HOMA	Higher degree of obesity and lower serum leptin concentration seen in IR patients compared with insulin sensitive (p=0.0053, p=0.0027).
(Hoffstedt et al., 2004)	77 obese (BMI ≥ 30 kg/m^2) and 23 non-obese subjects	Nil	Plasma insulin, glucose, serum adiponectin. HOMA-IR to estimate IR, subcutaneous fat biopsy for analysis of adiponectin mRNA and measurement of adiponectin secretion rates	Serum adiponectin concentrations highest in high insulin sensitivity group and lowest in low insulin sensitivity group (p<0.001). Adipose tissue secretion rate pre tissue weight reduced in most IR group (p<0.05)
(Plomgaard et al., 2005)	9 healthy men	Infusion of TNF-α or placebo	Hyperinsulinaemic-euglycaemic clamp with stable isotope tracer, vastus lateralis biopsies for protein phosphorylation	TNF-α infusion increased phosphorylation of p70S6kinase, ERK-1/2 and JNK in skeletal muscle (targets implicated in negative regulation of insulin signal transduction)
(Oki et al., 2007)	295 Japanese Americans enrolled in long-term epidemiological study	Nil	Anthropometrics, venous blood, HOMA-IR	Positive correlation between serum visfatin and Il-6 or CRP (r-0.271, p<0.001, r=0.118, p<0.05). No significant trend of visfatin with HOMA-IR, but increasing HOMA-IR correlated with increasing IL-6 and CRP.
(Jocken et al., 2007)	44 overweight-obese (BMI≥26kg/m^2) subjects selected from multicentre observational study	10 week dietary intervention (low or medium fat diet)	HOMA-IR, anthropometrics, SAT biopsy at baseline and after 10 weeks, plasma glucose, glycerol, fat cell weight, Western blot analysis, mRNA analysis	Diet resulted in loss of body weight (p<0.001), circulating fatty acids (p=0.016), leptin (p<0.001) but no difference in fasting glucose, insulin and HOMA-IR. Fasting insulin concentrations and degree of IR negatively correlated with HSL and ATGL protein expression. mRNA and protein levels of ATGL and HSL reduced in IR compared with insulin sensitive subjects (p<0.05). Weight reduction decreased

				ATGL and HSL mRNA and protein expression.
(Hung et al., 2008)	1094 subjects from community-based study	Nil	Anthropometrics, venous blood, HOMA	Concentrations of CRP, IL-6 were inversely related to adiponectin concentrations ($p<0.001$). Plasma adiponectin inversely related to IR ($p<0.001$)
(Frederiksen et al., 2009)	783 men aged 20-29 years	Nil	Anthropometrics, measurement of adiponectin, insulin and HOMA-IR, DXA, MRI	Serum adiponectin correlated negatively with waist circumference ($r=-0.21$, $p<0.001$) waist/hip ratio ($r=-0.23$, $p<0.001$), VAT mass from MRI ($r=-0.21$, $p<0.001$), SAT mass from MRI ($r=-021$, $p<0.001$). Adiponectin was an independent predictor of HOMA-IR ($r=-0.14$, $p<0.001$)
(Erdmann et al., 2009)	291 patients attending nutritional medicine outpatient department	Nil	HOMA-IR, venous blood at several time points	Basal glucose concentration was 8% higher in obese group compared with normal weight. Postprandial insulin clearance decreased with increasing body weight.
(Horakova et al., 2010)	53 healthy subjects, 29 overweight subjects ($25<BMI<30 kg/m^2$), 51 obese subjects ($BMI\geq 30 kg/m^2$)	Nil	Serum insulin, glucose, cholesterol, triacylglycerols, adiponectin, A-FABP. QUICKI and HOMA to estimate IR.	Adiponectin concentrations highest in healthy subjects and lowest in obese subjects, a-FABP lowest in healthy subjects. Correlation between a-FABP and BMI ($r=0.48$), adiponectin and QUICKI ($r=0.42$) and adiponectin and BMI ($r=-0.31$)
(Indulekha et al., 2011)	85 subjects with normal glucose tolerance (NGT), 49 subjects with IGT, 93 subjects with T2DM, all recruited from CURES	Nil	Anthropometrics, measurement of carotid intimal medial thickness, plasma glucose, serum cholesterol, triglycerides, adiponectin, CRP, TNFα, visfatin, leptin. HOMA-IR to estimate IR. CT scan analysis to determine visceral and subcutaneous fat.	Total abdominal fat ($p=0.041$) and visceral fat ($p=0.039$) progressively increased in NGT, IGT and T2DM subjects. Increasing quartiles of visceral fat correlated with increasing IR ($p=0.040$). Decrease in adiponectin ($p=0.043$), increase in TNFα, CRP ($p=0.043$) and visfatin ($p=0.040$) with increasing visceral fat.
(Sandqvist et al., 2011)	7 obese ($BMI \geq 30 kg/m^2$) postmenopausal women and 9 lean women ($BMI \leq 30 kg/m^2$)	Nil	Oral glucose tolerance test, microdialysis technique used to measure interstitial insulin concentration in subcutaneous adipose and muscle tissue, SAT biopsy	Mean arterial insulin concentrations higher in obese than lean group after oral glucose ingestion ($p<0.05$). Interstitial insulin concentrations in fat and muscle similar in both

				groups therefore proportion of circulating insulin transported significantly lower in obese than lean group
(Ackermann et al., 2011)	89 women with metabolic syndrome participating in dietary intervention trial	Nil (baseline)	Anthropometrics, dietary analysis, fasting blood samples, HOMA	Waist circumference correlated positively with CRP (r=0.307, p<0.01), IL-6 (r=0.228, p<0.05) and negatively with adiponectin (r=-0.309, p<0.001)
Studies in surgical patients				
(Stolic et al., 2002)	14 lean (BMI<25kg/m^2), 12 overweight (25<BMI<30kg/m^2) and 15 (BMI>30kg/m^2) obese patients undergoing elective abdominal surgery	Nil (samples taken at start of surgery)	Omental and SAT subject to glucose uptake assay	Lean subjects demonstrated increase in 2-deoxyglucose transport over basal in omental (p<0.0001) and SAT. In overweight and obese subjects, insulin-stimulated glucose uptake reduced in both fat depots. Reduced insulin action in omental adipose tissue in those with central compared with peripheral obesity (p=0.024).
(Lehrke et al., 2008)	37 non-diabetic patients undergoing cardiac surgery	Cardiac surgery	Insulin requirements to maintain euglycaemia (insulin glycaemic index calculated), blood sampling at baseline, 4 to 6 hours postoperatively, 10 to 12 hours and day 2 postoperatively	IR increased during observation period, peaked at 22h post operatively. IL-6 and TNF-α peaked at 4h postoperatively. Cortisol, resistin and leptin peaked at 22h and adiponectin declined and reached base at 22h. Cortisol was best predictor of IR, followed by IL-6, leptin and adiponectin.
(Cao et al., 2008)	32 obese (BMI ≥ 28 kg/m^2) subjects undergoing elective surgery	Nil (samples taken preoperatively)	Anthropometrics, Western blot analysis of paired omental and subcutaneous fat biopsies, blood samples	Omental adipose tissue showed strong protein expression of TNF-α compared to subcutaneous fat (p<0.01). Omental TNF-α protein concentrations correlated with HOMA-IR (p<0.01)
(Mikkelsen et al., 2010)	836 patients undergoing elective cardiac surgery	Cardiac surgery	Preoperative fasting blood samples taken and used to calculate HOMA-IR, outcomes analysis	Increased HOMA values not significantly associated with postoperative mortality or morbidity. Adiponectin concentrations correlated with BMI. High concentrations of adiponectin associated with all cause death (p<0.05, OR 2.9)

(Alvehus et al., 2010)	17 healthy women undergoing elective abdominal surgery	Nil (samples taken at start of surgery)	Anthropometrics, Body fat (DXA) and distribution (CT), HOMA-IR, venous blood, rt-PCR from VAT, superficial and deep SAT biopsies	Volume of superficial and deep SAT similar but VAT significantly smaller ($p<0.001$) Separation of VAT from SAT depots driven by chemotactic factors CCR2 and MIF
(Witasp et al., 2010)	7 women and 5 men undergoing elective abdominal surgery	Abdominal surgery	Blood samples preoperatively and at day 1 and day 2 postoperatively, subcutaneous and omental adipose tissue samples subjected to rt-PCR	Surgery induced up-regulation of IL-6, STAT3, SOSC3, NAMPT and NFKB1 genes and down-regulation of ADIPOQ, LEP< IRS1, PIK3R1, SLC2A4 and IKBKB. mRNAs encoding ADIPOQ and LEPR approximately 1.5-fold decreased in SAT and omental adipose tissue.
(Vendrell et al., 2011a)	22 morbidly obese patients scheduled for biliopancreatic diversion surgery and 73 healthy controls 3T3-L1 cell culture	Nil (samples taken at start of surgery)	Anthropometrics, venous bloods, subcutaneous and VAT samples at start of surgery subjected to Western blotting, immunohistochemistry Measurement of cAMP production in cultured cells, lipolysis assays, total RNA isolation, rt-PCR	Progressive decrease in GLP-1R gene expression in VAT depot from lean to morbidly obese. GLP-1R mRNA expression increased 2.5-fold in high insulin-resistant obese group.
(Hardy et al., 2011)	20 patients undergoing laparoscopic Roux-en-Y gastric bypass surgery	Nil (samples taken at start of surgery)	HOMA-IR, paired samples of omental and SAT used to extract total RNA and GeneChip expression array analysis performed, rt-PCR, adipose tissue histologic examination	Larger adipocyte size and increased macrophage infiltration associated with IR. Gene sets related to chemokine activity and chemokine receptor binding highly expressed in omental tissue of IR compared to insulin sensitive subjects.

Tumour necrosis factor alpha (TNF-α)

In the obese state, TNF-α was the first adipokine shown to be secreted by adipocytes and macrophages located within adipose tissue (Cao et al., 2008). Early studies demonstrated that when adipocytes were treated with TNF-α, their insulin signalling was impaired, mainly because of changes in the transcription of insulin signalling molecules, in particular the insulin receptor, IRS-1, and GLUT-4 (Stephens et al., 1997, Stephens and Pekala, 1992). TNF-α can induce IR in adipocytes, hepatocytes, and skeletal muscles (Hotamisligil et al., 1994, Rosenzweig et al., 2002, Plomgaard et al., 2005). Obese mice lacking TNF-α or its receptors were protected against developing IR (Uysal et al., 1997). Blocking functions of TNFα by genetic and pharmacological interventions improved obesity-induced IR (Ventre et al., 1997).

TNF-α mRNA and protein were highly expressed in adipose tissue of obese subjects (Hotamisligil et al., 1995). Serum TNF-α concentrations were elevated in obese compared with non-obese subjects (Moon et al., 2004). These links between TNF-α, obesity and IR suggested the novel concept that an immune pathway may regulate the development of obesity-induced IR.

Interleukin 6 (IL-6)

IL-6 is released by adipocytes and macrophages within fat tissues of obese humans and IL-6 concentrations are elevated in obesity (Karakelides et al., 2010). In cell cultures of adipocytes from insulin-resistant humans, overexpression of IL-6 was

demonstrated and insulin-stimulated glucose transport was reduced by IL-6 (Rotter et al., 2003). IL-6 was demonstrated to induce IR by inhibiting insulin signalling in human adipocytes and hepatocytes, however, there is conflicting evidence that it also has insulin-sensitising effects in humans and mice (Pedersen and Febbraio, 2007). In conscious mice subjected to the HEC, IL-6 treatment blunted the ability of insulin to suppress hepatic glucose production and reduced insulin-stimulated glucose uptake in skeletal muscle (Kim et al., 2004). In healthy humans, IL-6 increased glucose infusion rate and glucose oxidation without affecting suppression of endogenous glucose production during a HEC (Carey et al., 2006). IL-6 may be one of the mediators that links obesity with IR but recent evidence suggests that its effects are influenced by whether it is present acutely or chronically (Kim et al., 2009).

Resistin

Resistin is an adipokine which has been shown to mediate IR in mice (Steppan et al., 2001). In a study of adipocytes, overexpression of resistin affected adipogenesis, impaired the ability of insulin to stimulate glucose transport, led to a reduction in GLUT-4 gene expression and augmented the production of TNFα and IL-6 (Fu et al., 2006). In a study of 37 patients without diabetes undergoing cardiac surgery, resistin was not proven to be a prominent mediator of inflammatory IR (Lehrke et al., 2008). In 68 postmenopausal women undergoing elective surgery, resistin mRNA expression in SAT was significantly higher in obese compared with non-obese subjects. Mean serum resistin concentrations were also significantly higher in obese compared with non-obese women (13.92±6.32 ng/mL *vs*. 9.05±5.14 ng/mL, $p<0.001$). Resistin mRNA expression in SAT correlated with HOMA-IR in obese

(r=0.45, p<0.01) but not in non-obese (r=-0.04, p>0.05) participants (Sadashiv et al., 2012). The role of resistin in the pathogenesis of human IR remains unclear at present (Arner, 2005).

Visfatin

Visfatin is increased with visceral adiposity and is released from visceral fat via an unknown mechanism (Indulekha et al., 2011). It has been recognised as a pre-B-cell colony enhancing factor (PBEF), which acts synergistically with IL-7 and stem cell factors to promote the growth of B cell precursors (Samal et al., 1994). It has also been shown to activate NF-κB (Choi et al., 2011). Production of IL-6 in human monocytes was induced by visfatin via the p38 mitogen-activated protein kinase (MAPK) and MAPK kinase 1 (MEK1) pathways (Moschen et al., 2007). In a large population-based study of Japanese Americans, visfatin concentrations were positively correlated with serum IL-6 concentrations (Oki et al., 2007). Visfatin was not related to IR (measured by HOMA-IR) in this population (Oki et al., 2007). In a study of obese and non-obese patients undergoing surgery, plasma visfatin showed no correlation with BMI but in SAT, insulin resistant subjects demonstrated lower expression of visfatin. Therefore, it is possible that visfatin has a role as an adipokine which augments the action of insulin (Varma et al., 2007).

In 38 morbidly obese subjects undergoing gastric bypass surgery, omental visfatin gene expression and protein levels correlated positively with insulin sensitivity. In this study, HOMA-IR was used to estimate IR (Goktas et al., 2013). However, in 35 morbidly obese subjects (BMI 45±4.7 kg/m^2) undergoing bariatric surgery, serum

visfatin concentrations decreased and adiponectin concentrations increased after weight loss. There was no correlation between HOMA-IR and visfatin or adiponectin concentrations (Hosseinzadeh-Attar et al., 2013).

A meta-analysis sought to clarify the role of visfatin and found that plasma visfatin concentrations were significantly increased in subjects diagnosed with overweight/obesity, Type 2 diabetes mellitus, metabolic syndrome and cardiovascular diseases (Chang et al., 2011). Furthermore, the authors concluded that overall, plasma visfatin concentration was associated positively with IR, as assessed by HOMA-IR (Chang et al., 2011).

1.7 Adipose tissue: an endocrine organ

It appears evident that adipokines are an important link between obesity, inflammation and IR. They may also have a role in the pursuit of and maintenance of weight loss; all of these functions have significant implications for the obese surgical patient.

Adipose tissue is an active endocrine organ and modulator of immune function (John et al., 2006, Fawcett et al., 2010). Several recent studies have suggested that the site and distribution of adipose tissue may impact on the resultant degree of inflammation and IR (Stolic et al., 2002, Hsieh et al., 2014). Central or abdominal obesity appears to correlate more strongly with IR, the metabolic syndrome and the development of type 2 diabetes than total adiposity or BMI (Ross et al., 2002b, Ross et al., 2002a, Donohoe et al., 2011, Indulekha et al., 2011, Fawcett et al., 2010,

Smith et al., 2001, Thamer et al., 2010). Upper body 'fatness' and waist circumference predict development of type 2 diabetes and cardiovascular disease (McLaughlin et al., 2011). In a study of 40 overweight and obese (BMI 32.3±3.3 kg/m^2) premenopausal women (42±7 years), waist circumference correlated positively with the results of an oral glucose tolerance test (OGTT) and negatively with glucose disposal (Ross et al., 2002b). Korean women with abdominal waist circumference >80 cm were 1.6-3.4 times more likely to have at least two components of the metabolic syndrome *per* 1 standard deviation increase of abdominal fat measurement (Lee et al., 2008). In severely obese (BMI 33.7-59.8 kg/m^2) women, VAT volume, as estimated using multi-slice computed tomography, was a predictor of dysglycaemia (impaired fasting glucose or diabetes) (Gletsu-Miller et al., 2013).

The association between visceral fat and IR appears to be related to the metabolic properties of visceral fat (Jankiewicz-Wika et al., 2011, Donohoe et al., 2011, Fabbrini et al., 2013). In a study of 89 obese males, VAT was a significant correlate of glucose tolerance and IR ($p<0.005$, $r=0.50$ and -0.41 respectively) (Ross et al., 2002a). Similarly, in 115 moderately obese and overweight adults (BMI 25-36.9 kg/m^2) VAT mass was significantly higher in insulin resistant subjects compared with insulin sensitive ones (McLaughlin et al., 2011). In the same study, insulin sensitive individuals had greater SAT mass compared with insulin resistant individuals (McLaughlin et al., 2011). In a study of Asian Indians without diabetes, visceral but not subcutaneous fat was associated with metabolic syndrome and IR, independently of age (Sandeep et al., 2010).

Some studies have also divided the visceral fat component into deep and superficial compartments. Smith *et al.* (Smith et al., 2001) examined the metabolic differences between distribution of total body fat and deep and superficial SAT. SAT was divided in deep and superficial compartments by the *fascia superficialis*. They demonstrated that total body fat was a good predictor of overall metabolic risk and in men, deep SAT was responsible for a greater proportion of the variance in fasting insulin (r^2=0.528) than VAT (r^2=0.374), but this difference was not tested for statistical significance (Smith et al., 2001). In a study of healthy middle-aged men, the relationship of insulin sensitivity (measured by the HEC) was compared with total adiposity (measured using hydrodensitometry) and regional adiposity (measured using magnetic resonance imaging [MRI]) (Abate et al., 1995). Glucose disposal rate and residual hepatic glucose output correlated significantly with total body fat, subcutaneous abdominal fat and intraperitoneal fat but not retroperitoneal fat. The latter study concluded that subcutaneous truncal fat had a strong association with obesity-related IR in men and intraperitoneal/retroperitoneal fat played a lesser role (Abate et al., 1995). These contrasting results may reflect the methods used to divide deep and superficial compartments – some studies have used MRI techniques while others have used CT. There may also be differences in the cohorts studied.

The mechanisms underlying the association between fat distribution and IR may be related to secretion of adipokines (Kelley et al., 2000, Ross et al., 2002b, Hung et al., 2008, Buemann et al., 2005, Vohl et al., 2004). In Sprague-Dawley rats, a 48%

reduction in visceral fat was associated with a reduction in resistin by 26% and adiponectin by 30% (Borst et al., 2005). In this study, it was hypothesised that improved muscle insulin response occurred in spite of reduced serum adiponectin, rather than because of it (Borst et al., 2005). A genetic study in obese men found that IL-6 mRNA expression was significantly higher in visceral compared with subcutaneous fat (Vohl et al., 2004). In a study of obese Chinese subjects undergoing surgery, paired subcutaneous and omental adipose tissue biopsies showed significantly increased TNF-α protein expression in omental compared with subcutaneous adipose tissue (Cao et al., 2008). An observational study of overweight and obese women found a negative correlation between adiponectin and waist circumference (Ackermann et al., 2011) and a similar result was found in young lean Danish men (Frederiksen et al., 2009). As adiponectin is an insulin sensitiser, this supports the hypothesis that abdominal adiposity may be associated with IR.

Adipocytes from subcutaneous and visceral deposits exhibit different properties, subcutaneous having higher fatty acid uptake and lower glucose uptake than visceral adipocytes (Fawcett et al., 2010). VAT appears to be more susceptible to lipolysis and released greater quantities of free fatty acids per gram of tissue, compared with SAT (Gletsu et al., 2006). In obese white and black women, subcutaneous adipocytes were found to be less sensitive to the antilipolytic effects of insulin (Johnson et al., 2001). Insulin clearance is altered with increased weight (Erdmann et al., 2009), and it has been suggested that insulin metabolism may be different in SAT and VAT. In adipocytes taken from subcutaneous and visceral sites

in patients undergoing elective open abdominal surgery, there was more insulin degradation in VAT compared with SAT (Fawcett et al., 2010). Suggested mechanisms such as insulin receptor binding have been investigated but results are inconclusive. Insulin degrading enzyme is responsible for most cellular insulin degradation and this may be responsible for the differences in insulin degradation in VAT and SAT (Fawcett et al., 2010).

Another hypothesis to explain the correlation between IR and central adiposity relates to the properties of omental fat. Omental fat appears to have more macrophage infiltration than subcutaneous fat (Harman-Boehm et al., 2007, Hardy et al., 2011), a finding that is exaggerated by central obesity (Harman-Boehm et al., 2007).

In lean individuals, insulin sensitivity of omental and SAT was similar, but basal glucose uptake was significantly higher in omental adipose tissue. However, in obese subjects with a central distribution of adiposity, IR occurred at the site of omental adipose tissue, in contrast to those with peripheral obesity (Stolic et al., 2002). No difference in insulin-stimulated glucose uptake of SAT was demonstrated (Stolic et al., 2002).

VAT releases adipokines and free fatty acids directly into the portal vein for delivery to the liver (Ross et al., 2002b, Fabbrini et al., 2010). This has stimulated research into the beneficial effects of omentectomy. In Sprague-Dawley male rats, surgical removal of visceral fat resulted in increased response to insulin in skeletal muscle

and reduction in the serum concentration of several cytokines that have the potential to impair insulin responses (Borst et al., 2005).

1.8 Fatty Acids and insulin resistance

Adiposity is characterized by increased triacylglycerol (TAG) storage in adipose tissue and IR (Mitterberger et al., 2010, Jocken et al., 2007). Increased circulating free fatty acids (FFAs) inhibit the ability of insulin to stimulate muscle glucose uptake and suppress hepatic glucose production (Boden, 2009, Fabbrini et al., 2010). Mechanisms involved in FFA-induced IR include accumulation of lipids and lipid intermediates such as diacylglycerol in muscle and liver, activation of several protein kinase C isoforms, reduction in tyrosine phosphorylation of insulin receptor substrates 1 and 2 and activation of the proinflammatory nuclear factor-κB pathway (Figure 1.2).

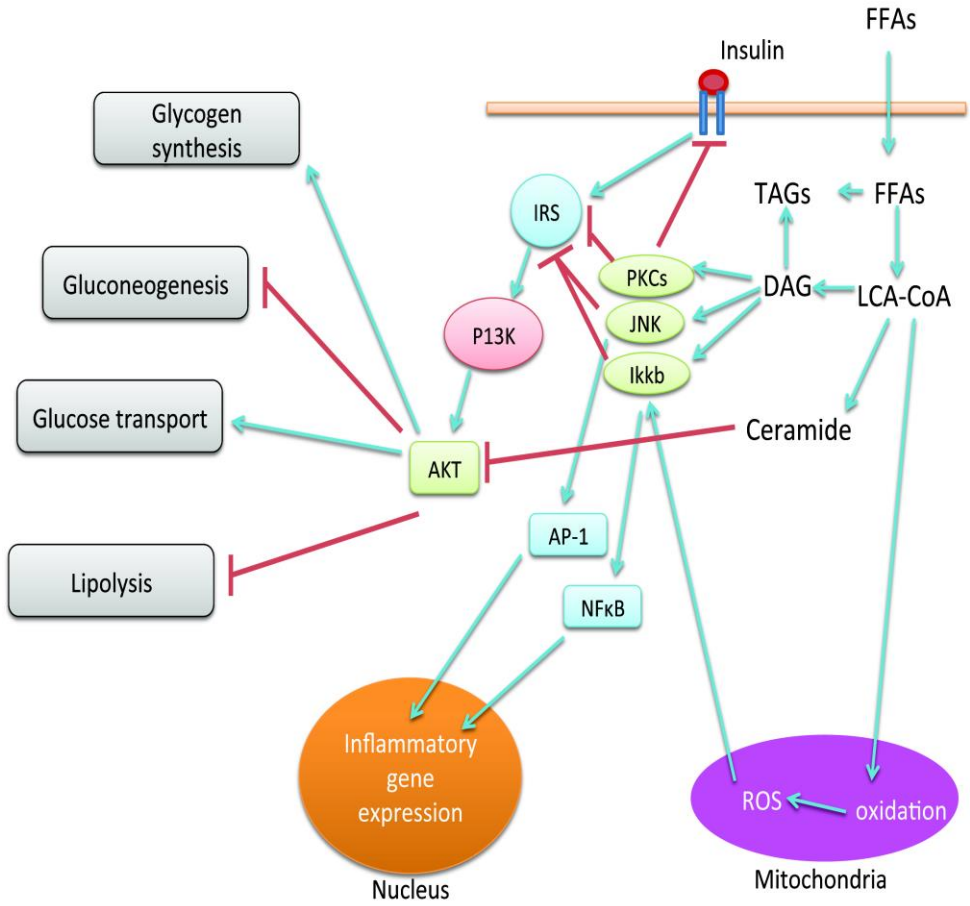


Figure 1.2. Mechanism of free fatty acids (FFAs) induced IR and enhanced inflammatory responses in adipose tissue

JNK = Jun N-terminal kinase. PKC = protein kinase C. IKKβ = inhibitor of nuclear factor kappa B kinase beta. ROS = reactive oxygen species. PI3K = phosphatidylinositol-3 kinase. DAG = diacylglycerol. TAG = triacylglycerols. LCA-CoA = long-chain acyl-CoAs. NFκB = nuclear factor kappa B. Akt is also known as protein kinase B (PKB).Modified, with permission from themedicalbiochemostrypage (King, 2013)

Decreased postprandial insulin sensitivity has been accompanied by higher postprandial TAG concentrations and increased TAG extraction across skeletal muscle during periods of impaired glucose tolerance (Frayn et al., 1996). These impairments may lead to increased fat storage in the skeletal muscle and contribute to the progression of overt type 2 diabetes mellitus in these individuals (Moors et al., 2012). The mobilisation of TAG is mediated by hormone-sensitive lipase (HSL) (Jocken et al., 2007) and adipose triglyceride lipase (ATGL) which are expressed in

highest concentrations in adipose tissue. Obesity is characterised by impaired catecholamine-induced lipolysis and reduced HSL expression in pre-adipocytes and differentiated adipocytes (Jocken et al., 2007). The major finding of a study on 44 overweight or obese subjects was that reduced ATGL and HSL mRNA and protein expression was associated with IR independent of fat mass (Jocken et al., 2007). When the obese state has already developed, IR and hyperinsulinaemia appear to be the major determinants for ATGL and HSL protein expression (Jocken et al., 2007).

Since the mid-1990s, it has been theorised that systemic lipid imbalances may give rise to glucose dysfunction (Muoio, 2012). Intramyocellular lipids were suspected to play a role in the aetiology of metabolic disease (Muoio, 2010). Studies using nuclear magnetic resonance spectroscopy suggested that IMCL content and muscle insulin sensitivity was found to be independent of BMI, fasting blood glucose and age (Krssak et al., 1999). The mechanisms by which IMCLs contribute IR in obesity are incompletely understood but lipid signalling metabolites such as long-chain acyl-Co-As, diacylglycerol and ceramides have been implicated (Muoio, 2012).

1.9 Body composition, sarcopenia, myosteatosis and insulin resistance

Sarcopenia refers to a decrease in muscle mass, and resultant low muscularity which is quantifiable using cross sectional imaging (Tsai, 2012). CT- based cut-offs for the definition of sarcopenia have previously been established using regression equations from a heterogeneous group of cancer patients (Prado et al., 2008, Mourtzakis et al., 2008). Sarcopenia is associated with poor clinical outcomes

including increased risk of infection, loss of function and increased mortality (Awad et al., 2012b, Tan et al., 2009, Prado et al., 2008). In addition, sarcopenic patients have an increased risk of chemotherapy-related toxicity (Baracos et al., 2010, Prado et al., 2007, Prado et al., 2009). Recent evidence, however, has suggested that the relationship between sarcopenia and cancer outcomes may be mostly relevant in overweight or obese patients (Tan et al., 2009). However, other studies have suggested that sarcopenia may not have a bearing on clinical outcome (Awad et al., 2012b, Dalal et al., 2012).

Myosteatosis is the process of infiltration of lipid into both the inter- and intramyocellular compartments (Miljkovic and Zmuda, 2010, Aubrey et al., 2014) and can be estimated by the attenuation of skeletal muscle Hounsfield Units (HU) on CT scanning. The conventional lower cut off for the normal attenuation of skeletal muscle is 30 HU (Goodpaster et al., 2000, Aubrey et al., 2014, Lee et al., 2005), which is estimated to be two standard deviations below the mean skeletal muscle HU of young healthy people. There are a range of factors associated with attenuated muscle density including obesity (Lee et al., 2005), increasing age (Anderson et al., 2013), male gender (Kalichman et al., 2010), type 2 diabetes mellitus (Kelley et al., 2003), inactivity (Taaffe et al., 2009) and malignancy. Myosteatosis has also been linked to the host systemic inflammatory response (Malietzis et al., 2015), particularly neutrophil-lymphocyte ratio (NLR) in patients with colorectal cancer, but this relationship is not yet understood fully. Attenuated skeletal muscle density is prognostic of reduced survival independently in patients with malignancy of the lung and gastrointestinal tract (Martin et al., 2013).

It is unclear what the underlying relationship between myosteatosis and systemic inflammation is. As with the lipotoxic hypothesis in type II diabetes, it may be that the infiltration of lipid into both the inter- and intramyocellular compartments has overall toxic effects and drives the presence of insulin resistance which, in turn, may exacerbate systemic inflammation (Miljkovic et al., 2013, Shaw et al., 2010). On the other hand, it is possible that the presence of increasing systemic inflammation, leads to the development of myosteatosis (Miljkovic et al., 2011).

1.10 Preoperative carbohydrate loading

Overnight fasting leads to thirst, hunger, headaches, and anxiety and preoperative treatment with oral complex carbohydrates can obtund these effects (Thorell et al., 1999, Awad and Lobo, 2012). Most people have breakfast shortly after waking up, leading to the release of insulin, which not only effects the storage of nutrients but also a change in the oxidation from fat to carbohydrates, while also activating glucose transport into muscle and fat and glycogen and fat storage as well as protein anabolism. The most obvious way to change metabolism from the overnight fasted state to the fed state is to give a carbohydrate load. This was initially done using 20% glucose intravenously at a dose of 5 mg/kg/min (Ljungqvist et al., 1994), and later using a mixture of complex carbohydrates as an oral drink (Nygren et al., 1995). Intake of such drinks completely reverses the setting of overnight fasting while stimulating glucose uptake, shutting down gluconeogenesis, and thus transferring the patient to a much more anabolic state before the onset of the surgical stress (Svanfeldt et al., 2005). This increase in insulin sensitivity continues in the postoperative period.

It is important to note that the carbohydrate used must be complex such as maltodextrins rather than glucose or sucrose and the minimum dose required is 50 g (in 400 ml). The recommended dose is 100 g (800 ml) on the night before surgery and 50 g (400 ml) 2 hours prior to the induction of anaesthesia.

A number of studies have demonstrated the safety (Nygren et al., 1995, Awad et al., 2010a, Lobo et al., 2009), metabolic effects (Svanfeldt et al., 2005, Awad et al., 2010a{Awad, 2011 #576), (Awad et al., 2011a) and effects on well-being (Awad et al., 2009, Thorell et al., 1999) of preoperative carbohydrate treatment. Preoperative ingestion of clear carbohydrate-based drinks is safe (the drinks empty from the stomach in 1.5-2 h (Nygren et al., 1995, Lobo et al., 2009), increases liver glycogen reserves (Awad et al., 2009, Awad et al., 2010b, reduces intraoperative plasma non-esterified fatty acid concentrations {Awad, 2010 #247), and attenuates the development of postoperative insulin resistance by up to 50% (Awad et al., 2009, Thorell et al., 1999). Recent studies have demonstrated the latter may occur secondary to effects on gene and protein expression, insulin signaling and mitochondrial β-oxidation (Awad et al., 2010a, Wang et al., 2010). Specifically, preoperative treatment with carbohydrate-based drinks in patients undergoing laparoscopic cholecystectomy was associated with a 4-fold ($p<0.001$) and 44% ($p<0.05$) lower intraoperative muscle pyruvate dehydrogenase kinase 4 (PDK4) mRNA and PDK4 protein expression, respectively (Awad et al., 2010a). PDK4 acts to inhibit the mitochondrial enzyme pyruvate dehydrogenase complex resulting in decreased carbohydrate oxidation (COX) (Awad et al., 2010b). As defective glucose

disposal (i.e. reduced COX) is a key feature of postoperative insulin resistance the aforementioned study (Awad et al., 2010a) suggested a possible cellular mechanism by which preoperative carbohydrate treatment attenuated postoperative insulin resistance. Another randomized study of 52 patients undergoing elective colorectal surgery demonstrated preoperative carbohydrate treatment resulted in enhanced tyrosine kinase activity, phosphatidyl-inositol 3-kinase and protein kinase B expression, the cellular pathway responsible for most of the metabolic actions of insulin, the aforementioned changes being associated with decreased postoperative insulin resistance (Wang et al., 2010). Finally, preventing excessive or incomplete mitochondrial β-oxidation, which is characterized by perturbed carnitine homeostasis resulting in elevated plasma carnitine concentrations, is suggested to be another mechanism by which preoperative carbohydrate treatment attenuates postoperative insulin resistance (Awad et al., 2012a). The risk of aspiration is related to the patient's comorbidity rather than the time of fasting, with the exception of patients with slow gastric emptying.

Several studies have examined the effects of preoperative carbohydrate treatment in different elective surgical situations and the results have been conflicting, with some showing benefit and others no benefit. A recent meta-analysis of 21 randomised controlled studies on patients undergoing elective surgery has helped clarify some of these issues (Awad et al., 2013). This meta-analysis demonstrated a significant reduction in length of stay amongst patients undergoing major open abdominal surgery. Preoperative carbohydrate treatment was safe (no occurrence of drink-related complications), associated with reduced development of

postoperative insulin resistance but the latter was not associated with any effect on surgical complications.

The main limitation of this meta-analysis was the relatively weak design of many of the included studies whose quality, as assessed by GRADEpro®, was rated as low to moderate. There were small numbers and significant heterogeneity in the design and magnitude of surgery in many studies of preoperative carbohydrate treatment that precluded their inclusion in this meta-analysis. The definitions of outcomes such as complications and reporting of events varied between studies.

Chapter 2 Hypotheses

The forthcoming thesis hypothesises that

differences in the distribution (central abdominal *versus* peripheral) and physiology of adipose tissue and skeletal muscle contribute to differences in the magnitude of inflammatory and insulin resistant responses.

Chapter 3 Common methods

3.1 Approvals from regulatory bodies

Ethical approval for the studies in the Muscle Fat IR and UGI Sarcopenia Studies was obtained from the Nottingham Research Ethics Committee East Midlands – Northampton. The respective approval numbers for these studies were as follows: Muscle fat IR (11/EM/0232), UGI Sarcopenia (11/EM/0419). The respective Research and Development approval numbers for the studies were 11EN005 and 11GA034. The studies in Chapters 4 and 6 were registered at www.controlled-trials.com (ISRCTN16597586 and ISRCTN15674981). All studies were performed in accordance with the Declaration of Helsinki of the World Medical Association.

3.2 Hyperinsulinaemic-euglycaemic clamp

The methodology for the hyperinsulinaemic-euglycaemic clamp was identical for patients in the muscle fat IR study and UGI Sarcopenia study. All studies were performed in the David Greenfield Human Physiology Unit at The University of Nottingham. Participants attended after an overnight fast. Two intravenous infusion lines were placed, one into an antecubital vein for the infusion of insulin (Actrapid, Novo Nordisk, Denmark) and 10% glucose, and the other retrograde into a dorsal hand vein which was kept warm in a hot box for the intermittent sampling of arterialised blood. A baseline blood sample for measurement of fasting blood glucose and inflammatory and hormonal markers was taken. After a 10 minute priming infusion, insulin infusion (50 units/50 ml of normal saline) was held constant at 120mU/m^2/min for 110 minutes. Blood glucose concentration was determined every five minutes using the YSI 2300 stat plus (YSI Incorporated, Yellow Springs, Ohio). Blood glucose concentration was clamped at 4.5 mmol/l by infusion

of variable amounts of glucose solution. The total body glucose disposal rate (M-value) was calculated during the final 15 minutes of the study (steady-state).

3.3 Anthropometrics

Height and weight

Height (cm) and weight (kg) (Avery 3306ABV scales (Avery Berkel, Royston, UK)) were measured with the subjects wearing light clothing and no shoes. Body mass index (BMI) (kg/m^2) was calculated.

Mid upper arm circumference

Mid upper arm circumference (MUAC) was measured to the nearest 1 mm with the subject's non-dominant arm flexed at 90° and the forearm parallel to the floor. The measurement was taken three times at the midway level between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process and the mean of three measurements recorded.

Triceps skinfold thickness

Triceps skinfold thickness (TSF) was measured using SlimGuide calliper (Creative Health Products, Michigan, USA) with the subject standing upright, at the midway level between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process. The mean of three measurements was noted to the nearest 1 mm.

Mid arm muscle circumference

The midarm muscle circumference (MAMC) was calculated using the following standard formula

MAMC =MUAC (cm)− ∏ × TSF thickness (cm) (Heymsfield et al., 1982).

Hand grip dynamometry

The forearm was placed with the elbow in 90° flexion and the wrist in the neutral position. Three measurements were made using a digital hand dynamometer (Jamar Plus+; Sammons Preston, Bolingbrook, USA) in the non-dominant hand with the shoulder adducted and neutrally, with elbow supported (Hillman et al., 2005). The mean of three measurements was recorded.

Waist circumference

Waist circumference was measured to the nearest mm midway between the costal margin and iliac crest, using a non-elastic tape measure, during mid-expiration.

Hip circumference

Hip circumference was measured to the nearest mm at the level of the iliac crests, using a non-elastic tape measure.

3.4 Dual energy x-ray absorptiometry (DXA)

In the muscle fat IR study, each patient underwent a DXA scan at the preoperative visit. In the sarcopenia/UGI study, DXA scans were performed at the pre-

chemotherapy visit and at the post-chemotherapy, pre-surgery visit. All scans were performed in the David Greenfield Human Physiology Unit using one DXA scanner (GE LUNAR II) by research personnel trained and certified in DXA. DXA analysis allowed whole body estimates of lean mass and fat mass. Whole body FFM was selected as the majority of nutritional literature uses FFM as the denominator (Heymsfield et al., 1982).

3.5 Body composition analysis of CT scans

Electronic copies of CT scans taken routinely for clinical reasons were obtained from the hospital Picture Archiving and Communication System (PACS). Once accessed, the scans were anonymised, and one CT image slice at the third lumbar vertebrae (L3) level was selected. The images were analysed using SliceOmatic V4.2 software (Tomovision, Montreal, Canada) to calculate the surface area of the specific tissue types: skeletal muscle tissue, visceral adipose tissue and subcutaneous/intramuscular adipose tissue. Within the L3 region are the following muscles: psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal obliques and rectus abdominus.

SliceOmatic software relies on the variation in density of the different tissue types to identify and thereby quantify the surface area of the tissue present. The different tissue densities are represented by specific HU thresholds. The HU thresholds used for skeletal muscle were -29 to +150 (Mitsiopoulos et al., 1998), for visceral adipose tissue were -150 to -50 (Vehmas et al., 1996) and for subcutaneous and intramuscular adipose were -190 to -30 (Kvist et al., 1986). Once the tissues were

identified, the cross-sectional surface area (cm^2) of each tissue was calculated automatically by the software (Prado et al., 2007). Any change in tissue area was expressed as absolute change (cm^2).

These data were used to estimate whole body stores of fat-free mass (FFM) and fat mass (FM) using the regression equations described previously of (Mourtzakis et al., 2008):

Total body fat-free mass (FFM) (kg) = 0.3 × [skeletal muscle at L3 (cm^2)] + 6.06 (r = 0.94)

Total body fat mass (FM) (kg) = 0.042 × [total adipose tissue at L3 (cm^2)] + 11.2 (r = 0.88)

The cross sectional area of skeletal muscle was normalised for patient height by calculating the skeletal muscle index (SMI) (cm^2/m^2). Patients were classified as sarcopenic according to cut offs established (Prado et al., 2008); L3 skeletal muscle index (SMI) ≤ 38.5 cm^2/m^2 for women and ≤ 52.4 cm^2/m^2 for men.

3.6 Bioelectrical impedance analysis

Bioelectrical impedance (BIA) was measured with a Bodystat®QuadScan 400 (Bodystat Ltd., Isle of Man) at 5, 50, 100 and 200 kHz. The physical principle behind

the BIA technique is that the body's lean compartment, comprising approximately 73% electrolytic water, conducts electricity far better than the body's fat compartment which is very low in body water content (between 5-10%) (Kyle et al., 2004a, Kyle et al., 2004b). These two compartments have, therefore, very different impedance (or resistance) values to a high frequency electrical current.

Whole body measurements were taken with the subject in a supine position on a nonconducting surface, with the arms slightly abducted from the trunk and the legs slightly separated. Surface electrodes were placed on the right side of the body on the dorsal surface of the hands and feet proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively, and also medially between the distal prominences of the radius and ulna and between the medial and lateral malleoli at the ankle. A tiny torch-battery operated electrical signal is sent through the supine body via electrodes attached to the right foot and hand. Standardised equations for males and females were used to calculate FFM from resistance measurements obtained by BIA (Lohman, 1988).

3.7 SF-36 and M-SGA questionnaires

SF-36 questionnaire was obtained on license from QualityMetric Incorporated, Lincoln, USA. Questionnaires were completed on paper by study participants individually who were able to ask research personnel to clarify meaning of the questions only. Results were analysed using QualityMetric Health Outcomes Scoring v4.5 software (QualityMetric Incorporated, Lincoln, USA). M-SGA questionnaires

(Kalantar-Zadeh et al., 1999) were administered at the same time as SF-36 and completed on paper by subjects individually.

3.8 Skeletal muscle and adipose tissue biopsies

The biopsy was performed under aseptic precautions using a Weil-Blakesley forceps (medizintechnik, Stuttgart, Germany), also known as conchotome. The skin of the thigh was shaved and cleaned with aqueous betadine. The biopsy site was infiltrated with 5 ml of 1% lignocaine, taking care to inject skin and subcutaneous tissue, but avoiding the muscle itself. A 5 mm skin incision was made, and the fascia penetrated with a single-use scalpel blade. The closed jaws of the conchotome were inserted through the incision with the long axis of the jaws parallel to the muscle fibres. The jaws were opened and the conchotome advanced 2-3 mm. With a single movement, the jaws were closed, twisted through 180˚ and withdrawn. A sterile needle was used to deliver the sample onto a gauze swab, it was quickly dried, placed in a pre-labelled cryovial and immediately frozen in a dewar of liquid nitrogen. A more superficial biopsy of fat was then taken, transferred to the appropriate cryovial and snap frozen. Pressure was applied to the biopsy site for 5-10 minutes to stop bleeding and prevent haematoma formation. The incision was closed using an undyed absorbable suture. The patient rested on the bed after the procedure – as it was either performed before a 2 hour HEC or in anaesthetised patients in theatre. The frozen samples were stored prior to further analysis in liquid nitrogen tanks.

3.9 RNA extraction

Biopsy samples were removed from liquid nitrogen and homogenised with 1 ml of TriReagent (SigmaAldrich, Dorset, UK) per sample. Large biopsy samples were cut down to around 50mg on ice prior to commencing RNA isolation. Samples were allowed to stand for 5 minutes at room temperature to allow complete dissociation of nucleoprotein complexes. 0.1mL of 1-bromo-3-chloropropane (1-BCP) was added per 1 mL of TriReagent. Samples were shaken vigorously for 15 seconds and stored overnight at -20˚C.

The resulting mixtures were centrifuged at 13000 *g* in a microcentrifuge for 15 minutes at 4˚C. The colourless upper aqueous phase (containing RNA) was removed into a fresh tube. To this 0.5 mL of ice-cold isopropanol (Sigma, I9516) was added. The samples were mixed and incubated at room temperature for 10 minutes, and then centrifuged at 10000 *g* for 10 minutes at 4˚C to pellet the precipitated RNA. Taking care not to disturb the RNA pellet, the supernatant was removed and the pellet was subsequently washed by the addition of 1 ml of 75% of ethanol and vortexed. The samples were centrifuged at 10000 *g* for 5 minutes at 4˚C. The supernatant was removed and the wash step was repeated. The RNA pellet was allowed to air-dry for 10 minutes and then re-suspended in 50 µL of RNase free water. To facilitate dissolution the sample was repeatedly pipetted.

Samples were centrifuged at 13000 *g* in a microcentrifuge for 10 minutes at 4˚C, then the pellet was washed twice with 70% ethanol and allowed to air dry. 87.5µL of RNase free water was added and samples incubated for 5 minutes at 65˚C. 2.5 µL of DNAse and 10 µL of RDD buffer were added and samples incubated at room

temperature for 10 minutes. 300 µl of RNAse free water and 400 µl of phenyl choloroform isoamyl alcohol were added per reaction. Samples were vortexed and centrifuged at 10000 *g* for 10minutes. The upper phase was removed and 40µl 3M Na acetate and 800 µl absolute ethanol were added before incubating samples at -20˚C overnight.

Samples were centrifuged at 13000 *g* for 10 minutes then washed in 70% ethanol. 50µl RNAse free water was added and samples incubated at 65˚C for 5 minutes before measuring RNA concentration using LVis plate (BMG Labtech, Ortenberg, Germany).

3.10 cDNA preparation

Reverse Transcription

Affinity Script Multiple Temperature Reverse Transcriptase (Agilent Technologies, Santa Clara, CA) was used for reverse transcription from RNA to cDNA, following the standard protocol and manufacturer's instructions, using random primers (Invitrogen, Paisley, UK). RNAse out Recombinant Ribonuclease Inhibitor (Life Technologies, Paisley, UK) was also added to avoid RNA degradation. 500 ng total RNA was used as a template for synthesis of first strand cDNA.

Real-time quantitative polymerase chain reaction (RT-qPCR)

The template cDNA generated from the reverse transcription reaction was used as the basis for RT-qPCR using TaqMan® Universal PCR Master Mix (Life Technologies).

RT-qPCR measures mRNA amplification of a target sequence as it occurs, so that it is possible to estimate the starting concentration of nucleic acid. During amplification using a TaqMan® probe (ThermoFisher Scientific, Paisley, UK), accumulation of PCR products is detected directly by monitoring the increase in fluorescence of a reporter dye. During exponential amplification, when the fluorescence level breaches the background threshold, the cycle number is recorded for each sample; this is the CT value that is used in quantitation.

3.11 TaqMan low density gene array analysis

A custom TaqMan® Array Micro Fluidic Card was designed for each tissue (Life Technologies) (Appendix 1). This was a 384-well card preloaded with TaqMan® gene expression tests from Life Technologies inventoried assays, in format 96a with 95 target assays and a mandatory reference gene. The reference gene which was automatically included on the card was Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH), transcript variant 1 assay reference Hs99999905_m1 (Life Technologies).

A 7900HT Fast Real-Time PCR System (Life Technologies) was used following the standard protocol (Applied Biosystems Part Number 4400263). 110 ng cDNA (from total RNA) was used per lane, in duplicate for each sample. TaqMan® Universal PCR Master Mix (Life Technologies) was used as recommended

Target genes were selected from background research and this list extended using IPA interactive pathways analysis software (Ingenuity Systems). Please see Appendix 1 for details of the target genes.

Quantification

The comparative CT method was used to compare the gene expression in two different samples (sample A and sample B); with each sample related to a geometric mean of housekeeping genes prior to comparison. The three housekeepers were checked for normal distribution and correlation to each other. Target gene delta-CTs were also checked for normal distribution. Fold change was calculated as described previously, using $2^{-\text{delta CT}}$ (Livak and Schmittgen, 2001, Schmittgen and Livak, 2008).

Interpretation of comparative results

As expression levels were already normalised against control genes prior to analyses their results could be negative or positive, dependent on the expression differential to the control. CT values that are larger indicate lower expression and later amplification, and lower CT values indicate higher expression and earlier amplification. ΔCT expression levels that are negative indicate expression of the transcript above that of the control and positive values indicate expression levels below that of the control. Therefore ΔCT values that are negative indicate expression levels higher than positive values. When correlations or association analyses are carried out, the direction of association has to take into account the inverse relationship between expression level and CT value.

3.12 Collection of blood samples

Peripheral venous blood was sampled either by venepuncture or via a 19G venous cannula (Venflon®, Ohmeda, Sweden) into the appropriate Vacutainer®blood collection tube (Becton Dickinson & Co, Oxford, UK). In the studies detailed in Chapters 6 and 7, arterialised venous blood was sampled by the heated-hand technique as described previously (Brookset al., 1989). In brief, a retrograde venous cannula was sited into the dorsum of the hand which was placed into a hand warmer (55°C) for at least 15 minutes prior to each blood sampling time point. As was standard practice in our laboratory, we did not measure the temperature of the heated hand placed inside the 'hot-box'.

Omental venous blood was sampled in patients in whom sizeable omental veins could be identified. After opening the abdomen, a lateral omental vein was cannulated with a 22-gauge cannula (Venflon®, Ohmeda, Sweden) in a retrograde fashion (Samra et al., 2005). After taking an omental venous blood sample, the cannula was removed and the vessel ligated. At the end of the operation, prior to closing the abdomen, another lateral omental vein was cannulated in the same way and a further omental venous blood sample collected. The cannula was removed and the vessel ligated.

3.13 Analysis of blood samples

Serum or plasma was collected in appropriate vacutainers and centrifuged at 4000 *g* for 10 minutes. The serum or plasma was removed and stored in aliquots at -80˚C until analysis.

All assays were performed by trained laboratory staff using standard operating procedures and calibrated equipment.

Serum TNF-α was measured using a Sandwich enzyme-linked immunosorbent assay (ELISA) method (BD BioSciences, San Diego, California, USA). The inter-assay coefficient of variation was less than 15%.

IL-6 was measured using a Sandwich ELISA method (BD BioSciences).

For CRP, TAG, FFA and glucose, the methods were carried out on a Pentra 400 Clinical Chemistry analyser (Horiba Medical, Montpelier, France).

C-reactive Protein (CRP) was measured using an immunoturbidimetric assay, triacylglycerol (TAG) using a spectrophotometric assay and glucose using a spectrophotometric assay (all Horiba Medical).

Free fatty acids (FFA) were measured using a spectrophotometric assay (Wako, Neuss, Germany).

Cortisol was measured in serum using competitive solid phase ELISA (Alpha Diagnostics International, San Antonio, Texas, USA). The patient sample competes with enzyme-linked Cortisol for a fixed and limited number of antibody-binding sites on the coated plates.

Resistin was measured using a sandwich ELISA method (EMD Millipore, Billerica, Massachusetts, USA).

Visfatin was measured in serum using a competitive ELISA (Sigma Aldrich, St Louis, Missouri, USA). After a blocking step and incubation of the plate with anti-Visfatin antibody, both biotinylated Visfatin peptide and standard or targeted peptide in samples interacts competitively with the Visfatin antibody. Unbound biotinylated Visfatin peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP) which catalyses a colour development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide SA-HRP complex and inversely proportional to the amount of Visfatin in the standard or samples. This is due to the competitive binding to Visfatin antibody between biotinylated Visfatin peptide and peptides in standard or samples. A standard curve of known concentration of Visfatin peptide can be established and the concentration of Visfatin peptide in the samples can be calculated accordingly.

Serum insulin was measured using a solid-phase ^{125}I radioimmunoassay (Coat-a-count insulin kit, Siemens Medical Solutions Diagnostics, Camberley, UK). The inter-assay coefficient of variation for this assay was 6.3%.

Nonesterified/free fatty acid assays (serum NEFA/FFA) were measured using an automated immunoassay analyser (Olympus AU5400, Olympus Corporation, Tokyo, Japan) and a commercially available kit (Randox®kit NEFA RB1007, County Antrim, UK). The inter-assay coefficient of variation for this assay was 3.5%.

3.14 Composition of study drinks

The constituents and physical properties of a 100ml serving of study drinks (Nutricia preOp®, Nutricia Clinical Care, Trowbridge, UK) and placebo (Tovali Sugar Free Whole Lemon Drink diluted 1:4 with water, Tovali Ltd, Carmarthen, UK) are given in Table 3.1 .

Table 3.1. Composition of study drinks

	Unit	Nutricia preOp®	Placebo
Energy	kcal kJ	50 215	1.6 7
Protein	g	0	0.02
Carbohydrate	g	12.6	0.04
Sugars	g	2.1	0.04
Fat	g	0	Trace
Sodium	mg (mmol)	50 (22)	20 (8.8)
Potassium	mg (mmol)	122 (3.1)	Unavailable
Chloride	mg (mmol)	6 (0.2)	Unavailable
Calcium	mg (mmol)	6 (0.1)	Unavailable
Phosphorus	mg (mmol)	1 (0.0)	Unavailable
Magnesium	mg (mmol)	1 (0.0)	Unavailable
Water	g	92	80

In the randomised double-blind study described in this thesis, the study drinks (preOp®) and placebo were prepared by a person not involved in the studies and served to the study subjects in identical opaque bottles.

3.14 Statistical analyses

Statistical analysis was performed with SPSS®for Windows™ v22 software (IBM, New York, USA) and GraphPad Prism v6.04 (GraphPad Software Inc., La Jolla, CA, USA). The Shapiro-Wilkes test was used to determine normality of distribution. Data are presented as mean (SE or 95% CI) or median (IQR) as appropriate. The paired and independent samples t-tests were used for parametric paired and unpaired data, respectively. The Wilcoxon signed rank and Mann-Whitney U tests were used for non-parametrically distributed paired and unpaired data, respectively. The Chi square test was used for categorical data.

Comparisons between the study time points for parametric and non-parametric data were made using the repeated measures (one-way, two-way or three-way as appropriate) ANOVA or the Kruskal-Wallis tests, respectively. The appropriate post-hoc test (paired t-test or Wilcoxon signed rank test) was used to locate any differences. Two-tailed p values, unadjusted for multiple testing, are reported and differences were considered significant at $p<0.050$.

3.15 Funding for studies

The studies in this thesis were supported by the following grants:

CORE/ Nutritional Research Foundation

Nottingham University Hospitals Charity Pump Priming Grant

ESPEN Research Fellowship

The funders of these studies had no role in the design and execution of the studies, data analysis, writing or decision to submit the manuscripts for publication.

Chapter 4 Investigating the relationship between the distribution and physiology of adipose tissue and skeletal muscle and postoperative inflammation and insulin resistance

4.1 Background

Up to 3 million surgical procedures are performed annually in the UK (Health & Social Care Information, 2013-2014). Current practice is to withhold food from patients for 6 hours preoperatively and clear liquids for 2 hours but in reality delays and changes in operating schedules mean patients are fasted for longer, even up to 18 hours. Preoperative fasting and surgery, as previously discussed, induce 'metabolic-stress', inflammation and insulin resistance (IR) (Awad et al., 2009, Thorell et al., 1999, Witasp et al., 2009, Witasp et al., 2010). Measures to attenuate IR may, therefore, be of clinical benefit. Such measures include administering carbohydrate-based drinks preoperatively (Awad et al., 2009, Awad et al., 2010a, Ljungqvist et al., 2002).

In a healthy volunteer study, it was demonstrated, using magnetic resonance spectroscopy, that a 24-hour fast was associated with a 57% decrease in liver glycogen reserves and a 23% increase in intramyocellular lipid (IMCL) in calf muscle (Awad et al., 2010b). The latter was a novel finding that supported the hypothesis that IMCL accumulation (secondary to mitochondrial dysfunction) interfered with insulin signalling and led to IR. This study also demonstrated, for the first time, that fasting for 24 hours significantly decreased mitochondrial membrane complex I (-75%) and II (-43%) activities (Awad et al., 2010b). Refeeding with a 'preoperative' carbohydrate-based drink that contained glutamine and antioxidants (ONS, Fresenius Kabi, Germany) partially reversed these changes (Awad et al., 2010b).

A subsequent randomised placebo-controlled study of preoperative conditioning with ONS in patients undergoing laparoscopic cholecystectomy, demonstrated that ONS led to increased intraoperative liver glycogen (44%) and plasma glutamine and antioxidant concentrations, the latter two remaining elevated into the first postoperative day (Awad et al., 2010a). Another novel finding was that muscle pyruvate dehydrogenase kinase 4 (PDK4) mRNA, PDK4 protein expression and metallothionein-1A (marker of cellular oxidative stress) mRNA expression were, respectively, 4-fold, 44% and 1.5-fold lower in the ONS group than the placebo group (Awad et al., 2010a). Mitochondrial pyruvate dehydrogenase complex (PDC) controls the rate of carbohydrate oxidation (COX) and is inhibited by PDK4. Reduced expression of PDK4 could, therefore, enhance PDC activity and COX.

In patients with colorectal cancer, patients were assigned to preoperative oral carbohydrate, fasting or placebo (Wang et al., 2010). The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signalling pathway was investigated in rectus abdominus muscle as a possible mechanism related to postoperative IR (estimated using HOMA). By the end of surgery, muscle protein tyrosine kinase (PTK) activity, PI3K and PKB mRNA and protein concentrations were increased in the carbohydrate group compared with fasting or placebo (Wang et al., 2010). In patients undergoing major abdominal surgery, biopsies from rectus abdominus at the start and end of surgery were analysed for gene expression. There was increased expression of several genes associated with inflammation, such as IL-6 and TNF-α (Witasp et al., 2009). The results from previous studies suggested

possible cellular mechanisms underlying the development of postoperative IR and the potential effects of preconditioning carbohydrate-based drinks.

4.2 Study hypothesis

This study hypothesised that

a) the magnitude of the postoperative inflammatory and insulin resistance response is related to the distribution of adipose tissue and skeletal muscle
b) postoperative inflammation and insulin resistance is more pronounced in obese compared with non-obese individuals
c) preoperative carbohydrate loading ameliorates the postoperative inflammatory and insulin resistance response

4.3 Methods

This randomised, double-blinded, placebo-controlled pilot study was set in a university teaching hospital. Adult patients between 18-80 yeas undergoing elective open abdominal surgery were invited to participate. Exclusion criteria are shown in Table 4.1.

Table 4.1. Exclusion criteria

History of significant preoperative weight loss (>10% over preceding 3 months)
Clinical history of pulmonary aspiration
Known gastro oesophageal reflux disease or hiatus hernia
History of difficult intubation or conditions leading to latter such as: previous neck radiotherapy/ rheumatoid arthritis/ presence of thyroid goitre
Metabolic disorders (e.g. diabetes mellitus, thyroid disease, Cushing's syndrome, phaeochromocytoma, carcinoid tumour/ syndrome)

Simultaneous participation in another clinical study or involvement in a clinical study within the preceding 3 months
Patients with suspicion of alcohol or drug abuse
Pregnancy

Subjects were classified as obese (n=16) or non-obese (n=16) according to body mass index (BMI) or waist circumference. Those with BMI >30 kg/m^2 or waist circumference ≥94 cm in men or 80 cm in women were classed as obese. Participants underwent a preoperative screening visit (P1) in the week before surgery (1-7days preoperatively) which included height and weight measurement, measurement of hip and waist circumference, dual X-ray absorptiometry (DXA) scan and hyperinsulinaemic clamp (HEC). Subjects were randomised to receive a preoperative oral carbohydrate drink containing 50 g carbohydrate in 400 ml (Nutricia preOp®, Nutricia Clinical Care, Trowbridge, UK) or placebo and consumed 800 ml on the evening before surgery and 400 ml on the morning of surgery. A second HEC was performed on the first postoperative day (P2). This was a pragmatic decision – as the patients had already undergone major surgery and spent several hours on the operating table, it was felt to be too much of a burden to also put them through a 2 hour HEC. As most could not be fed on the day of surgery, following the clamp, a glucose infusion would have to be continued. The postoperative IR seen in the majority of studies peaks on the first postoperative day (Thorell et al., 1994) therefore the results from this study would be comparable to the majority of literature in this field. The study outline is given in Table 4.2.

Table 4.2. Study outline

	1-7days before surgery	Evening before surgery	2 h before surgery	Start of surgery	End of surgery	1-day Postop
Peripheral venous blood sampling	X			X	X	X
Thigh vastus lateralis muscle biopsy	X			x	X	X
Thigh subcutaneous fat biopsy	X			x	X	X
Hyperinsulinaemic-euglycaemic clamp	X					X
Ingestion of study drinks		X	X			
Rectus muscle biopsy				X	X	
Abdominal subcutaneous fat biopsy				X	X	X
Omental blood sampling				X	X	
Omental fat biopsy				X	X	

Body composition analysis

CT scans were analysed for sarcopenia using Sliceomatic Software, as described in Chapter 3. In addition, CT scans were analysed for myosteatosis. The mean HU measurement of all skeletal muscles within the L3 cross-section was recorded as a measure of myosteatosis, which was defined operationally as a mean skeletal muscle radiodensity of <33 HU in those with a BMI ≥ 25 kg/m^2 and < 41 HU in those with a BMI < 25 kg/m^2 across the axial orthogonal view (Martin et al., 2013).

Statistical analyses

Statistical analysis was performed as described in Chapter 3. Multiple linear regression analysis was performed to determine whether age, sex, BMI, surgery or chemotherapy influenced the association between each surrogate index and clamp-derived M value. No previous studies have investigated whether differences in skeletal muscle and adipose tissue distribution affect the magnitude of postoperative IR. However, part of the 2x2 factorial design of this study involved preoperative carbohydrate loading versus no carbohydrate loading. Based on the results of a previous study (Nygren et al., 1998) and with an estimated standard deviation of 10% in measurements of insulin sensitivity, 8 participants were required to detect pair-wise differences of 27% between the two groups (carbohydrate loading versus no carbohydrate loading) with a power of 80% and a significance level of 0.05. This study therefore had N=8 participants in each of the four study arms.

Gene expression data was analysed using one way ANOVA and Welch's post-test correction. Data were entered into Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA). It is recognised that there is the potential for bias in this method of functional enrichment analysis (Timmons et al., 2015). Therefore, gene enrichment analysis was also undertaken against the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (DAVID: Database for Annotation, Visualization, and Integrated Discovery 6.7, NIH)(Huang et al., 2009).

4.4 Results

All eligible patients undergoing open abdominal surgery during the study period under the care of the 3 surgeons involved in the study were approached by their clinical team. The CONSORT diagram is shown in Figure 4.1.

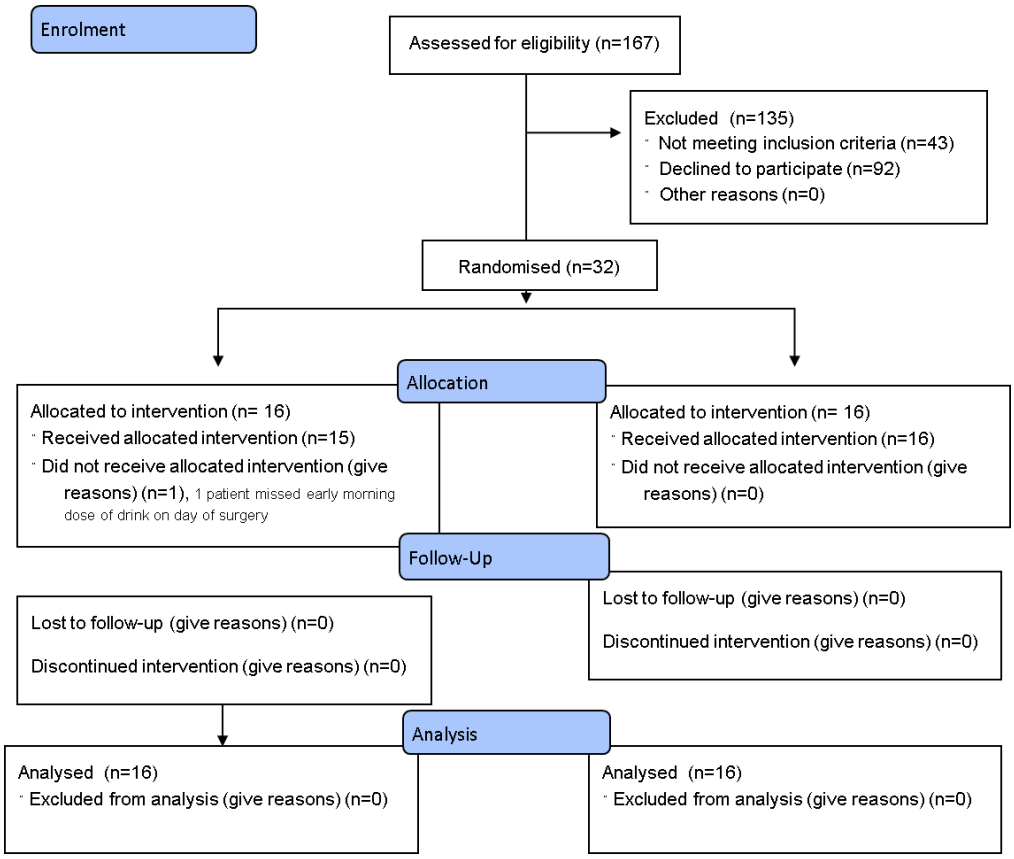


Figure 4.1. Consort diagram

A total of 167 patients were assessed for eligibility, 135 did not meet inclusion criteria or declined to participate and 32 were randomised, 16 obese and 16 non-obese, 16 to receive preoperative carbohydrate and 16 to receive placebo.

A total of 32 patients were recruited to the study and randomised via a computer-aided randomisation programme (Nottingham Clinical Trials Unit) to receive preoperative carbohydrate loading or placebo. The demographic details of study patients are given in Table 4.3.

	Total	Obese (n=16)	Non-obese (n=16)	p value
Age (median, IQR)yrs Carbohydrate No carbohydrate p value	61 (50-67) 61 (48-67) 61 (50-67) 0.6883	61 (58-66) 62 (59-70) 60 (54-63) 0.2539	63 (46-68) 56 (41-65) 66 (49-73) 0.2028	0.9159 0.1186 0.4504
Gender				
Male Carbohydrate No carbohydrate	22 14 8	12 8 4	10 6 4	
Female Carbohydrate No carbohydrate	10 2 8	4 0 4	6 2 4	
BMI (mean ± SD)kg/m^2 Carbohydrate No carbohydrate p value	28.3 ± 5.449 28.36 ± 5.719 28.27 ±5.352 0.9647	30.98 ± 6.10 31.00 ± 6.87 30.95 ± 5.69 0.9876	25.65 ± 3.01 25.71 ± 2.62 25.56 ± 3.54 0.9371	0.0049 0.0615 0.0401
Waist circumference (median, IQR)cm Carbohydrate No carbohydrate p value	93 (86.5-106.5) 98.5 (92.2-107.0) 90 (85.0-99.8) 0.1776	106.5 (98.55-113) 107 (105.3-111.3) 98.6 (92.5-116.3) 0.1585	87.5 (79-92.35) 92.4 (81-93) 85.5 (79-87.8) 0.2269	<0.0001 0.0010 0.0026
Operating time (median, IQR)mins Carbohydrate No carbohydrate p value	180 (128-240) 160 (137-234) 155 (113-236) 0.6007	160 (128-246) 155 (113-236) 182 (128-370) 0.5823	182 (133-222.5) 172 (145-232) 184 (114-231) 0.9015	0.8685 0.9803 0.6512

Blood loss (mean ±SD)mls Carbohydrate No carbohydrate p value	542 ±465 371 (108-695) 546 (349-857) 0.4103	631 ± 533 400 (100-776) 540 (349-1065) 0.9205	469 ± 405 267 (119-515) 546 (174-771) 0.2315	0.3606 0.3201 0.8200
Epidural insertion Carbohydrate No carbohydrate	24 13 11	12 7 5	12 6 6	
Surgery type				
Pancreatic bypass procedure Carbohydrate No carbohydrate	9 5 4	3 2 1	6 3 3	
Whipples procedure Carbohydrate No carbohydrate	6 3 3	2 1 1	4 2 2	
Colorectal resection Carbohydrate No carbohydrate	6 3 3	5 3 2	1 0 1	
Incisional hernia Repair Carbohydrate No carbohydrate	3 3 0	2 2 0	1 1 0	
Formation of/ surgery to ileoanal J Pouch Carbohydrate No carbohydrate	2 1 1	0 0 0	2 1 1	

Reversal of colostomy Carbohydrate No carbohydrate	2 0 2	1 0 1	1 0 1	
Abdominal rectopexy Carbohydrate No carbohydrate	1 0 1	1 0 1	0 0 0	
Completion proctectomy Carbohydrate No carbohydrate	1 1 0	0 0 0	1 1 0	
Distal pancreatectomy/ splenectomy Carbohydrate No carbohydrate	1 0 1	1 0 1	0 0 0	
Open bile duct exploration Carbohydrate No carbohydrate	1 0 1	1 0 1	0 0 0	

Table 4.3. Demographic details of study patients

Results of DXA, CT and bioelectrical impedance analysis

31/32 (96.9%) patients underwent DXA scanning and bioelectrical impedance analysis (BIA) at the preoperative visit. One patient was too heavy for the DXA scanner and one patient had lower limb wasting as a result of childhood illness so declined BIA. CT scans were available for analysis in 27/32 (84.4%) of patients. The results of body composition analyses are given in Table 4.4. Further results are detailed in Chapter 7.

Table 4.4. Results of different methods of body composition analysis

	DXA measurement (Mean±SD)(kg)	CT measurement (Mean±SD)(kg)	BIA measurement
Overall			
Fat mass	27.14 ± 7.93	26.52 ± 7.69	48.39 ± 128.46
Lean/ Fat free mass	48.52 ± 10.26	54.80 ± 11.78	
Obese			
Fat mass	31.38 ± 6.43	30.51 ± 5.75	74.34 ± 184.21
Lean / Fat free mass	51.70 ± 10.72	60.89 ± 12.54	
Non-obese			
Fat mass	23.17 ± 7.23	22.82 ± 4.12	24.07 ± 5.48
Lean / Fat free mass	45.54 ± 9.16	49.14 ± 7.80	
Difference between obese and non-obese (p value)			0.3086
Fat mass	0.0024	0.0066	
Lean / Fat free mass	0.0955	0.0069	

Hyperinsulinaemic euglycaemic clamps

During the last 15 minutes of insulin clamps blood glucose concentration was maintained between 4.0 and 5.0 mmol/L. Glucose consumption rose steadily up until 105th min and only thereafter a steady state was achieved (Figure 4.2). In the light of this, the M-value calculated during the last 15 minutes of clamp was utilised as a measure of insulin sensitivity.

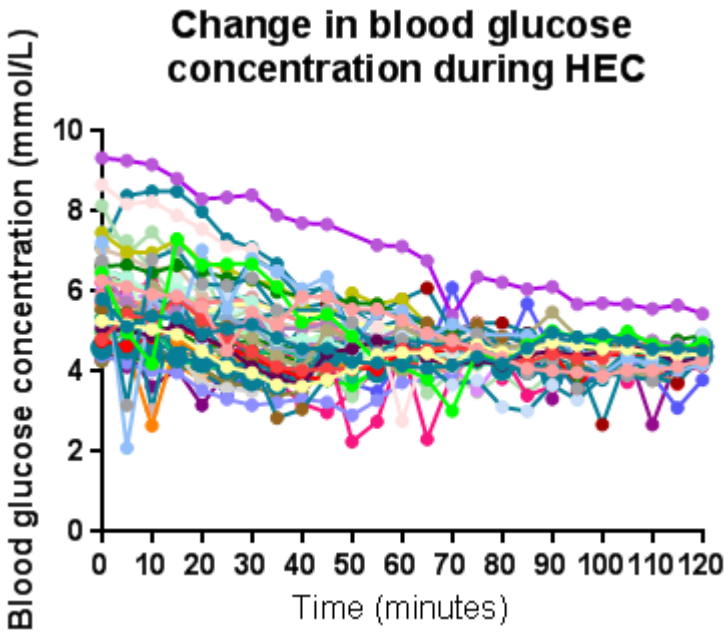


Figure 4.2. Change in blood glucose concentration during HEC
Glucose was administered intravenously during the hyperinsulinaemic euglycaemic clamp (HEC) to maintain blood glucose concentration bwtween 4.0 and 5.0mmol/L. Steady state was achieved in the last 15 minutes of the clamp and M value calculated during this period was utilised as a measure of insulin sensitivity.

There was a significant ($p<0.001$) decrease in insulin sensitivity (Figure 4.3) in both obese and non-obese subjects between visit 1 (before surgery) and visit 2 (first postoperative day) and this was not attenuated by carbohydrate drink/placebo. There was no influence of sex, age, cancer status or length of surgical procedure on the perioperative change of insulin sensitivity.

24/32 patients, 12 in the placebo arm and 12 in the carbohydrate arm, were treated with epidural analgesia. There was no observed difference in change in perioperative insulin sensitivity with epidural compared to no epidural.

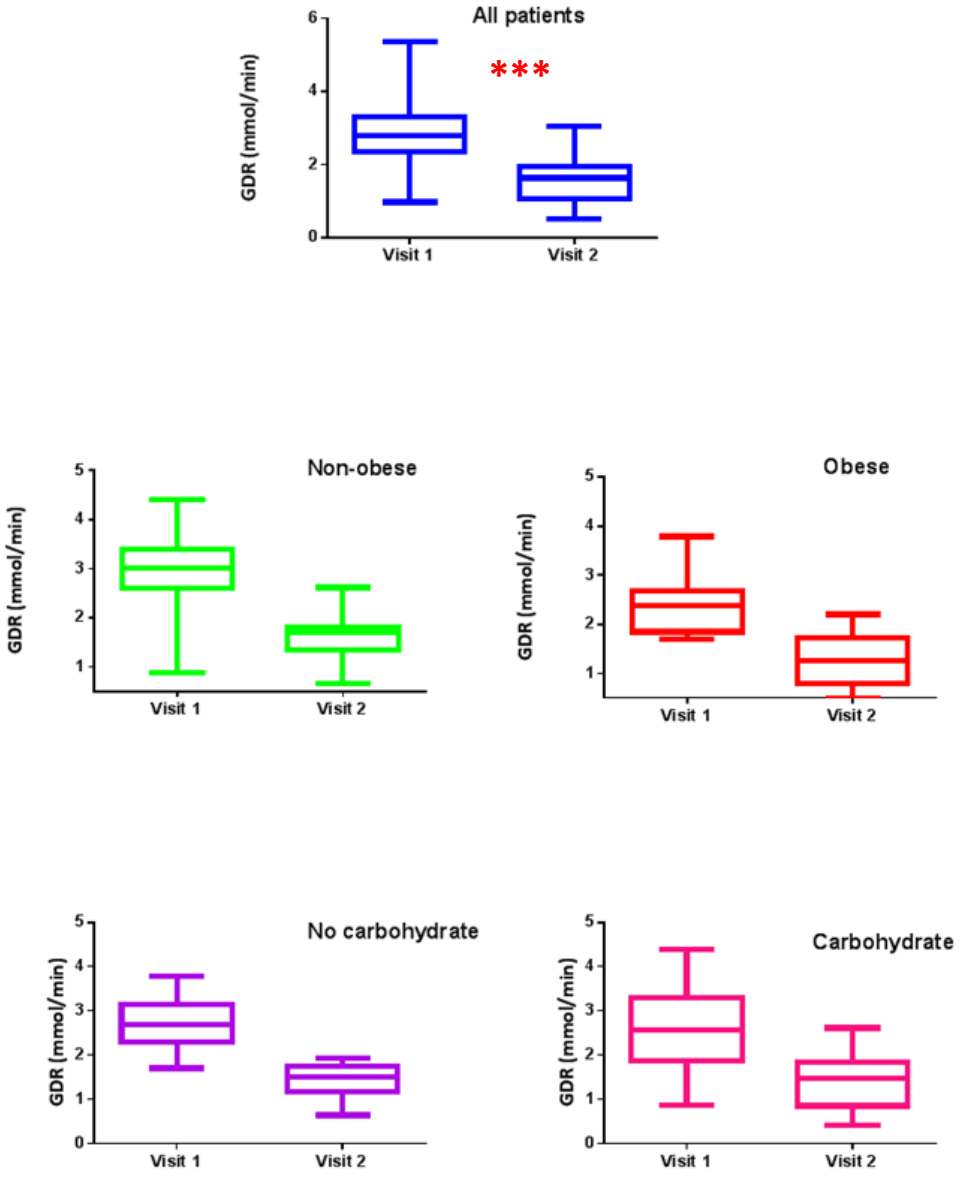


Figure 4.3. Change in insulin sensitivity with surgery *** statistically significant
In all patients, there was a significant decrease in insulin sensitivity, as measured by the HEC with surgery ($p<0.001$). There was no difference in perioperative change in insulin sensitivity between obese and non–obese patients. There was no difference in perioperative change in insulin sensitivity between those receiving carbohydrate treatment preoperatively or placebo.

Body composition and insulin sensitivity

There was no significant correlation between presence of sarcopenia and preoperative (p=0.594) or postoperative (p=0.120) insulin resistance. Myosteatosis was present in 8/27 (29.6%) patients who had preoperative CT scans available for analysis. There was no significant correlation between myosteatosis and sarcopenia (p=0.152). There was, however, significant correlation between presence of myosteatosis and postoperative insulin resistance (p=0.012, r^2=0.746).

Length of stay and complications

The mean ± SD length of hospital stay was 14± 16.1 days. There was no difference in length of stay between obese and non-obese participants. There was no difference in length of stay in the carbohydrate treated group (8.6 ± 4.8 days) compared with placebo (19.2 ± 21.3 days) (p=0.061). There was no significant difference in complications, classified according to Clavien-Dindo (Dindo et al., 2004), between obese and non-obese or carbohydrate loaded and placebo groups.

Metabolic data

The concentrations of several metabolites were measured in peripheral venous blood at four time points, which are described in Table 4.5.

Table 4.5. Time points for blood sampling

P1	S1	S2	P2
Before surgery (at commencement of preoperative clamp)	Day of surgery, before knife to skin	Day of surgery, after closure of the abdomen	Post-operative day 1 (at commencement of postoperative clamp)

Concentrations of each metabolite during these time points are given in Figure 4.4.

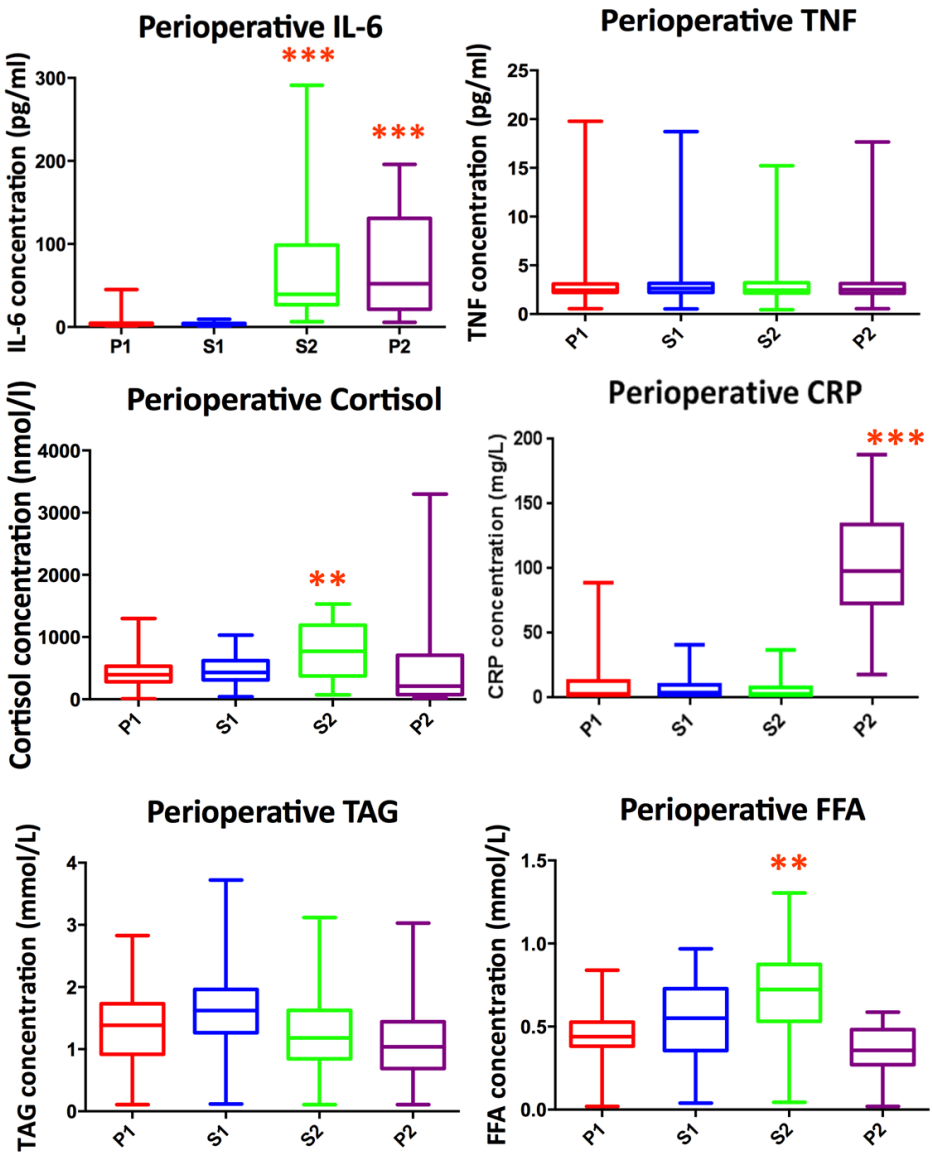


Figure 4.4. Concentrations of metabolites
P1 Preoperative **S1** start of surgery **S2** end of surgery **P2** Postoperative
** statistically significant *** highly statistically significant

There was a significant increase in IL-6 concentration from the preoperative time point to the end of surgery ($p<0.0001$) and there was a significant increase in CRP between the end of surgery and the first post-operative day ($p<0.001$). There was no significant difference between obese and non-obese patients in IL-6 ($p=0.2026$), CRP ($p=0.6452$), FFA concentration ($p=0.4465$), cortisol concentration ($p=0.3224$) or TNF ($p=0.101$) but the difference in TAG ($p=0.0097$) was significant.

There was no significant difference in perioperative concentrations of catecholamines, namely noradrenaline and adrenaline (Figure 4.5).

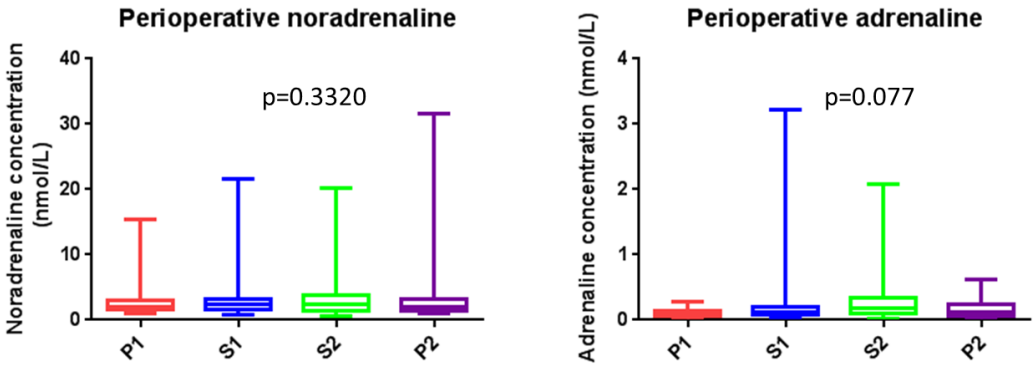


Figure 4.5. Perioperative catecholamines
P1 Preoperative **S1** start of surgery **S2** end of surgery **P2** Postoperative

Blood was also taken from an omental vein, if available, at the start (O1) and end of surgery (O2). Adequate volumes for analysis in paired samples were available in 19 patients (12 non obese, 17 obese, 9 placebo and 10 carbohydrate loaded). This has been analysed for resistin and visfatin concentrations and compared with peripheral venous blood taken at the start (S1) and end of surgery (S2) (Figure 4.6). The increase in peripheral venous blood resistin concentrations between start and end of surgery was not significant (p=0.181). However, there was a significant increase in omental resistin concentrations between start and end of surgery (p=0.0005). There was no significant intraoperative change in visfatin concentration.

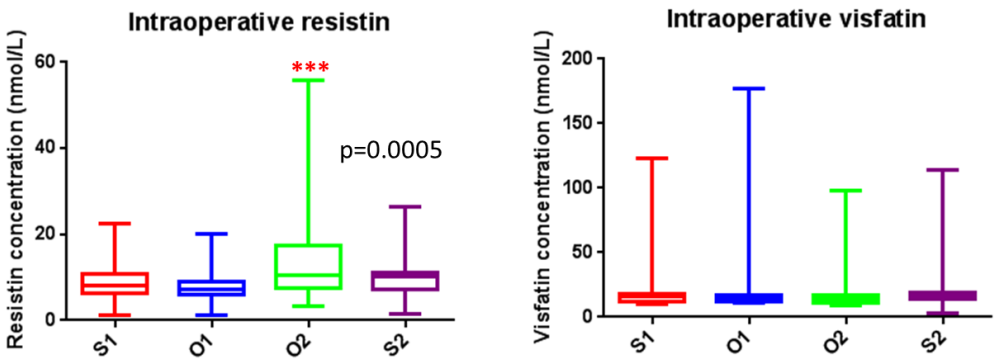


Figure 4.6. Omental and venous resistin and visfatin

S1 Peripheral venous blood at start of surgery **O1** Omental venous blood at start of surgery
O2 Omental venous blood at end of surgery **S2** Peripheral venous blood at end of surgery
** **statistically significant** *** **highly statistically significant**

There was excellent correlation between peripheral venous and omental resistin concentration at start of surgery, but this was less pronounced, although statistically significant, at the end of surgery. Similarly, there was excellent correlation between peripheral venous and omental resistin concentration at the start of surgery and a significant correlation remained at the end of surgery (Figure 4.7).

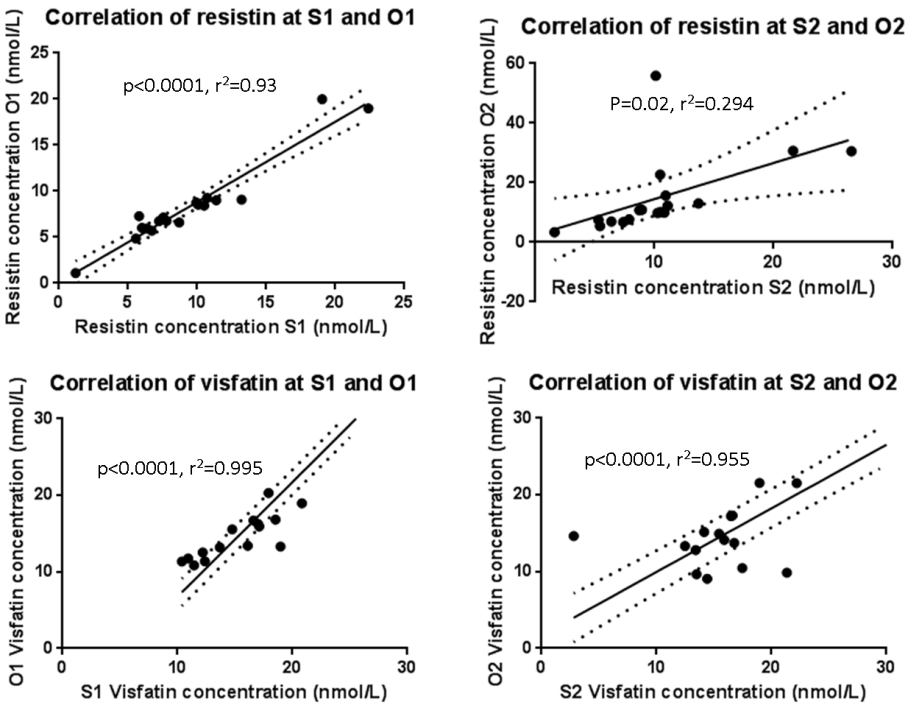


Figure 4.7.Correlation between omental and venous resistin and visfatin

Peripheral venous blood and omental blood resistin and visfatin concentrations were tested at S1/O1 start of surgery, S2/O2 end of surgery. The correlation was statistically significant at both timepoints for both resistin and visfatin.

Gene expression analysis of muscle and fat biopsies using Taqman low density arrays

VL samples taken in the week preoperatively were compared with those taken on the first postoperative day. Both were taken prior to starting the HEC and subjects had been fasted for more than 6 hours. A small volume of local anaesthetic (3 ml of 1% lignocaine) was infiltrated to the biopsy site to minimise discomfort in conscious subjects.

There was a decrease in expression of Tripartite Motif Containing 63 (TRIM 63) gene, also known as muscle ring finger 1 (MURF-1). Expression of several other genes namely Activating transcription factor 3 and 4 (ATF3 and ATF4), DNA-damage inducible transcript 3 and 4 (DDIT3 and DDIT4), also decreased. On initial analysis using Ingenuity Pathway Analysis, results suggested that genes involved in endoplasmic reticulum(ER) stress were involved in the response to surgery. However, on reanalysis using DAVID, ER stress gene pathways were not significantly affected.

Expression of Stearoyl-Co-A Desaturase (SCD) increased very significantly and there was associated increase in ATP citrate lyase (ACLY) and Forkhead box protein 03 (FOXO3) gene expression.

In postoperative VL samples, gene expression differences indicated activation of the PPAR-α/ RXR-α pathway in obese compared with non-obese patients (Figure 4.8). This was demonstrated by a negative z score on IPA (z-score = -1.000, $p<0.0001$). The z score in IPA infers the activation state of predicted transcriptional

regulators based on experimentally observed gene expression or transcription events. This was confirmed on DAVID analysis which highlighted 'fatty acid metabolism' ($p=2.8x10^{-14}$), 'peroxisome proliferator-activated receptor (PPAR) signalling pathway ($p=6.0x10^{-13}$) and 'insulin signalling pathway' ($p=2.0x10^{-10}$) as the top three enriched pathways from the KEGG.

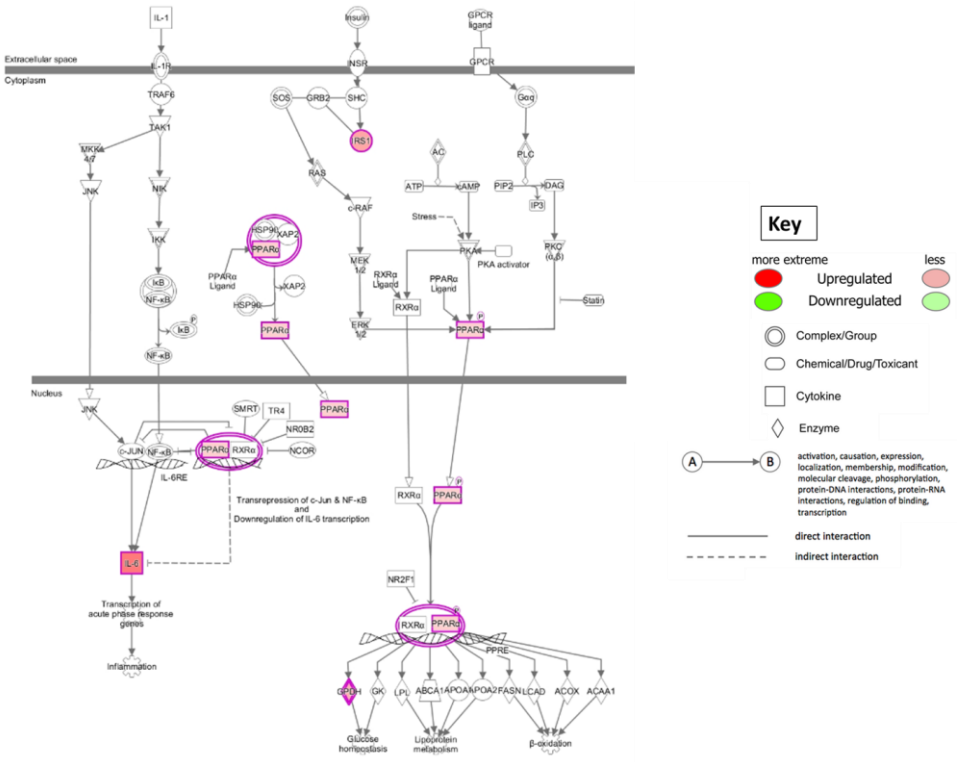


Figure 4.8. PPARα/ RXRα pathway

PPARs regulate gene expression by binding with RXR (Retinoid X Receptor) as heterodimeric partner to specific DNA sequence elements termed PPRE (Peroxisome Proliferator Response Element) (Ogata et al., 2002). Heterodimeric transcription factor complex binds to cognate sequences in promoter regions of target genes involved in the catabolism of fatty acids. PPARα regulates expression of genes involved in peroxisomal and mitochondrial ß-oxidation pathways, regulates FATP (Fatty Acid Transport Protein), the FAT/CD36 (Fatty Acid Translocase), L-FABP (Liver Cytosolic Fatty Acid-Binding Protein) and UCP2 and UCP3 (Uncoupling Proteins-2 and 3), leads to increased breakdown of triglycerides and fatty acids, increased cellular fatty acid uptake, and reduced triglyceride and fatty acid synthesis.

Overall, in all VL samples, there were very few significant changes in gene expression associated with carbohydrate loading.

There were no significant differences in postoperative gene expression levels between carbohydrate loaded patients and placebo. However, there were many

differences in gene expression in obese compared with non-obese patients. These are listed in Table 4.6 below.

Table 4.6. Gene changes in obese compared with non-obese

Gene	Expanded name	Mean postop non obese	Mean postop obese	Fold difference	Up/ down	p value
GRB14	Growth factor receptor bound protein 14	0.028584	0.060085	2.1021	UP	0.045
GPD2	Glyceraldehyde – 3 – phosphate dehydrogenase	1.361965	2.504954	1.8392	UP	0.015
NRF1	Nuclear respiratory factor	0.1114105	0.183942	1.6510	UP	0.001
NUAK2	NUAK family SNF-1 like kinase1	0.020911	0.031479	1.5054	UP	0.031
FSTL1	Follistatin-like 1	1.175079	3.938313	3.3515	UP	0.036
SLC2A1	Solute carrier family 2 (Facilitated Glucose Transporter)	0.030419	0.061983	2.0376	UP	0.049
IL6	Interleukin 6	0.012767	0.040618	3.1815	UP	0.005
PPARα	Peroxisome proliferator-activated receptorα	1.079697	1.265584	1.1722	UP	0.04
TAT	Tyrosine Aminotransferase	0.000628	0.003073	4.8933	UP	0.02

ELOVL6	Fatty Acid Elongase 6	0.014215	0.030255	2.1284	UP	0.028
IRS2	Insulin Receptor Substrate 2	0.735154	1.351409	1.8383	UP	0.042
PPARδ	Peroxisome proliferator-activated receptorδ	0.912487	1.078186	1.1816	UP	0.042
IRS1	Insulin Receptor Substrate 1	0.160136	0.334458	2.0886	UP	0.001
TRIB3	Tribbles homolog 3	0.009891	0.030856	3.1196	UP	0.023
BHLHE40	Basic loop helix loop helix family member e40	1.56474	2.640537	1.6875	UP	0.003
PPP1R3A	Protein phosphatase 1 regulatory subunit 1	0.997656	1.410117	1.4134	UP	0.037

In obese patients, there were no differences seen postoperatively between carbohydrate loaded and placebo and there was an increase in Angiopoietin-like 4 (ANGPTL4) (126%) and SCD5 (121%) in non-obese carbohydrate loaded compared with placebo. These changes are small and affect only 2 genes in isolation, so may have occurred due to chance and do not indicate any major changes in gene expression or related pathways.

In patients who received placebo, there were changes seen in a number of genes associated with carbohydrate metabolism pathways in obese compared with non-

obese patient post-operative samples, namely BHLHE40, Protein Kinase C Epsilon (PRKCE), IRS1, NRF1 and NUAK2. In carbohydrate loaded patients, NRF1 was similarly upregulated in obese compared with non-obese patients postoperatively, but the magnitude of change was greater (45.9% in placebo and 74.7% in carbohydrate loaded).

In preoperative VL samples, there were no significant gene expression changes associated with carbohydrate loading overall. There were significant differences in individual genes between obese and non-obese patients, but they did not significantly affect any particular metabolic pathway.

In omental fat samples, activation of the triggering receptor expressed on myeloid cells (TREM1) pathway was seen in response to surgery (Figure 4.9). This was demonstrated by a positive z score on IPA (z score = 2.646, p<0.0001).

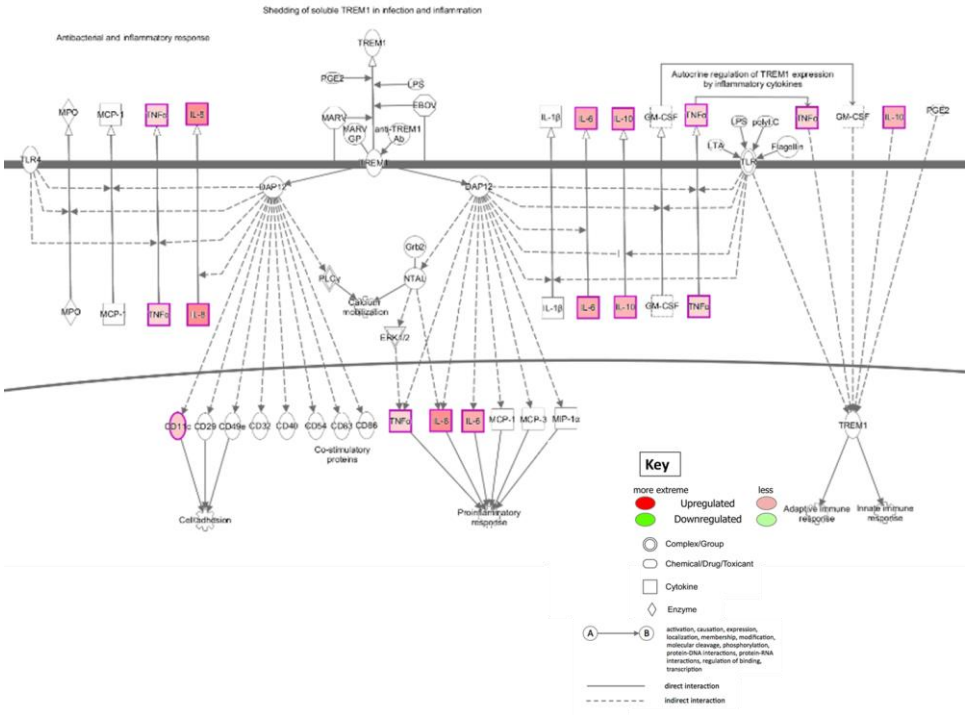


Figure 4.9. TREM 1 pathway

TREM1 activation triggers the Janus kinase 2 (JAK2), protein kinase B (PKB/AKT) and extracellular signal related kinase (ERK1/2) pathways leading to the phosphorylation of signal transducers of activation of transcription (STAT3, 5) and NF kappa B (NF-κB), upregulate expression of genes involved in inflammatory response. Stimulation of TREM by its ligand or toll like receptor (TLR) by lipopolysaccharide (LPS) can lead to an association of TREM1 and TLR, leads to activation of interleukin-1 receptor-associated kinase 1(IRAK1), triggers NF-κB and proinflammatory response. Engagement and activation of TREM-1 triggers expression and secretion of chemokines and cytokines like monocyte chemotactic protein 1(MCP-1) macrophage inflammatory protein-1alpha (MIP-1a), interleukins (IL-6,-8) and tumour necrosis factor (TNF) (Tessarz and Cerwenka, 2008).

When omental fat sample results were subjected to DAVID analysis, the top two enriched KEGG pathways highlighted were 'regulation of cytokine production' (NLRP3, IRAK3, IL1B, IL18, C3, HIF1A, CASP1, TGFB1, ADIPOQ, MYD88, IL6, PPARG, IL10, TNF, SOD1, CIDEA; $p=9.1x10^{-16}$) and 'positive regulation of cytokine production' (IRAK3, CEBPB, C3, ADIPOQ, PPARG, IL10, CIDEA; $p=2.1x10^{-10}$).

In omental fat, there were few changes in gene expression perioperatively in association with carbohydrate loading. However, there were several differences associated with obesity. The most significant difference was seen in Complement Factor D (CFD), in which there was a 998.3% perioperative increase in non-obese, as compared to a 125.9% increase in obese subjects. There was no significant difference in baseline preoperative levels of expression of this gene between obese and non-obese subjects.

Preoperatively, there were few significant differences in omental fat gene expression, between carbohydrate loaded and placebo patients, obese and non-obese, or any combination of these factors. In postoperative samples, there were no differences associated with carbohydrate loading. Obese patients had greater expression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A) and T-Box 21 (TBX21) compared with non-obese. In those who received placebo, there were some significant differences in gene expression between obese and non-obese (Table 4.7). In those who received carbohydrate, no postoperative differences in gene expression between obese and non-obese were noted.

Table 4.7. Postoperative gene expression differences in omental fat placebo obese vs non-obese subjects

Gene	Expanded name	Mean postop no carb non obese	Mean postop no carb obese	Fold difference	Up/ down	p value
acaca	Acetyl CoA carboxylase alpha	1.577549	0.86215	0.546512343	down	0.033
alox15b	Arachidonate 15-lipoxygenase	0.087131	0.007555	0.086708519	down	0.033
atf6	Activating Transcription Factor 6	1.384045	0.769211	0.555770224	down	0.047
il6	Interleukin 6	1.233944	0.258414	0.209421173	down	0.049
irak3	Interleukin Receptor associated kinase 6	0.60124	0.328184	0.545845253	down	0.031
mmp12	Matrix metalloproteinase 12	0.0102323	0.001674	0.163599582	down	0.006
socs3	Suppressor of cytokine signalling 3	31.2007	10.10934	0.324010038	down	0.047
sod1	Superoxide dismutase 1	0.661186	0.318722	0.482045899	down	0.003
cebpd	CCAAT/ enhancer binding protein	2.838484	0.778156	0.274144931	down	0.001
irs2	Insulin receptor substrate 2	5.146531	2.045932	0.397536127	down	0.035
tbx21	T-box protein 21	0.017787	0.008469	0.476134255	down	0.044

In abdominal fat, there were few significant gene expression changes associated with obesity or carbohydrate loading overall. There was also little influence of carbohydrate loading or obesity on perioperative changes in gene expression. However, overall, surgery was associated with a number of significant changes in gene expression. The most striking was an increase in zinc finger protein 664 (ZNF 664), which increased 17 249% between the preoperative and postoperative samples.

4.5 Discussion

Although previous reports suggest increased risk of morbidity in obese patients undergoing surgery (Doyle et al., 2010), in the present study, there were no differences in complications or length of stay. This may be due to improvements in perioperative care of obese patients but no detailed data comparing care regimes currently exists. Additionally, the obese group included only patients who were deemed fit enough for elective open major abdominal surgery, and therefore, may be judged relatively 'low risk' for complications despite their obese state. Interestingly, there were no differences in perioperative change in insulin sensitivity between obese and non-obese patients.

This was a pragmatic study of patients undergoing major abdominal surgery. Therefore, there was heterogeneity in terms of patient age (although no statistically significant differences between subgroups) and comorbidities. In addition, there was variability in surgical and anaesthetic technique which may have affected results from hyperinsulinaemic-euglycaemic clamp studies as well as

other outcomes tested. Every effort was made to ensure that procedures were comparable, in terms of length of procedure and blood loss, but in this relatively small patient group, the variability may have affected results. It is perhaps surprising that there were no significant differences in perioperative insulin sensitivity between obese and non-obese patients. A standard technique of calculation of insulin dose using body surface area was used (DeFronzo et al., 1979) but this may have overestimated the insulin dose required per unit of lean mass in obese individuals and thereby resulted in greater glucose disposal. However, when the M value was corrected for the circulating insulin concentration during the clamp, there remained no significant difference in change in perioperative insulin sensitivity between lean and obese individuals.

It is well established that surgery is associated with a reduction in insulin sensitivity (Thorell et al., 1994, Black et al., 1982). Some previous studies have suggested that the administration of preoperative carbohydrate attenuates this response (Nygren et al., 1998). In a small study in colorectal surgery (n=14), administration of 150 g of oral carbohydrate prior to surgery was associated with less reduction in whole body glucose disposal compared with the fasted group (Nygren et al., 1998). There have been two other small studies utilising the HEC which have demonstrated a reduction in the change in insulin sensitivity in preoperative carbohydrate treated patients compared with placebo (Nygren et al., 1999, Ljungqvist et al., 1994). In a recent animal study, postoperative whole body glucose disposal rate was higher in carbohydrate treated subjects than fasted and

this correlated negatively with muscle PDK4 protein expression (Gjessing et al., 2015). The same study group demonstrated, in pigs, that a single dose of carbohydrate preoperatively was sufficient to reduce postoperative insulin resistance, as measured by the HEC (Gjessing et al., 2014).

Preoperative oral carbohydrates have also been associated with reduced length of stay in patients undergoing surgery (Thorell et al., 1999). A previous study in patients undergoing colorectal operations or liver resection demonstrated no difference in length of stay or postoperative fatigue between groups receiving preoperative carbohydrate or placebo (Mathur et al., 2010). A recent study in patients undergoing open cholecystectomy and colorectal operations demonstrated no significant improvements in clinical status or length of hospital stay in the carbohydrate group compared with placebo (Sada et al., 2014). This study did report improved well-being in patients undergoing cholecystectomy associated with administration of carbohydrate drinks, a finding that was reproduced in a recent study in day-case laparoscopic cholecystectomy (Singh et al., 2015).

In the present study, although it was not the primary outcome measure, there was no significant difference in length of stay or postoperative complications between patients receiving carbohydrate drinks or placebo. There may truly be no difference in postoperative outcomes in patients receiving preoperative carbohydrate drinks or placebo. A metanalysis of trials using preoperative carbohydrate concluded that existing studies were of low to moderate quality

(Awad et al., 2013). It is also possible that the dose of carbohydrate used in the present study was not sufficient to produce a clinically significant effect. Although the dose selected (150g carbohydrate) is used in clinical practice in enhanced recovery after surgery (ERAS) protocols (Gustafsson et al., 2013), it is possible that the obese patients in this study particularly may have required a higher dose.

There were a number of metabolic changes occurring during surgery. As expected, serum metabolites associated with the inflammatory response increased with surgery. Although serum TAGs did not increase overall, there was an increase in obese compared with non-obese patients. In this context, increased TAG probably represents part of the acute phase response, which is potentially more marked in obese compared with non-obese individuals (Esteve et al., 2005).

Omental resistin concentration increased markedly between start and end of surgery. As numbers were small, analysis of serum and omental resistin and visfatin was not performed in obese and non-obese subgroups separately. Previous studies have evaluated the role of resistin in insulin resistance and obesity (Degawa-Yamauchi et al., 2003). To my knowledge, there are no studies in the non-bariatric population which have evaluated intraoperative serum and omental resistin. Although animal and human studies of resistin have shown conflicting results to date, this finding of increased omental resistin during surgery merits further investigation as an adipokine mediating the acute phase response.

Perhaps the most striking findings of the present study relate to gene expression analysis of abdominal fat, vastus lateralis and omental fat samples taken before and after surgery. Although there were few differences between obese and non-

obese subgroups and carbohydrate loaded and placebo clinically, or demonstrated by the HEC, there were many significant changes in gene expression.

Initial analysis using IPA suggested that genes involved in the unfolded protein response (UPR) demonstrated perioperative changes. When the ER becomes stressed due to the accumulation of newly synthesized unfolded proteins, the unfolded protein response (UPR) is activated. The three branches of the canonical UPR intersect with a variety of inflammatory and stress signalling systems including the NF-κB-IκB kinase (IKK) and JNK-AP1 pathways, as well as networks activated by oxidative stress (Ron and Walter, 2007). In the present study, decreased expression of ATF4 and ATF3 were seen in postoperative compared to preoperative VL samples. This is in contrast to previous studies which have reported activation of UPR and ER stress associated with increased ATF4 in the postoperative period (Hotamisligil, 2010). As these findings were not confirmed by further enrichment analysis, it is unlikely to represent a true downregulation of ER stress pathways, which would be in contrast to expected results based on the evidence available. Therefore further studies in different patient cohorts are required to investigate these mechanisms further.

In the present study, Stearoyl CoA Desaturase (SCD) expression was markedly increased in postoperative compared to preoperative VL samples. SCD is an endoplasmic reticulum enzyme that catalyses the biosynthesis of monounsaturated fatty acids from saturated fatty acyl Co-As. These monounsaturated fatty acids are used as substrates for the synthesis of triglycerides and esters (Kurikawa et al., 2013). It is possible that the findings of

the present study reflect the synthesis of fatty acids in skeletal muscle to be used as a substrate during surgical stress. A previous study in cell culture lines demonstrated that inhibition of SCD induced ATF4 mRNA, phosphorylated ATF4 protein and total ATF4 protein (Masuda et al., 2012). However, there are no previous studies in humans to corroborate the findings, therefore further evaluation, perhaps by examining protein expression is warranted.

Muscle atrophy has been observed in response to protein and/or energy malnutrition and fasting, but is also a feature of immobility, diabetes, cancer cachexia and sepsis (Glass, 2003). An increased expression of TRIM63 gene, also known as MURF-1 has been observed in models of muscle wasting, including fasting (Lecker et al., 2004) However, in a human study examining skeletal muscle of volunteers subjected to a short-term fast, there was no significant change in MURF-1 expression. Therefore, the changes observed in the present study in TRIM63 and related genes are unlikely to be a result of fasting alone (Larsen et al., 2006). Induction of TRIM63 has been observed in immobilisation-induced muscle atrophy (Stitt et al., 2004), but the length of surgery (2-3hrs) in this study is unlikely to have been sufficient to induce atrophy. Additionally, TRIM 63 gene expression was decreased in postoperative compared with preoperative VL samples. FOXO3 gene expression increased and FBOX032 (atrogin) expression decreased. Atrogin belongs to the ubiquitin proteasome pathway, the primary pathway involved in intracellular protein degradation in skeletal muscle (Lecker et al., 2004). A decrease in TRIM 63 (MURF 1) and atrogin (FBOX032) would counteract myofibril degradation (Figure 4.10).

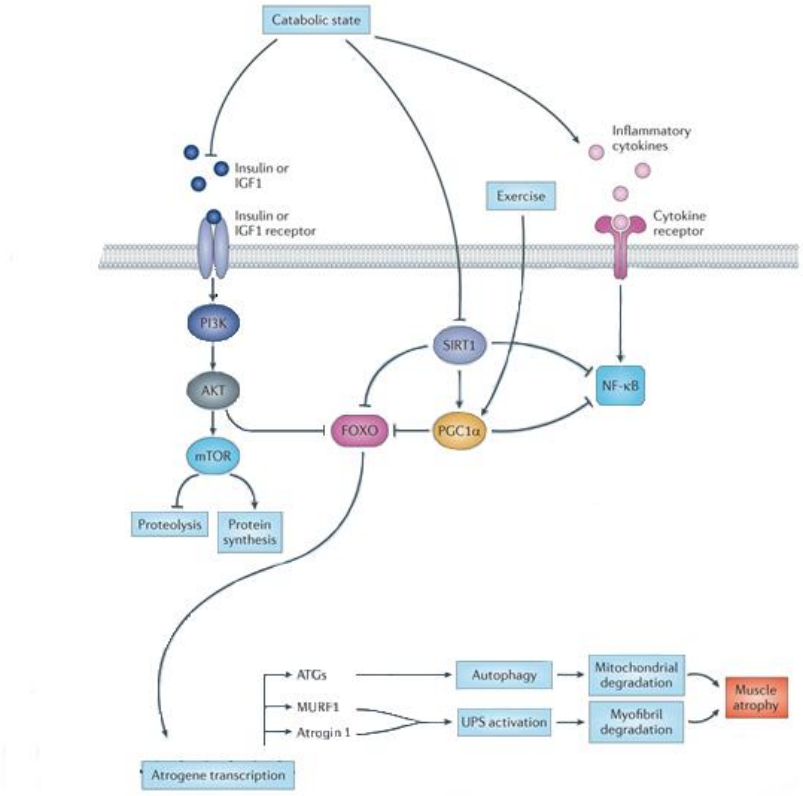


Figure 4.10 Effects of catabolism on muscle atrophy pathway

Catabolism stimulates the release of inflammatory cytokines. The binding of insulin or IGF 1 to its receptor triggers the P13K pathway. Both actions result in effects on FOXO and lead to atrogene transcription which results in muscle atrophy**. IGF1** Insulin like growth factor**, SIRT1** sirtuin 1, **ATGs** autophagy related genes**, UPS** ubiquitin proteasome system

There are no reported studies of changes in these genes in skeletal muscle of patients undergoing surgery. However, there have been some studies of TRIM63 and atrogin expression in skeletal muscle of mice. Transgenic mice overexpressing TRIM63 do not present with muscle atrophy but hyperinsulinaemia and reduced hepatic glycogen stores (Hirner et al., 2008). Currently, there is evidence that genes in this pathway are contributing to metabolism in skeletal muscle. The changes demonstrated in the present study have been described following stimulation of a catabolic state resulting in muscle atrophy (Cohen et al., 2015) but their precise role in the postoperative response is unclear.

Changes in the PPARα/RXRα pathway were demonstrated in postoperative VL samples of obese compared with non-obese patients. RXR or retinoid X receptor is an obligate heterodimeric partner for nuclear receptor PPAR and helps maintain homeostasis in metabolism (Shulman and Mangelsdorf, 2005). PPARα has a crucial role in controlling fatty acid oxidation (Reddy and Hashimoto, 2001) and is induced during fasting. In mouse models, PPARα agonists reduced adiposity, improved muscle and hepatic steatosis and consequently improved insulin sensitivity (Guerre-Millo et al., 2000, Chou et al., 2002, Kim et al., 2003). It is possible that activation of this pathway in the present study is reflective of preoperative fasting. However, this does not account for the increased activation in obese compared with non-obese patients. There are some parallels with the Randle hypothesis which suggested in rats that substrate competition between non esterified fatty acids (NEFAs) and glucose served as an energy source for muscle (Randle et al., 1963). These studies observed a relative increase in fat oxidation compared with carbohydrate metabolism in response to increased NEFAs. The findings of the present study could be further evaluated in bariatric patients undergoing surgery.

The most striking finding in omental fat was activation of the TREM1 pathway in postoperative compared to pre-operative samples. TREM1 activation results in production of pro-inflammatory cytokines such as IL-1ß and TNFα as well as chemokines such as IL-8 and MCAP-1. Surface expression of TREM1 in peripheral blood mononuclear cells from patients undergoing surgery has been compared in septic patients and healthy volunteers in a previous study. There was a significant increase in TREM1 expression with surgery but the highest TREM1 expression level

was seen in septic patients (Gonzalez-Roldan et al., 2005). In acute pancreatitis, expression of TREM-1 mRNA on myeloid cells correlates with the severity of disease (Wang et al., 2004). The finding of activation of the TREM1 pathway in omental samples in response to surgery is likely to represent the inflammatory response to surgery. It is unclear from the present study whether the finding of increase TREM-1 expression in omental fat reflects expression in adipocytes or resident immune cells. There is some interest in the potential role of TREM1 as a therapeutic target in sepsis-induced inflammation (Palazzo et al., 2012) so this novel finding certainly warrants further investigation.

This study does have some limitations. The hyperinsulinaemic-euglycaemic clamp was performed with an intentionally high insulin dose in order to suppress hepatic gluconeogenesis (although this was not measured directly with tracer infusions) and also to take account of the potentially lower insulin sensitivity of the obese subgroup. However, as a result, the present findings are not directly comparable with those in the literature using HEC to evaluate insulin sensitivity changes in surgical patients. In addition, because of the practicalities of performing the post-operative HEC on the surgical ward or high dependency unit, we were unable to augment the results with indirect calorimetry which would have enabled partitioning of whole body glucose disposal into oxidative and non-oxidative components. It is possible that the dose of carbohydrate used (150 g) was insufficient to produce significant differences, particularly in the obese subgroup. However, this was selected as the standard dose used in ERAS protocols (Fearon et al., 2005). Finally, the gene expression analysis was performed using a low

density gene array which has been reported in some studies to produce less reproducible and reliable results than quantitative PCR when measuring mRNA of genes with low levels of expression (Lu et al., 2008). However, to ensure the robustness of the method, it was determined that the housekeeping genes were expressed at nearly constant levels in each individual tissue. Results were normalised to the geometric mean of the housekeepers and the implementation of pathway analysis ensured that changes seen were described in context.

Overall, this intensive study of body composition and surgery has demonstrated that the perioperative environment is metabolically highly active. The reduction of insulin sensitivity associated with major abdominal surgery was confirmed but there were no changes or differences associated with preoperative carbohydrate loading or obesity. A number of changes in gene expression and serum metabolites have been demonstrated, many of which are novel.

Chapter 5 A comparison of surrogate indices of insulin sensitivity against the hyperinsulinaemic-euglycaemic clamp in two study populations

5.1 Background

Insulin sensitivity varies both acutely and chronically. The hyperinsulinaemic-euglycaemic clamp (HEC) is considered the gold standard method for assessment of insulin sensitivity (DeFronzo et al., 1979). However, it is a time-consuming technique and has therefore not been used routinely in large cohort studies. A number of other methods such as homeostatic model assessment (HOMA) (Matthews et al., 1985) and quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000) have been used to provide surrogate indices of insulin sensitivity in large studies. Many studies of preoperative carbohydrate treatment in surgery use surrogate markers of insulin sensitivity (Awad et al., 2013), but there is no evidence that the results are equivalent in a population undergoing major abdominal surgery. This study aimed to evaluate the correlation between two indices of insulin sensitivity, namely HOMA-IR and QUICKI, against the M value obtained from the HEC.

5.2 Study hypothesis

The hypothesis of this study was that surrogate markers of insulin sensitivity, namely HOMA-IR and QUICKI do not provide the same assessment of insulin sensitivity as the gold standard hyperinsulinaemic euglycaemic clamp.

5.3 Methods

Subjects were participants in two separate clinical trials. Based on the results of a previous study (Ljunggren et al., 2014) and with an estimated standard deviation of 10% in measurements of body composition, 21 participants would be required

to detect pair-wise differences of 7.8% in insulin sensitivity between HOMA-IR and HEC-derived M value with a power of 80% and a significance level of 0.05. Study 1 (Chapter 4) evaluated the influence of preoperative carbohydrate loading on perioperative change in insulin sensitivity. Study 2 (Chapter 6) assessed changes in insulin sensitivity and body composition during neoadjuvant chemotherapy (NAC) for oesophagogastric (OG) cancer. 32 subjects from the first study and 15 subjects from the second study were included.

Hyperinsulinaemic-euglycaemic clamp

The HEC was performed as described in Chapter 3.

Estimation of surrogate indices

HOMA-IR was calculated according to the formula:

HOMA-IR = fasting glucose (mmol/L) * fasting insulin (mU/ml) / 22.5 (Matthews et al., 1985).

QUICKI was calculated as:

1/ [log (fasting insulin (mmol/L)) + log (fasting insulin (mg/dl)] (Katz et al., 2000).

5.4 Results

Insulin Sensitivity

A total of 90 sets of results were available. 4 patients from Study 1 and 1 patient from Study 2 did not undergo the second HEC.

The median M value, HOMA-IR and QUICKI overall and for each study are given in Table 5.1.

Table 5.1. Results of indices of insulin sensitivity

	Overall	Study 1	Study 2
M value (mg/kg/min) (median, IQR)	4.76 (3.75-6.81)	4.75 (3.75-6.81)	5.31 (3.80-6.93)
HOMA-IR (median, IQR)	3.80 (2.63-6.35)	3.80 (2.63-6.35)	4.70 (2.96-6.74)
QUICKI (median, IQR)	0.31 (0.29-0.33)	0.32 (0.29-0.33)	0.30 (0.29-0.33)

Using the cut-off values for insulin resistance (IR) of HOMA-IR >3.6 (Stern et al., 2005), QUICKI < 0.33 (Ascaso et al., 2003) and M-value < 5 mg/kg/min (Borai et al., 2007) numbers of subjects with IR overall and at each study timepoint were calculated (Table 5.2).

Table 5.2. Proportion of subjects with IR estimated with each technique

	Number of subjects with IR (%)				
	Study 1 P1	Study 1 P2	Study 2 P1	Study 2 C1	Study 2 S1
HEC	10/32 (31.3)	23/28 (82.1)	6/15 (40.0)		7/14 (50.0)
HOMA-IR	9/32 (28.1)	21/28 (75.0)	9/15 (60.0)	14/15 (93.3)	8/15 (53.3)
QUICKI	18/32 (56.3)	25/28 (89.2)	10/15 (66.7)	15/15 (100)	9/15 (60.0)
P1 First study visit pre chemotherapy/ surgery, P2 First postoperative day, C1 During chemotherapy, S1 Post chemotherapy, before surgery					

QUICKI overestimated IR compared with HEC at all timepoints, while the results obtained from HOMA-IR were generally more variable. The difference between proportions of patients with IR estimated by HEC and QUICKI was only significant at baseline in Study 1 ($p=0.0489$).

Correlations between methods of estimating insulin sensitivity

HOMA-IR and QUICKI are both derived from fasting insulin and fasting glucose concentrations. Therefore, as would be expected, correlation between these methods was strong ($r < -0.98$ and $r > 0.98$ respectively, $p<0.001$).

Correlations between the surrogate markers of insulin sensitivity and M values derived from the HEC were very weak and non-significant (Figure 5.1).

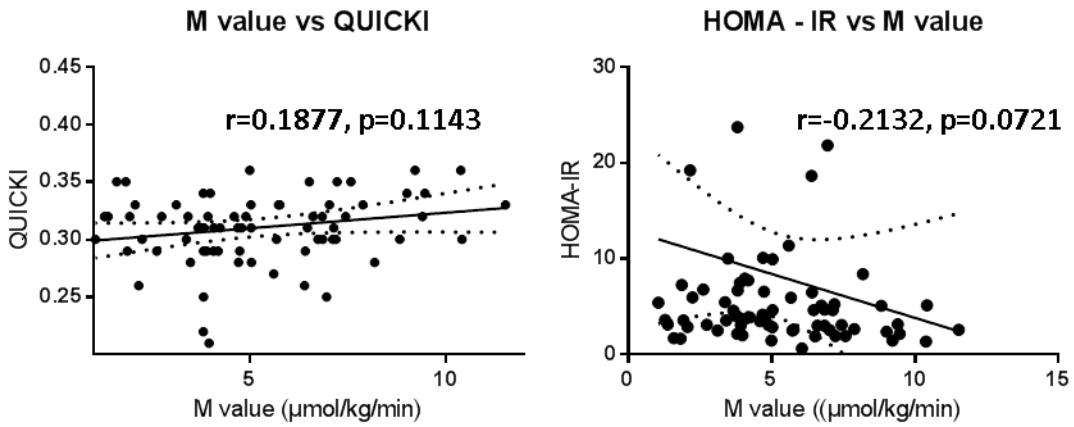


Figure 5.1. Comparison of methods of determining insulin sensitivity

Correlation between M value from HEC and QUICKI is shown in the first graph. Correlation between M value from HEC and HOMA-IR is shown in the second graph. Neither correlation was statistically significant.

Correlations of HEC with the other methods of estimating insulin sensitivity are shown in Table 5.3.

Table 5.3. Correlations of HEC and QUICKI/HOMA-IR at each timepoint

Visit no.	Study 1		Study 2	
	HOMA-IR vs M	QUICKI vs M	HOMA-IR vs M	QUICKI vs M
P1	r=-0.032, p-0.870	r=0.055, p=0.781	r=-0.661, p-0.009	r=0.612, p=0.017
P2/ S1	r=-0.148, p=0.463	r=0.260, p=0.302	r=-0.688, p=0.008	r=0.556, p=0.042

Change in Insulin Sensitivity during study

M value decreased by 45% between timepoints in Study 1 ($p<0.001$) and 13.9% ($p<0.0001$) in Study 2. In Study 1, HOMA-IR increased by 120% ($p=0.0002$) and QUICKI decreased by 7.7% ($p=0.0001$). The change in HOMA-IR and QUICKI was not statistically significant in Study 2 ($p=0.3303$ and $p=0.2090$ respectively).

Correlation between methods in obese and non-obese

Correlation between the surrogate markers, HOMA-IR and QUICKI, and M value from the HEC was poor in obese and non-obese subgroups in Study 1 (Figure 5.2). In study 2 (Figure 5.3) there was good correlation between methods in non-obese but not in obese subgroups.

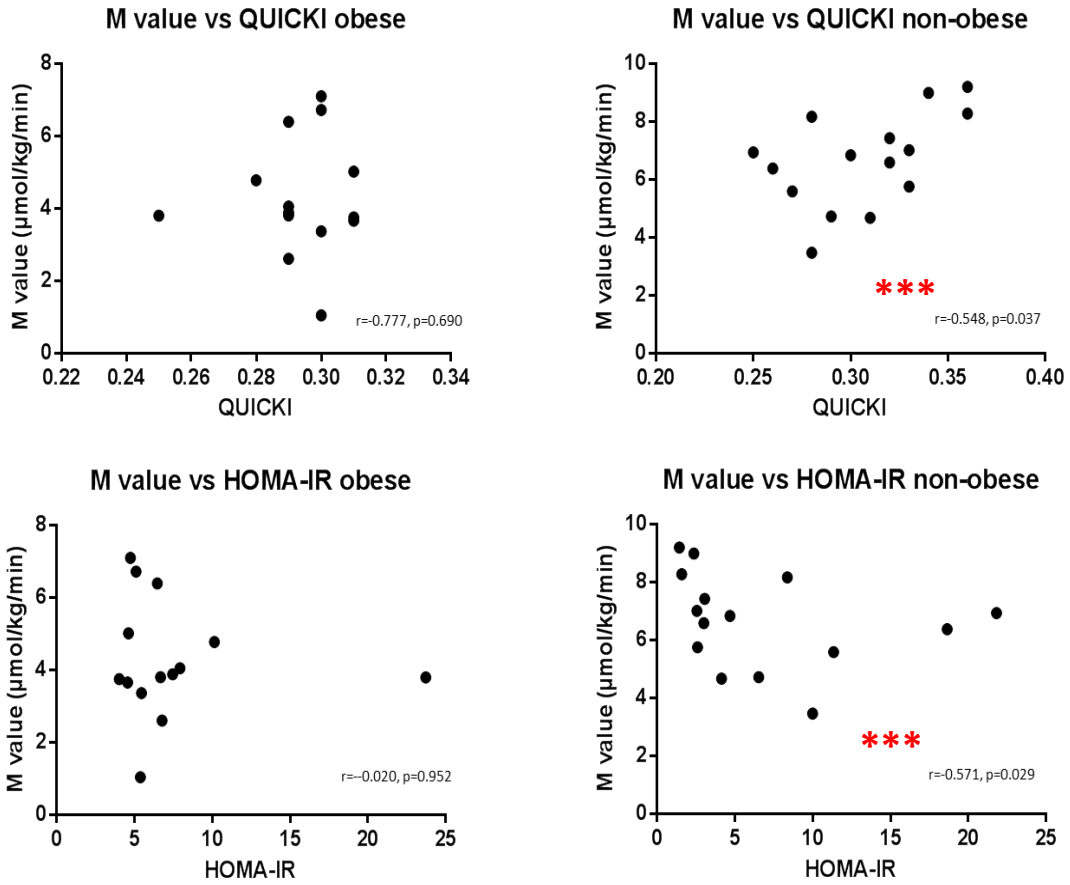


Figure 5.2. Correlation between methods in obese and non-obese subgroups in Study 1 *** statistically significant

Correlation between M value, HOMA-IR and QUICKI is shown in subgroups from the perioperative patients from the study in Chapter 4. These correlations were statistically significant only in non-obese patients.

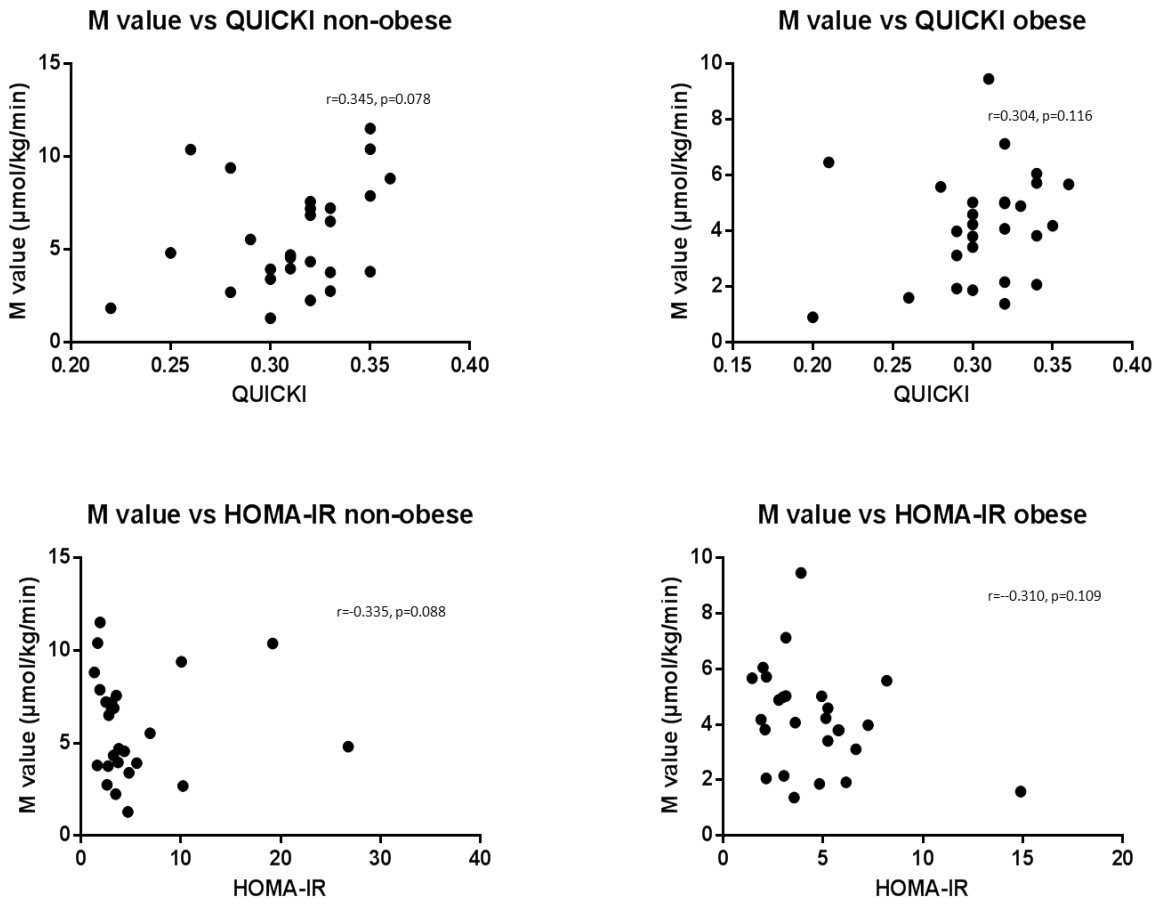


Figure 5.3. Correlation between methods in obese and non-obese subgroups in Study 2

Correlation between M value, HOMA-IR and QUICKI is shown in subgroups from the chemotherapy patients from the study in Chapter 6. These correlations were not statistically significant.

5.4 Discussion

The hyperinsulinaemic-euglycaemic clamp (HEC) technique was first described by Ralph De Fronzo in 1979 (DeFronzo et al., 1979). The plasma insulin concentration is raised and maintained by a continuous infusion of insulin. Meanwhile, the plasma glucose concentration is held constant at basal levels by a variable glucose infusion. When the steady-state is achieved, the glucose infusion rate equals glucose uptake by all the tissues in the body and is therefore a measure of tissue

insulin sensitivity (DeFronzo et al., 1979). It is the reference method for quantifying insulin sensitivity in humans because it measures directly the effects of insulin in promoting glucose utilisation under steady-state conditions *in vivo* (DeFronzo et al., 1979). The method is time-consuming and laborious and may therefore not be suitable for use in very large scale studies.

Several surrogate markers of insulin sensitivity have been proposed for use in clinical studies when the gold-standard HEC is not feasible. Mathematical modelling of the normal physiological dynamics of insulin and glucose produced the homeostasis model assessment (HOMA), which provided equations for estimating insulin resistance (HOMA-IR) and β-cell function from simultaneous fasting measures of insulin and glucose levels (Matthews et al., 1985). The quantitative insulin sensitivity check index (QUICKI), derived from logarithmically-transformed fasting plasma glucose and insulin levels has, in some studies, compared favourably with data obtained from the HEC (Mather et al., 2001).

A recent meta-analysis suggested that the revised QUICKI method could be used as a reasonable alternative to the HEC in large scale studies (Otten et al., 2014). The revised QUICKI requires fasting blood samples for analysis of insulin and glucose as well as analysis of non-esterified fatty acids (NEFA). The authors of the meta-analysis acknowledge that the strength of correlation with HEC is moderate (Otten et al., 2014). More recently, a large study in non-diabetic patients undergoing elective surgery, has confirmed poor agreement between results obtained from HOMA and HEC (Baban et al., 2015).

In the present studies, in the whole study group, there was poor correlation between QUICKI or HOMA-IR and M value. At certain time points, and in some subgroups, the correlation was more convincing, but these results suggest that there is no alternative to the HEC which will produce a reliable estimate of insulin sensitivity in all patient groups and all situations. This is likely to reflect the fact that HOMA-IR and QUICKI assess insulin resistance in the fasting state rather than in the postprandial state and fasting indices measure mainly hepatic insulin sensitivity while the HEC measures mainly skeletal muscle insulin sensitivity (Matsuda and DeFronzo, 1999).

However, in both studies, the trend in insulin sensitivity was accurately reflected by all three methods. Surgery was associated with a significant reduction in insulin sensitivity and accordingly, there was a significant decrease in insulin sensitivity between the preoperative and postoperative values in all methods. The magnitude of the change varied, but all reached statistical significance. In the OG cancer study, there was no significant change in insulin sensitivity between the pre-chemotherapy and post-chemotherapy visits and this was reflected in the results of all three methods.

This study has some limitations. The intensiveness of HEC as a technique has restricted the number of participants and this also confines the possibility of performing meaningful subgroup analysis. Subjects were recruited from studies examining the effect of obesity and chemotherapy on IR. As such, participants may not be representative of the general population.

However, we have demonstrated in a large number of studies, that the results obtained from QUICKI and HOMA-IR are not equivalent to the gold standard HEC. Surrogate markers of insulin sensitivity may be useful in large scale studies to determine trends. However, investigators should note that these methods cannot provide a measure of post-prandial whole body insulin sensitivity in the same way as the hyperinsulinaemic-euglycaemic clamp.

Chapter 6 The effects of an intensive nutritional support programme on body composition, insulin resistance and outcomes during neoadjuvant chemotherapy for oesophagogastric cancer: A before and after pilot study.

6.1 Background

Oesophageal and gastric cancer are among the top ten causes of cancer death in the UK. The prognosis is often poor with overall UK 5-year survival rates being approximately 15% and 19% for oesophageal and stomach cancer (UK, 2010-2011), respectively. The majority of patients present with advanced disease and many have significant co-morbidities. Patients presenting with locally advanced resectable disease typically undergo 3 cycles of neoadjuvant chemotherapy (NAC) over 2 months followed by surgery, a regimen based on the MAGIC trial (Cunningham et al., 2006), which leads to down-staging of tumours and significantly improves progression-free and overall survival (Cunningham et al., 2006).

Whilst nutritional depletion is commonly encountered in patients with oesophagogastric (OG) cancers, most patients undergoing NAC do not receive nutritional support. Furthermore, there are limited data on preoperative nutritional support of patients with OG cancer undergoing NAC, the majority of previous studies utilising parenteral nutrition (Sikora et al., 1998), which is expensive, invasive and carries risks of infectious morbidity.

It has previously been demonstrated, in 40 patients with resectable OG cancers undergoing NAC (Awad et al., 2011b), that significant loss of muscle tissue occurs in 85% of patients, mean (SD) estimated loss of fat free mass (FFM) was 3.1 (4) kg over the 2-month course of NAC. Loss of fat free mass is of clinical importance for a number of reasons. Firstly, previous studies (Baracos et al., Prado et al., 2007, Prado et al., 2009, Baracos et al., 2010) in patients with colorectal, breast and lung cancer demonstrated that sarcopenic (loss of fat free muscle mass) patients had a

higher incidence of chemotherapy toxicity. Secondly, preoperative loss of FFM increases surgical morbidity (infectious and non-infectious complications) (Windsor and Hill, 1988). Finally, as muscle is the main site of glucose utilisation, the development of sarcopenia would be expected to lead to an insulin resistant state. Development of the latter is of importance as for every unit increase in intraoperative insulin resistance, the incidence of postoperative major complications, including infectious complications, was increased (odds ratio 2.23, p=0.004) (Sato et al., 2010).

6.2 Study Hypothesis

This study hypothesised that changes in fat free mass (FFM) and fat mass (FM) are associated with changes in insulin sensitivity during neoadjuvant chemotherapy for oesophagogastric cancer.

6.3 Methods

Study design

This 'before and after' pilot interventional study was set in a University teaching hospital. Adult patients aged between 18 and 80 years with confirmed oesophagogastric cancer and due to undergo NAC followed by resectional surgery were identified at the Mid-Trent Upper GI cancer multidisciplinary team (MDT) meeting. They were provided with study information by the clinical team, and contacted by the study team after a minimum of 24 hours. Study exclusion criteria are given in Table 6.1.

Table 6.1. Exclusion criteria

Patients with GIST tumours
Presence of severe organ-specific disease (e.g. heart/respiratory/renal/liver failure)
Presence of inherited metabolic disorders
Simultaneous participation in another clinical study
Patients with suspicion of alcohol/drug abuse
Diabetes mellitus or other endocrine disorders (e.g. thyroid disease, Cushing's syndrome)
For second study cohort receiving intensive nutritional support programme (INSP) • Allergy to any constituent of the nutritional supplements • Total dysphagia (inability to take oral liquids or solids) • Clinical evidence of aspiration

Study regimen

Two cohorts of patients were followed successively. The first cohort was observed prior to commencing chemotherapy, during chemotherapy and at the end of

chemotherapy treatment. The second cohort was enrolled into an intensive nutritional support programme (INSP). The study regimen is outlined in Figure 6.1.

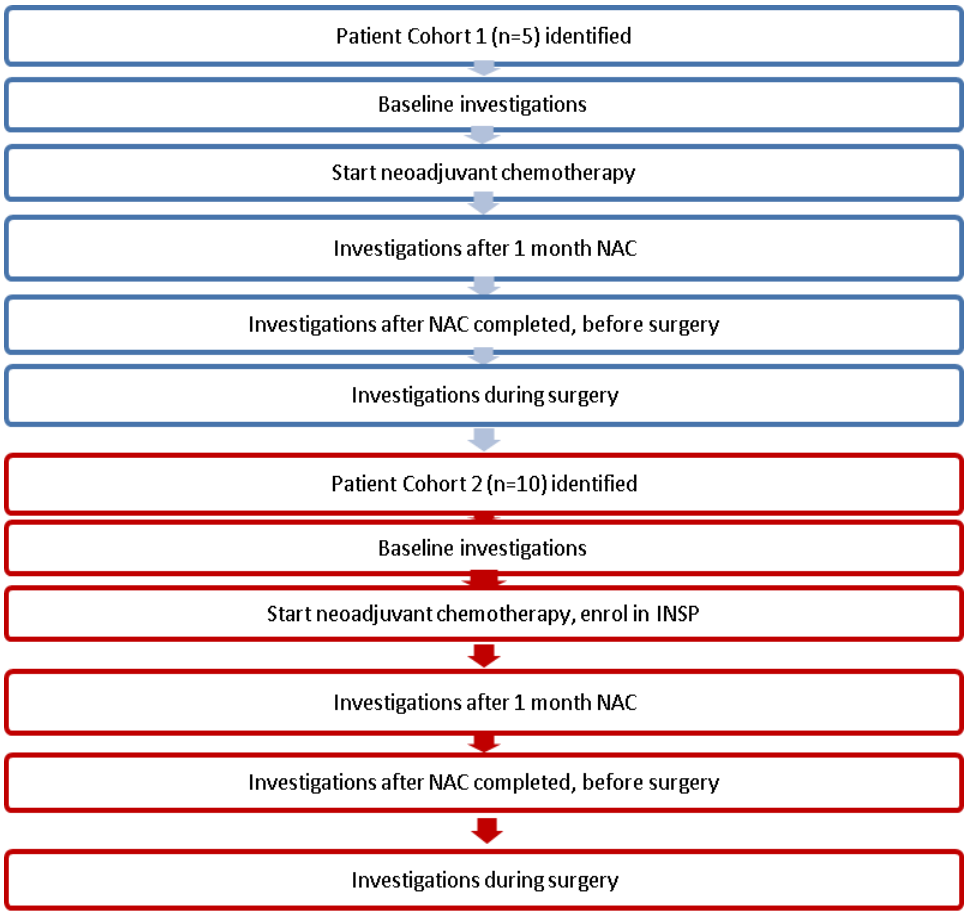


Figure 6.1. Study regimen

Interventions in the INSP included dietetic assessment, dietary advice, oral nutritional supplementation and nasojejunal supplementation, as deemed appropriate by the study dietitian.

Table 6.2 compares standard dietetic care, as provided in cohort 1 and the INSP received by cohort 2.

Table 6.2. INSP vs standard care

Study Interventions	Standard Hospital Care	Intensive Nutritional Support Programme
Dietetic assessment	If ward staff or upper GI specialist nurses are concerned about nutritional status	All patients
Keeping a food diary	All patients	All patients
Anthropometric measurements	Performed by research team	Performed by research team
Dietary advice (improving dietary intake)	If referred to dietitian as above and deemed necessary after assessment	If dietitian deems appropriate after nutritional assessment
Oral nutritional supplements	At patient request or if ward staff/ upper GI specialist nurse deem it appropriate, or via dietitian referral (as above)	If dietitian deems appropriate after nutritional assessment
Nasojejunal feeding	If ward staff/ upper GI specialist nurse deem it appropriate, or via dietitian referral (as above)	If dietitian deems appropriate after nutritional assessment
Regular dietetic review	If referred to dietitian as above and deemed necessary after assessment	All patients

Interventions

Study visit 1

At baseline, following an overnight fast, the following data/ samples were collected from all patients: BMI, anthropometric measurements including mid-arm circumference and triceps skinfold thickness, hand-grip dynamometry, bioelectrical impedance analysis, nutritional assessment comprising history taking and completion of M-SGA, completion of SF-36 questionnaire and sampling of 30ml venous blood. DXA scans were performed and participants underwent spirometry testing. A vastus lateralis (VL) thigh muscle and subcutaneous fat biopsy were taken, followed by the hyperinsulinaemic-euglycaemic clamp and indirect calorimetry. Details of the methods which were also used in previous chapters are given in Chapter 3.

Indirect calorimetry

Subjects were rested supine for 15 minutes in a temperature controlled environment (23°C), prior to measurement of baseline resting energy expenditure (REE), respiratory quotient (RQ), and carbon dioxide production (VCO_2) using a ventilated hood indirect calorimetry system. Further measurements were taken after 1 hour and 2 hours (during the HEC). Results were compared against control patients, recruited as part of a healthy volunteer study (thanks to Carolyn Chee), who underwent calorimetry at baseline and after 2 to 3 hours of a HEC. Although these controls were not matched, there were no significant differences in distribution of BMI or gender between study patients and controls. The insulin

infusion dose in the control group was 60 mU/m^2/min (compared with 120mU/m^2/min in study patients).

Study visit 2

After two cycles of NAC, all participants underwent the same assessments in the oncology outpatient department, but DXA, biopsies and HEC/ indirect calorimetry were not performed.

Study visit 3

After completion of NAC and before planned resectional surgery, after an overnight fast, all assessments performed at visit 1, except spirometry testing, were repeated.

Surgery

Rectus abdominus and abdominal subcutaneous fat biopsies were taken at the start of surgery. A thigh VL and fat biopsy were also taken at this time. Those patients undergoing thoracotomy as part of their planned surgery underwent intercostal muscle biopsy and those not having thoracotomy had a biopsy taken from the diaphragm.

Nutritional Assessment and Intensive Nutritional Support Programme (INSP)

Patients in the first cohort were assessed by a dietitian but underwent standard hospital nutritional care, i.e. further contact with the nutritional team was on *an ad hoc* basis. The second cohort entered an Intensive Nutritional Support Programme (INSP). Baseline nutritional assessment was performed by the study dietitian at the first visit, prior to commencing NAC. Patients were then contacted

by the dietitian, either by telephone, or in person during hospital visits, on a weekly basis. Further nutritional assessments were performed at each study visit. Additional Nutritional Support was provided, either by oral supplementation or nasojejunal feeding to meet nutritional requirements. All patients in Cohort 1 and Cohort 2 were asked to complete food diaries at two timepoints during NAC.

Analysis

The progress of patients during NAC was followed, taking account of the chemotherapy regimen and doses tolerated and the development of side effects/ toxicity reactions. Changes in body composition were analysed from DXA and Sliceomatic analysis of CT images taken as part of routine patient care (method described previously in Chapter 3).

Laboratory analyses

Frozen plasma and serum was used to determine concentrations of inflammatory cytokines according to the methods described in Chapter 3. Fasting insulin and glucose concentrations were used to calculate HOMA-IR.

Statistical analysis

There has been no previous study of change in insulin sensitivity during neoadjuvant chemotherapy for oesophagastric cancer. Therefore, this study was designed as a pilot, to assess the feasibility of such an intensive study protocol in

this patient group. As such, it was not possible to perform a power calculation. However, it was determined that large changes in insulin sensitivity should be detectable in a sample of 10 patients per group. Because of problems with recruitment, at the midpoint of recruitment to the first cohort, this sample size was reduced to 5 patients in cohort 1 and 10 patients in cohort 2.

6.4 Results

During the study period, 84 patients were identified at the UGI MDT meeting as planned for curative treatment including NAC and resectional surgery for OG cancer. All were approached by the clinical team and 12 of these were diabetic and therefore not eligible for inclusion in the study. The remaining 72 patients were approached by the study team. 19 patients agreed to participate in the study. One patient in Cohort 1 underwent the first study visit before planned chemotherapy, but then underwent emergency surgery for bleeding from gastric cancer so NAC was never commenced and the patient was excluded further from the study. A second patient in Cohort1 attended for the first study visit and commenced chemotherapy, but did not attend for any subsequent visits and could not be contacted by the study team, so was also excluded from the study. Two patients in Cohort 2 withdrew after recruitment but before the first study visit. 15 patients, 5 in cohort 1 and 10 in cohort2, completed the study. All had at least 2 cycles of NAC. 12/15 patients underwent surgical resection. Two patients in Cohort 2 were found to have inoperable disease at the time of surgery and one patient declined surgery after completing NAC.

Demographics

Patient demographics are shown in Table 6.3. There was no significant difference in postoperative survival (to the time of data analysis) in those undergoing resectional surgery in those from Cohort 1 compared with Cohort 2 (p=0.11). Median (IQR) length of stay was 9 (7-13) days overall and in Cohort 1 was 13 (7.5-17) and Cohort 2, it was 9 (6-11.5) days.

Table 6.3. Demographic data

	Overall	Cohort 1	Cohort 2
Gender			
Male	14	5	9
Female	1	0	1
Median (IQR) age (years)	66.5 (58.5-70.0)	63.1 (58.1-66.8)	70.0 (67.0-72.2)
Mean (SD) % weight loss last 3 months before study commencement	4.9 (4.5)	4.4 (4.7)	5.8 (4.3)
Comorbidities			
Ischaemic heart disease	1	1	0
Minor surgery	2	1	1
Peripheral vascular disease	1	1	0
Cerebrovascular disease	1	0	1
Previous cancer (BCC)	1	0	1
Smoking history			
Current smoker	1	1	0

Ex-smoker	11	4	7
Never smoked	3	0	3
Alcohol consumption			
None	3	2	1
<20 units/week	9	2	7
20-40 units/week	2	0	2
>40 units/week	1	1	0
Tumour location			
Mid oesophagus	2	0	2
Distal oesophagus	5	2	3
Oesophagogastric junction	7	3	4
Stomach	1	0	1
Pathology			
Adenocarcinoma	13	4	9
Squamous cell carcinoma	2	1	1
pTNM classification			
$pT_{is}N_1M_0$	1	1	0
$pT_2N_0M_0$	2	1	1
$pT_2N_1M_0$	1	0	1
$pT_3N_0M_0$	3	2	1
$pT_3N_1M_0$	2	0	2
$pT_3N_2M_0$	1	0	1
$pT_3N_3M_0$	1	1	0
$T_{4a}N_1M_0$	1	0	1
Inoperable (locally advanced)	2	0	2

Declined surgery after NAC	1	0	1
Mandard tumour regression grade			
1	0	0	0
2	2	1	1
3	1	1	0
4	4	1	3
5	5	2	3
Mean (SD) postoperative survival (days) for curative resection	613.17 (271.20)	762.60(263.61)	506.43 (238.17)

Anthropometrics

There were few differences in anthropometric measures over the time of chemotherapy or between cohorts (Table 6.4). There was, however a significant reduction in mid arm circumference during NAC in all patients, and in Cohort 2. There was also a significant decrease in MAMC in Cohort 2 during NAC, which was reversed afterwards. There was no significant difference in MSGA score during chemotherapy or between cohorts.

Table 6.4. Anthropometrics

	Pre chemo	Mid chemo	Post chemo	p value
Waist hip ratio (Mean ± SD)				
All	0.955 ± 0.057	0.935 ± 0.058	0.950 ± 0.039	0.561
Cohort 1	0.959 ± 0.054	0.949 ± 0.057	0.960 ± 0.034	0.862
Cohort 2	0.947 ± 0.069	0.906 ± 0.055	0.928 ± 0.041	0.538
Mid arm circumference (cm) (Mean ± SD)				

All	29.456 ± 3.222	21.131 ± 8.890	26.477 ± 7.288	0.007
Cohort 1	30.184 ± 3.570	22.929 ± 8.800	25.904 ± 8.909	0.114
Cohort 2	28.002 + 1.927	17.534 ± 8.845	27.622 ± 2.199	0.015
Triceps skin fold thickness (mm) (Mean ±SD)				
All	16.938 ± 4.900	12.580 ± 5.730	16.934 ± 8.336	0.118
Cohort 1	16.174 ± 4.081	11.804 ± 5.964	15.434 ± 7.413	0.234
Cohort 2	18.468 ± 6.500	14.132 ± 5.511	19.934 ± 10.135	0.481
MAMC (cm) (Mean ±SD)				
All	22.347 ± 6.520	17.178 ± 8.400	21.156 ± 6.204	0.126
Cohort 1	22.420 ± 8.055	19.220 ± 7.900	21.055 ± 7.426	0.659
Cohort 2	22.200 ± 1.660	13.094 ± 8.681	21.359 ± 3.246	0.039
Hand grip strength (kg)(Mean +/- SD)				
All	30.320 ± 7.125	31.113 ± 8.907	29.647 ± 8.439	0.887
Cohort 1	31.227 ± 7.950	31.847 ± 10.016	30.640 ± 9.668	0.958
Cohort 2	28.506 ± 5.411	29.646 ± 6.924	27.660 ± 5.619	0.873
Body mass index (kg/m^2) (Mean ±SD)				
All	26.240 ± 4.864	26.247 ± 5.066	26.480 ± 5.102	0.989
Cohort 1	27.140 ± 5.531	27.300 ± 5.568	27.540 ± 5.606	0.987
Cohort 2	24.440 ± 2.813	24.140 ± 3.427	24.360 ± 3.457	0.989
MSGA score (Median, IQR)				
All	12 (9-15)	10 (8-11)	9 (8-13)	0.324
Cohort 1	13 (9-15)	10 (9-11)	10 (7.5-14)	0.333
Cohort 2	9 (7-15)	8 (7-12.5)	18 (7.5-15.5)	0.855
Overweight				
All	9	9	9	
Cohort 1	3	3	3	
Cohort 2	6	6	6	
Obese				

All	3	3	3	
Cohort 1	2	2	2	
Cohort 2	1	1	1	
Sarcopenia (CT analysis)				
All	6		9	
Cohort 1	2		3	
Cohort 2	4		6	

Study patients vs controls

The mean ± SD BMI of the control group was 26.24 ± 4.86 and in the study group was 26.240 ± 4.864 (p = 0.656). The median (IQR) age of controls was 70.35 (69.2-72.3) years and in the study group was 66.5 (58.5-70.0) (p = 0.039).

SF-36

SF-36 scores were analysed using Quality Metric Incorporated Health Outcomes Scoring Software©. There were individual differences in Physical Health and Mental Health Scores, but overall there was no trend or significant differences during NAC (Figure 6.2).

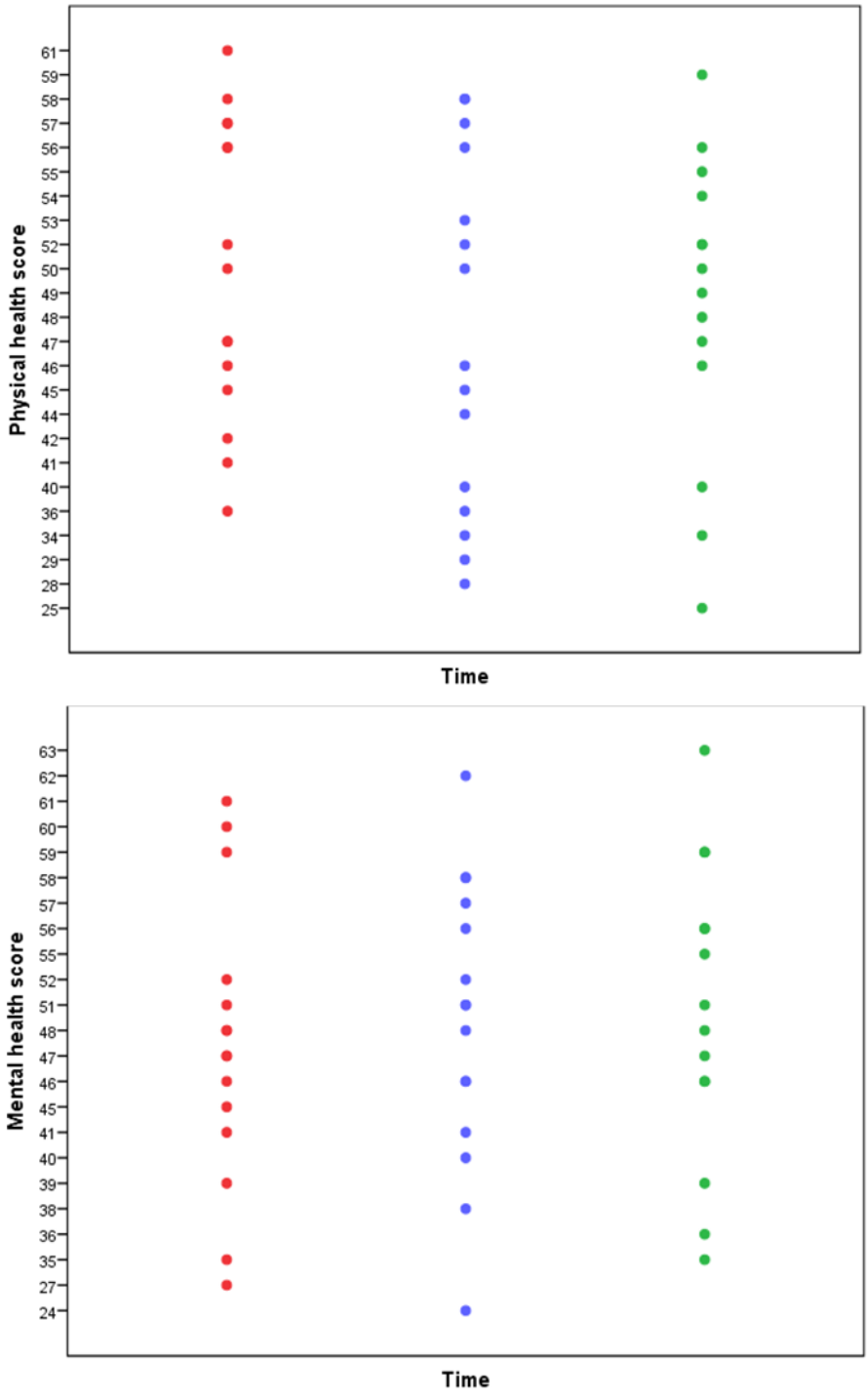


Figure 6.2. SF-36 individual scores

SF-36 questionnaires were administered before starting chemotherapy, during chemotherapy and at the end of chemotherapy. There were no significant differences between SF-36 scores at any of the timepoints.

There were no differences in each of the SF-36 domains during the course of NAC (Table 6.5).

Table 6.5. SF-36 domain scores

	PF	RP	BP	GH	VT	SF	RE	MH	PCS	MCS
Prechemo	52.309 ± 5.898	44.133 ± 10.437	51.113 ± 12.627	51.822 ± 6.464	51.409 ± 12.645	45.308 ± 10.852	46.884 ± 10.080	48.601 ± 9.580	50.710 ± 7.479	47.093 ± 9.218
Midchemo	48.355 ± 8.565	44.433 ± 11.023	51.327 ± 9.822	48.209 ± 9.186	48.242 ± 10.645	43.971 ± 10.995	49.670 ± 10.435	51.392 ± 8.630	47.073± 9.643	49.461 ± 10.041
Postchemo	48.519 ± 9.597	41.437 ± 9.528	51.056 ± 11.127	47.516 ± 10.727	50.264 ± 10.170	41.585 ± 12.570	47.961 ± 7.799	51.055 ± 9.261	46.309 ± 10.066	48.761 ± 8.664
p value (ANOVA)	0.335	0.698	0.998	0.380	0.738	0.680	0.725	0.665	0.383	0.777

PF Physical Functioning, **RP** Role Limitations due to Physical Problems, **GH** General Health Perceptions, **VT** Vitality ,**SF** Social Functioning, **RE** Role Limitations due to Emotional Problems, **MH** General Mental Health, **PCS** Physical Component Summary Score, **MCS** Mental Component Summary Score

Dietetic results

In cohort 1, all patients were failing to meet energy requirements at baseline (prior to commencing NAC) and 4/5 patients (80%) were failing to meet protein requirements. In cohort 2, 4/10 (40%) patients were failing to meet energy requirements and 7/10 (70%) patients were failing to meet protein requirements. The mean energy and protein deficits in those failing to meet requirements are given in Table 6.6.

Table 6.6. Daily energy and protein deficits prior to commencing NAC

	Cohort 1	Cohort 2
Energy Deficit (kcal)	506	476.25
(MJ)	2.12	1.99
Protein Deficit (g)	31.9	23.4

After two cycles of chemotherapy, one patient in cohort 1 could not be reassessed as no food diary was completed. Three patients were still failing to meet energy requirements with a mean deficit of 488.5 kcal (2.04 MJ) and three patients failed to meet protein requirements with a mean deficit of 13.7 g. All patients in cohort 2 were meeting or exceeding energy requirements but 2/10 (20%) were still failing to meet protein requirements with a mean deficit of 18.5 g.

After completion of chemotherapy, there was no available information on energy or protein intake for cohort 1 as no food diaries were returned. In cohort 2, 9/10 patients (90%) were meeting energy requirements – the deficit in one patient was 301.5 kcal (1.26 MJ). All patients were meeting protein requirements.

In cohort 1, patients had a median of 1 dietetic contact, while in cohort 2, the median number of contacts was 8 (p=0.0017). Type of supplementation given in each cohort is given in Table 6.7.

Table 6.7. Nutritional supplementation during NAC

Supplement Type	Cohort 1	Cohort 2
Oral nutritional supplements	2/5 (40%)	5/10 (50%)
Nasojejunal feeding	0/5	3/10 (30%)

Body composition analysis

DXA results were available in 14/15 patients pre-NAC and 13/15 post-NAC. There was no significant difference in fat mass (p=0.6355) or lean mass (p=0.2661) as measured on pre-chemotherapy and post-chemotherapy DXA scans (Figure 6.3).

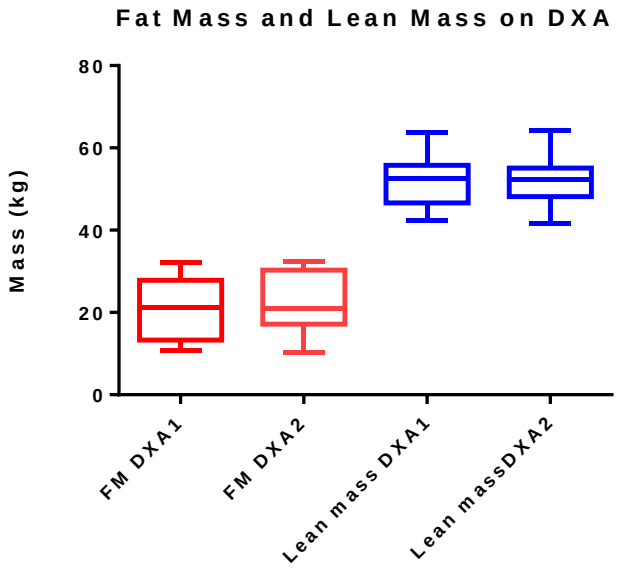


Figure 6.3. Lean mass and fat mass (FM) on DXA before (DXA1) and after (DXA2) NAC

There was no significant difference between lean mass assessed on DXA before and after neoadjuvant chemotherapy (NAC) (p= 0.2661). There was no significant difference in fat mass assessed on DXA before and after NAC (p=0.6335).

Analysis of body composition – CT, DXA and BIA

CT scans were available for 11/15 patients before and after NAC. There was a significant decrease in fat free mass during chemotherapy (p=0.042) but no change in fat mass (p=0.6377) (Figure 6.4).

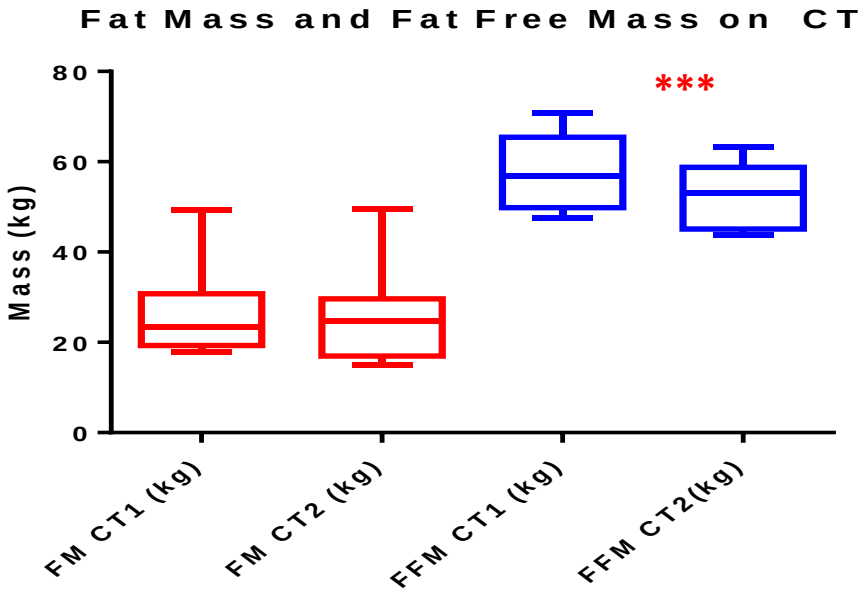


Figure 6.4. Fat mass (FM) and fat free mass (FFM) on CT before (CT1) and after (CT2) NAC *** statistically significant

There was no significant difference in fat mass tested assessed on CT analysis before and after neoadjuvant chemotherapy (NAC) (p=0.6377).There was a significant decrease in fat free mass assessed on CT analysis before and after NAC (p= 0.0.042).

From the CT scans available for analysis, sarcopenia was present pre-chemotherapy in 2/4 (50%) in cohort 1 and 4/8 (50%) in cohort 2. Post chemotherapy sarcopenia was present in 3/4 (75%) in cohort 1, 6/8 (75%) in cohort 2.

8/15 (53.3%) completed all planned cycles of chemotherapy. Of those who did not complete chemotherapy, 4/7 (57.1%) were sarcopenic at baseline and 5/7 (71.4%) were sarcopenic at the end of chemotherapy. Of those who completed

chemotherapy, 2/8 (25%) were sarcopenic at baseline and 2/8 (25%) were sarcopenic at the end of NAC.

Insulin sensitivity

There was a significant decrease in insulin sensitivity (M value) between pre-chemo and post-chemo clamps (p=0.049) (Figure 6.5).

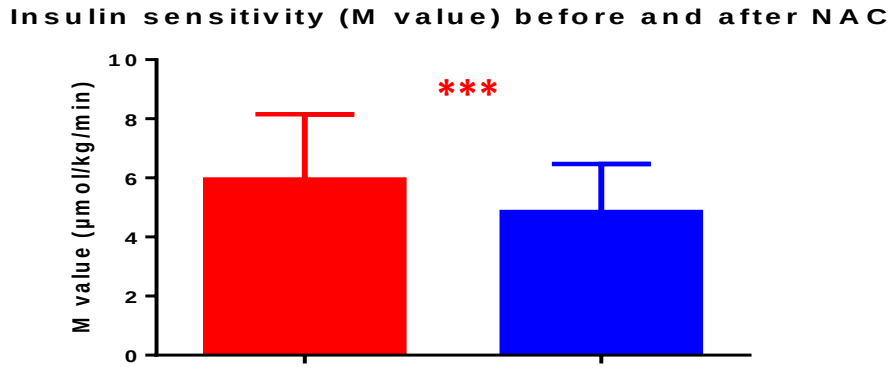


Figure 6.5. Insulin sensitivity before and after NAC

*** statistically signficant

Insulin sensitivity, measured by M value from the HEC, decreased significantly between the pre chemotherapy study and the post chemotherapy study (p=0.049).

There was no difference in insulin sensitivity between sarcopenic and non-sarcopenic patients pre-NAC (p=0.5686) or post NAC (p=0.8925). Insulin resistance was present in 6/15 (40%) of patients at Visit 1 and 8/15 (53.3%) patients at Visit 2. These results are shown per cohort in Table 6.8.

Table 6.8. Insulin resistance per cohort and per visit

Insulin Resistance	Visit 1	Visit 2
Cohort 1	2/5 (40%)	3/5 (60%)
Cohort 2	4/10 (40%)	5/10 (50%)

Indirect calorimetry

There was no significant change in fat oxidation during the pre-NAC clamp (p=0.2874) but there was a decrease in fat oxidation during the post-NAC clamp (p=0.0025) (Figure 6.6). Similarly, there was no significant change in carbohydrate oxidation during the pre-NAC clamp (p=0.0904) but there was an increase in carbohydrate oxidation during the post-NAC clamp (p=0.0003). Non-oxidative glucose disposal rate was calculated by subtracting the glucose oxidation rate from the whole body glucose disposal rate.

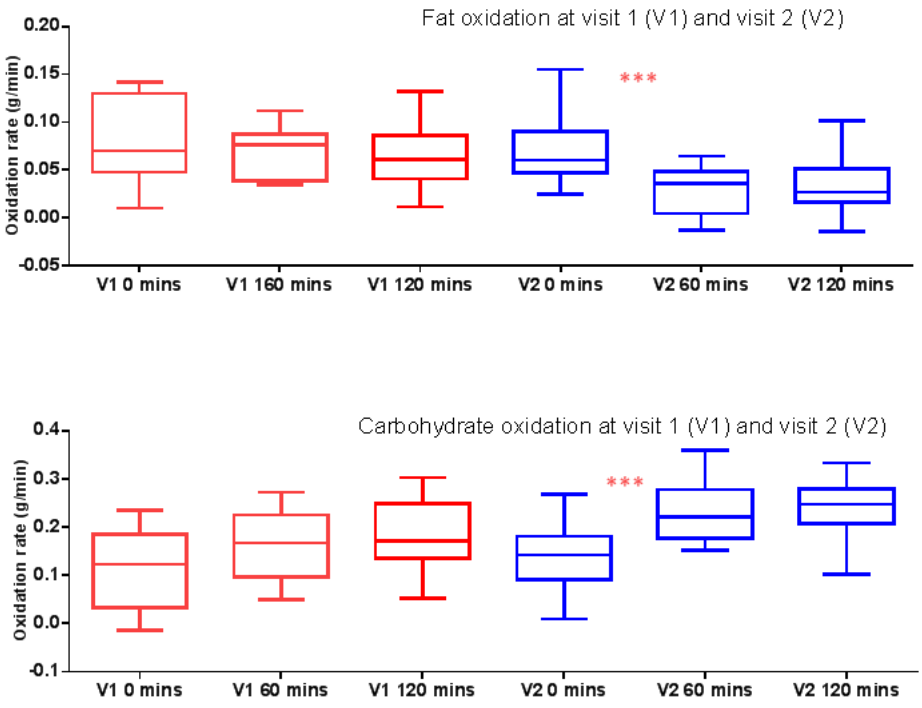


Figure 6.6. Carbohydrate and fat oxidation during clamps pre and post NAC *** Statistically significant

Carbohydrate and fat oxidation was measured prior to commencing the clamp, at 60mins, and at 120 mins. Measurements were taken at the pre-neoadjuvant chemotherapy (NAC) visit and at the post-NAC visit. There was no significant change in fat oxidation during the pre-NAC clamp (p=0.2874) but there was a decrease in fat oxidation during the post-NAC clamp (p=0.0025). There was no significant change in carbohydrate oxidation during the pre-NAC clamp (p=0.0904) but there was an increase in carbohydrate oxidation during the post-NAC clamp (p=0.0003).

Figure 6.7 shows a significant decrease in non-oxidative glucose disposal between the pre and post-NAC visits (p=0.0355).

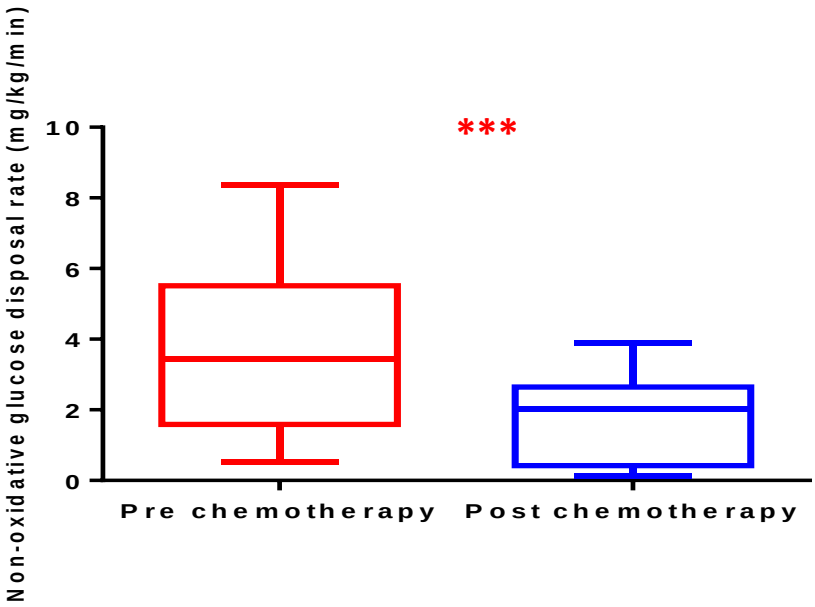


Figure 6.7. Non-oxidative glucose disposal pre and post NAC

*** statistically significant

Non oxidative glucose disposal was calculated by calculating glucose oxidation rate from whole body glucose disposal rate at 120mins. There was a significant decrease in non-oxidative glucose disposal between pre-chemotherapy and post chemotherapy studies (p=0.0355).

Calorimetry in control subjects

In healthy volunteers used as control subjects, taken from a study performed by Carolyn Chee, there was a decrease in fat oxidation during the clamp and an increase in carbohydrate oxidation (Figure 6.8).

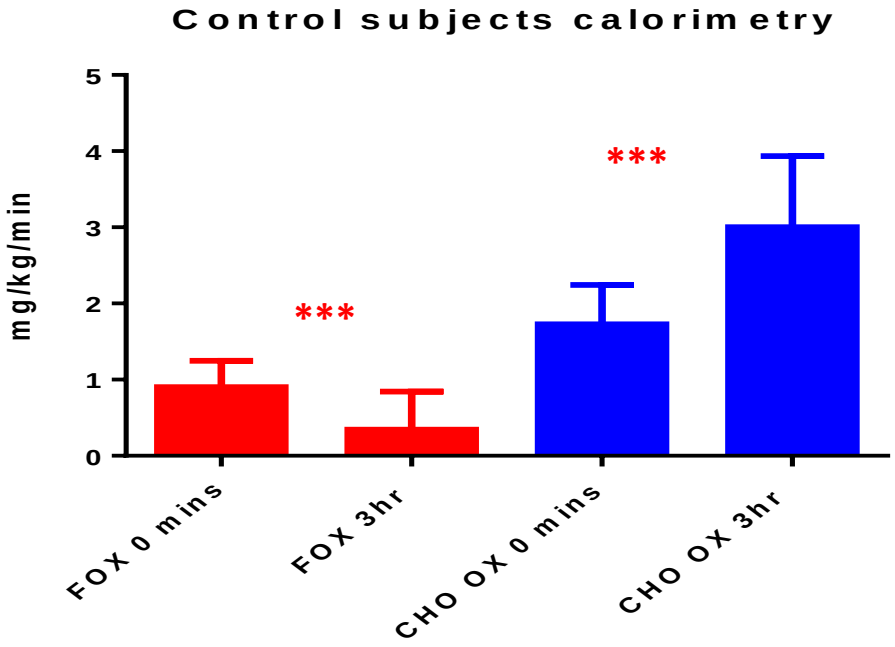


Figure 6.8. Calorimetry in control subjects *** statistically significant
In healthy volunteer data, used as controls, there was a decrease in fat oxidation (FOX) (p=0.0156) during the clamp and an increase in carbohydrate oxidation (CHO OX) (p-0.0313).

There was a significant difference in non-oxidative glucose disposal (Figure 6.9)

between cancer patients and controls but no significant difference in fat oxidation.

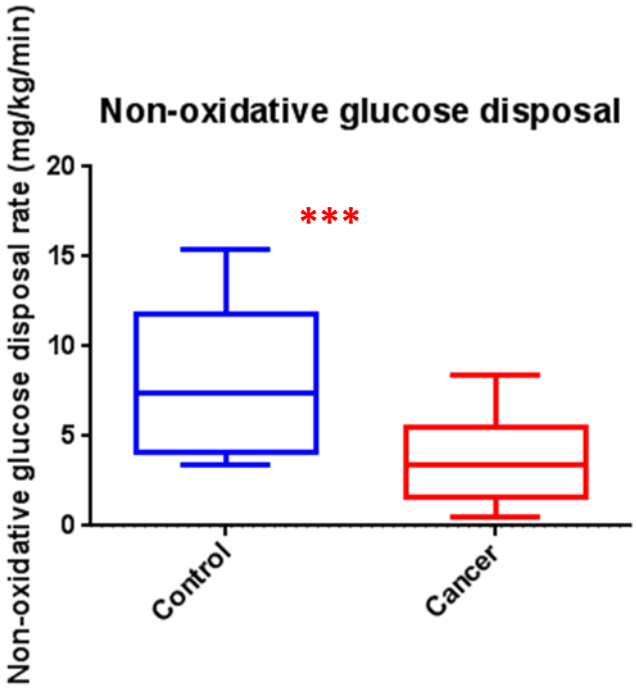


Figure 6.9. Non-oxidative glucose disposal in cancer patients and controls

*** statistically significant

There was a significant difference in non-oxidative glucose disposal between cancer patients and controls (p=0.0159)

Metabolites

Serum metabolites including cytokines, inflammatory markers and catecholamines were measured at each study visit. There was a significant change in TAG and FFA during NAC (Figure 6.100).

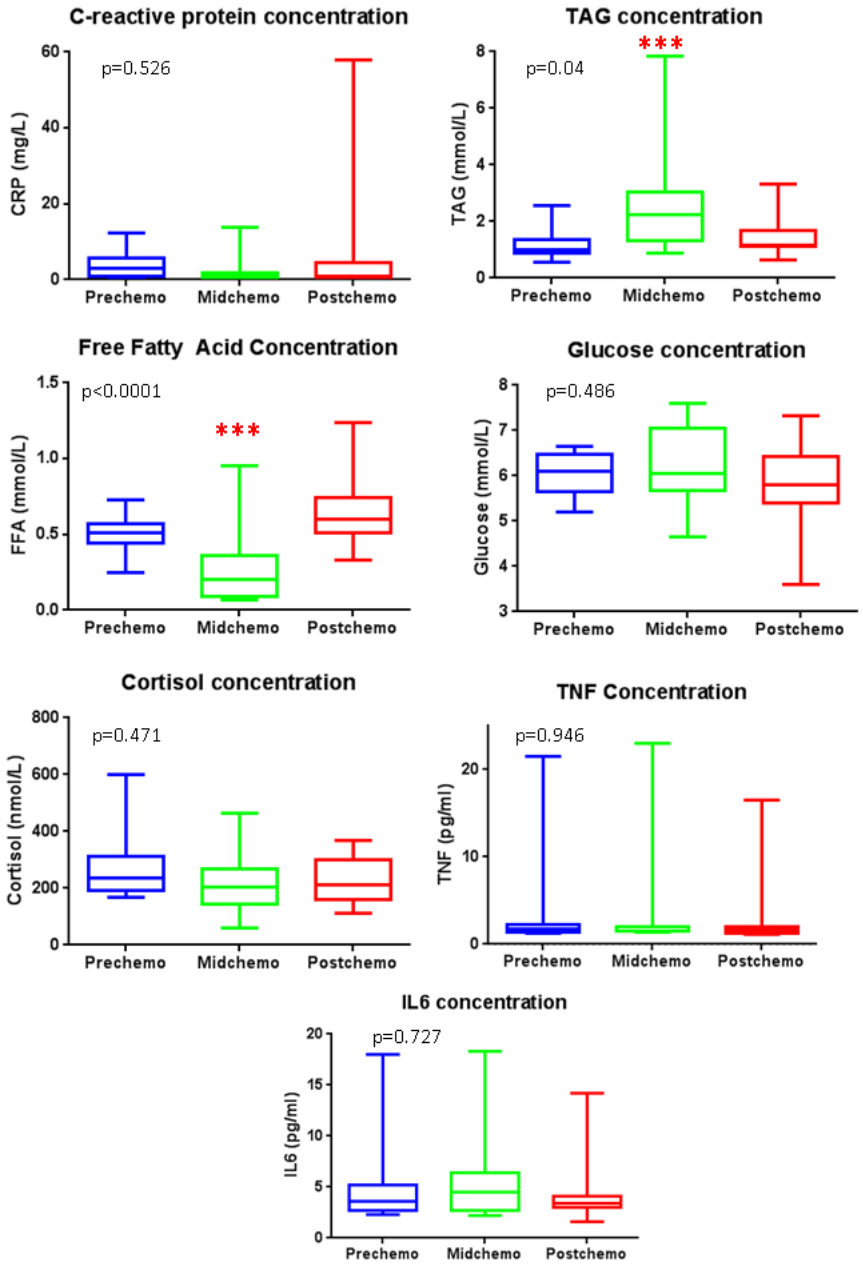


Figure 6.100. Metabolite concentrations *** statistically significant
Serum metabolites were measured pre-chemotherapy, during chemotherapy and after chemotherapy. There was a significant increase in triacylcglycerol concentration (TAG) during chemotherapy which fell back to baseline after chemotherapy finished (p=0.04). There was a significant increase in free fatty acid concentration during chemotherapy which rose to higher than baseline concentration after chemotherapy finished (p<0.0001).

There was a significant change in noradrenaline concentration during NAC (Figure 6.111).

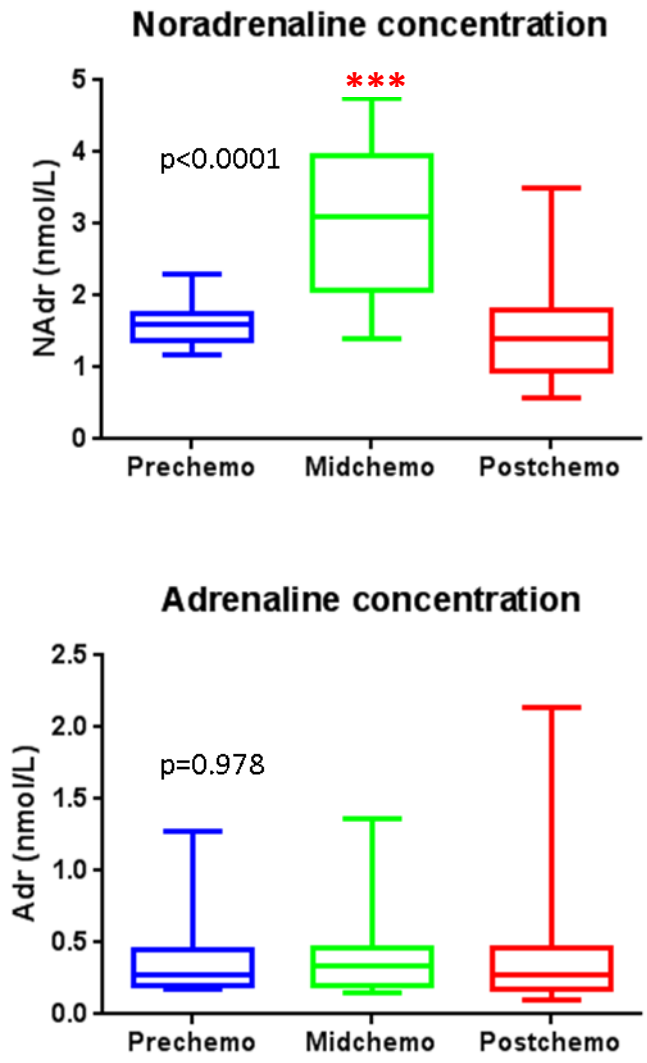


Figure 6.111. Metabolite concentrations during NAC *** statistically significant. There was a significant increase in noradrenaline concentration during chemotherapy and this decreased again post chemotherapy (p<0.0001). There was no significant change in adrenaline concentration during chemotherapy (p=0.978).

6.5 Discussion

This was an intensive study of body composition and metabolic changes in patients undergoing neoadjuvant chemotherapy for oesophagogastric cancer. It has demonstrated that, despite few significant changes in anthropometric measures, body mass index or body composition, sarcopenia is prevalent and there are associated changes in insulin sensitivity, carbohydrate oxidation and

several metabolites. In particular, it is interesting to note that there was a greater prevalence of sarcopenia in those who did not complete chemotherapy compared with those who did. Previous studies have demonstrated that sarcopenic patients had a higher incidence of chemotherapy-related toxicity (Prado et al., 2009) and shorter time to tumour progression (Prado et al., 2008). In oesophageal cancer, patients with dose-limiting toxicity (DLT) had lower skeletal muscle mass than those without DLT (Anandavadivelan et al., 2015). Sarcopenic obesity was also associated with increased risk of DLT (Anandavadivelan et al., 2015). There were no patients in the present study who were sarcopenic and obese.

It was hypothesised that the mechanism underlying the association between body composition and outcomes in cancer may be related to insulin resistance. In the present study, there was no association between sarcopenia and IR. Previous studies have shown that cancer cachexia is accompanied by metabolic abnormalities, including alterations in protein and lipid metabolism and insulin resistance (Argiles et al., 1997, Tisdale, 1999). These alterations may not necessarily be accompanied by a significant change in body mass, a finding that is demonstrated in the present study. There was a demonstrated a reduction in insulin sensitivity over the period of NAC. Interestingly, on DXA, which is considered the 'gold standard' for assessment of body composition, there was no difference in fat mass or fat free mass during NAC. However CT analysis did demonstrate a reduction in FM. There is further discussion of the utility of different methods of assessing body composition in Chapter 7.

A recent study using BIA found reduced FFM in some patients undergoing NAC for OG cancer and there was some evidence of an association with postoperative complications in those with greater loss of FFM (Ida et al., 2014). In another study using CT, the prevalence of sarcopenia before NAC was 26% and this increased to 43% after NAC (Yip et al., 2014). Although there was no association between body composition and survival, loss of FM was associated with risk of circumferential resection margin positivity (Yip et al., 2014).

In the present study, there were several significant metabolic changes associated with NAC, notably a reduction in insulin sensitivity based on the M value obtained from the post chemotherapy HEC compared with the pre chemotherapy HEC. A potential causative association between adiposity and oesophageal carcinoma has been reported (Renehan et al., 2008). The risk of abdominal obesity appears to be independent of BMI (Corley et al., 2008, O'Doherty et al., 2012). It is accepted that obesity is a state of chronic-low-grade systemic inflammation and is associated with IR as well as increased risk of many cancers (Yeung et al., 2013). There is no literature available on change in insulin sensitivity using the HEC during NAC. However, the present findings of a reduction in insulin sensitivity are supported by the associated reduction in non-oxidative glucose disposal. This suggests that there is decreased whole body glucose utilisation. In addition, the finding of reduced non-oxidative glucose disposal in cancer patients compared with controls supports the theory that cancer, like obesity, is a pro-inflammatory, insulin-resistant state. In the absence of significant changes in body composition in the present study, and with the paucity of literature in this area, it is difficult to fully

explain the metabolic results. However, it is certainly an interesting finding worthy of future research.

There were also effects on fat oxidation, which did not change during the pre-NAC clamp, but decreased during the post-NAC clamp. In healthy controls, fat oxidation decreased during the HEC. This is in line with results reported in healthy volunteer studies (Gjedsted et al., 2007). It is also supported by the finding of increased serum FFAs and TAGs during the course of NAC. A study performed more than 30 years ago in patients with oesophageal cancer demonstrated normal resting energy expenditure and plasma catecholamines (Klein and Wolfe, 1990), The rate of triglyceride-cycling in cancer patients were similar to their cachectic control group and 50% greater than normal weight volunteers. They concluded that the increase in lipolysis in cachexic cancer patients was due to alterations in nutritional status (Klein and Wolfe, 1990).

In the present study, subjective assessment of nutritional status using the M-SGA did not suggest a significant nutritional deficit for most patients. However, more detailed dietetic assessment revealed that all patients were failing to meet energy requirements at baseline. It is notable though that the study population did not have low average BMI and their BMI did not change significantly over the period of NAC. This confirms that BMI is a poor assessor of nutritional status in this population. It is likely that poor nutritional status contributed to the observed alterations in metabolic parameters. This study aimed to improve nutritional status in the second patient cohort through provision of an INSP. Unfortunately, poor return of patient questionnaires and data loss did not allow INSP to be

adequately compared with standard dietetic care. In addition, all patients in this study received more intensive assessment and support than the standard patients undergoing NAC for OG cancer, simply by participating in the study. This may have contributed to the finding of little change in nutritional status during NAC. Previous studies in cancer have shown better nutritional status to be associated with better survival outcomes (Rodrigues et al., 2015, Lee et al., 2015).

The main limitation of this study is small numbers which make any of the findings difficult to interpret. Patients deemed suitable for curative treatment for OG cancer in our institution are required to attend for up to 7 hospital appointments prior to commencing an intensive regime of chemotherapy followed by major surgery. Therefore, it is unsurprising that the study was extremely difficult to recruit to. Although it may never be feasible to perform such an intensive study in a larger cohort from this patient group, it may be possible to reproduce some aspects of the study to confirm findings and guide changes to management. Another limitation was the loss of follow-up data in the first cohort not receiving INSP. Again, this was probably related to the numerous demands placed on OG cancer patients prior to commencing NAC. However, the very good recording of results in the INSP group lends support to the hypothesis that intensive dietetic input is beneficial for OG cancer patients.

In conclusion, this study has demonstrated that significant metabolic changes occur during NAC for OG cancer, independent of changes in BMI or body composition. Oesophagogastric cancer prognosis is poor, even for patients in

whom curative treatment is possible. Therefore, it is important to identify any interventions which could reduce morbidity and improve quality of life. The results of this study provide further evidence that nutritional support may be beneficial in this patient group.

Chapter 7 A comparison of three methods of assessing body composition

7.1 Background

The assessment of fat free mass (FFM) and fat mass (FM) has been shown to correlate better with relevant reduction in muscle mass than change in body weight or body mass index (BMI) (Prado et al., 2008). Sarcopenia has recently been defined as reduced skeletal muscle mass associated with low muscle strength and/or poor physical performance (Cruz-Jentoft et al., 2010). Skeletal muscle index (SMI) at the L3 level, normalised for height, of ≤ 38.5 cm^2/m^2 in women and ≤52.4 cm^2/m^2 in men (Prado et al., 2008, Fearon et al., 2011) has been used as part of the definition of sarcopenia. Several studies have suggested that presence of sarcopenia, particularly in obese patients, is associated with poor outcomes in some cancers (Tan et al., 2009, Prado et al., 2008). Therefore, the analysis of body composition is translating from a largely research tool to a potentially important clinical prognosticator.

A number of techniques have been used to assess body composition. Dual X-ray absorptiometry (DXA) is the gold-standard non-invasive method of measuring FM and FFM (Lee and Gallagher, 2008). However, it requires exposure to radiation, albeit at a low dose. Recently, analysis of CT or MRI scans using software to assess surface areas of different tissues at a certain level, has emerged as a useful technique for assessing FFM and FM, and calculating SMI (Awad et al., 2012b). Scans which are taken for clinical indications are used usually, to avoid exposing the patient to repeated imaging and, in the case of CT, high radiation doses. Bioelectrical impedance analysis (BIA) has been used as a simple, non-invasive tool for assessment of body composition over the last 40 years (Lukaski et al., 1985).

Results have been reported as comparable to those obtained from other methods in some studies (Beshyah et al., 1995), but there are also reports which question its accuracy. DXA analysis of body composition remains the gold standard but there is a large volume of emerging literature on the use of alternative methods, such as CT analysis (Mourtzakis et al., 2008). Therefore, it is important when conducting research using any of these methods to ensure they are comparable in the relevant study population. This study sought to compare results obtained for measures of body composition from CT analysis and BIA with DXA.

7.2 Study hypothesis

This study hypothesised that estimation of fat free mass from dual X-ray absorptiometry was comparable to CT analysis and bioelectrical impedance analysis in patients undergoing abdominal surgery or neoadjuvant chemotherapy for oesophagogastric cancer.

7.3 Methods

Results were taken from the two previously described clinical trials on metabolic effects of surgery and neoadjuvant chemotherapy for oesophagogastric cancer. Results of CT analysis, DXA and BIA were compared in their estimation of FM (DXA and CT) and FFM (BIA, DXA and CT).

Statistical analysis

Bland Altman plots were used to estimate agreement between methods and bias. Statistical analysis was performed using GraphPad Prism v6.04 (GraphPad Software Inc, La Jolla, USA). Based on the results of a previous study (Mourtzakis

et al., 2008) and with an estimated standard deviation of 10% in measurements of body composition, 17 participants would be required to detect pair-wise differences of 13% in fat free mass between methods with a power of 80% and a significance level of 0.05.

7.4 Results

A total of 56 DXA scans, 48 CTs and 61 BIAs were performed in 47 patients. 47 sets of paired results of DXA-CT were available, and 56 paired DXA-BIA results were available. There was good correlation between FM on DXA and CT and FFM on DXA and CT (Figure 7.1).

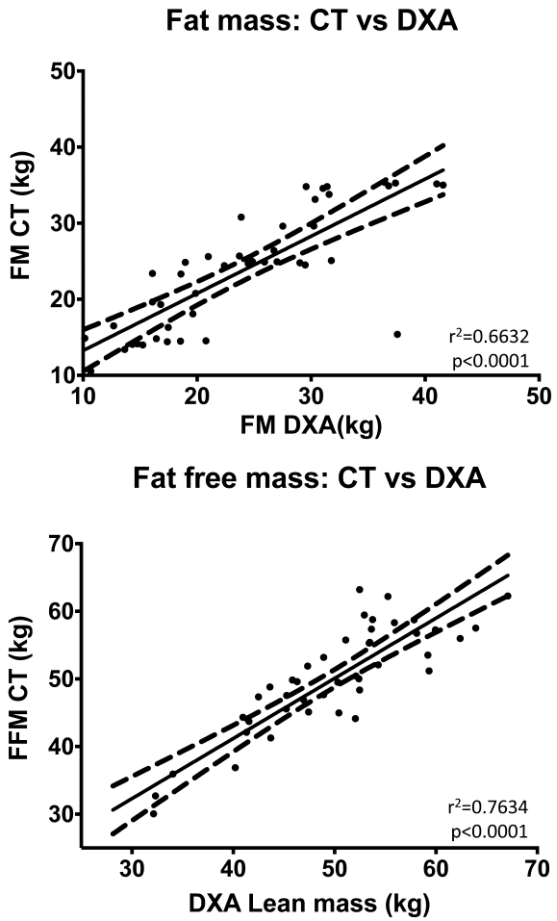


Figure 7.1. Correlation between CT and DXA

There was excellent correlation between fat mass measured on CT and DXA (r^2=0.6632, p<0.0001) and fat free mass measured on CT and DXA (r^2=0.7634, p<0.0001).

There was significant correlation between FFM on DXA and BIA. Correlation between FFM on CT and BIA was also statistically significant (Figure 7.2).

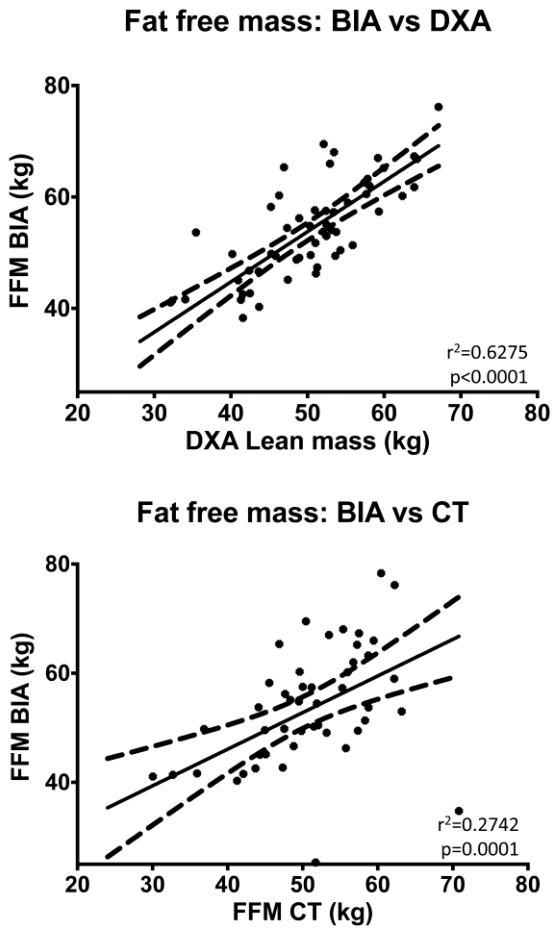


Figure 7.2. Correlation between BIA and DXA/CT

There was statistically significant correlation between fat free mass measured on BIA and DXA (r^2=0.6275, p<0.0001) and between fat free mass measured on BIA and CT (r^2=0.2742, p=0.0001).

On Bland Altman analysis, average bias for FM on DXA and CT was 0.2564, 95% limits of agreement (LOA) were -9.451 to 9.964. For FFM on DXA and CT, average bias was -0.1477, LOA were -8.621 to 8.325. For FFM on DXA and BIA, average bias

was -3.792, LOA were -15.52 to 7.936 and FFM on CT and BIA, average bias was -2.661, LOA were -22.71 to 17.39 (Figure 7.3).

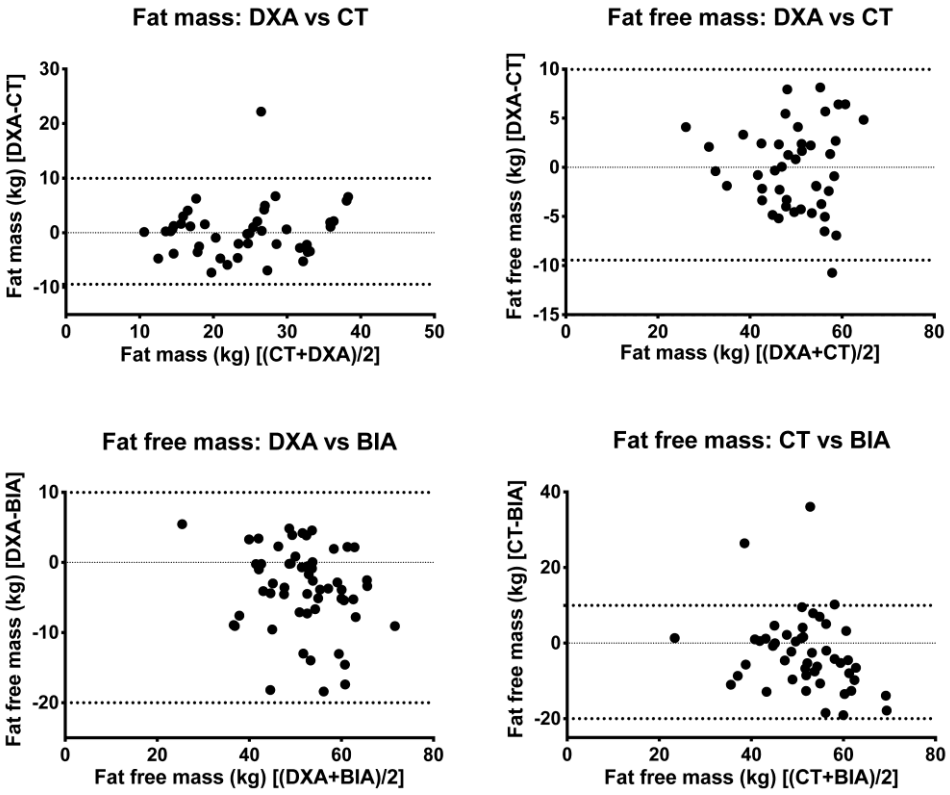


Figure 7.3. Bland Altman analysis of DXA, CT and BIA

On Bland Altman analysis, average bias for FM on DXA and CT was 0.2564, 95% limits of agreement (LOA) were -9.451 to 9.964. For FFM on DXA and CT, average bias was -0.1477, LOA were -8.621 to 8.325. For FFM on DXA and BIA, average bias was -3.792, LOA were -15.52 to 7.936 and FFM on CT and BIA, average bias was -2.661, LOA were -22.71 to 17.39.

7.5 Discussion

Body Mass Index (BMI) has been used as an indicator of risk related to obesity in a variety of studies, but it allows no differentiation between adipose tissue and lean mass. Therefore, methods of determining body composition more accurately have emerged. These include water dilution, densitometry, anthropometry, DXA, analysis of CT and MRI and BIA. The use of DXA is limited in some situations due to the cost of equipment, the need for trained operators, the lack of portability and need for exposure to ionising radiation. CT also requires expensive equipment, trained operators and exposes the subject to a high dose of radiation. The analysis of body composition from CT and MRI necessitates specialist software and training. BIA is practical, non-invasive and easy to perform.

However, studies evaluating BIA have provided inconsistent findings, some reporting good accuracy (Jebb et al., 2007, Andreoli et al., 2002), while others have shown poor results, particularly in obese populations (Frisard et al., 2005, Deurenberg, 1996, Segal et al., 1988). There are several equations available for the estimation of body composition from BIA. Many are gender specific and some have corrections of hydration constants for obese populations. The adoption of an inappropriate equation may explain these conflicting results. In addition, there is variability in the equipment used to measure BIA, some studies reporting single frequency (SF-BIA) and, more recently, some reporting multi-frequency (MF-BIA).

In this study there was excellent correlation between results obtained from DXA and analysis of CT scans. There was also very strong correlation between results obtained from DXA and MF-BIA. The correlation between CT analysis and results

obtained from BIA was poorer, but still statistically significant. This was also reflected in the Bland Altman analysis. Average bias was low for CT/DXA but higher for comparisons with BIA. Limits of agreement were much wider for CT vs BIA compared with DXA vs BIA and higher for DXA v BIA than for DXA v CT.

The subjects in our current study were a heterogeneous group, with both obese and lean patients undergoing elective abdominal surgery, while others were patients with oesophagogastric cancer. Therefore, their hydration status may have been inconsistent and this may have impacted on readings obtained from BIA. A previous study has shown that MF-BIA underestimated body fat to a greater extent in obese men compared to women (Sun et al., 2005). However, this could also have had an impact on DXA readings, and even CT measurements.

Although BIA may not be as accurate for estimation of body composition as other more invasive methods, a recent systematic review has suggested that it is useful if implemented as a longitudinal tool, e.g. before and after an intervention, as long as hydration status does not alter (Haverkort et al., 2015). In the present study, BIA was used to estimate body composition, in combination with DXA and analysis of CT scans, before and after surgical intervention or chemotherapy. In this context, it is a useful tool for research and particularly for clinical purposes.

Chapter 8 Critique of thesis

Development of marked IR has been demonstrated to correlate with poor surgical outcomes (Awad et al., 2013, Sato et al., 2010, Awad et al., 2009). Obesity-induced IR appears, in part, to be associated with inflammatory changes associated with increased adiposity (Samaras et al., 2010, Festa et al., 2001). Certainly, obese individuals without proinflammatory adipose tissue changes display more favourable clinical characteristics, such as arterial function similar to normal weight individuals (Farb et al., 2011). Obesity-associated inflammation has also been shown to be a risk factor for colorectal cancers (Moghaddam et al., 2007), colorectal adenomas (Sato et al., 2011) and components of the metabolic syndrome. Individuals with increased central adiposity may constitute an 'at-risk' phenotype. However, the mechanisms underlying obesity-associated IR are as yet poorly understood (Ballantyne et al., 2005). Obesity certainly has implications for the surgical patient, both with regards to the increased risk of developing pathology and the potential increased risks of obesity- associated IR.

Adipose tissue has been identified as an active endocrine organ and the chemokines secreted as a result of macrophage infiltration appear to play a role in the pathogenesis of IR. VAT appears to be the most metabolically active, although results across studies are not consistent. In addition, oxidative stress, mitochondrial dysfunction and a number of other components of the metabolic pathway have been implicated.

However, results from animal and human studies often provide conflicting results which have rendered the pursuit of a common mechanistic pathway challenging.

This thesis has confirmed that open abdominal surgery is associated with a significant reduction in insulin sensitivity in both obese and non-obese individuals. The magnitude of decrease in insulin sensitivity was not affected by obesity, preoperative carbohydrate loading, cancer status, length of surgery or age. It should be noted in stating this, that all operations included in the perioperative study detailed in Chapter 4 were major open operations and there was little significant difference overall in operating time. The numbers with active cancer were small and there were few very elderly patients, as expected in a population who were deemed fit for elective major surgery.

The original hypothesis, that body composition, specifically distribution and physiology of adipose tissue, would affect insulin resistant and inflammatory response has not been proven by the trial in Chapter 4. There was no association between sarcopenia and IR. There does appear to be some relationship between myosteatosis and postoperative IR. In the past decade, a number of studies have reported an association between intramyocellular fat and IR. The relationship has been seen in healthy volunteers (Brehm et al., 2006), obese and lean subjects, diabetic individuals and offspring of diabetic people (Perseghin et al., 1999). A

causative relationship remains to be established and the mechanism by which intramyocellular fat is associated with IR is unclear. It has been proposed that accumulation of intramyocellular lipid may impair the insulin receptor substrate 1 (IRS1)/phosphatidylinositol 3-kinase (PI3K) pathway and growth-factor-regulated protein kinase B (Akt/PKB) pathway of insulin signalling (Petersen and Shulman, 2006). An alternative hypothesis is that increased accumulation of lipid intermediates, such as diacylglycerol, long-chain fatty acyl-CoA species, ceramides, and oxidized lipid mediators, due to increased accumulation of fat in the myocytes, may be responsible for suppressing the insulin signalling (Timmers et al., 2008). In the study in Chapter 4, although we there were no changes in this whole pathway, surgery was associated with increased expression of FOXO3 in VL samples. There was a significant increase in serum FFA during the course of surgery. TAG concentrations were not significantly altered overall by surgery, but there was an increase in TAGs in obese compared with non-obese individuals. There is sufficient evidence to support a relationship between myosteatosis and IR and this certainly warrants further investigation.

The study described in Chapter 4 did not demonstrate any differences in postoperative IR, measured by the HEC, in obese or non-obese individuals, or with carbohydrate loading. However, there were a number of significant changes demonstrated through genecard analysis of muscle and fat samples. The finding of significantly increased expression of SCD

gene in postoperative compared with preoperative VL samples is novel. Mice with SCD-1 deficiency display improved glucose tolerance and insulin sensitivity (Ntambi et al., 2002, Jiang et al., 2005). There have been few studies of SCD in humans; one examined SCD1 protein in VAT and SAT of morbidly obese subjects and found that there was a strong association with IR (Garcia-Serrano et al., 2011). There have been no studies reported of SCD in perioperative patients but this novel finding certainly warrants further investigation as a contributor to the mechanism underlying postoperative IR.

The findings of activation of the PPAR-α/RXR-α pathway in obese compared with non-obese patients postoperatively has never previously been described in human studies but support the hypothesis that postoperative metabolism differs with body composition. The novel finding of activation of inflammatory pathway genes in omental fat is biologically significant and promotes the theory that adipose tissue acts as an endocrine organ.

Many of these mechanisms have been studied recently in obese populations undergoing metabolic surgery. After gastric bypass surgery, plasma concentrations of adiponectin and IL-6 were demonstrably lower (both $p<0.001$) and at 12 months after surgery, correlations were seen between IL-6 and HOMA-IR (Illan-Gomez et al., 2012). In the same study, hs-CRP concentrations correlated with BMI ($r^2=0.40$, $p=0.004$) and HOMA-IR ($r^2=0.46$, p-0.002) (Illan-Gomez et al., 2012). In subcutaneous

and omental adipose tissue from obese insulin sensitive and insulin resistant patients undergoing gastric bypass surgery, elevated chemokine and chemokine receptor activity distinguished the omental adipose tissue of insulin-resistant obese patients from the omental adipose tissue of insulin-sensitive obese individuals (Gauthier et al., 2011). However, in obese men and women undergoing gastric bypass surgery with or without omentectomy, no differences between groups were found in metabolic variables or insulin sensitivity (Fabbrini et al., 2010). A more recent study of gastric bypass surgery with or without omentectomy confirmed the findings that omentectomy did not enhance the effect of Roux-en-Y gastric bypass on insulin sensitivity or adipokine concentration (Lima et al., 2013).

In obese women who underwent gastric bypass surgery a significant reduction of ER stress upon weight loss was seen, as evidenced by the suppression of biochemical indicators of ER stress such as JNK phosphorylation (Gregor et al., 2009). A study of women undergoing gastric bypass surgery and age-matched controls undergoing cholecystectomy, demonstrated a marked increase in mRNA expression of genes involved in glucose transport in SAT adipocytes of obese compared to non-obese controls and decreased adiponectin in VAT adipocytes of obese compared to controls (Dolinkova et al., 2008). In a study of 22 morbidly obese patients undergoing biliopancreatic diversion, there was an improvement in HOMA index at 6 months post-operatively,

which correlated positively with GLP-1 receptor mRNA and protein expression in VAT (Vendrell et al., 2011b). In a study of 77 morbidly obese women, plasma concentrations of adipocyte fatty acid binding protein (a-FABP), proinflammatory cytokines and insulin sensitivity (estimated by HOMA-IR) were measured before and 1 year after surgical gastric bypass. After massive weight loss, a-FABP concentrations decreased significantly ($p<0.001$) and HOMA-IR improved. There was no association with circulating adipokines or proinflammatory cytokines (Simon et al., 2009). The current epidemic of obesity and the continuing popularity of bariatric and metabolic surgery affords the opportunity for further research.

The studies included in this thesis have demonstrated results which dispute some of those published previously. Although confirming the fall in insulin sensitivity associated with surgery, there was no effect of preoperative carbohydrate. It therefore appears unclear whether carbohydrate treatment has a true effect on outcome after surgery. This highlights the importance of clear reporting of methodology and comparison of like with like. The gold standard method for measuring insulin sensitivity should be regarded as the hyperinsulinaemic-euglycaemic clamp, as evidenced by the study in Chapter 5.

In Chapter 6, the intensive study of body composition changes in oesophagogastric cancer demonstrated that sarcopenia is prevalent, even in subjects with normal body mass index. There are changes in insulin sensitivity during the course of NAC and these have not previously

been reported. It is unclear from this pilot study whether these changes, and the implementation of nutritional support in an attempt to counteract them, will be beneficial in terms of outcome. Certainly, the theme of body composition changes in association with metabolic alterations was further highlighted in this patient group.

A number of methods of assessment of body composition were assessed in Chapter 7. It was shown that analysis of CT scans provides results comparable to DXA. BIA may have a place in clinical practice but its accuracy as a research tool is still unconfirmed.

Overall, although the studies reported in this thesis have not proven the hypotheses stated in Chapter 2, there have certainly been important and novel findings. The two clinical trials reported in Chapter 4 and Chapter 6 have resulted in the generation of new questions related to body composition and metabolism.

Chapter 9 Future Directions

The studies described in this thesis have demonstrated that surgery and NAC are associated with a number of metabolic changes. The association with body composition is not clear. However, there is a possible association between myosteatosis and IR and demonstrable changes in gene expression associated with surgery and obesity. The impact of nutritional status on metabolic changes associated with NAC has not been clearly demonstrated and requires further evaluation. Some questions remain:

1. What is the significance of the substantial changes seen in omental fat of obese compared to non-obese patients? As this has never been previously reported, this requires confirmation and further evaluation – a bariatric population undergoing surgery would serve as an ideal sample group.
2. Does preoperative carbohydrate treatment have any effect on postoperative insulin sensitivity? As the present study has found no effect and previous studies using the HEC demonstrating positive effects were in small numbers of patients, a large scale study is required.
3. Is myosteatosis involved in the mechanism underlying insulin resistance? This requires evaluation in a larger study of perioperative patients and in patients undergoing NAC.
4. Do patients undergoing NAC for OG cancer require routine intensive nutritional assessment and support? There is a suggestion from this pilot study that this is the case, but the results are difficult to interpret in such a small sample. A larger, less intensive randomised trial focusing on clinical outcomes only would help to clarify these findings.

Bibliography

Abate, N., Garg, A., Peshock, R. M., Stray-Gundersen, J. & Grundy, S. M. 1995. Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest,* 96**,** 88-98.

Ackermann, D., Jones, J., Barona, J., Calle, M. C., Kim, J. E., Lapia, B., Volek, J. S., Mcintosh, M., Kalynych, C., Najm, W., Lerman, R. H. & Fernandez, M. L. 2011. Waist circumference is positively correlated with markers of inflammation and negatively with adiponectin in women with metabolic syndrome. *Nutr Res,* 31**,** 197-204.

Akintola, D. F., Sampson, B., Burrin, J., Fleck, A., Price, C. & Hall, G. 1997. Changes in plasma metallothionein-1, interleukin-6, and C-reactive protein in patients after elective surgery. *Clin Chem,* 43**,** 845-7.

Alberti, K. G. & Zimmet, P. Z. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med,* 15**,** 539-53.

Alvehus, M., Buren, J., Sjostrom, M., Goedecke, J. & Olsson, T. 2010. The human visceral fat depot has a unique inflammatory profile. *Obesity (Silver Spring),* 18**,** 879-83.

Anandavadivelan, P., Brismar, T. B., Nilsson, M., Johar, A. M. & Martin, L. 2015. Sarcopenic obesity: A probable risk factor for dose limiting toxicity during neo-adjuvant chemotherapy in oesophageal cancer patients. *Clin Nutr*.

Anderson, D. E., D'agostino, J. M., Bruno, A. G., Demissie, S., Kiel, D. P. & Bouxsein, M. L. 2013. Variations of CT-based trunk muscle attenuation by age, sex, and specific muscle. *J Gerontol A Biol Sci Med Sci,* 68**,** 317-23.

Andreoli, A., Melchiorri, G., De Lorenzo, A., Caruso, I., Sinibaldi Salimei, P. & Guerrisi, M. 2002. Bioelectrical impedance measures in different position and vs dual-energy X-ray absorptiometry (DXA). *J Sports Med Phys Fitness,* 42**,** 186-9.

Apovian, C. M., Bigornia, S., Mott, M., Meyers, M. R., Ulloor, J., Gagua, M., Mcdonnell, M., Hess, D., Joseph, L. & Gokce, N. 2008. Adipose macrophage infiltration is associated with insulin resistance and vascular endothelial dysfunction in obese subjects. *Arterioscler Thromb Vasc Biol,* 28**,** 1654-9.

Argiles, J. M., Alvarez, B. & Lopez-Soriano, F. J. 1997. The metabolic basis of cancer cachexia. *Med Res Rev,* 17**,** 477-98.

Arner, P. 2005. Resistin: yet another adipokine tells us that men are not mice. *Diabetologia,* 48**,** 2203-5.

Ascaso, J. F., Pardo, S., Real, J. T., Lorente, R. I., Priego, A. & Carmena, R. 2003. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care,* 26**,** 3320-5.

Aubrey, J., Esfandiari, N., Baracos, V. E., Buteau, F. A., Frenette, J., Putman, C. T. & Mazurak, V. C. 2014. Measurement of skeletal muscle radiation attenuation and basis of its biological variation. *Acta Physiol (Oxf),* 210**,** 489-97.

Awad, S., Constantin-Teodosiu, D., Constantin, D., Rowlands, B. J., Fearon, K. C., Macdonald, I. A. & Lobo, D. N. 2010a. Cellular

mechanisms underlying the protective effects of preoperative feeding: a randomized study investigating muscle and liver glycogen content, mitochondrial function, gene and protein expression. *Ann Surg,* 252**,** 247-53.

Awad, S., Constantin-Teodosiu, D., Macdonald, I. A. & Lobo, D. N. 2009. Short-term starvation and mitochondrial dysfunction - a possible mechanism leading to postoperative insulin resistance. *Clin Nutr,* 28**,** 497-509.

Awad, S., Fearon, K. C., Macdonald, I. A. & Lobo, D. N. 2011a. A randomized cross-over study of the metabolic and hormonal responses following two preoperative conditioning drinks. *Nutrition,* 27**,** 938-42.

Awad, S. & Lobo, D. N. 2012. Metabolic conditioning to attenuate the adverse effects of perioperative fasting and improve patient outcomes. *Curr Opin Clin Nutr Metab Care,* 15**,** 194-200.

Awad, S., Stephens, F., Shannon, C. & Lobo, D. N. 2012a. Perioperative perturbations in carnitine metabolism are attenuated by preoperative carbohydrate treatment: Another mechanism by which preoperative feeding may attenuate development of postoperative insulin resistance. *Clin Nutr,* 31**,** 717-20.

Awad, S., Stephenson, M. C., Placidi, E., Marciani, L., Constantin-Teodosiu, D., Gowland, P. A., Spiller, R. C., Fearon, K. C., Morris, P. G., Macdonald, I. A. & Lobo, D. N. 2010b. The effects of fasting and refeeding with a 'metabolic preconditioning' drink on substrate reserves and mononuclear cell mitochondrial function. *Clin Nutr,* 29**,** 538-44.

Awad, S., Tan, B. H., Cui, H., Bhalla, A., Fearon, K. C., Parsons, S. L., Catton, J. A. & Lobo, D. N. 2012b. Marked changes in body composition following neoadjuvant chemotherapy for oesophagogastric cancer. *Clin Nutr,* 31**,** 74-7.

Awad, S., Tan, B. H., Cui, H., Bhalla, A., Fearon, K. C. H., Parsons, S. L., Catton, J. A. & Lobo, D. N. 2011b. Marked changes in body composition following neoadjuvant chemotherapy for oesophagogastric cancer. *Clinical nutrition (Edinburgh, Scotland), Epub date 31/8/11, DOI10.1016/J.clnu.2011.08.008*.

Awad, S., Varadhan, K. K., Ljungqvist, O. & Lobo, D. N. 2013. A meta-analysis of randomised controlled trials on preoperative oral carbohydrate treatment in elective surgery. *Clin Nutr,* 32**,** 34-44.

Baban, B., Thorell, A., Nygren, J., Bratt, A. & Ljungqvist, O. 2015. Determination of insulin resistance in surgery: the choice of method is crucial. *Clin Nutr,* 34**,** 123-8.

Ballantyne, G. H., Gumbs, A. & Modlin, I. M. 2005. Changes in insulin resistance following bariatric surgery and the adipoinsular axis: role of the adipocytokines, leptin, adiponectin and resistin. *Obes Surg,* 15**,** 692-9.

Baracos, V. E., Reiman, T., Mourtzakis, M., Gioulbasanis, I. & Antoun, S. Body composition in patients with non-small cell lung cancer: a contemporary view of cancer cachexia with the use of computed tomography image analysis. *Am J Clin Nutr,* 91**,** 1133S-1137S.

Baracos, V. E., Reiman, T., Mourtzakis, M., Gioulbasanis, I. & Antoun, S. 2010. Body composition in patients with non-small cell lung cancer: a contemporary view of cancer cachexia with the use of computed tomography image analysis. *Am J Clin Nutr,* 91**,** 1133S-1137S.

Beshyah, S. A., Freemantle, C., Thomas, E. & Johnston, D. G. 1995. Comparison of measurements of body composition by total body potassium, bioimpedance analysis, and dual-energy X-ray absorptiometry in hypopituitary adults before and during growth hormone treatment. *Am J Clin Nutr,* 61**,** 1186-94.
Bigornia, S. J., Farb, M. G., Mott, M. M., Hess, D. T., Carmine, B., Fiscale, A., Joseph, L., Apovian, C. M. & Gokce, N. 2012. Relation of depot-specific adipose inflammation to insulin resistance in human obesity. *Nutr Diabetes,* 2**,** e30.
Bisgaard, T., Kristiansen, V. B., Hjortso, N. C., Jacobsen, L. S., Rosenberg, J. & Kehlet, H. 2004. Randomized clinical trial comparing an oral carbohydrate beverage with placebo before laparoscopic cholecystectomy. *Br J Surg,* 91**,** 151-8.
Black, P. R., Brooks, D. C., Bessey, P. Q., Wolfe, R. R. & Wilmore, D. W. 1982. Mechanisms of insulin resistance following injury. *Ann Surg,* 196**,** 420-35.
Boden, G. 2009. Endoplasmic reticulum stress: another link between obesity and insulin resistance/inflammation? *Diabetes,* 58**,** 518-9.
Bonneau, G. A., Pedrozo, W. R. & Berg, G. 2014. Adiponectin and waist circumference as predictors of insulin-resistance in women. *Diabetes Metab Syndr,* 8**,** 3-7.
Borai, A., Livingstone, C. & Ferns, G. A. 2007. The biochemical assessment of insulin resistance. *Ann Clin Biochem,* 44**,** 324-42.
Borst, S. E., Conover, C. F. & Bagby, G. J. 2005. Association of resistin with visceral fat and muscle insulin resistance. *Cytokine,* 32**,** 39-44.
Brehm, A., Krssak, M., Schmid, A. I., Nowotny, P., Waldhausl, W. & Roden, M. 2006. Increased lipid availability impairs insulin-stimulated ATP synthesis in human skeletal muscle. *Diabetes,* 55**,** 136-40.
Bremer, A. A., Devaraj, S., Afify, A. & Jialal, I. 2011. Adipose tissue dysregulation in patients with metabolic syndrome. *J Clin Endocrinol Metab,* 96**,** E1782-8.
Buemann, B., Sorensen, T. I., Pedersen, O., Black, E., Holst, C., Toubro, S., Echwald, S., Holst, J. J., Rasmussen, C. & Astrup, A. 2005. Lower-body fat mass as an independent marker of insulin sensitivity--the role of adiponectin. *Int J Obes (Lond),* 29**,** 624-31.
Cahill, G. F., Jr., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A., Jr. & Kipnis, D. M. 1966. Hormone-fuel interrelationships during fasting. *J Clin Invest,* 45**,** 1751-69.
Can, M. F., Yagci, G., Dag, B., Ozturk, E., Gorgulu, S., Simsek, A. & Tufan, T. 2009. Preoperative administration of oral carbohydrate-rich solutions: Comparison of glucometabolic responses and tolerability between patients with and without insulin resistance. *Nutrition,* 25**,** 72-7.
Cao, Y. L., Hu, C. Z., Meng, X., Wang, D. F. & Zhang, J. 2008. Expression of TNF-alpha protein in omental and subcutaneous adipose tissue in obesity. *Diabetes Res Clin Pract,* 79**,** 214-9.
Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., Prelovsek, O., Hohnen-Behrens, C., Watt, M. J., James, D. E., Kemp, B. E., Pedersen, B. K. & Febbraio, M. A. 2006. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation

in vitro via AMP-activated protein kinase. *Diabetes,* 55**,** 2688-97.
Chang, Y. H., Chang, D. M., Lin, K. C., Shin, S. J. & Lee, Y. J. 2011. Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. *Diabetes Metab Res Rev,* 27**,** 515-27.
Choban, P. S., Heckler, R., Burge, J. C. & Flancbaum, L. 1995. Increased incidence of nosocomial infections in obese surgical patients. *Am Surg,* 61**,** 1001-5.
Choi, Y. J., Choi, S. E., Ha, E. S., Kang, Y., Han, S. J., Kim, D. J., Lee, K. W. & Kim, H. J. 2011. Involvement of visfatin in palmitate-induced upregulation of inflammatory cytokines in hepatocytes. *Metabolism,* 60**,** 1781-9.
Chou, C. J., Haluzik, M., Gregory, C., Dietz, K. R., Vinson, C., Gavrilova, O. & Reitman, M. L. 2002. WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice. *J Biol Chem,* 277**,** 24484-9.
Clarke, R. S. 1970. The hyperglycaemic response to different types of surgery and anaesthesia. *Br J Anaesth,* 42**,** 45-53.
Cohen, S., Nathan, J. A. & Goldberg, A. L. 2015. Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat Rev Drug Discov,* 14**,** 58-74.
Corley, D. A., Kubo, A. & Zhao, W. 2008. Abdominal obesity and the risk of esophageal and gastric cardia carcinomas. *Cancer Epidemiol Biomarkers Prev,* 17**,** 352-8.
Cruz-Jentoft, A. J., Baeyens, J. P., Bauer, J. M., Boirie, Y., Cederholm, T., Landi, F., Martin, F. C., Michel, J. P., Rolland, Y., Schneider, S. M., Topinkova, E., Vandewoude, M., Zamboni, M. & European Working Group on Sarcopenia in Older, P. 2010. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing,* 39**,** 412-23.
Cunningham, D., Allum, W. H., Stenning, S. P., Thompson, J. N., Van De Velde, C. J., Nicolson, M., Scarffe, J. H., Lofts, F. J., Falk, S. J., Iveson, T. J., Smith, D. B., Langley, R. E., Verma, M., Weeden, S., Chua, Y. J. & Participants, M. T. 2006. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med,* 355**,** 11-20.
Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M. E., Pratipanawatr, T., Defronzo, R. A., Kahn, C. R. & Mandarino, L. J. 2000. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest,* 105**,** 311-20.
Cuthbertson, D. P. 1929. The influence of prolonged muscular rest on metabolism. *Biochem J,* 23**,** 1328-45.
Cuthbertson, D. P. 1930. The disturbance of metabolism produced by bony and non-bony injury, with notes on certain abnormal conditions of bone. *Biochem J,* 24**,** 1244-63.
Cuthbertson, D. P. 1980a. Alterations in metabolism following injury: part I. *Injury,* 11**,** 175-89.
Cuthbertson, D. P. 1980b. Alterations in metabolism following injury: part II. *Injury,* 11**,** 286-303.
Dalal, S., Hui, D., Bidaut, L., Lem, K., Del Fabbro, E., Crane, C., Reyes-Gibby, C. C., Bedi, D. & Bruera, E. 2012. Relationships

among body mass index, longitudinal body composition alterations, and survival in patients with locally advanced pancreatic cancer receiving chemoradiation: a pilot study. *J Pain Symptom Manage,* 44**,** 181-91.
Damsbo, P., Vaag, A., Hother-Nielsen, O. & Beck-Nielsen, H. 1991. Reduced glycogen synthase activity in skeletal muscle from obese patients with and without type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia,* 34**,** 239-45.
Dancea, H. C., Obradovic, V., Sartorius, J., Woll, N. & Blansfield, J. A. 2012. Increased complication rate in obese patients undergoing laparoscopic adrenalectomy. *JSLS,* 16**,** 45-9.
Defronzo, R. A., Tobin, J. D. & Andres, R. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol,* 237**,** E214-23.
Defronzo, R. A. & Tripathy, D. 2009. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care,* 32 Suppl 2**,** S157-63.
Degawa-Yamauchi, M., Bovenkerk, J. E., Juliar, B. E., Watson, W., Kerr, K., Jones, R., Zhu, Q. & Considine, R. V. 2003. Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab,* 88**,** 5452-5.
Desborough, J. P. 2000. The stress response to trauma and surgery. *Br J Anaesth,* 85**,** 109-17.
Deurenberg, P. 1996. Limitations of the bioelectrical impedance method for the assessment of body fat in severe obesity. *Am J Clin Nutr,* 64**,** 449S-452S.
Dindo, D., Demartines, N. & Clavien, P. A. 2004. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg,* 240**,** 205-13.
Dohm, G. L., Tapscott, E. B., Pories, W. J., Dabbs, D. J., Flickinger, E. G., Meelheim, D., Fushiki, T., Atkinson, S. M., Elton, C. W. & Caro, J. F. 1988. An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin Invest,* 82**,** 486-94.
Dolinkova, M., Dostalova, I., Lacinova, Z., Michalsky, D., Haluzikova, D., Mraz, M., Kasalicky, M. & Haluzik, M. 2008. The endocrine profile of subcutaneous and visceral adipose tissue of obese patients. *Mol Cell Endocrinol,* 291**,** 63-70.
Donohoe, C. L., Doyle, S. L. & Reynolds, J. V. 2011. Visceral adiposity, insulin resistance and cancer risk. *Diabetol Metab Syndr,* 3**,** 12.
Doyle, S. L., Lysaght, J. & Reynolds, J. V. 2010. Obesity and post-operative complications in patients undergoing non-bariatric surgery. *Obes Rev,* 11**,** 875-86.
Drapeau, S., Doucet, E., Rabasa-Lhoret, R., Brochu, M., Prud'homme, D. & Imbeault, P. 2011. Improvement in insulin sensitivity by weight loss does not affect hyperinsulinemia-mediated reduction in total and high molecular weight adiponectin: a MONET study. *Appl Physiol Nutr Metab,* 36**,** 191-200.
Duska, F., Andel, M., Kubena, A. & Macdonald, I. A. 2005. Effects of acute starvation on insulin resistance in obese patients with and without type 2 diabetes mellitus. *Clin Nutr,* 24**,** 1056-64.
Echahidi, N., Mohty, D., Pibarot, P., Despres, J. P., O'hara, G., Champagne, J., Philippon, F., Daleau, P., Voisine, P. & Mathieu, P. 2007a. Obesity and metabolic syndrome are independent

risk factors for atrial fibrillation after coronary artery bypass graft surgery. *Circulation,* 116**,** I213-9.
Echahidi, N., Pibarot, P., Despres, J. P., Daigle, J. M., Mohty, D., Voisine, P., Baillot, R. & Mathieu, P. 2007b. Metabolic syndrome increases operative mortality in patients undergoing coronary artery bypass grafting surgery. *J Am Coll Cardiol,* 50**,** 843-51.
Erdmann, J., Mayr, M., Oppel, U., Sypchenko, O., Wagenpfeil, S. & Schusdziarra, V. 2009. Weight-dependent differential contribution of insulin secretion and clearance to hyperinsulinemia of obesity. *Regul Pept,* 152**,** 1-7.
Esteve, E., Ricart, W. & Fernandez-Real, J. M. 2005. Dyslipidemia and inflammation: an evolutionary conserved mechanism. *Clin Nutr,* 24**,** 16-31.
Fabbrini, E., Cella, M., Mccartney, S. A., Fuchs, A., Abumrad, N. A., Pietka, T. A., Chen, Z., Finck, B. N., Han, D. H., Magkos, F., Conte, C., Bradley, D., Fraterrigo, G., Eagon, J. C., Patterson, B. W., Colonna, M. & Klein, S. 2013. Association between specific adipose tissue CD4+ T-cell populations and insulin resistance in obese individuals. *Gastroenterology,* 145**,** 366-74 e1-3.
Fabbrini, E., Tamboli, R. A., Magkos, F., Marks-Shulman, P. A., Eckhauser, A. W., Richards, W. O., Klein, S. & Abumrad, N. N. 2010. Surgical removal of omental fat does not improve insulin sensitivity and cardiovascular risk factors in obese adults. *Gastroenterology,* 139**,** 448-55.
Farb, M. G., Bigornia, S., Mott, M., Tanriverdi, K., Morin, K. M., Freedman, J. E., Joseph, L., Hess, D. T., Apovian, C. M., Vita, J. A. & Gokce, N. 2011. Reduced adipose tissue inflammation represents an intermediate cardiometabolic phenotype in obesity. *J Am Coll Cardiol,* 58**,** 232-7.
Faria, M. S., De Aguilar-Nascimento, J. E., Pimenta, O. S., Alvarenga, L. C., Jr., Dock-Nascimento, D. B. & Slhessarenko, N. 2009. Preoperative fasting of 2 hours minimizes insulin resistance and organic response to trauma after video-cholecystectomy: a randomized, controlled, clinical trial. *World J Surg,* 33**,** 1158-64.
Fawcett, J., Sang, H., Permana, P. A., Levy, J. L. & Duckworth, W. C. 2010. Insulin metabolism in human adipocytes from subcutaneous and visceral depots. *Biochem Biophys Res Commun,* 402**,** 762-6.
Fearon, K., Strasser, F., Anker, S. D., Bosaeus, I., Bruera, E., Fainsinger, R. L., Jatoi, A., Loprinzi, C., Macdonald, N., Mantovani, G., Davis, M., Muscaritoli, M., Ottery, F., Radbruch, L., Ravasco, P., Walsh, D., Wilcock, A., Kaasa, S. & Baracos, V. E. 2011. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol,* 12**,** 489-95.
Fearon, K. C., Ljungqvist, O., Von Meyenfeldt, M., Revhaug, A., Dejong, C. H., Lassen, K., Nygren, J., Hausel, J., Soop, M., Andersen, J. & Kehlet, H. 2005. Enhanced recovery after surgery: a consensus review of clinical care for patients undergoing colonic resection. *Clin Nutr,* 24**,** 466-77.
Ferrannini, E., Bjorkman, O., Reichard, G. A., Jr., Pilo, A., Olsson, M., Wahren, J. & Defronzo, R. A. 1985. The disposal of an oral glucose load in healthy subjects. A quantitative study. *Diabetes,* 34**,** 580-8.
Ferrannini, E., Natali, A., Bell, P., Cavallo-Perin, P., Lalic, N. & Mingrone, G. 1997. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J Clin Invest,* 100**,** 1166-73.

Festa, A., D'agostino, R., Jr., Williams, K., Karter, A. J., Mayer-Davis, E. J., Tracy, R. P. & Haffner, S. M. 2001. The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord,* 25**,** 1407-15.

Fjeldborg, K., Pedersen, S. B., Moller, H. J., Christiansen, T., Bennetzen, M. & Richelsen, B. 2014. Human adipose tissue macrophages are enhanced but changed to an anti-inflammatory profile in obesity. *J Immunol Res,* 2014**,** 309548.

Frayn, K. N., Williams, C. M. & Arner, P. 1996. Are increased plasma non-esterified fatty acid concentrations a risk marker for coronary heart disease and other chronic diseases? *Clin Sci (Lond),* 90**,** 243-53.

Frederiksen, L., Nielsen, T. L., Wraae, K., Hagen, C., Frystyk, J., Flyvbjerg, A., Brixen, K. & Andersen, M. 2009. Subcutaneous rather than visceral adipose tissue is associated with adiponectin levels and insulin resistance in young men. *J Clin Endocrinol Metab,* 94**,** 4010-5.

Friedman, J. E., Caro, J. F., Pories, W. J., Azevedo, J. L., Jr. & Dohm, G. L. 1994. Glucose metabolism in incubated human muscle: effect of obesity and non-insulin-dependent diabetes mellitus. *Metabolism,* 43**,** 1047-54.

Frisard, M. I., Greenway, F. L. & Delany, J. P. 2005. Comparison of methods to assess body composition changes during a period of weight loss. *Obes Res,* 13**,** 845-54.

Fu, Y., Luo, L., Luo, N. & Garvey, W. T. 2006. Proinflammatory cytokine production and insulin sensitivity regulated by overexpression of resistin in 3T3-L1 adipocytes. *Nutr Metab (Lond),* 3**,** 28.

Gallagher, D. & Delegge, M. 2011. Body composition (sarcopenia) in obese patients: implications for care in the intensive care unit. *JPEN J Parenter Enteral Nutr,* 35**,** 21S-8S.

Garcia-Serrano, S., Moreno-Santos, I., Garrido-Sanchez, L., Gutierrez-Repiso, C., Garcia-Almeida, J. M., Garcia-Arnes, J., Rivas-Marin, J., Gallego-Perales, J. L., Garcia-Escobar, E., Rojo-Martinez, G., Tinahones, F., Soriguer, F., Macias-Gonzalez, M. & Garcia-Fuentes, E. 2011. Stearoyl-CoA desaturase-1 is associated with insulin resistance in morbidly obese subjects. *Mol Med,* 17**,** 273-80.

Gatica, R., Bertinat, R., Silva, P., Kairath, P., Slebe, F., Pardo, F., Ramirez, M. J., Slebe, J. C., Campistol, J. M., Nualart, F., Caelles, C. & Yanez, A. J. 2015. Over-expression of muscle glycogen synthase in human diabetic nephropathy. *Histochem Cell Biol,* 143**,** 313-24.

Gauthier, M. S., O'brien, E. L., Bigornia, S., Mott, M., Cacicedo, J. M., Xu, X. J., Gokce, N., Apovian, C. & Ruderman, N. 2011. Decreased AMP-activated protein kinase activity is associated with increased inflammation in visceral adipose tissue and with whole-body insulin resistance in morbidly obese humans. *Biochem Biophys Res Commun,* 404**,** 382-7.

Gjedsted, J., Gormsen, L. C., Nielsen, S., Schmitz, O., Djurhuus, C. B., Keiding, S., Orskov, H., Tonnesen, E. & Moller, N. 2007. Effects of a 3-day fast on regional lipid and glucose metabolism in human skeletal muscle and adipose tissue. *Acta Physiol (Oxf),* 191**,** 205-16.

Gjessing, P. F., Constantin-Teodosiu, D., Hagve, M., Lobo, D. N., Revhaug, A. & Irtun, O. 2015. Preoperative carbohydrate supplementation attenuates post-surgery insulin resistance via

reduced inflammatory inhibition of the insulin-mediated restraint on muscle pyruvate dehydrogenase kinase 4 expression. *Clin Nutr,* 34**,** 1177-83.
Gjessing, P. F., Hagve, M., Fuskevag, O. M., Revhaug, A. & Irtun, O. 2014. Single-dose carbohydrate treatment in the immediate preoperative phase diminishes development of postoperative peripheral insulin resistance. *Clin Nutr*.
Glass, D. J. 2003. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat Cell Biol,* 5**,** 87-90.
Gletsu-Miller, N., Kahn, H. S., Gasevic, D., Liang, Z., Frediani, J. K., Torres, W. E., Ziegler, T. R., Phillips, L. S. & Lin, E. 2013. Sagittal abdominal diameter and visceral adiposity: correlates of beta-cell function and dysglycemia in severely obese women. *Obes Surg,* 23**,** 874-81.
Gletsu, N., Lin, E., Zhu, J. L., Khaitan, L., Ramshaw, B. J., Farmer, P. K., Ziegler, T. R., Papanicolaou, D. A. & Smith, C. D. 2006. Increased plasma interleukin 6 concentrations and exaggerated adipose tissue interleukin 6 content in severely obese patients after operative trauma. *Surgery,* 140**,** 50-7.
Goktas, Z., Owens, S., Boylan, M., Syn, D., Shen, C. L., Reed, D. B., San Francisco, S. & Wang, S. 2013. Associations between tissue visfatin/nicotinamide, phosphoribosyltransferase (Nampt), retinol binding protein-4, and vaspin concentrations and insulin resistance in morbidly obese subjects. *Mediators Inflamm,* 2013**,** 861496.
Gonzalez-Roldan, N., Ferat-Osorio, E., Aduna-Vicente, R., Wong-Baeza, I., Esquivel-Callejas, N., Astudillo-De La Vega, H., Sanchez-Fernandez, P., Arriaga-Pizano, L., Villasis-Keever, M. A., Lopez-Macias, C. & Isibasi, A. 2005. Expression of triggering receptor on myeloid cell 1 and histocompatibility complex molecules in sepsis and major abdominal surgery. *World J Gastroenterol,* 11**,** 7473-9.
Goodpaster, B. H., Thaete, F. L. & Kelley, D. E. 2000. Composition of skeletal muscle evaluated with computed tomography. *Ann N Y Acad Sci,* 904**,** 18-24.
Goodpaster, B. H., Thaete, F. L., Simoneau, J. A. & Kelley, D. E. 1997. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes,* 46**,** 1579-85.
Gregor, M. F., Yang, L., Fabbrini, E., Mohammed, B. S., Eagon, J. C., Hotamisligil, G. S. & Klein, S. 2009. Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. *Diabetes,* 58**,** 693-700.
Greisen, J., Juhl, C. B., Grofte, T., Vilstrup, H., Jensen, T. S. & Schmitz, O. 2001. Acute pain induces insulin resistance in humans. *Anesthesiology,* 95**,** 578-84.
Guerre-Millo, M., Gervois, P., Raspe, E., Madsen, L., Poulain, P., Derudas, B., Herbert, J. M., Winegar, D. A., Willson, T. M., Fruchart, J. C., Berge, R. K. & Staels, B. 2000. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem,* 275**,** 16638-42.
Gustafsson, U. O., Scott, M. J., Schwenk, W., Demartines, N., Roulin, D., Francis, N., Mcnaught, C. E., Macfie, J., Liberman, A. S., Soop, M., Hill, A., Kennedy, R. H., Lobo, D. N., Fearon, K., Ljungqvist, O., Enhanced Recovery after Surgery Society, F. P. C., European Society for Clinical, N., Metabolism, International Association for Surgical, M. & Nutrition 2013. Guidelines for

perioperative care in elective colonic surgery: Enhanced Recovery After Surgery (ERAS((R))) Society recommendations. *World J Surg,* 37**,** 259-84.
Hammes, T. O., Costa Cdos, S., Rohden, F., Margis, R., De Almeida, J. C., Padoin, A. V., Mottin, C. C. & Guaragna, R. M. 2012. Parallel down-regulation of FOXO1, PPARgamma and adiponectin mRNA expression in visceral adipose tissue of class III obese individuals. *Obes Facts,* 5**,** 452-9.
Hardy, O. T., Perugini, R. A., Nicoloro, S. M., Gallagher-Dorval, K., Puri, V., Straubhaar, J. & Czech, M. P. 2011. Body mass index-independent inflammation in omental adipose tissue associated with insulin resistance in morbid obesity. *Surg Obes Relat Dis,* 7**,** 60-7.
Harman-Boehm, I., Bluher, M., Redel, H., Sion-Vardy, N., Ovadia, S., Avinoach, E., Shai, I., Kloting, N., Stumvoll, M., Bashan, N. & Rudich, A. 2007. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab,* 92**,** 2240-7.
Haverkort, E. B., Reijven, P. L., Binnekade, J. M., De Van Der Schueren, M. A., Earthman, C. P., Gouma, D. J. & De Haan, R. J. 2015. Bioelectrical impedance analysis to estimate body composition in surgical and oncological patients: a systematic review. *Eur J Clin Nutr,* 69**,** 3-13.
Health & Social Care Information, C. 2013-2014. Hospital Episode Statistics

Admitted Patient Care, England, 2013-14. http://www.hscic.gov.uk/catalogue/PUB16719/hosp-epis-stat-admi-summ-rep-2013-14-rep.pdf.
Heymsfield, S. B., Mcmanus, C., Smith, J., Stevens, V. & Nixon, D. W. 1982. Anthropometric measurement of muscle mass: revised equations for calculating bone-free arm muscle area. *Am J Clin Nutr,* 36**,** 680-90.
Hillman, T. E., Nunes, Q. M., Hornby, S. T., Stanga, Z., Neal, K. R., Rowlands, B. J., Allison, S. P. & Lobo, D. N. 2005. A practical posture for hand grip dynamometry in the clinical setting. *Clin Nutr,* 24**,** 224-8.
Hirner, S., Krohne, C., Schuster, A., Hoffmann, S., Witt, S., Erber, R., Sticht, C., Gasch, A., Labeit, S. & Labeit, D. 2008. MuRF1-dependent regulation of systemic carbohydrate metabolism as revealed from transgenic mouse studies. *J Mol Biol,* 379**,** 666-77.
Hoffstedt, J., Arvidsson, E., Sjolin, E., Wahlen, K. & Arner, P. 2004. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. *J Clin Endocrinol Metab,* 89**,** 1391-6.
Hollenbeck, C. & Reaven, G. M. 1987. Variations in insulin-stimulated glucose uptake in healthy individuals with normal glucose tolerance. *J Clin Endocrinol Metab,* 64**,** 1169-73.
Horakova, D., Stejskal, D., Pastucha, D., Muchova, L. & Janout, V. 2010. Potential markers of insulin resistance in healthy vs obese and overweight subjects. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub,* 154**,** 245-9.
Hosseinzadeh-Attar, M. J., Golpaie, A., Janani, L. & Derakhshanian, H. 2013. Effect of weight reduction following bariatric surgery on

serum visfatin and adiponectin levels in morbidly obese subjects. *Obes Facts,* 6**,** 193-202.
Hotamisligil, G. S. 2010. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell,* 140**,** 900-17.
Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L. & Spiegelman, B. M. 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest,* 95**,** 2409-15.
Hotamisligil, G. S., Murray, D. L., Choy, L. N. & Spiegelman, B. M. 1994. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A,* 91**,** 4854-8.
Hsieh, C. J., Wang, P. W. & Chen, T. Y. 2014. The relationship between regional abdominal fat distribution and both insulin resistance and subclinical chronic inflammation in non-diabetic adults. *Diabetol Metab Syndr,* 6**,** 49.
Hu, F. B., Meigs, J. B., Li, T. Y., Rifai, N. & Manson, J. E. 2004. Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes,* 53**,** 693-700.
Huang, D., Bt, S. & Ra, L. 2009. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protocols,* 4**,** 44-57.
Hung, J., Mcquillan, B. M., Thompson, P. L. & Beilby, J. P. 2008. Circulating adiponectin levels associate with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity. *Int J Obes (Lond),* 32**,** 772-9.
Ida, S., Watanabe, M., Karashima, R., Imamura, Y., Ishimoto, T., Baba, Y., Iwagami, S., Sakamoto, Y., Miyamoto, Y., Yoshida, N. & Baba, H. 2014. Changes in body composition secondary to neoadjuvant chemotherapy for advanced esophageal cancer are related to the occurrence of postoperative complications after esophagectomy. *Ann Surg Oncol,* 21**,** 3675-9.
Illan-Gomez, F., Gonzalvez-Ortega, M., Orea-Soler, I., Alcaraz-Tafalla, M. S., Aragon-Alonso, A., Pascual-Diaz, M., Perez-Paredes, M. & Lozano-Almela, M. L. 2012. Obesity and inflammation: change in adiponectin, C-reactive protein, tumour necrosis factor-alpha and interleukin-6 after bariatric surgery. *Obes Surg,* 22**,** 950-5.
Indulekha, K., Anjana, R. M., Surendar, J. & Mohan, V. 2011. Association of visceral and subcutaneous fat with glucose intolerance, insulin resistance, adipocytokines and inflammatory markers in Asian Indians (CURES-113). *Clin Biochem,* 44**,** 281-7.
Jankiewicz-Wika, J., Kolomecki, K., Cywinski, J., Piestrzeniewicz, K., Swietoslawski, J., Stepien, H. & Komorowski, J. 2011. Impact of vertical banded gastroplasty on body weight, insulin resistance, adipocytokine, inflammation and metabolic syndrome markers in morbidly obese patients. *Endokrynol Pol,* 62**,** 109-19.
Jebb, S. A., Siervo, M., Murgatroyd, P. R., Evans, S., Fruhbeck, G. & Prentice, A. M. 2007. Validity of the leg-to-leg bioimpedance to estimate changes in body fat during weight loss and regain in overweight women: a comparison with multi-compartment models. *Int J Obes (Lond),* 31**,** 756-62.
Jiang, G., Li, Z., Liu, F., Ellsworth, K., Dallas-Yang, Q., Wu, M., Ronan, J., Esau, C., Murphy, C., Szalkowski, D., Bergeron, R., Doebber, T. & Zhang, B. B. 2005. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J Clin Invest,* 115**,** 1030-8.

Jocken, J. W., Langin, D., Smit, E., Saris, W. H., Valle, C., Hul, G. B., Holm, C., Arner, P. & Blaak, E. E. 2007. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. *J Clin Endocrinol Metab,* 92**,** 2292-9.

John, B. J., Irukulla, S., Abulafi, A. M., Kumar, D. & Mendall, M. A. 2006. Systematic review: adipose tissue, obesity and gastrointestinal diseases. *Aliment Pharmacol Ther,* 23**,** 1511-23.

Johnson, J. A., Fried, S. K., Pi-Sunyer, F. X. & Albu, J. B. 2001. Impaired insulin action in subcutaneous adipocytes from women with visceral obesity. *Am J Physiol Endocrinol Metab,* 280**,** E40-9.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. *Metabolism,* 27**,** 1893-902.

Kalantar-Zadeh, K., Kleiner, M., Dunne, E., Lee, G. H. & Luft, F. C. 1999. A modified quantitative subjective global assessment of nutrition for dialysis patients. *Nephrol Dial Transplant,* 14**,** 1732-8.

Kalichman, L., Hodges, P., Li, L., Guermazi, A. & Hunter, D. J. 2010. Changes in paraspinal muscles and their association with low back pain and spinal degeneration: CT study. *Eur Spine J,* 19**,** 1136-44.

Karakelides, H., Irving, B. A., Short, K. R., O'brien, P. & Nair, K. S. 2010. Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. *Diabetes,* 59**,** 89-97.

Katz, A., Nambi, S. S., Mather, K., Baron, A. D., Follmann, D. A., Sullivan, G. & Quon, M. J. 2000. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab,* 85**,** 2402-10.

Katz, L. D., Glickman, M. G., Rapoport, S., Ferrannini, E. & Defronzo, R. A. 1983. Splanchnic and peripheral disposal of oral glucose in man. *Diabetes,* 32**,** 675-9.

Kelley, D. E., Mckolanis, T. M., Hegazi, R. A., Kuller, L. H. & Kalhan, S. C. 2003. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab,* 285**,** E906-16.

Kelley, D. E., Thaete, F. L., Troost, F., Huwe, T. & Goodpaster, B. H. 2000. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *Am J Physiol Endocrinol Metab,* 278**,** E941-8.

Kim, H., Haluzik, M., Asghar, Z., Yau, D., Joseph, J. W., Fernandez, A. M., Reitman, M. L., Yakar, S., Stannard, B., Heron-Milhavet, L., Wheeler, M. B. & Leroith, D. 2003. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes,* 52**,** 1770-8.

Kim, H. J., Higashimori, T., Park, S. Y., Choi, H., Dong, J., Kim, Y. J., Noh, H. L., Cho, Y. R., Cline, G., Kim, Y. B. & Kim, J. K. 2004. Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. *Diabetes,* 53**,** 1060-7.

Kim, J. H., Bachmann, R. A. & Chen, J. 2009. Interleukin-6 and insulin resistance. *Vitam Horm,* 80**,** 613-33.

King, M. W. 2013. Insulin Function, Insulin Resistance and Food Intake Control of Secretion. *http://themedicalbiochemistrypage.org/insulin.php*

Accessed 22.7.14

Klein, S. & Wolfe, R. R. 1990. Whole-body lipolysis and triglyceride-fatty acid cycling in cachectic patients with esophageal cancer. *J Clin Invest,* 86**,** 1403-8.

Kloting, N., Fasshauer, M., Dietrich, A., Kovacs, P., Schon, M. R., Kern, M., Stumvoll, M. & Bluher, M. 2010. Insulin-sensitive obesity. *Am J Physiol Endocrinol Metab,* 299**,** E506-15.

Kovacova, Z., Tencerova, M., Roussel, B., Wedellova, Z., Rossmeislova, L., Langin, D., Polak, J. & Stich, V. 2012. The impact of obesity on secretion of adiponectin multimeric isoforms differs in visceral and subcutaneous adipose tissue. *Int J Obes (Lond),* 36**,** 1360-5.

Krssak, M., Falk Petersen, K., Dresner, A., Dipietro, L., Vogel, S. M., Rothman, D. L., Roden, M. & Shulman, G. I. 1999. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia,* 42**,** 113-6.

Kubota, N., Terauchi, Y., Yamauchi, T., Kubota, T., Moroi, M., Matsui, J., Eto, K., Yamashita, T., Kamon, J., Satoh, H., Yano, W., Froguel, P., Nagai, R., Kimura, S., Kadowaki, T. & Noda, T. 2002. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem,* 277**,** 25863-6.

Kurikawa, N., Takagi, T., Wakimoto, S., Uto, Y., Terashima, H., Kono, K., Ogata, T. & Ohsumi, J. 2013. A novel inhibitor of stearoyl-CoA desaturase-1 attenuates hepatic lipid accumulation, liver injury and inflammation in model of nonalcoholic steatohepatitis. *Biol Pharm Bull,* 36**,** 259-67.

Kvist, H., Sjostrom, L. & Tylen, U. 1986. ADIPOSE-TISSUE VOLUME DETERMINATIONS IN WOMEN BY COMPUTED-TOMOGRAPHY - TECHNICAL CONSIDERATIONS. *International Journal of Obesity,* 10**,** 53-67.

Kyle, U. G., Bosaeus, I., De Lorenzo, A. D., Deurenberg, P., Elia, M., Gomez, J. M., Heitmann, B. L., Kent-Smith, L., Melchior, J. C., Pirlich, M., Scharfetter, H., Schols, A. M., Pichard, C. & Composition of The, E. W. G. 2004a. Bioelectrical impedance analysis--part I: review of principles and methods. *Clin Nutr,* 23**,** 1226-43.

Kyle, U. G., Bosaeus, I., De Lorenzo, A. D., Deurenberg, P., Elia, M., Manuel Gomez, J., Lilienthal Heitmann, B., Kent-Smith, L., Melchior, J. C., Pirlich, M., Scharfetter, H., A, M. W. J. S., Pichard, C. & Espen 2004b. Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clin Nutr,* 23**,** 1430-53.

Laakso, M., Edelman, S. V., Olefsky, J. M., Brechtel, G., Wallace, P. & Baron, A. D. 1990. Kinetics of in vivo muscle insulin-mediated glucose uptake in human obesity. *Diabetes,* 39**,** 965-74.

Larsen, A. E., Tunstall, R. J., Carey, K. A., Nicholas, G., Kambadur, R., Crowe, T. C. & Cameron-Smith, D. 2006. Actions of short-term fasting on human skeletal muscle myogenic and atrogenic gene expression. *Ann Nutr Metab,* 50**,** 476-81.

Lecker, S. H., Jagoe, R. T., Gilbert, A., Gomes, M., Baracos, V., Bailey, J., Price, S. R., Mitch, W. E. & Goldberg, A. L. 2004. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J,* 18**,** 39-51.

Lee, J. L., Leong, L. P. & Lim, S. L. 2015. Nutrition intervention approaches to reduce malnutrition in oncology patients: a systematic review. *Support Care Cancer*.
Lee, K., Lee, S., Kim, Y. J. & Kim, Y. J. 2008. Waist circumference, dual-energy X-ray absortiometrically measured abdominal adiposity, and computed tomographically derived intra-abdominal fat area on detecting metabolic risk factors in obese women. *Nutrition,* 24**,** 625-31.
Lee, S., Kuk, J. L., Davidson, L. E., Hudson, R., Kilpatrick, K., Graham, T. E. & Ross, R. 2005. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. *J Appl Physiol (1985),* 99**,** 1220-5.
Lee, S. Y. & Gallagher, D. 2008. Assessment methods in human body composition. *Curr Opin Clin Nutr Metab Care,* 11**,** 566-72.
Lehrke, M., Broedl, U. C., Biller-Friedmann, I. M., Vogeser, M., Henschel, V., Nassau, K., Goke, B., Kilger, E. & Parhofer, K. G. 2008. Serum concentrations of cortisol, interleukin 6, leptin and adiponectin predict stress induced insulin resistance in acute inflammatory reactions. *Crit Care,* 12**,** R157.
Lima, M. M., Pareja, J. C., Alegre, S. M., Geloneze, S. R., Kahn, S. E., Astiarraga, B. D., Chaim, E. A., Baracat, J. & Geloneze, B. 2013. Visceral fat resection in humans: effect on insulin sensitivity, beta-cell function, adipokines, and inflammatory markers. *Obesity (Silver Spring),* 21**,** E182-9.
Liu, S., Tinker, L., Song, Y., Rifai, N., Bonds, D. E., Cook, N. R., Heiss, G., Howard, B. V., Hotamisligil, G. S., Hu, F. B., Kuller, L. H. & Manson, J. E. 2007. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Arch Intern Med,* 167**,** 1676-85.
Livak, K. J. & Schmittgen, T. D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods,* 25**,** 402-408.
Ljunggren, S., Nystrom, T. & Hahn, R. G. 2014. Accuracy and precision of commonly used methods for quantifying surgery-induced insulin resistance: Prospective observational study. *Eur J Anaesthesiol,* 31**,** 110-6.
Ljungqvist, O., Nygren, J. & Thorell, A. 2002. Modulation of post-operative insulin resistance by pre-operative carbohydrate loading. *Proc Nutr Soc,* 61**,** 329-36.
Ljungqvist, O., Thorell, A., Gutniak, M., Haggmark, T. & Efendic, S. 1994. Glucose infusion instead of preoperative fasting reduces postoperative insulin resistance. *J Am Coll Surg,* 178**,** 329-36.
Lobo, D. N., Hendry, P. O., Rodrigues, G., Marciani, L., Totman, J. J., Wright, J. W., Preston, T., Gowland, P., Spiller, R. C. & Fearon, K. C. 2009. Gastric emptying of three liquid oral preoperative metabolic preconditioning regimens measured by magnetic resonance imaging in healthy adult volunteers: a randomised double-blind, crossover study. *Clin Nutr,* 28**,** 636-41.
Lohman, T. 1988. Anthropometric standardization reference manual. *Human Kinetics Books; Champaign, IL*.
Lu, B., Xu, J., Chen, J., Yu, J., Xu, E. & Lai, M. 2008. TaqMan low density array is roughly right for gene expression quantification in colorectal cancer. *Clin Chim Acta,* 389**,** 146-51.
Lukaski, H. C., Johnson, P. E., Bolonchuk, W. W. & Lykken, G. I. 1985. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr,* 41**,** 810-7.

Malietzis, G., Johns, N., Al-Hassi, H. O., Knight, S. C., Kennedy, R. H., Fearon, K. C., Aziz, O. & Jenkins, J. T. 2015. Low Muscularity and Myosteatosis Is Related to the Host Systemic Inflammatory Response in Patients Undergoing Surgery for Colorectal Cancer. *Ann Surg*.

Mansell, P. I. & Macdonald, I. A. 1990. The effect of starvation on insulin-induced glucose disposal and thermogenesis in humans. *Metabolism,* 39**,** 502-10.

Marques-Vidal, P., Bastardot, F., Von Kanel, R., Paccaud, F., Preisig, M., Waeber, G. & Vollenweider, P. 2013. Association between circulating cytokine levels, diabetes and insulin resistance in a population-based sample (CoLaus study). *Clin Endocrinol (Oxf),* 78**,** 232-41.

Martin, L., Birdsell, L., Macdonald, N., Reiman, T., Clandinin, M. T., Mccargar, L. J., Murphy, R., Ghosh, S., Sawyer, M. B. & Baracos, V. E. 2013. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *J Clin Oncol,* 31**,** 1539-47.

Masuda, M., Ting, T. C., Levi, M., Saunders, S. J., Miyazaki-Anzai, S. & Miyazaki, M. 2012. Activating transcription factor 4 regulates stearate-induced vascular calcification. *J Lipid Res,* 53**,** 1543-52.

Mather, K. J., Hunt, A. E., Steinberg, H. O., Paradisi, G., Hook, G., Katz, A., Quon, M. J. & Baron, A. D. 2001. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab,* 86**,** 5457-64.

Mathur, S., Plank, L. D., Mccall, J. L., Shapkov, P., Mcilroy, K., Gillanders, L. K., Merrie, A. E., Torrie, J. J., Pugh, F., Koea, J. B., Bissett, I. P. & Parry, B. R. 2010. Randomized controlled trial of preoperative oral carbohydrate treatment in major abdominal surgery. *Br J Surg,* 97**,** 485-94.

Matsuda, M. & Defronzo, R. A. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care,* 22**,** 1462-70.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. & Turner, R. C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia,* 28**,** 412-9.

Mclaughlin, T., Lamendola, C., Liu, A. & Abbasi, F. 2011. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. *J Clin Endocrinol Metab,* 96**,** E1756-60.

Mikkelsen, M. M., Hansen, T. K., Gjedsted, J., Andersen, N. H., Christensen, T. D., Hjortdal, V. E. & Johnsen, S. P. 2010. Insulin resistance, adiponectin and adverse outcomes following elective cardiac surgery: a prospective follow-up study. *J Cardiothorac Surg,* 5**,** 129.

Miljkovic, I., Cauley, J. A., Wang, P. Y., Holton, K. F., Lee, C. G., Sheu, Y., Barrett-Connor, E., Hoffman, A. R., Lewis, C. B., Orwoll, E. S., Stefanick, M. L., Strotmeyer, E. S. & Marshall, L. M. 2013. Abdominal myosteatosis is independently associated with hyperinsulinemia and insulin resistance among older men without diabetes. *Obesity (Silver Spring),* 21**,** 2118-25.

Miljkovic, I., Kuipers, A. L., Kammerer, C. M., Wang, X., Bunker, C. H., Patrick, A. L., Wheeler, V. W., Kuller, L. H., Evans, R. W. & Zmuda, J. M. 2011. Markers of inflammation are heritable and

associated with subcutaneous and ectopic skeletal muscle adiposity in African ancestry families. *Metab Syndr Relat Disord,* 9**,** 319-26.
Miljkovic, I. & Zmuda, J. M. 2010. Epidemiology of myosteatosis. *Curr Opin Clin Nutr Metab Care,* 13**,** 260-4.
Miller, R. A., Chu, Q., Le Lay, J., Scherer, P. E., Ahima, R. S., Kaestner, K. H., Foretz, M., Viollet, B. & Birnbaum, M. J. 2011. Adiponectin suppresses gluconeogenic gene expression in mouse hepatocytes independent of LKB1-AMPK signaling. *J Clin Invest,* 121**,** 2518-28.
Mitsiopoulos, N., Baumgartner, R. N., Heymsfield, S. B., Lyons, W., Gallagher, D. & Ross, R. 1998. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *Journal of Applied Physiology,* 85**,** 115-122.
Mitterberger, M. C., Mattesich, M., Klaver, E., Lechner, S., Engelhardt, T., Larcher, L., Pierer, G., Piza-Katzer, H. & Zwerschke, W. 2010. Adipokine profile and insulin sensitivity in formerly obese women subjected to bariatric surgery or diet-induced long-term caloric restriction. *J Gerontol A Biol Sci Med Sci,* 65**,** 915-23.
Moghaddam, A. A., Woodward, M. & Huxley, R. 2007. Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. *Cancer Epidemiol Biomarkers Prev,* 16**,** 2533-47.
Moon, Y. S., Kim, D. H. & Song, D. K. 2004. Serum tumor necrosis factor-alpha levels and components of the metabolic syndrome in obese adolescents. *Metabolism,* 53**,** 863-7.
Moors, C. C., Van Der Zijl, N. J., Diamant, M., Blaak, E. E. & Goossens, G. H. 2012. Impaired insulin sensitivity is accompanied by disturbances in skeletal muscle fatty acid handling in subjects with impaired glucose metabolism. *Int J Obes (Lond),* 36**,** 709-17.
Moschen, A. R., Kaser, A., Enrich, B., Mosheimer, B., Theurl, M., Niederegger, H. & Tilg, H. 2007. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. *J Immunol,* 178**,** 1748-58.
Mourtzakis, M., Prado, C. M. M., Lieffers, J. R., Reiman, T., Mccargar, L. J. & Baracos, V. E. 2008. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme,* 33**,** 997-1006.
Mowery, N. T., Dortch, M. J., Dossett, L. A., Norris, P. R., Diaz, J. J., Jr., Morris, J. A., Jr. & May, A. K. 2009. Insulin resistance despite tight glucose control is associated with mortality in critically ill surgical patients. *J Intensive Care Med,* 24**,** 242-51.
Muoio, D. M. 2010. Intramuscular triacylglycerol and insulin resistance: guilty as charged or wrongly accused? *Biochim Biophys Acta,* 1801**,** 281-8.
Muoio, D. M. 2012. Revisiting the connection between intramyocellular lipids and insulin resistance: a long and winding road. *Diabetologia,* 55**,** 2551-4.
Nilsson, L. H. & Hultman, E. 1973. Liver glycogen in man--the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. *Scand J Clin Lab Invest,* 32**,** 325-30.
Ntambi, J. M., Miyazaki, M., Stoehr, J. P., Lan, H., Kendziorski, C. M., Yandell, B. S., Song, Y., Cohen, P., Friedman, J. M. & Attie, A.

D. 2002. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci U S A,* 99**,** 11482-6.
Nygren, J., Soop, M., Thorell, A., Efendic, S., Nair, K. S. & Ljungqvist, O. 1998. Preoperative oral carbohydrate administration reduces postoperative insulin resistance. *Clin Nutr,* 17**,** 65-71.
Nygren, J., Soop, M., Thorell, A., Sree Nair, K. & Ljungqvist, O. 1999. Preoperative oral carbohydrates and postoperative insulin resistance. *Clin Nutr,* 18**,** 117-20.
Nygren, J., Thorell, A., Efendic, S., Nair, K. S. & Ljungqvist, O. 1997. Site of insulin resistance after surgery: the contribution of hypocaloric nutrition and bed rest. *Clin Sci (Lond),* 93**,** 137-46.
Nygren, J., Thorell, A., Jacobsson, H., Larsson, S., Schnell, P. O., Hylen, L. & Ljungqvist, O. 1995. Preoperative gastric emptying. Effects of anxiety and oral carbohydrate administration. *Ann Surg,* 222**,** 728-34.
O'doherty, M. G., Freedman, N. D., Hollenbeck, A. R., Schatzkin, A. & Abnet, C. C. 2012. A prospective cohort study of obesity and risk of oesophageal and gastric adenocarcinoma in the NIH-AARP Diet and Health Study. *Gut,* 61**,** 1261-8.
O'keefe, S. J., Sender, P. M. & James, W. P. 1974. "Catabolic" loss of body nitrogen in response to surgery. *Lancet,* 2**,** 1035-8.
Ogata, T., Miyauchi, T., Sakai, S., Irukayama-Tomobe, Y., Goto, K. & Yamaguchi, I. 2002. Stimulation of peroxisome-proliferator-activated receptor alpha (PPAR alpha) attenuates cardiac fibrosis and endothelin-1 production in pressure-overloaded rat hearts. *Clin Sci (Lond),* 103 Suppl 48**,** 284S-288S.
Ogden, C. L., Carroll, M. D. & Flegal, K. M. 2014. Prevalence of obesity in the United States. *JAMA,* 312**,** 189-90.
Oki, K., Yamane, K., Kamei, N., Nojima, H. & Kohno, N. 2007. Circulating visfatin level is correlated with inflammation, but not with insulin resistance. *Clin Endocrinol (Oxf),* 67**,** 796-800.
Otten, J., Ahren, B. & Olsson, T. 2014. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic-euglycaemic clamp: a meta-analysis. *Diabetologia*.
Palazzo, S. J., Simpson, T. & Schnapp, L. M. 2012. Triggering receptor expressed on myeloid cells type 1 as a potential therapeutic target in sepsis. *Dimens Crit Care Nurs,* 31**,** 1-6.
Pandey, A. K., Bhardwaj, V. & Datta, M. 2009. Tumour necrosis factor-alpha attenuates insulin action on phosphoenolpyruvate carboxykinase gene expression and gluconeogenesis by altering the cellular localization of Foxa2 in HepG2 cells. *FEBS J,* 276**,** 3757-69.
Papadia, F., Marinari, G. M., Camerini, G., Civalleri, D., Scopinaro, N. & Adami, G. F. 2003. Leptin and insulin action in severely obese women. *Obes Surg,* 13**,** 241-4.
Pedersen, B. K. & Febbraio, M. A. 2007. Point: Interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. *J Appl Physiol (1985),* 102**,** 814-6.
Perseghin, G., Scifo, P., De Cobelli, F., Pagliato, E., Battezzati, A., Arcelloni, C., Vanzulli, A., Testolin, G., Pozza, G., Del Maschio, A. & Luzi, L. 1999. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes,* 48**,** 1600-6.
Petersen, K. F. & Shulman, G. I. 2006. Etiology of insulin resistance. *Am J Med,* 119**,** S10-6.

Pikarsky, A. J., Saida, Y., Yamaguchi, T., Martinez, S., Chen, W., Weiss, E. G., Nogueras, J. J. & Wexner, S. D. 2002. Is obesity a high-risk factor for laparoscopic colorectal surgery? *Surg Endosc,* 16**,** 855-8.

Plomgaard, P., Bouzakri, K., Krogh-Madsen, R., Mittendorfer, B., Zierath, J. R. & Pedersen, B. K. 2005. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes,* 54**,** 2939-45.

Prado, C. M., Baracos, V. E., Mccargar, L. J., Mourtzakis, M., Mulder, K. E., Reiman, T., Butts, C. A., Scarfe, A. G. & Sawyer, M. B. 2007. Body composition as an independent determinant of 5-fluorouracil-based chemotherapy toxicity. *Clin Cancer Res,* 13**,** 3264-8.

Prado, C. M., Baracos, V. E., Mccargar, L. J., Reiman, T., Mourtzakis, M., Tonkin, K., Mackey, J. R., Koski, S., Pituskin, E. & Sawyer, M. B. 2009. Sarcopenia as a determinant of chemotherapy toxicity and time to tumor progression in metastatic breast cancer patients receiving capecitabine treatment. *Clin Cancer Res,* 15**,** 2920-6.

Prado, C. M., Lieffers, J. R., Mccargar, L. J., Reiman, T., Sawyer, M. B., Martin, L. & Baracos, V. E. 2008. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol,* 9**,** 629-35.

Raji, A., Seely, E. W., Arky, R. A. & Simonson, D. C. 2001. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab,* 86**,** 5366-71.

Randle, P. J., Garland, P. B., Hales, C. N. & Newsholme, E. A. 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet,* 1**,** 785-9.

Rebelos, E., Muscelli, E., Natali, A., Balkau, B., Mingrone, G., Piatti, P., Konrad, T., Mari, A., Ferrannini, E. & Investigators, R. S. 2011. Body weight, not insulin sensitivity or secretion, may predict spontaneous weight changes in nondiabetic and prediabetic subjects: the RISC study. *Diabetes,* 60**,** 1938-45.

Reddy, J. K. & Hashimoto, T. 2001. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr,* 21**,** 193-230.

Renehan, A. G., Tyson, M., Egger, M., Heller, R. F. & Zwahlen, M. 2008. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet,* 371**,** 569-78.

Rodrigues, C. S., Lacerda, M. S. & Chaves, G. V. 2015. Patient Generated Subjective Global Assessment as a prognosis tool in women with gynecologic cancer. *Nutrition,* 31**,** 1372-8.

Ron, D. & Walter, P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol,* 8**,** 519-29.

Rosenzweig, T., Braiman, L., Bak, A., Alt, A., Kuroki, T. & Sampson, S. R. 2002. Differential effects of tumor necrosis factor-alpha on protein kinase C isoforms alpha and delta mediate inhibition of insulin receptor signaling. *Diabetes,* 51**,** 1921-30.

Ross, R., Aru, J., Freeman, J., Hudson, R. & Janssen, I. 2002a. Abdominal adiposity and insulin resistance in obese men. *Am J Physiol Endocrinol Metab,* 282**,** E657-63.

Ross, R., Freeman, J., Hudson, R. & Janssen, I. 2002b. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *J Clin Endocrinol Metab,* 87**,** 5044-51.
Rotter, V., Nagaev, I. & Smith, U. 2003. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem,* 278**,** 45777-84.
Sada, F., Krasniqi, A., Hamza, A., Gecaj-Gashi, A., Bicaj, B. & Kavaja, F. 2014. A randomized trial of preoperative oral carbohydrates in abdominal surgery. *BMC Anesthesiol,* 14**,** 93.
Sadashiv, Tiwari, S., Paul, B. N., Kumar, S., Chandra, A., Dhananjai, S. & Negi, M. P. 2012. Over expression of resistin in adipose tissue of the obese induces insulin resistance. *World J Diabetes,* 3**,** 135-41.
Samal, B., Sun, Y., Stearns, G., Xie, C., Suggs, S. & Mcniece, I. 1994. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol,* 14**,** 1431-7.
Samaras, K., Botelho, N. K., Chisholm, D. J. & Lord, R. V. 2010. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. *Obesity (Silver Spring),* 18**,** 884-9.
Samra, J. S., Sved, P., Sullivan, D., Hugh, T. J. & Smith, R. C. 2005. Lipid metabolism in omental adipose tissue during operative surgery. *J Surg Res,* 124**,** 23-8.
Sandeep, S., Gokulakrishnan, K., Velmurugan, K., Deepa, M. & Mohan, V. 2010. Visceral & subcutaneous abdominal fat in relation to insulin resistance & metabolic syndrome in non-diabetic south Indians. *Indian J Med Res,* 131**,** 629-35.
Sandqvist, M., Strindberg, L., Schmelz, M., Lonnroth, P. & Jansson, P. A. 2011. Impaired delivery of insulin to adipose tissue and skeletal muscle in obese women with postprandial hyperglycemia. *J Clin Endocrinol Metab,* 96**,** E1320-4.
Sato, H., Carvalho, G., Sato, T., Lattermann, R., Matsukawa, T. & Schricker, T. 2010. The association of preoperative glycemic control, intraoperative insulin sensitivity, and outcomes after cardiac surgery. *J Clin Endocrinol Metab,* 95**,** 4338-44.
Sato, T., Takeda, H., Sasaki, Y. & Kawata, S. 2011. Increased homeostasis model assessment-insulin resistance is a risk factor for colorectal adenoma in Japanese males. *Tohoku J Exp Med,* 223**,** 297-303.
Schmittgen, T. D. & Livak, K. J. 2008. Analyzing real-time PCR data by the comparative CT method. *Nat. Protocols,* 3**,** 1101-1108.
Segal, K. R., Van Loan, M., Fitzgerald, P. I., Hodgdon, J. A. & Van Itallie, T. B. 1988. Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. *Am J Clin Nutr,* 47**,** 7-14.
Shaw, C. S., Clark, J. & Wagenmakers, A. J. 2010. The effect of exercise and nutrition on intramuscular fat metabolism and insulin sensitivity. *Annu Rev Nutr,* 30**,** 13-34.
Shulman, A. I. & Mangelsdorf, D. J. 2005. Retinoid x receptor heterodimers in the metabolic syndrome. *N Engl J Med,* 353**,** 604-15.
Sikora, S. S., Ribeiro, U., Kane, J. M., 3rd, Landreneau, R. J., Lembersky, B. & Posner, M. C. 1998. Role of nutrition support during induction chemoradiation therapy in esophageal cancer. *JPEN J Parenter Enteral Nutr,* 22**,** 18-21.

Silha, J. V., Krsek, M., Skrha, J. V., Sucharda, P., Nyomba, B. L. & Murphy, L. J. 2003. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol,* 149**,** 331-5.

Simon, I., Escote, X., Vilarrasa, N., Gomez, J., Fernandez-Real, J. M., Megia, A., Gutierrez, C., Gallart, L., Masdevall, C. & Vendrell, J. 2009. Adipocyte fatty acid-binding protein as a determinant of insulin sensitivity in morbid-obese women. *Obesity (Silver Spring),* 17**,** 1124-8.

Singh, B. N., Dahiya, D., Bagaria, D., Saini, V., Kaman, L., Kaje, V., Vagadiya, A., Sarin, S., Edwards, R., Attri, V. & Jain, K. 2015. Effects of preoperative carbohydrates drinks on immediate postoperative outcome after day care laparoscopic cholecystectomy. *Surg Endosc*.

Sites, C. K., Calles-Escandon, J., Brochu, M., Butterfield, M., Ashikaga, T. & Poehlman, E. T. 2000. Relation of regional fat distribution to insulin sensitivity in postmenopausal women. *Fertil Steril,* 73**,** 61-5.

Smith, S. R., Lovejoy, J. C., Greenway, F., Ryan, D., Dejonge, L., De La Bretonne, J., Volafova, J. & Bray, G. A. 2001. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism,* 50**,** 425-35.

Stephens, J. M., Lee, J. & Pilch, P. F. 1997. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *J Biol Chem,* 272**,** 971-6.

Stephens, J. M. & Pekala, P. H. 1992. Transcriptional repression of the C/EBP-alpha and GLUT4 genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. Regulations is coordinate and independent of protein synthesis. *J Biol Chem,* 267**,** 13580-4.

Stepien, M., Rosniak-Bak, K., Paradowski, M., Misztal, M., Kujawski, K., Banach, M. & Rysz, J. 2011. Waist circumference, ghrelin and selected adipose tissue-derived adipokines as predictors of insulin resistance in obese patients: preliminary results. *Med Sci Monit,* 17**,** PR13-18.

Stepien, M., Stepien, A., Wlazel, R. N., Paradowski, M., Rizzo, M., Banach, M. & Rysz, J. 2014. Predictors of insulin resistance in patients with obesity: a pilot study. *Angiology,* 65**,** 22-30.

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel, H. R., Ahima, R. S. & Lazar, M. A. 2001. The hormone resistin links obesity to diabetes. *Nature,* 409**,** 307-12.

Stern, S. E., Williams, K., Ferrannini, E., Defronzo, R. A., Bogardus, C. & Stern, M. P. 2005. Identification of individuals with insulin resistance using routine clinical measurements. *Diabetes,* 54**,** 333-9.

Stitt, T. N., Drujan, D., Clarke, B. A., Panaro, F., Timofeyva, Y., Kline, W. O., Gonzalez, M., Yancopoulos, G. D. & Glass, D. J. 2004. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell,* 14**,** 395-403.

Stolic, M., Russell, A., Hutley, L., Fielding, G., Hay, J., Macdonald, G., Whitehead, J. & Prins, J. 2002. Glucose uptake and insulin action in human adipose tissue--influence of BMI, anatomical

depot and body fat distribution. *Int J Obes Relat Metab Disord,* 26**,** 17-23.
Strissel, K. J., Stancheva, Z., Miyoshi, H., Perfield, J. W., 2nd, Defuria, J., Jick, Z., Greenberg, A. S. & Obin, M. S. 2007. Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes,* 56**,** 2910-8.
Sun, G., French, C. R., Martin, G. R., Younghusband, B., Green, R. C., Xie, Y. G., Mathews, M., Barron, J. R., Fitzpatrick, D. G., Gulliver, W. & Zhang, H. 2005. Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of percentage body fat in a large, healthy population. *Am J Clin Nutr,* 81**,** 74-8.
Svanfeldt, M., Thorell, A., Hausel, J., Soop, M., Nygren, J. & Ljungqvist, O. 2005. Effect of "preoperative" oral carbohydrate treatment on insulin action--a randomised cross-over unblinded study in healthy subjects. *Clin Nutr,* 24**,** 815-21.
Swinburn, B. A., Nyomba, B. L., Saad, M. F., Zurlo, F., Raz, I., Knowler, W. C., Lillioja, S., Bogardus, C. & Ravussin, E. 1991. Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest,* 88**,** 168-73.
Taaffe, D. R., Henwood, T. R., Nalls, M. A., Walker, D. G., Lang, T. F. & Harris, T. B. 2009. Alterations in muscle attenuation following detraining and retraining in resistance-trained older adults. *Gerontology,* 55**,** 217-23.
Tan, B. H., Birdsell, L. A., Martin, L., Baracos, V. E. & Fearon, K. C. 2009. Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. *Clin Cancer Res,* 15**,** 6973-9.
Tateya, S., Kim, F. & Tamori, Y. 2013. Recent advances in obesity-induced inflammation and insulin resistance. *Front Endocrinol (Lausanne),* 4**,** 93.
Tessarz, A. S. & Cerwenka, A. 2008. The TREM-1/DAP12 pathway. *Immunol Lett,* 116**,** 111-6.
Thamer, C., Machann, J., Staiger, H., Mussig, K., Schwenzer, N., Ludescher, B., Machicao, F., Claussen, C., Fritsche, A., Schick, F., Stefan, N. & Haring, H. U. 2010. Interscapular fat is strongly associated with insulin resistance. *J Clin Endocrinol Metab,* 95**,** 4736-42.
Thorand, B., Lowel, H., Schneider, A., Kolb, H., Meisinger, C., Frohlich, M. & Koenig, W. 2003. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. *Arch Intern Med,* 163**,** 93-9.
Thorell, A., Efendic, S., Gutniak, M., Haggmark, T. & Ljungqvist, O. 1993. Development of postoperative insulin resistance is associated with the magnitude of operation. *Eur J Surg,* 159**,** 593-9.
Thorell, A., Efendic, S., Gutniak, M., Haggmark, T. & Ljungqvist, O. 1994. Insulin resistance after abdominal surgery. *Br J Surg,* 81**,** 59-63.
Thorell, A., Loftenius, A., Andersson, B. & Ljungqvist, O. 1996. Postoperative insulin resistance and circulating concentrations of stress hormones and cytokines. *Clin Nutr,* 15**,** 75-9.
Thorell, A., Nygren, J. & Ljungqvist, O. 1999. Insulin resistance: a marker of surgical stress. *Curr Opin Clin Nutr Metab Care,* 2**,** 69-78.

Timmers, S., Schrauwen, P. & De Vogel, J. 2008. Muscular diacylglycerol metabolism and insulin resistance. *Physiol Behav,* 94**,** 242-51.
Timmons, J. A., Szkop, K. J. & Gallagher, I. J. 2015. Multiple sources of bias confound functional enrichment analysis of global -omics data. *Genome Biol,* 16**,** 186.
Tisdale, M. J. 1999. Wasting in cancer. *J Nutr,* 129**,** 243S-246S.
Tsai, S. M. D. M. H. S. 2012. Importance of Lean Body Mass in the Oncologic Patient. *Nutrition in Clinical Practice,* 27**,** 593-598.
Uchida, I., Asoh, T., Shirasaka, C. & Tsuji, H. 1988. Effect of epidural analgesia on postoperative insulin resistance as evaluated by insulin clamp technique. *Br J Surg,* 75**,** 557-62.
Uk, C. R. 2010-2011. *http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/stomach-cancer/survival#heading-Zero***,** Accessed 21.6.15.
Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. S. 1997. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature,* 389**,** 610-4.
Valdez, R., Mitchell, B. D., Haffner, S. M., Hazuda, H. P., Morales, P. A., Monterrosa, A. & Stern, M. P. 1994. Predictors of weight change in a bi-ethnic population. The San Antonio Heart Study. *Int J Obes Relat Metab Disord,* 18**,** 85-91.
Van Beek, L., Lips, M. A., Visser, A., Pijl, H., Ioan-Facsinay, A., Toes, R., Berends, F. J., Willems Van Dijk, K., Koning, F. & Van Harmelen, V. 2014. Increased systemic and adipose tissue inflammation differentiates obese women with T2DM from obese women with normal glucose tolerance. *Metabolism,* 63**,** 492-501.
Varma, V., Yao-Borengasser, A., Rasouli, N., Bodles, A. M., Phanavanh, B., Lee, M. J., Starks, T., Kern, L. M., Spencer, H. J., 3rd, Mcgehee, R. E., Jr., Fried, S. K. & Kern, P. A. 2007. Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation. *J Clin Endocrinol Metab,* 92**,** 666-72.
Vehmas, T., Kairemo, K. J. A. & Taavitsainen, M. J. 1996. Measuring visceral adipose tissue content from contrast enhanced computed tomography. *International Journal of Obesity,* 20**,** 570-573.
Vendrell, J., El Bekay, R., Peral, B., Garcia-Fuentes, E., Megia, A., Macias-Gonzalez, M., Fernandez Real, J., Jimenez-Gomez, Y., Escote, X., Pachon, G., Simo, R., Selva, D. M., Malagon, M. M. & Tinahones, F. J. 2011a. Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. *Endocrinology,* 152**,** 4072-9.
Vendrell, J., El Bekay, R., Peral, B., Garcia-Fuentes, E., Megia, A., Macias-Gonzalez, M., Real, J. F., Jimenez-Gomez, Y., Escote, X., Pachon, G., Simo, R., Selva, D. M., Malagon, M. M. & Tinahones, F. J. 2011b. Study of the Potential Association of Adipose Tissue GLP-1 Receptor with Obesity and Insulin Resistance. *Endocrinology,* 152**,** 4072-9.
Ventre, J., Doebber, T., Wu, M., Macnaul, K., Stevens, K., Pasparakis, M., Kollias, G. & Moller, D. E. 1997. Targeted disruption of the tumor necrosis factor-alpha gene: metabolic consequences in obese and nonobese mice. *Diabetes,* 46**,** 1526-31.
Vohl, M. C., Sladek, R., Robitaille, J., Gurd, S., Marceau, P., Richard, D., Hudson, T. J. & Tchernof, A. 2004. A survey of genes

differentially expressed in subcutaneous and visceral adipose tissue in men. *Obes Res,* 12**,** 1217-22.
Wagenknecht, L. E., Langefeld, C. D., Scherzinger, A. L., Norris, J. M., Haffner, S. M., Saad, M. F. & Bergman, R. N. 2003. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes,* 52**,** 2490-6.
Wakefield, H., Vaughan-Sarrazin, M. & Cullen, J. J. 2012. Influence of obesity on complications and costs after intestinal surgery. *Am J Surg,* 204**,** 434-40.
Wang, D. Y., Qin, R. Y., Liu, Z. R., Gupta, M. K. & Chang, Q. 2004. Expression of TREM-1 mRNA in acute pancreatitis. *World J Gastroenterol,* 10**,** 2744-6.
Wang, X., Yang, Z., Xue, B. & Shi, H. 2011. Activation of the cholinergic antiinflammatory pathway ameliorates obesity-induced inflammation and insulin resistance. *Endocrinology,* 152**,** 836-46.
Wang, Z. G., Wang, Q., Wang, W. J. & Qin, H. L. 2010. Randomized clinical trial to compare the effects of preoperative oral carbohydrate versus placebo on insulin resistance after colorectal surgery. *Br J Surg,* 97**,** 317-27.
Webber, J., Taylor, J., Greathead, H., Dawson, J., Buttery, P. J. & Macdonald, I. A. 1994. Effects of fasting on fatty acid kinetics and on the cardiovascular, thermogenic and metabolic responses to the glucose clamp. *Clin Sci (Lond),* 87**,** 697-706.
Weisberg, S. P., Mccann, D., Desai, M., Rosenbaum, M., Leibel, R. L. & Ferrante, A. W., Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest,* 112**,** 1796-808.
Wentworth, J. M., Naselli, G., Brown, W. A., Doyle, L., Phipson, B., Smyth, G. K., Wabitsch, M., O'brien, P. E. & Harrison, L. C. 2010. Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. *Diabetes,* 59**,** 1648-56.
Windsor, J. A. & Hill, G. L. 1988. Weight loss with physiologic impairment. A basic indicator of surgical risk. *Ann Surg,* 207**,** 290-6.
Witasp, A., Nordfors, L., Schalling, M., Nygren, J., Ljungqvist, O. & Thorell, A. 2009. Increased expression of inflammatory pathway genes in skeletal muscle during surgery. *Clin Nutr,* 28**,** 291-8.
Witasp, A., Nordfors, L., Schalling, M., Nygren, J., Ljungqvist, O. & Thorell, A. 2010. Expression of inflammatory and insulin signaling genes in adipose tissue in response to elective surgery. *J Clin Endocrinol Metab,* 95**,** 3460-9.
Wolfe, R. R. 1998. Metabolic interactions between glucose and fatty acids in humans. *Am J Clin Nutr,* 67**,** 519S-526S.
Ye, J. 2013. Mechanisms of insulin resistance in obesity. *Front Med,* 7**,** 14-24.
Yeung, C. Y., Tso, A. W., Xu, A., Wang, Y., Woo, Y. C., Lam, T. H., Lo, S. V., Fong, C. H., Wat, N. M., Woo, J., Cheung, B. M. & Lam, K. S. 2013. Pro-inflammatory adipokines as predictors of incident cancers in a Chinese cohort of low obesity prevalence in Hong Kong. *PLoS One,* 8**,** e78594.
Yip, C., Goh, V., Davies, A., Gossage, J., Mitchell-Hay, R., Hynes, O., Maisey, N., Ross, P., Gaya, A., Landau, D. B., Cook, G. J., Griffin, N. & Mason, R. 2014. Assessment of sarcopenia and

changes in body composition after neoadjuvant chemotherapy and associations with clinical outcomes in oesophageal cancer. *Eur Radiol,* 24**,** 998-1005.

Appendix 1. Adipose and skeletal muscle gene cards

Adipose Tissue Gene Card	Expanded gene name/ Protein product
ACACA	Acetyl CoA Carboxylase Alpha
ALOX15B	Arachidonate 15-lipoxygenase type B
ALOX15	Arachidonate 15-lipoxygenase
ATF6	Activating Transcription Factor 6
ATG5	Autophagy Related Gene 5
ATG7	Autophagy Related Gene 7
BCL2	B-cell lymphoma 2
BECN1	Beclin 1
C3	Complement component 3
CASP1	Caspase 1
GAPDH	**Glyceraldehyde 3-phosphate dehydrogenase**
CASP3	Caspase 3
CASP7	Caspase 7
CD36	Cluster of differentiation 36
CEBPA	CCAAT enhancer binding protein alpha
CEBPB	CCAAT enhancer binding protein beta
CES1	Carboxylesterase 1
CLEC7A	C-type lectin domain family 7
CRAT	Carnitine acetyltransferase
DDIT3	DNA damage inducible transcript 3
DERL2	Degradation in endoplasmic reticulum 2
DLK1	Delta-like 1 homolog
EDEM2	ER degradation enhancer, mannosidase alpha-like 1

EPHX2	Epoxide hydrolase 2
FAS	TNF receptor superfamily member 6
FOXO1	Forkhead box 01
HIF1A	Hypoxia inducible factor 1A
IGFBP2	Insulin-like growth factor binding protein 2
IL10	Interleukin 10
IL1B	Interleukin 1B
IL18	Interleukin 18
IL6	Interleukin 6
IL8	Interleukin 8
IRAK1	Interleukin receptor associated kinase 1
IRAK3	Interleukin receptor associated kinase 3
LCN2	Lipocalin 2
LPCAT1	Lysophosphatidylcholine acyltransferase 1
LPCAT2	Lysophosphatidylcholine acyltransferase 2
MAP1LC3A	Microtubule associated protein 1 light chain 3 alpha
MAP1LC3B	Microtubule associated protein 1 light chain 3 beta
MMP12	Matrix metallopeptidase 12
MMP3	Matrix metallopeptidase 3
MYD88	Myeloid differentiation primary response 88
NAMPT	Nicotinamide phosphoribosyltransferase
NFE2L2	Nuclear factor (erythroid-2-like-2)
NLRP3	NLR family, pyrin domain containing 3
PDK1	Pyruvate dehydrogenase kinase 1

PNPLA2	Patatin like phospholipase domain containing 2
PNPLA3	Patatin like phospholipase domain containing 3
PPARD	Peroxisome proliferator activated receptor delta
SOCS3	Suppressor of cytokine signalling 3
SOD1	Superoxide dismutase 1
SOD2	Superoxide dismutase 2
SREBF1	Sterol regulatory element binding transcription factor 1
TGFB1	Transforming growth factor beta 1
TIMP1	TIMP metallopeptidase inhibitor 1
TNF	Tumour necrosis factor
TNFAIP6	Tumour necrosis factor, alpha induced protein 6
ADIG	Adipogenin
ADIPOQ	Adioponectin c1Q and collagen domain containing
CCL2	Chemokine (C-C motif) ligand 2
CD68	Cluster of differentiation 68
CEBPD	CCAAT enhancer binding protein delta
CFD	Complement factor d
CIDEA	Cell death inducing DFFA-like effector a
FABP4	Fatty acid binding protein 4
FAM101A	Family with sequence similarity 101, member A
IRS1	Insulin receptor substrate 1
IRS2	Insulin receptor substrate 2
IRS4	Insulin receptor substrate 4
ITGAX	Integrin, alpha X

LEP	Leptin
LPIN1	Lipin 1
LPL	Lipoprotein lipase
MTX1	Metaxin 1
PLIN1	Perilipin 1
PPARG	Peroxisome proliferator activated receptor gamma
PPARGC1A	Peroxisome proliferator activated receptor gamma, co-activator 1 alpha
RETN	Resistin
RPUSD3	RNA pseudouridylate synthase domain containing 3
SLC2A4	Solute carrier family 2, member 4
CD3E	Epsilon
GATA3	GATA binding protein 3
GSTK1	Glutathione S-transferase kappa 1
HERPUD1	Homocysteine-inducible endoplasmic reticulum stress inducible, ubiquitin-like domain receptor 1
HP	Haptoglobin
LIPE	Hormone sensitive lipase
RORC	**RAR-related orphan receptor C**
TBX21	T-box 21
TBP	**TATA box binding protein**
BLANK	
TRPV4	Transient receptor potential cation channel, subfamily V member 4
BLANK	
ACTB	**Beta-actin**

BLANK	
HMBS	Hydroxymethylbilane synthase

Housekeeping genes are highlighted in bold

Skeletal Muscle Gene Card	Expanded gene name/ Protein product
GRB14	Growth factor receptor bound protein 1
GPD2	**Glycerol-3-phosphate dehydrogenase 2**
FGF21	Fibroblast growth factor 21
FOXO3	Forkhead box 03
SCD	Stearoyl Co A Desaturase
ANGPTL4	Angiopoietin-like 4
FASN	Fatty acid synthase
NRF1	Nuclear respiratory factor 1
NUAK2	NUAK family, SNF-1 like kinase
LCP1	Lymphocytic cytosolic protein 1
FSTL1	Follistatin-like 1
G6PD	Glucose 6 phosphate dehydrogenase
ERN1	Endoplasmic reticulum to nucleus signalling 1
SCD5	Stearoyl Co A Desaturase 5
ACLY	ATP citrate lyase
GREM1	Gremlin 1
NR4A3	Nuclear receptor subfamily 4, group A, member 3
NR4A1	Nuclear receptor subfamily 1, group A, member 1
IGF1	Insulin-like growth factor 1
MYEF2	Myelin expression factor 2
CD36	Thrombospondin receptor
CPT1B	Carnitine palmitoyltransferase 1B

SLC2A1	Solute carrier family 2 member 1
ATF3	Activating transcription factor 3
ME1	Malic enzyme 1
RPLP2	**Ribosomal protein P2**
PRKCE	Protein kinase C, epsilon
PPARGC1B	Peroxisome proliferator activated receptor gamma, coactivator 1 beta
TDO2	Tryptophan 2-3, dioxygenase
IL6	Interleukin 6
UCP3	Uncoupling protein 3
ABL1	Abelson murine leukaemia 1
ACACA	Acetyl CoA Carboxylase Alpha
RRAD	Ras related associated with diabetes
PKM	Pyruvate kinase, muscle
MLXIPL	MLX interacting protein like
CASP3	Caspase 3
ACADL	Acyl CoA dehydrogenase
ACADVL	Acyl CoA dehydrogenase, very long chain
PDK2	Pyruvate dehydrogenase kinase 2
SLC7A11	Solute carrier family 7 (anionic amino acid transporter y+ system), member 11
PPARA	Peroxisome proliferated activated receptor alpha
SLC2A4	Solute carrier family 2 (facilitated glucose transporter), member 4
LCN2	Lipocalin
FOXO1	Forkhead box 01

IL8	Interleukin 8
ARRDC4	Arrestin domain containing 4
KSR2	Kinase suppressor of ras 2
TAT	Tyrosine aminotransferase
ELOVL6	Fatty acid elongase 6
DDIT4	DNA damage inducible transcript 4
NDUFA7	NADH dehydrogenase alpha subcomplex subunit 7
PRKAA1	Protein kinase AMP activated alpha 1 catalytic subunit
TBP	**TATA box binding protein**
FABP3	Fatty acid binding protein 3
PPARGC1A	Peroxisome proliferated activated receptor gamma, coactivator 1 alpha
TRIM63	Tripartite motif containing 63
IRS2	Insulin receptor substrate 2
ACACB	Acetyl CoA Carboxylase Beta
CEBPB	CCAAT enhancer binding protein B
KLF10	Kruppel-like factor 10
PDK4	Pyruvate dehydrogenase kinase 4
MLYCD	Malonyl Ca O decarboxylase
DDIT3	DNA damage inducible transcript 3
PPARD	Peroxisome proliferated activated receptor delta
IL15	Interleukin 15
ERO1L	Endoplasmic reticulum oxidoreductase -like
HPRT1	**Hypoxanthine phosphoribosyltransferase 1**

HSPA5	Heat shock 70kDa protein 5
UCP2	Uncoupling protein 2
SREBF1	Sterol regulatory binding transcription factor 1
GAPDH	Glyceraldehyde 3 phosphate dehydrogenase
CHI3L1	Chitonase 3 like 1
MYOG	Myogenin
IRS1	Insulin receptor substrate 1
EGR1	Early growth response 1
TRIB3	Tribbles homolog 1
TNF	Tumour necrosis factor
STAT3	Signal transducer and activator of transcription
CRTC2	CREB regulated transcription coactivator 2
BHLHE40	Basic loop helix family member E40
IL6R	Interleukin 6 receptor
HK2	Hexokinase 2
CARM1	Coactivator associated methyltransferase 1
HAS2	Hyaluronan synthase 2
TXNIP	Thioredoxin interacting protein
PPP1R3A	Protein phosphatase 1 regulatory subunit 3A
SREBF2	Sterol regulatory binding transcription factor 1
ACSL4	Acyl CoA Synthetase long chain family member 4
RASD1	Ras, dexamethasone induced 1
SIRT1	Sirtuin 1

FBX032	F-Box protein 32/ Atrogin
ATF4	Activating transcription factor 4
SOCS3	Suppressor of cytokine signalling 3
TFE3	Transcription factor binding to IGHM enhancer 3
GSTA5	Glutathione S transferase alpha 5

Housekeeping genes are highlighted in bold