Effects of caloric restriction and bariatric surgery on non-alcoholic fatty liver disease (NAFLD)

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1 Abstract

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Non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation of the metabolic syndrome. It encompasses a broad spectrum of severity, from steatosis through to inflammation (non-alcoholic steatohepatitis) and subsequent progressive liver fibrosis. It is rapidly becoming the leading cause of cirrhosis and liver transplantation worldwide and, even in advanced disease, liver biochemistry is often normal. No pharmaceutical agents are currently licensed to treat NAFLD; its clinical management is focused on weight loss (via reduced energy intake and exercise-induced increased energy expenditure), and addressing metabolic comorbidities and liver disease risk factors.

Bariatric surgery is recognised as a highly effective treatment for obesity and type 2 diabetes (T2D). Additionally, it appears to usually improve NAFLD, although it is not currently recommended in the UK's clinical guidelines for NAFLD management. In contrast to T2D, where improved postoperative glycaemic control is expected, cases of postoperative liver dysfunction have been reported when advanced NAFLD was present at the time of surgery. The mechanisms underlying changes in NAFLD after bariatric surgery and the optimal surgical intervention for its treatment are unknown.

In order to evaluate current clinical practice and the views of key stakeholders in NAFLD management, I developed parallel surveys for patients, bariatric surgeons and hepatologists. These identified that most patients would consider bariatric surgery as an intervention for the combined treatment of their obesity and NAFLD, but that this option is usually not discussed by health professionals. Most centres did not have defined protocols for NAFLD staging and monitoring in patients undergoing bariatric surgery, and surgeons rarely used non-invasive biomarkers to predict NAFLD severity and guide clinical care. Hepatologists identified bariatric surgery's omission from NAFLD guidelines as the major barrier to the wider implementation of bariatric surgery as a treatment for NAFLD.

Advanced fibrotic NAFLD is associated with adverse clinical outcomes such as liver and cardiovascular morbidity and mortality. Since isolated liver blood tests poorly predict liver histology, non-invasive biomarkers have previously been developed to improve diagnostic accuracy. However, they have undergone limited validation in patients with severe obesity and NAFLD. Therefore, I performed a comprehensive assessment of the performance of a range of existing non-invasive biomarkers and found that these performed relatively poorly in this patient population. Machine learning approaches were then used to develop and validate a novel model that incorporated routine clinical parameters and offered improved diagnostic accuracy for advanced fibrotic NAFLD prediction.

Clinical outcomes after conventional medical or bariatric surgical treatment of NAFLD have not previously been compared. Therefore, a retrospective cohort study was designed for this purpose. Reduced mortality and greater improvements in a range of key clinical targets in NAFLD management were found after management with bariatric surgery.

Patients undergoing bariatric surgery are often prescribed preoperative low energy diets. These are designed to reduce liver volume and improve surgical visualisation, but it is unclear to what extent these may also improve NAFLD. A prospective study was performed to characterise the effects of a routine preoperative diet in non-diabetic females with severe obesity. Significant improvements in liver and metabolic health outcome measures were observed but these remained elevated relative to non-obese control participants.

5-10% total body weight loss is often recommended for patients with NAFLD, but it is unclear whether this is sufficient for people with severe obesity. I randomised such individuals to undergo either sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB) surgery and studied them after matched 20% postoperative weight loss. Whilst postoperative improvements were observed, these were still insufficient to reverse NAFLD relative to non-obese control participants. Preliminary results show normalisation of liver steatosis 1 year after surgery. Similar changes occurred after SG and RYGB surgery and I conclude that these interventions represent promising interventions for the combined treatment of obesity and NAFLD.

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6 Abbreviations

%B: Pancreatic Beta Cell Activity

%S: Insulin Sensitivity

95% CI: 95% Confidence Interval

AGB: Adjustable Gastric Band

ALT: Alanine aminotransferase

ANOVA: Analysis of Variance

ApoB: Apolipoprotein B

APRI: AST to Platelet Ratio Index

AST: Aspartate aminotransferase

AUC: Area Under the Curve

AUROC: Area Under the Receiver Operating Characteristic

BARD: BMI, AST/ALT Ratio, Diabetes

BMI: Body Mass Index

BPD-DS: Biliopancreatic Diversion with Duodenal Switch

BS: bariatric surgery

CO_2: Carbon Dioxide

CRU: Clinical Research Unit

CT: Computed Tomography

CTIMP: Controlled Trial of Investigational Medicinal Product

CTRG: Clinical Trials and Research Governance

DNL: De Novo Lipogenesis

DXA: Dual Energy X-ray Absorptiometry

ECM: Extracellular Matrix

EDISON: Effects of Dietary Intervention and Surgery on NAFLD

EDTA: Ethylenediaminetetraacetic acid

ELF: Enhanced Liver Fibrosis

EU: European Union

Fib-4: Fibrosis 4

FSH: Follicle-Stimulating Hormone

g: gram

GDPR: General Data Protection Regulation

GLP-1: Glucagon-Like Peptide 1

GORD: Gastro-Oesophageal Reflux Disease

GP: General Practitioner

H_2O: Dihydrogen Oxide

HA: Hyaluronic Acid

HbA_1c: Glycated Haemoglobin

HCC: Hepatocellular Carcinoma

HCV: Hepatitis C Virus

HDL: High Density Lipoprotein

HIV: Human Immunodeficiency Virus

HOMA: Homeostatic Model Assessment

HR: Hazard Ratio

IDEAL: Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation

IFG: Impaired Fasting Glucose

IL: Interleukin

IQR: Interquartile Range

kg: kilogram

l: litre

LDL: Low Density Lipoprotein

LPL: Lipoprotein Lipase

m: metre

MBOAT7: Membrane Bound O-Acyltransferase Domain Containing 7

MH: metabolic hepatology

Mmol: millimole

mol: mole

MOLLI: Modified Look-Locker Inversion Recovery

MRI: Magnetic Resonance Imaging

MRS: Magnetic Resonance Spectroscopy

n: nano

NAFLD: Non-Alcoholic Fatty Liver Disease

NAS: NAFLD Activity Score

NASH: Non-Alcoholic Steatohepatitis

NEFA: Non-Esterified Fatty Acids

NFS: NAFLD Fibrosis Score

NHS: National Health Service

NICE: National Institute for Health and Care Excellence

NIT: Non-Invasive Test

NOC: Non-Obese Control

NPV: Negative Predictive Value

OAGB: One Anastomosis Gastric Bypass

OCDEM: Oxford Centre for Diabetes, Endocrinology and Metabolism

OCMR: Oxford Centre for Clinical Magnetic Resonance Research

OHB: Hydroxybutyrate

OHS: Obesity Hypoventilation Syndrome

ONS: Office of National Statistics

OSA: Obstructive Sleep Apnoea

OUHFT: Oxford University Hospitals NHS Foundation Trust

PDFF: Proton Density Fat Fraction

PIIINP: Type III Procollagen Peptide

PMSF: Phenylmethylsulfonyl fluoride

PNPLA3: Patatin-like phospholipase domain-containing protein 3

PPI: Patient and Public Involvement

PPV: Positive Predictive Value

PUFA: Polyunsaturated Fatty Acids

PYY: Peptide YY

QC: Quality Control

RCT: Randomised Controlled Trial

ROC: Receiver Operating Characteristic

RYGB: Roux-en-Y Gastric Bypass

SADI-S: Single Anastomosis Duodenoileal bypass with Sleeve gastrectomy

SD: Standard Deviation

SEM: Standard Error of Mean

SG-TB: Sleeve Gastrectomy with Transit Bipartition

SG: Sleeve Gastrectomy

SGLT-2: Sodium-Glucose Co-Transporter-2

SOP: Standard Operating Procedure

T2D: Type 2 Diabetes

TAG: Triacylglycerol

TBW: Total Body Weight

THIN: The Healthcare Improvement Network

TIMP-1: Tissue Inhibitor of Metalloproteinases-1

TM6SF2: Transmembrane 6 Superfamily member 2

TNF: Tumour Necrosis Factor

TTR: Tracer to Tracee Ratio

U: Units

UK: United Kingdom

UN: United Nations

USA: United States of America

US: Ultrasound

USS: Ultrasound Scan

VLDL: Very Low Density Lipoprotein

WEOG: Western European and Others Group

YI: Youden's Index

7 Introduction

7.1 Obesity and the metabolic syndrome

7.1.1 Obesity: Definition

Body Mass Index (BMI) is the most frequently used method for categorising body anthropometry, with BMI ≥30 conventionally used to define obesity[1]. It is calculated using the formula: BMI = total body weight (kilogram (kg)) / height (metres (m))^2. The most common thresholds for categorising BMI are shown in Table 7-1[2]:

Table 7-1: BMI classification thresholds

BMI (kg/m^2)	Anthropometric Category
<18.5	Underweight
18.5 to 24.9	Healthy
25.0 to 29.9	Overweight
30.0 to 34.9	Class 1 Obesity
35.0 to 39.9	Class 2 Obesity
≥40.0	Class 3 Obesity

Subdivision of Class 3 obesity has also been proposed in the surgical literature to reflect the BMI range encountered in the bariatric surgical population, although the BMI cut-offs and nomenclature are less well established.

Obesity-associated disease is more common in Asian populations at a lower BMI, where lower thresholds have been applied for overweight (BMI 23 to <28) and obesity (BMI ≥28)[3]. It has been suggested that this increased risk may be due to higher mean percentage body fat in Asian individuals compared to Western individuals of the same BMI[4].

7.1.2 Obesity: Epidemiology

Obesity is increasingly prevalent worldwide, with around 29% of English adults diagnosed with the disease[5]. Half of United Kingdom (UK) adults are projected to have obesity by 2050[6] and is estimated that the UK's National Health Service (NHS) spends £5.1 billion

annually on obesity-associated healthcare costs[7]. Obesity rates are also increasing in children and in regions where under-nutrition was more common until relatively recently[8,9].

7.1.3 Obesity: Pathophysiology

An energy imbalance between energy intake and the expenditure of absorbed calories fundamentally underlies weight gain. Historically, nutrition was inconsistently available, and it is likely that evolutionary adaptations occurred to favour genotypes that promote overeating, energy absorption and reduced energy expenditure. A genetic predisposition to obesity is supported by studies showing a close correlation in the BMI of identical twins, even when raised serparately[10].

Neurohormonal signals regulate appetite, which increases following a period of weight loss, in order to promote increased caloric intake. Metabolic adaptation also occurs, with a reduction in basal metabolic rate in order to reduce energy expenditure and preserve energy stores[11]. It has been proposed that after weight gain, this new body mass represents an individual's modified 'set point', and weight-defending mechanisms are triggered by subsequent reductions below this[12]. Physical inactivity has become more common over recent decades and is the major contributor to reduced energy expenditure[13].

7.1.4 Obesity: Clinical implications

Obesity is now regarded as a primary disease rather than solely the underlying cause for a cluster of associated comorbidities[14]. The clinical implications of obesity, as defined by BMI, vary according to an individual's muscle and fat mass proportions. Increased muscle mass is inversely associated with obesity-associated mortality[15], whilst fat mass increases the risk of obesity-associated disease. Additionally, fat distribution is clinically relevant, as central obesity increase the risk of all-cause mortality whilst gynoid fat has been found to be associated with a reduced mortality rate[16].

7.1.4.1 The Metabolic Syndrome

The metabolic syndrome consists of a combination of cardiometabolic risk factors that are interlinked and associated with obesity. These factors include android obesity, hyperlipidaemia, hyperglycaemia and hypertension[13].

Adipocytes may respond to overnutrition by hyperplasia or by hypertrophy, which is associated with the development of the metabolic syndrome[17]. Adipose tissue dysfunction occurs when subcutaneous adipose tissue depots become saturated, leading to ectopic fat accumulation in abdominal organs and viscera. This leads to a state of chronic low-grade inflammation, insulin resistance and to subsequent organ dysfunction[17,18].

Type 2 Diabetes

Type 2 Diabetes (T2D) results from several pathophysiological abnormalities that impair glucose homeostasis. Increased central, hepatic and peripheral insulin resistance is initially observed, leading to hyperinsulinaemia[19]. Subsequently there is reduced insulin secretion in response to glucose ingestion due to inadequate pancreatic beta cell function[20]. This results in hyperglycaemia, leading to a range of T2D-associated complications such as neuropathy[21].

Hyperlipidaemia

The increased plasma triglyceride concentrations causes ectopic fat to be stored in the liver and muscle of individuals with obesity, contributing to hepatic and peripheral insulin resistance[22]. The products of intracellular free fatty acid metabolism have been shown to oppose insulin action and to directly impair pancreatic beta cell function[23]. Hyperlipidaemia also increases the risk of developing other obesity-associated diseases including pancreatitis.

Cardiovascular Disease

Central obesity is a risk factor for the development of cardiovascular disease and cardiac mortality risk is increased[24,25]. Obesity may affect the heart and vasculature since dyslipidaemia promotes atherosclerosis through proinflammatory and prothrombotic

mechanisms, and as a result of increased cardiac workload. Increased haemodynamic demand leads to initial cardiac muscle hypertrophy and subsequent ventricular dysfunction[26]. Fat accumulation within the heart also directly impairs its function and leads to cardiomyopathy[27].

Non-alcoholic Fatty Liver Disease

Non-alcoholic Fatty Liver Disease (NAFLD) represents the hepatic manifestation of the metabolic syndrome and is characterised by the ectopic accumulation of liver fat as a result of overnutrition. It will be discussed in more detail later in this chapter.

7.1.4.2 Obstructive Sleep Apnoea and Obesity Hypoventilation Syndrome

Obstructive sleep apnoea (OSA) is diagnosed when upper airway collapse occurs whilst sleeping. This leads to intermittent hypoxia, hypercapnia and sympathetic response activation[28]. When this occurs at least 5 times per hour and there is significant associated daytime fatigue, this is known as obstructive sleep apnoea syndrome[29].

Obesity hypoventilation syndrome (OHS) is a disorder in which there is type 2 respiratory failure due to disordered breathing during sleep in people with obesity[30]. There is significant overlap between patients with OSA and with OHS[29].

7.1.4.3 Osteoarthritis

It is estimated that 15% of the world's population have OA and individuals with a Body Mass Index (BMI) of ≥40kg/m^2 are at significantly higher risk of requiring total knee arthroplasty (TKA) than those with a BMI of < 25kg/m^2[2;31]. Obesity results in an immediate functional mobility impairment and increased mechanical joint load and damage that leads to osteoarthritis over a longer period. The pain and reduced mobility that result from joint osteoarthritis make exercise-induced energy expenditure more challenging and may lead to obesity progression.

7.1.4.4 Cancer

More than 10% of malignant disease worldwide can be attributed to obesity[31]. Underlying mechanisms are thought to include chronic low-grade inflammation and oxidative stress, insulin resistance and dysfunctional adipose tissue[32]. Obesity-linked alterations to the gut microbiome[33] and to circadian rhythms[32] are also the subject of considerable scientific interest at present.

Cancers at a wide range of anatomic sites have now been linked to obesity. Site-specific mechanisms have also been proposed, such as hormonal effects in oestrogen-driven cancers like breast, ovarian and endometrial cancer[34], gastrooesophageal reflux leading to cellular metaplasia and ultimately oesophageal cancer[35], and the local effects of dietary metabolites in colorectal cancer[36]. Additional sites include (steatohepatitis-driven) hepatocellular carcinoma, gallbladder, gastric and pancreatic carcinomas and renal, thyroid and meningeal malignancies[34].

Somewhat counterintuitively, obesity has also been linked to improved survival in individuals with cancer, which has been termed the 'obesity paradox'. It has, however, been suggested that this finding relates to the aforementioned limitations of BMI as a measure of body anthropometry and to methodological limitations of the studies reporting improved survival in individuals with obesity[37,38]. Studies that include more precise body composition measures will be important in determining the link between adiposity and cancer survival.

7.1.4.5 Gallstone Disease

Obesity is associated with an increased prevalence of gallstone disease. This has been linked to increased hepatic synthesis of cholesterols that then saturate bile, resulting in cholesterol stone formation[39]. Furthermore, weight loss resulting from the treatment of obesity can cause gallstones to form, although this risk may be mitigated by ursodeoxycholic acid treatment[39]. Proposed mechanisms for stone formation during weight loss include biliary stasis due to decreased dietary fat intake, and increased hepatic cholesterol export[40].

7.1.4.6 Pancreatitis

Pancreatitis is more common in individuals with obesity than non-obese people as it can be triggered by several obesity-associated pathologies. Gallstone disease complicated by a stone in the common bile duct (choledocholithiasis) and hyperlipidaemia are common precipitants, as is glucagon-like peptide 1 (GLP-1) agonist use, which is a licensed treatment for T2D and obesity[41]. Furthermore, clinical outcomes have been reported to be worse in people with pancreatitis who also have obesity due to increased proinflammatory cytokines, lipotoxicity and pancreatic (and peripancreatic fat) necrosis[42].

7.1.4.7 Impaired Mental Health and Quality of Life

Obesity has been associated with decreased health-related quality of life[43], both due to the symptoms of obesity-linked disease and due to more direct factors such as body image[44]. Symptoms resulting from gastro-oesophageal reflux disease, biliary disease, irritable bowel syndrome (including bloatedness and abdominal pain) are more prevalent in individuals with obesity than non-obese controls, leading to poorer gastrointestinal quality of life[45,46]. Longitudinal studies have also found associations between the presence of obesity and the subsequent development of impaired mental health and depression[47]. Their bidirectional relationship may be explained by common underlying homeostatic and neuroendocrine alterations, that when targeted may improve both obesity and impaired mental health[48].

7.2 The clinical investigation of obesity

7.2.1 Obesity investigation: Diagnosis of obesity and the metabolic syndrome

Obesity is usually diagnosed by calculating an individual's BMI[1]. As previously discussed, this measure has several limitations. Alternative measures such as waist circumference or waist-hip ratio may be more informative in predicting metabolic syndrome risk, but these are not frequently incorporated into clinical care pathways. The metabolic syndrome is diagnosed if ≥3 of the following are present[49]:

- Increased waist circumference
- Elevated plasma triglycerides
- Low High Density Lipoprotein (HDL) cholesterol levels

- Hypertension
- Fasting hyperglycaemia

Insulin resistance, inflammation and a hypercoagulable state are also often present and are included in some definitions[50].

7.2.2 Obesity investigation: Investigation and risk stratification in clinical care pathways

The investigation of obesity should include a thorough clinical history and examination. Factors precipitating weight gain should be sought and previous weight reduction interventions (and their efficacy) should be discussed along with motivation for change. Dietary history should be recorded, and food diaries may be beneficial along with an assessment of physical activity levels. Mental health status and eating disorders should be explored. It should be ascertained whether an individual has developed complications of obesity or is taking medications that may promote weight gain[1]. Family history may suggest either a genetic predisposition or shared environmental risk factors for obesity and metabolic syndrome development[51].

The clinical examination should incorporate body anthropometry, assess whether signs of obesity-related complications are present and whether obesity may be secondary to other pathology such as hypothyroidism or Cushing's disease.

Blood tests should include routine full blood count and biochemistry along with lipid profiles, thyroid function tests, and glycated haemoglobin or fasting glucose levels. Additional investigations may be indicated depending on history and examination findings[1]. Obesity is usually polygenic, so genetic testing is rarely indicated except in cases of severe childhood or syndromic obesity[52].

Following this initial assessment, patients are risk assessed and stratified to optimise their management and prevent obesity-related complications. In the UK, there are 4 tiers to weight management services[1]:

- Tier 1 includes health promotion and primary care
- Tier 2 focuses on lifestyle interventions
- Tier 3 provides specialist weight management services and pharmacotherapy
- Tier 4 involves bariatric surgical services

7.3 The clinical management of obesity

7.3.1 Obesity management: Dietary and lifestyle interventions

Dietary and lifestyle interventions have long been the mainstay of obesity management. These are promoted at a societal level as part of a national public health approach as well as directly to individuals with obesity. Wider public health tools include restricting the advertisement of unhealthy foods and increasing their taxation[53]. Improving the quality of food in schools, school meal provision and education are other strategies that have been designed to promote healthy eating[54]. The limited training of medical students and clinicians in obesity prevention and management has also been highlighted and targeted for improvement[55]. However, it appears from the rising obesity rates in children and adults over recent decades that these strategies have been incompletely effective.

7.3.1.1 Diet

The most important contributor to weight loss is caloric restriction, with a daily net shortfall of 600Kcal recommended by the UK's National Institute for Health and Care Excellence (NICE) guidelines for obesity management[1]. It has been suggested that this modest calorie deficit is more sustainable than more intensive diets, and may lead to better longer term weight loss[56]. A range of strategies to reduce intake have been studied, including time restricted feeding and intermittent fasting. Reported outcomes are variable, and the dietary method appears less influential than the caloric deficit[57].

Alterations in macronutrient proportions have also been studied. Early weight loss is observed with carbohydrate-restricted diets, in part due to the depletion of hepatic and muscle glycogen and associated water[58]. In ketogenic diets, there is an associated feeling of euphoria[59] that may positively reinforce early compliance with this intervention. However, depletion of glycogen stores has been associated with fatigue[60], and a consequently

reduced energy expenditure may limit the success of this approach over the longer term. 'Mediterranean diets' have also been the subject of scientific interest due to the observation that inhabitants of this region have relatively low rates of obesity and metabolic syndrome[61]. This diet is rich in fruits and vegetables, unsaturated fats lean meat and fish. Whilst there is evidence for the short term efficacy of a range of dietary interventions[62-65], weight regain is observed over time[66] and there is a general trend of increasing weight with age[67].

7.3.1.2 Exercise

Increased physical activity is the other component of lifestyle modification as a treatment for obesity. The aim of exercise is to increase energy expenditure sufficiently to achieve a negative energy balance. In addition to calories burned during exercise, physical activity has also been shown to increase an individual's basal metabolic rate[68]. Improvements in physical activity can be achieved through modifications to existing daily activities. Examples of recent public health recommendations include promoting cycling or walking to work rather than motor transportation and taking stairs instead of lifts. These measures can be supplemented by dedicated exercise programmes, which may broadly be categorised as aerobic or anaerobic in nature depending on their intensity. Advantages of more intensive approaches, such as high-intensity intermittent training, have been suggested as energy expenditure is more rapid and its time-efficiency may improve adherence[69].

However, evidence from the existing literature suggests that the overall contribution of exercise to weight loss, particularly in individuals with severe obesity, is modest[70]. Such individuals may have functional limitations that limit exercise ability, such as physical mobility restrictions and joint pain. There may be additional psychological barriers to physical activity, such as exercising in public venues or in a group setting, so it is important that programmes are adapted to each individual. The role of exercise in weight loss maintenance is more established, and some of these functional limitations may be diminished after a period of dietary or medical weight loss.

7.3.2 Obesity management: Pharmacological interventions

Several medications have been licensed for the treatment of obesity, and some have additional evidence of benefit in the treatment of other aspects of the metabolic syndrome. In general, their use has been limited by cost, side effects and the potential need for lifelong therapy in order to prevent weight regain after treatment withdrawal. An initial monitored pharmacotherapy trial period, with the aim of weight loss of ≥5% total body weight, has been recommended to assess the probability of benefit against the side effects associated with continued prescribing. The following medications have been approved for use in the European Union (EU):

7.3.2.1 Malabsorptive

Orlistat inhibits the action of gastric and pancreatic lipase to reduce the intestinal absorption of ingested fat by approximately a third. It has been approved for long term use in paediatric and adult populations and is the only licensed medication with a solely peripheral effect. There is evidence of its efficacy in reducing body weight by approximately 3kg whilst improving other markers of metabolic health[71]. Adherence with orlistat treatment is, however, limited by gastrointestinal side effects such as steatorrhoea, faecal urgency and incontinence. It is contraindicated in individuals with intestinal malabsorption and it is necessary to combine with fat-soluble vitamin supplementation. Acarbose acts to reduce carbohydrate absorption and is licensed for use in diabetic patients. It also has been found to be promote modest weight loss[72], and there is a suggestion that its combination with orlistat may have additional weight loss and metabolic benefits over orlistat monotherapy[73].

7.3.2.2 Lorcaserin

Lorcaserin is a selective serotonin 5-hydroxytryptamine receptor 2C agonist. It has been associated with weight loss of approximately 5.8kg after 1 year of therapy, although lost weight was largely regained by 2 years despite continued use[74]. Side effects of lorcaserin include headache, fatigue dry mouth and eyes, headache and constipation.

7.3.2.3 Naltrexone/buprione

Naltrexone is an opioid-receptor antagonist, and bupropion is a noradrenaline/dopamine reuptake inhibitor. Bupropion's selective inhibition of neuronal catecholamine reuptake results in an antidepressant effect in addition to weight reduction. Side effects of this drug combination include nausea and vomiting, headache, dry mouth and diarrhoea and may lead to drug cessation in over a quarter of patients[75].

7.3.2.4 Phentermine/topiramate

This drug combination has level 1 evidence of its efficacy in reducing body weight and metabolic risk factors. The chemical structure of phentermine is related to that of amphetamine and it is approved for short term use. Weight loss of 5-10% of total body weight may be achieved[76,77]. Common side effects include tachycardia, dry mouth, constipation, paraesthesia and dizziness, and the psychiatric and cognitive adverse effects seen with higher dose topiramate are still present with this combination therapy[75].

7.3.2.5 GLP-1 agonists

Liraglutide and semaglutide are glucagon-like peptide 1 (GLP-1) agonists designed for the treatment of obesity and T2D. Liraglutide is currently licensed and requires daily injection, whilst semaglutide is injected weekly. A daily oral semaglutide formulation is currently being evaluated with promising reported outcomes[78]. A range of doses have been studied with dose-dependent beneficial weight loss effects. Weight loss appears to be higher with semaglutide, where up to 13.8% weight reduction may be observed at 1 year[79]. Side effects of GLP-1 agonists may include nausea, vomiting, constipation or diarrhoea. Abdominal pain, feadache, fatigue, hypoglycaemia and pancreatitis have also been described[75].

7.3.3 Obesity management: Endoscopic and surgical interventions

7.3.3.1 Endoscopic procedures

A range of endoscopic procedures have been designed to promote weight loss. Endoscopic intragastric balloon placement (Figure 7-1A) is perhaps the most established and leads to early satiety and reduced oral intake. Disadvantages of intragastric balloons include the need for their replacement and weight regain on balloon removal. They are also prone to

side effects such as vomiting due to pyloric obstruction[80]. Additional endoscopic procedures such as endoscopic sleeve gastropexy and enteric coating or liners have been described but their use is considered experimental at present[80].

7.3.3.2 Bariatric Surgery

National bariatric surgery guidelines include patient eligibility criteria such as a period of engagement with non-surgical weight management services and a BMI of ≥40kg/m^2, or ≥35kg/m^2 with a comorbidity, such as type 2 diabetes (T2D), that is improved by surgery[1]. Almost all bariatric operations are performed laparoscopically, with low complication rates[81]. Surgery usually involves an overnight hospital stay, and diet is gradually re-established over the following weeks. Lifelong adjustments in diet and lifestyle and regular postoperative follow-up are required to optimise clinical outcomes.

A range of mechanisms of action have been proposed, which have been termed the 'BRAVE effects'. These are:

- Bile acid composition and flow modification
- Reduction of gastric volume
- Anatomical rearrangement and altered nutrient flow
- Vagal manipulation
- Enteric hormone modulation[82]

More recently alterations in the gut microbiome have also been suggested to have mechanistic importance in promoting weight loss, including weight loss after bariatric surgery. The intestinal microbial flora contributes to nutrient absorption, metabolic processes, defence from pathogens and the development of the immune system in humans. However, alterations in the composition of the microbiota have been linked to the development of obesity and associated comorbidities including non-alcoholic fatty liver disease.

Bariatric surgery has been shown to induce changes in the composition of the intestinal microbiota. For example, a decrease of the ratio of Firmicutes to Bacteroidetes and an

increase of Proteobacteria have been described after Roux-en-Y Gastric Bypass (RYGB)[83]. Experimental studies with faecal transplant from RYGB-treated mice to germ-free mice lead to weight loss[84] and similar findings have been reported after faecal transplant from a human donor who has undergone RYGB surgery[85].

Adjustable gastric banding

Adjustable gastric banding (AGB) involves the insertion of an inflatable band around the gastric body, which is connected to a subcutaneous adjustment port (Figure 7-1B). This allows the volume of fluid within the band to be titrated against an individual's satiety and symptoms levels. This procedure was previously the most frequently performed bariatric intervention, but in recent years has become less common. Reasons for this include inferior weight loss and diabetes remission outcomes, the need for band volume adjustments and relatively high complication and revisional surgery rates[86,87].

Roux-en-Y gastric bypass

Roux-en-Y gastric bypass (RYGB) largely superseded the gastric band[88] as it was found to be more effective in the treatment of obesity and metabolic disease, with lower revision rates. In this procedure, a stapling device is used to divide the stomach, creating an approximately 30cm gastric pouch. The jejunum is divided and a gastrojejunal anastomosis is created in an ante-colic ante-gastric fashion, usually with suture reinforcement of the staple line to reduce the risk of anastomotic leak. The proximal end of divided jejunum is then anastomosed to distal jejunum in a similar fashion, creating a Roux limb (Figure 7-1C).

Sleeve gastrectomy

Sleeve gastrectomy (SG) involves the creation of a tubular gastric sleeve, with removal of the resected stomach (Figure 7-1D). A bougie is inserted into the stomach and a linear cutting stapling device is used just beyond this to divide along the stomach's greater curve, resulting in an approximately 80% reduction in gastric volume. This results in earlier satiety and gut hormone alterations that may result in greater weight loss than the mechanical effects alone. Sleeve gastrectomy is currently the most frequently performed bariatric

operation globally[89]. Complication rates are low, but specific complications include staple line leak and postoperative *de novo* Gastro-Oesophageal Reflux Disease (GORD)[90].

One-anastomosis gastric bypass

This procedure is becoming more common since it is relatively straightforward, with reduced early complications and operating time compared to RYGB. A gastric pouch is created and gastrojejunal anastomosis formed, bypassing 150-200cm of small intestine, without jejunal division and Roux limb creation (Figure 7-1E). It offers similar advantages to RYGB over SG in terms of weight loss and T2D remission[91]. However, there are concerns that bile reflux into the oesophagus may lead to Barrett's oesophagus and oesophageal carcinoma in the longer term[92]. Depending on limb lengths, it is also more malabsorptive than RYGB, and cases of postoperative liver dysfunction have been reported[93].

Biliopancreatic diversion with duodenal switch

The biliopancreatic diversion with duodenal switch (BPD-DS) operation is infrequently performed. A gastric tube is formed in a relatively similar fashion to a SG and the duodenum is divided beyond the gastric pylorus. A duodenoileostomy is formed along with an ileoileostomy (Figure 7-1F). This more distal bypass with a short common channel means that BPD-DS is more malabsorptive than the previously discussed bariatric operations and it is also a more technically challenging operation. It is relatively uncommon for these reasons, and because there is a greater probability of macronutrient and micronutrient deficiencies[94], requiring careful patient selection, close monitoring and adherence to dietary guidelines. However, excellent weight loss and T2D remission outcomes are reported[95,96].

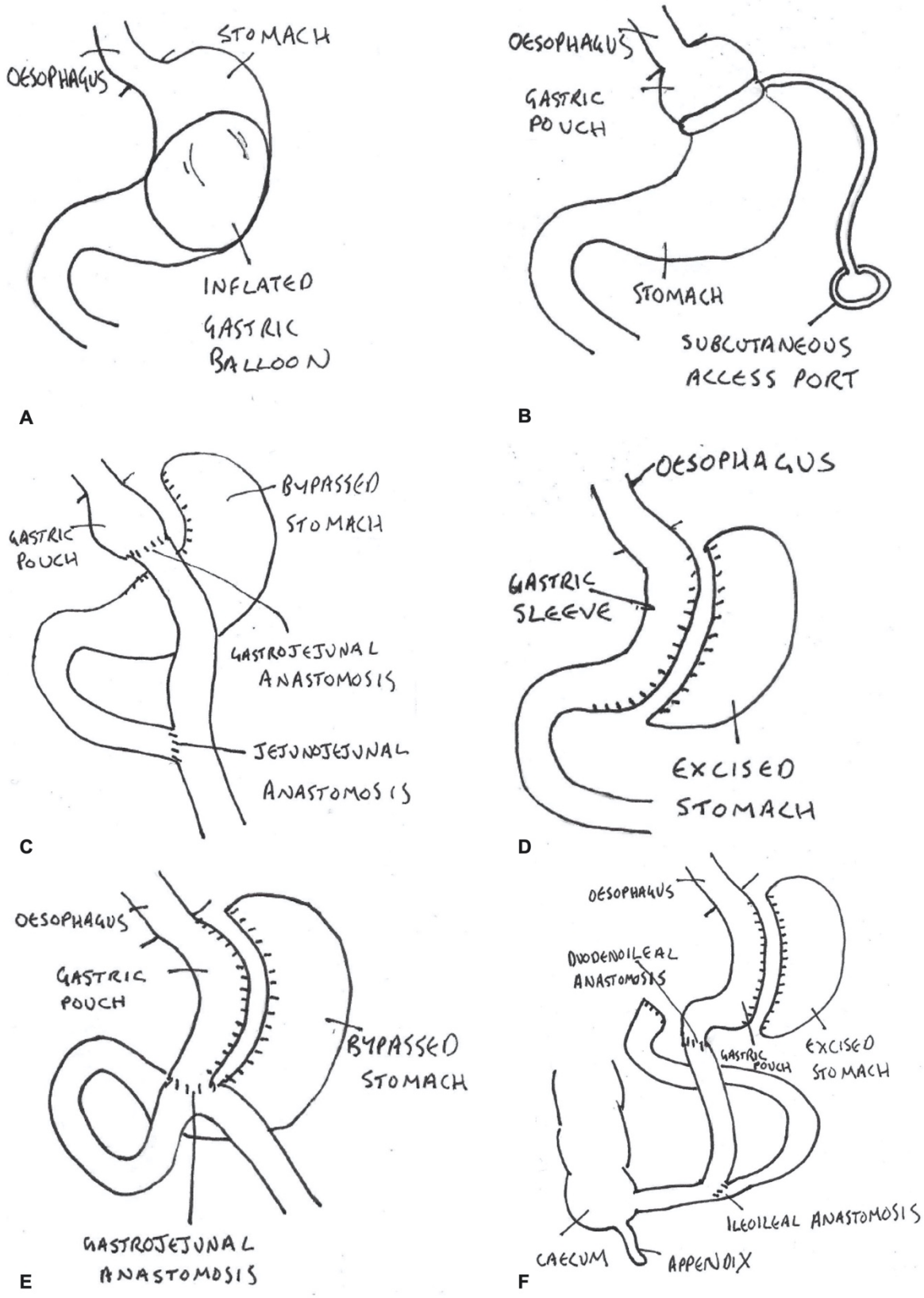


Figure 7-1 Schematic representation of bariatric procedures and operations: (A) intragastric balloon, (B) adjustable gastric band, (C) Roux-en-Y gastric bypass, (D) sleeve gastrectomy, (E) one anastomosis gastric bypass, (F) biliopancreatic diversion with duodenal switch

Comparative studies

It has now been established that bariatric surgery is superior to intensive medical therapy for improving a range of metabolic health outcomes including T2D and body weight[44,97]. There is however a need for more prospective randomised comparisons of the benefits and risks of different bariatric procedures. The By-Band-Sleeve study is one such study that has completed recruitment and aims to compare outcomes after RYGB, SG or AGB surgery. Additionally, it has been highlighted that bariatric operations should be standardised[98], as postoperative outcomes may vary with technical modifications, for example in limb lengths in bypass operations.

Despite evidence that bariatric surgery is the most effective and durable intervention for obesity and that it is cost effective, it is not widely implemented and there are regional variations in service provision[99]. The UK has the second highest obesity rate in Europe but its annual number of bariatric operations per head of population is particularly low and has decreased in recent years[100]. Despite limited evidence of efficacy, the focus of UK governments has been largely on obesity prevention and on its management through dietary and lifestyle modification[99]. Bariatric surgery is not viewed as an effective treatment option by all individuals with obesity, and its scalability would require increased investment in the health professionals and physical resources required for functional bariatric surgical units. Therefore, whilst it is likely to have an increasing role in the management of obesity, ongoing research is required to identify effective dietary, lifestyle and pharmacological interventions. Additionally, there is a need to investigate the potential additive or synergistic benefits of combined surgical, lifestyle and pharmaceutical approaches to weight loss and weight maintenance.

7.4 Non-alcoholic fatty liver disease (NAFLD)

7.4.1 NAFLD: Definition

NAFLD is diagnosed histologically when greater than 5% of hepatocytes are steatotic in the absence of excessive alcohol intake or other causes of liver fat accumulation[101]. Inflammation and hepatocyte injury are driven by factors such as proinflammatory adipocytokines and occur in 10- 30% of cases[102], leading to non-alcoholic steatohepatitis

(NASH) development. With repeated cycles of inflammation, hepatic fibrosis then develops[103]. Fibrosis stage is clinically important since it is the strongest predictor of disease-specific and all-cause mortality in NAFLD[104]. Cirrhosis is a state of advanced liver fibrosis in which liver function is impaired and risks of liver failure and hepatocellular carcinoma development are elevated.

7.4.2 NAFLD: Epidemiology

NAFLD is increasingly recognised as the leading cause of chronic liver disease worldwide but it is often undiagnosed as it is largely asymptomatic, with normal liver biochemistry even in its more advanced stages. It is reported to be present in approximately 30% of the general population[102,105,106]. Its reported prevalence in people with severe obesity undergoing bariatric surgery ranges from less than 10% in the UK's National Bariatric Surgery Registry Report[107] to 95% in other published series[108]. Its prevalence is increasing in parallel with the escalating obesity epidemic.

7.4.3 NAFLD: Pathophysiology

7.4.3.1 Genetic and demographic risk factors

Several genetic risk factors for the development of NAFLD have been established. A missense mutation in the Patatin-like phospholipase domain-containing protein 3 (*PNPLA3)* gene is not only associated with liver fat accumulation but with the probability of subsequent development of NASH and fibrosis[109]. This effect is observed independently of obesity and T2D.The risk allele of the Transmembrane 6 Superfamily 2 (*TM6SF2)* gene has been linked with increased liver fibrosis risk in people with NAFLD[110]. More recently, a genetic variant in the Membrane Bound O-Acyltransferase Domain Containing 7 (*MBOAT7)* gene has also been linked with increased prevalence and severity of NAFLD[111].

Increasing age has been associated with greater rates of NAFLD, although it is uncertain to what extent this observation is independent of the recognised increase in obesity rates with age. Male sex, also associated with central obesity, is another recognised risk factor for NAFLD development[112]. Variations in prevalence are also observed in different ethnic

groups, with Hispanic people in the United States of America (USA) at higher risk than white or black populations[113].

7.4.3.2 Clinical risk factors

Obesity, particularly abdominal obesity, is the key risk factor for NAFLD development. The chronic low grade inflammation seen in obesity has been implicated, with visceral adiposity thought to be particularly harmful due to its drainage into the hepatic portal vein[114]. There is also a close relationship between T2D and NAFLD. T2D increases adipose tissue lipolysis, delivering FFAs to the liver and drives progressive fibrosis in NAFLD[115]. OSA and sleep deprivation are also thought to contribute to NAFLD development. The mechanism for this relates to the release of inflammatory cytokines such as interleukin (IL)-6 and Tumour Necrosis Factor (TNF)-α, and intermittent hypoxia results in a catecholamine response that impairs glucose homeostasis and leads to hepatic steatosis[116].

7.4.4 NAFLD: Hepatic lipid flux and its measurement

7.4.4.1 Liver fat metabolism

Fat can accumulate in the liver via 4 metabolic pathways[117]. Free fatty acids (FFA) are produced by lipolysis and lipoprotein lipase (LPL) spillover and are transported to the liver where they are synthesised into hepatic triglyceride (pathways 1 and 3 respectively in Figure 7-2). Exogenous lipids can also be absorbed and packaged into chylomicrons, the remnants of which are eventually transported to the liver and synthesised into triglyceride (TAG) (pathway 4 in Figure 7-2). Finally, triglycerides accumulate via synthesis from non-lipid precursors, a process known as *de novo* lipogenesis (DNL, pathway 2 in Figure 7-2). Hepatic DNL is known to increase in NAFLD[118], but the changes that occur in metabolic pathways after bariatric surgical weight loss are unknown.

Fatty acids can be disposed of via oxidation to ketone bodies and carbon dioxide (CO_2). Malonyl CoA, which is an intermediate enzyme in DNL, inhibits fatty acid oxidation and leads to a further increase in intrahepatic lipid[119]. Hepatic fatty acids can also be disposed of in secreted Very Low Density Lipoprotein (VLDL).

7.4.4.2 The measurement of liver fat metabolism

It is possible to define the relative contributions of the pathways involved in lipid homeostasis using stable isotope 'tracers'. These are naturally-occurring non-radioactive elements that have a different mass to their more abundant form in the human body[120]. The change in mass is due to an increased number of neutrons, and this difference enables stable isotopes to be measured in clinical samples using mass spectrometry techniques. They can be ingested or infused intravenously and are then used in metabolic processes. ^{13}C-palmitate can be used in combination with mathematic modelling for the measurement of fatty acid oxidation as it is expired in breath as $^{13}CO_2$[121]. $^{2}H_2O$ can be used for the measurement of hepatic *de novo* lipogenesis (pathway 2 in Figure 7-2) by measuring the appearance of ^{2}H in VLDL—palmitate-TAG[122].

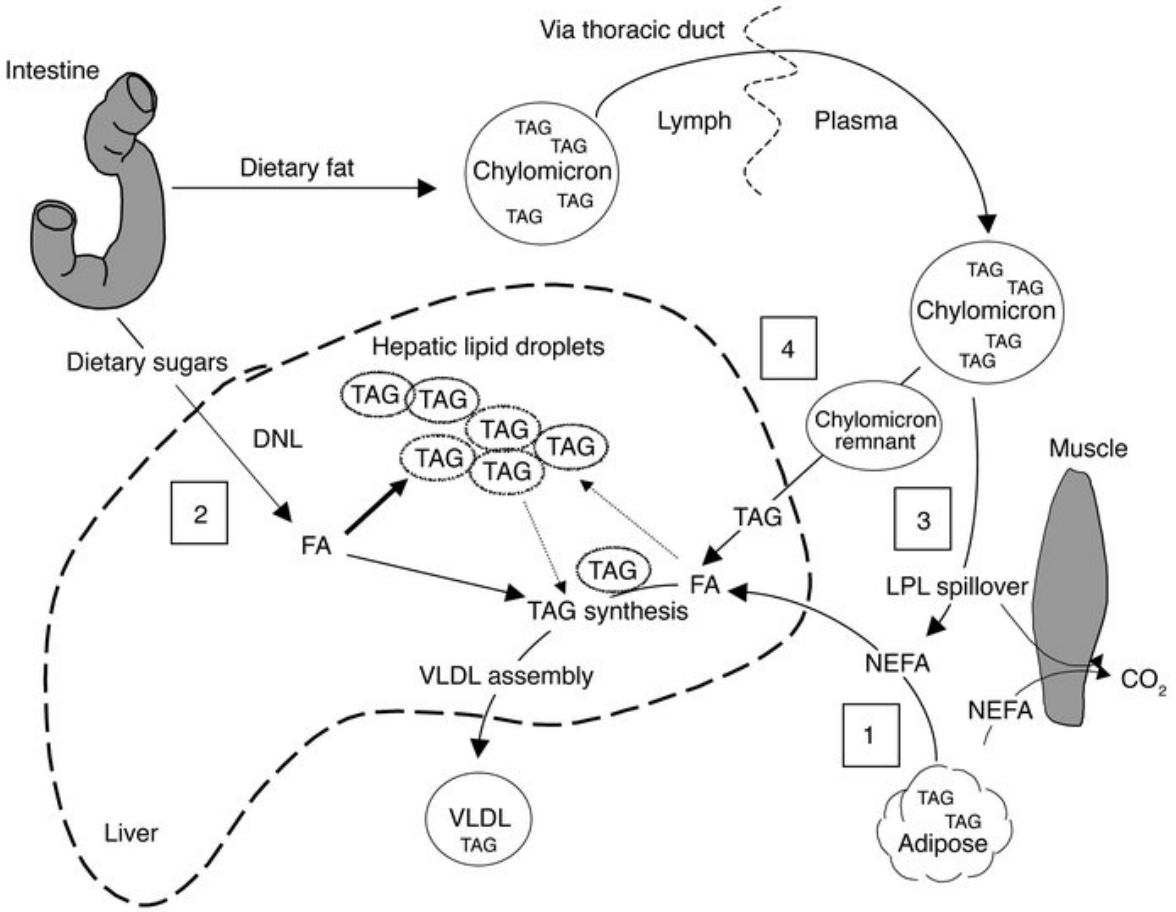


Figure 7-2 Schematic diagram of hepatic lipid flux. The numbers indicate pathways that can be traced using stable isotopes. Reproduced with from J Clin Invest DOI:10.1172/JCI23621 with permission.

7.4.5 NAFLD: Clinical and economic implications

The presence of NAFLD increases the risk of liver failure, and this risk increases with progressive liver fibrosis[104]. The risk of hepatocellular carcinoma development also increases in a similar way[123]. Liver steatosis in patients with NAFLD leads to hepatic insulin resistance, increasing the severity of T2D. Whilst NAFLD increases all-cause mortality rates, most people with NAFLD die from cardiovascular rather than liver disease[124]. For this reason, evidence of cardiovascular benefit is emphasised in the licensing of candidate drugs for the treatment of obesity and NAFLD.

There is a considerable economic burden associated with NAFLD, which is estimated to be similar to that of diabetes or cardiovascular disease. Estimated annual direct medical costs in the United States of America are over $100 billion, and around €35 billion amongst the Europe-4 countries (the UK, Germany, France and Italy). With consideration of additional societal costs, the annual burden of NAFLD increases almost 3-fold in the USA and to approximately €225 billion amongst the Europe-4 countries[125].

7.5 The clinical investigation of NAFLD and risk stratification

7.5.1 NAFLD investigation: Diagnosis

7.5.1.1 Liver histology

The primary method for diagnosing NAFLD is through histological analysis. Kleiner's NAFLD Activity Score (NAS) and fibrosis classification[126] provides semi-quantitative assessment of the NAFLD spectrum of histological features. It is commonly used to diagnose Non-Alcoholic Steatohepatitis (NASH) and to stage liver fibrosis. However, liver biopsy histology is regarded as an imperfect "gold standard" for diagnosing and staging NAFLD since it is invasive, costly and associated with procedural complications and sampling error[127]. As a result, there is a clinical need for accurate non-invasive biomarkers of NAFLD severity.

7.5.1.2 Liver Imaging

Several imaging modalities may be used for non-invasively diagnosing NAFLD. However, their ability to guide clinical decision-making and to differentiate between different stages of NAFLD severity is variable.

Ultrasound

Fatty liver is often diagnosed incidentally on abdominal ultrasound (US). Its strengths are in the diagnosis of steatosis, which can be graded according to its severity, although there is an element of subjectivity[121]. Additionally, ultrasound can detect cirrhosis and ultrasound surveillance is offered to patients with NASH-cirrhosis to screen for hepatocellular carcinoma (HCC) development.

Ultrasound elastography aims to quantify liver fibrosis. Its rationale is that elasticity reduces with increased liver fibrosis. Transient elastography, such as in the Fibroscan™ device (EchoSens, Paris, France), has been validated for its accuracy in identifying individuals with advanced fibrotic NAFLD[128]. However, operator training is required and it has undergone limited validation in individuals with severe obesity. In this patient group, obtaining valid readings is more challenging due to an increased probe-liver distance, although specialised probes have been developed for use in people with severe obesity[129]. Recently, controlled attenuation parameter readings have become available on the Fibroscan device, offering quantification of liver steatosis. In contrast with transient elastography, where live-imaging is not available, shear-wave elastography uses acoustic energy to create shear waves, allowing the quantification of hepatic fibrosis. It is similarly challenging in individuals with obesity and both inflammation and fibrosis increase shear wave velocity, confounding fibrosis staging.

Computed Tomography

Unenhanced computed tomography (CT) images can be used to make a diagnosis of hepatic steatosis[130]. The attenuation value of the liver tissue is measured in Hounsfield units, and can be compared against reference values or against the attenuation of splenic tissue. Additionally, CT can identify the irregular contour of a cirrhotic liver and signs of portal

hypertension. Dual-energy CT scanning is a recent development with the potential to quantify hepatic steatosis more accurately and to stage fibrosis.

Magnetic Resonance Imaging

Magnetic resonance imaging modalities offer the potential for accurate NAFLD diagnosis and staging[130]. Their use is, however, limited by cost and by limited scanner and radiographer availability. Additionally, since patients must be able to fit within the scanner, this modality is not suitable for people with the most severe obesity. Magnetic resonance spectroscopy (MRS) is well-established for quantifying liver steatosis. Fat and water molecules result in peaks observed on liver spectroscopy. Sequences with and without water suppression are obtained, and fat peaks are quantified from these. Magnetic Resonance Imaging (MRI)-Proton density fat fraction (MRI-PDFF) can also be used to quantify steatosis with high diagnostic accuracy[121]. It measures the fraction of visible protons bound to fat and divides these by the total number of visible protons. Multiparametric MRI imaging has also been developed to stage liver fibrosis in NAFLD[131]. LiverMultiScan (Perspectum Diagnostics, Oxford, United Kingdom) utilises this approach, and it has been shown that the T1 relaxation time, corrected for iron, may be used as an imaging biomarker for fibrosis in NAFLD. However, additional factors such as fat may also affect T1 relaxation times and further validation is required. Nevertheless, this software is increasingly used in clinical trials research and in some healthcare settings. Elastography can also be combined with MR for the quantification of liver fibrosis. It has been shown to be relatively accurate for this purpose, but its use is limited by the cost of purchasing the required elastography probe[132].

7.5.1.3 Liver biochemistry

Liver biochemistry is available at low cost and is frequently measured in clinical practice. Unfortunately, isolated liver enzymes have not been shown to accurately predict the presence liver steatosis, inflammation of fibrosis[133]. They can, however, be used for non-invasive liver fibrosis staging when incorporated as variables into predictive models. These are also known as serological non-invasive biomarkers.

7.5.2 NAFLD investigation: Non-Invasive biomarkers and their use in NAFLD histology prediction

7.5.2.1 Approaches to non-invasive biomarker development

Predictive models are frequently used in clinical practice to improve diagnosis. The initial step is to define the area of clinical need to ensure that the model will inform decision-making. The identification of existing high-quality data sources with sufficient numbers of cases and controls, or establishing a protocol for prospective data collection, is also key. Database creation, including defining input variables, data entry and handling missing data, are common subsequent steps. Input variables may be identified through literature review or by an expert panel, although there is some risk of bias with this approach. An alternative method would be to include all available clinical data and then determine the contribution of each variable to the predictive model. Example predictor variables include demographic, anthropometric, biochemical and imaging data. Included variables can be continuous or categorical, but transforming available continuous data into categories reduces statistical power and should be avoided. Missing data may be handled by excluding cases or variables, inserting mean or median values for that variable, or by more sophisticated approaches such as multiple imputation. Once the database has been prepared in this way, quality control is performed before model development.

It is common for the data to be split into at least two distinct portions – one for developing the predictive model and the other for validating it[134]. In clinical studies, a validation cohort may be formed from patients in a different hospital, or the dataset can alternatively be randomly partitioned to create an 'unseen' validation group. There is some debate around the proportion of the whole dataset that should be allocated to development and to validation, but most studies use 70-90% of included cases for model development.

The presence or absence of a clinical diagnosis or event is a binary outcome. Predicting a binary outcome has commonly been performed using logistic regression[135], but machine learning approaches have been more recently developed and may improve diagnostic accuracy[136]. They can be flexibly deployed for diverse purposes ranging from diagnosing pathology on x-rays[137] to highlighting patients at risk of clinical deterioration and intensive

care unit admission[138]. Dedicated algorithms have been designed to optimise performance in specific scenarios, such as for the diagnosis of a rare clinical event[139].

Whichever approach is used, the input variables are evaluated according to their contribution to the multivariable model. It is possible to reduce the number of predictors (known as 'feature selection') by excluding or combining highly correlated variables and excluding those that weakly contribute to a model's diagnostic accuracy[134].

Once the final model, or candidate models, have been developed they should then be tested in the independent testing cohort. A range of statistical tests can then be applied to evaluate and compare their performance in order to assess their clinical utility. The receiver operating characteristic (ROC) curve plots the true positive rate (sensitivity) against the false positive rate (1-specificity). The area under the receiver operating characteristic (AUROC) curve is then calculated; a value of 1 indicates perfect discrimination and a value of 0.5 indicates that the model performs no better than chance[140]. ≥0.8 is a suggested minimum AUROC threshold for the incorporation of developed models into clinical practice range[141]. Depending on the desired characteristics for the predictive model, a cut-off value on the ROC curve can be selected and a range of statistics are often calculated at this point. Informative statistics include sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The formulae for these are[142]:

Sensitivity = true positive / true positive + false negative

Specificity = true negative / true negative + false positive

Positive Predictive Value (PPV) = true positive / true positive + false positive

Negative Predictive Value (NPV) = true negative / true negative + false negative

The fully evaluated model should be published and undergo subsequent independent external validation in order to determine its generalisability. At this point, it may require updating, recalibration (for example modifying the adopted cut-off score), or even rejection.

A potential advantage of machine learning over regression-based approaches is that there is the potential to host the model on an online server, where data entered to generate a predictive response can then be used in a continuous cycle of feedback and model refinement. A prospective model impact study should be performed to assess its contribution to clinical practice[143].

7.5.2.2 Non-invasive biomarkers of NAFLD histology

Although serum and imaging-based biomarkers have been developed for predicting NAFLD histology, they lack validation in cohorts of individuals with severe obesity and this has limited their adoption in the bariatric surgical community[144]. Serum-based biomarkers include scoring systems derived from routine biochemistry tests as well as the Enhanced Liver Fibrosis (ELF) test (Siemens/iQur Ltd, London, United Kingdom). This test has recently been recommended by the UK's National Institute for Health and Care Excellence (NICE) for staging and monitoring NAFLD[145]. The formulae of currently used biomarkers for NAFLD fibrosis prediction are:

Enhanced Liver Fibrosis (ELF) Score[146]: 2.278+0.851 ln(Hyaluronic Acid (*HA)*) +0.751 ln*(*Type III Procollagen Peptide (*PIIINP)*)+0.394 ln(Tissue Inhibitor of Metalloproteinases-1 (*TIMP-1)*)

Aspartate aminotransferase (AST)/(Alanine aminotransferase) ALT Ratio[147]: =*AST*/*ALT*

AST to Platelet Ratio Index (APRI)[148]: =*AST*/42/*Platelet count**100

BMI, AST/ALT Ratio, Diabetes (BARD) Score[149]: =(1 point if *BMI≥28*)+(2 points if *AST/ALT ratio≥0.8*}+(1 point if *T2D*)

Fibrosis 4 (Fib-4) Score[150]: =(*age***AST*)/(*Platelet count**SQRT(*ALT*))

NAFLD Fibrosis Score (NFS)[151]: = -1.675+(0.037**age*)+(0.094**BMI*)+(1.13*(1 point if Impaired Fasting Glucose (*IFG)/T2D*)+(0.99*(*AST/ALT ratio*)-(0.013**platelet count*)-(0.66*(*albumin)*)

7.5.3 NAFLD investigation: Risk stratification in clinical care pathways

These biomarkers can then be incorporated into care pathways for NAFLD management in order to improve and standardise clinical decision-making. The largest study to have evaluated the inclusion of non-invasive tests in care pathways proposed initial use of the Fib-4 test, followed by ELF testing in indeterminate cases[152]. It was reported that this approach increased the detection of advanced liver fibrosis and was cost effective, reducing unnecessary referrals for specialist NAFLD management by 80%[153].

7.6 The clinical management of NAFLD

7.6.1 NAFLD management: Dietary and lifestyle interventions

Currently, the clinical management of NAFLD is largely focused on the promotion of weight loss through dietary and lifestyle modification. This is principally achieved by increasing caloric restriction and physical activity[154,155], although improving food quality has also been shown to be beneficial[156]. The management of relevant comorbidities such as Type 2 Diabetes (T2D), which has been associated with NAFLD progression, and other liver disease risk factors (such as excessive alcohol intake) is also important[157]. The DIRECT study reported that an 800kcal liquid diet leads to liver fat reduction at 12 months in individuals with T2D, even after regular food reintroduction[158]. Similar findings were reported at 1 year in the Look AHEAD study[159]. It is recommended that individuals aim to lose at least 5-10% of their total body weight, as this has been shown to improve NAFLD histology[160].

There is some evidence that food choices within macronutrient groups may be important in addition to calorie reduction. For example, it has been found that isocaloric consumption of polyunsaturated fatty acids leads to less liver fat accumulation than saturated fatty acid intake[161]. A Mediterranean diet that is rich in omega-3 Polyunsaturated Fatty Acids (PUFA) is recommended by several national bodies for NAFLD management[155] whilst reduced dietary fructose intake is advised since it has been implicated in NAFLD development and progression[162]. Alcohol intake should be within national guideline limits and avoided in patients with cirrhosis. Exercise has also been shown to reduce liver steatosis, even without body weight loss[163].

7.6.2 NAFLD management: Pharmacological interventions

Whilst there is existing evidence for the effectiveness of pharmaceutical agents such as GLP-1 agonists in the combined treatment of obesity and NAFLD[164,165], the required magnitude of response and need for sustained treatment adherence means that long-term improvements are often modest amongst people with severe obesity. Currently, no pharmaceutical agent is licensed specifically for the treatment of NAFLD. Current medical management focuses on reducing cardiovascular disease risk, for example by using statins to optimise lipid profile[166]. In patients with co-existent T2D and NAFLD, it may also be possible to tailor therapy in a manner that promotes weight loss. For example, metformin, GLP-1 agonists and Sodium-Glucose Co-Transporter-2 (SGLT-2) inhibitors are licensed for T2D treatment and are also associated with reductions in body weight[167]. In contrast, insulin treatment has the effect of increasing body weight, and its dose should be reduced where possible in favour of weight-reducing treatment options.

7.6.3 NAFLD management: Endoscopic and surgical interventions

Bariatric surgery is recognised as the most effective treatment for durable and effective weight loss[168]. Additionally, long-term benefits after Roux-en-Y Gastric Bypass (RYGB) or Sleeve Gastrectomy (SG) surgery compared to lifestyle intervention and intensive medical therapy have been established in T2D[44].

Bariatric surgery not only improves obesity-associated morbidity and mortality[97], but also results in histological NAFLD improvement, with reductions in inflammation, scarring and fibrosis[169]. In addition to histological improvement in NAFLD on paired liver biopsy studies[170-172], it has previously been shown that bariatric surgery improves liver fat as measured on MR imaging[173,174]. Meta-analysis of studies assessing the effects of bariatric surgery on the spectrum of NAFLD suggests that inflammation and fibrosis reduction may occur in addition to improvements in steatosis[169]. There has been an additional suggestion that these histological changes are associated with reduced hepatic oxidative stress[175], and one study reported improved liver function, as measured by determining the enzymatic capacity of cytochrome P450 1A2[176]. Given the relatively rapid changes in liver fat with modest weight loss that have been demonstrated in low energy diet studies[177], it may be that body weight normalisation is not required for NAFLD remission after bariatric surgery.

Additionally, since low energy diet is often prescribed before bariatric surgery, defining the relative contributions of this diet and the surgery itself on liver fat metabolism and other liver-related outcome measures would be beneficial.

It is also currently unknown whether the differences in liver fat metabolism that exist pre-operatively (when individuals with obesity are compared to non-obese control participants) are eliminated post-operatively. The mechanisms underpinning the histological improvement in patients with NAFLD have not been examined, but an enhanced understanding of these pathways would provide an important mechanistic insight into the ways in which bariatric surgery can improve liver outcome.

Differential effects in T2D remission after SG and RYGB have been previously described[178]. It is also currently unknown whether there is a differential effect of SG and RYGB on NAFLD since previous studies have used surrogate markers with poor diagnostic accuracy to measure NAFLD improvement. Some studies have suggested that SG has a greater effect[179], whilst others found no difference between procedures[180]; others have suggested RYGB is more beneficial[181]. These studies have concluded that there is a need for randomised studies designed to assess whether differential changes in NAFLD occur after alternative types of bariatric surgery but no such study has previously been conducted. Thus, there is a need to assess if differences in NAFLD improvement exist (using robust non-invasive biomarkers) between operations, which will aid clinical practice by informing the choice of surgical intervention according to patients' metabolic disease profiles. Additionally, the relative contribution of the routine pre-operative very low calorie diet to the improvements in NAFLD seen post-operatively has not been defined.

In contrast to other metabolic diseases such as T2D, which is universally associated with early improvement after bariatric surgery, postoperative liver dysfunction has been reported after a range of procedures[182-185]. Risk factors and underlying mechanisms are unclear but may include advanced pre-existing disease, oxidative stress due to rapid weight loss and protein energy malnutrition, especially after malabsorptive operations.

7.7 Summary, hypotheses and aims

7.7.1 Summary of why the series of studies in this thesis are required

There is emerging evidence that bariatric surgery may represent an effective intervention for the treatment on NAFLD. However, bariatric surgery is currently performed infrequently in order to treat NAFLD. It is not clear whether this is because surgery is not considered to be an acceptable intervention to people with NAFLD or healthcare professionals managing NAFLD, or whether there are other barriers to its wider implementation. In the absence of effective pharmacotherapies for treating NAFLD, it would be beneficial to determine whether bariatric surgery, if confirmed to be effective, could feasibly be implemented more widely as a treatment option for the combined treatment of obesity and NAFLD.

Non-invasive biomarkers have also been developed to stage NAFLD and identify patients who may benefit from clinical interventions. However, these have had limited validation in people with severe obesity so their diagnostic accuracy in this patient group is uncertain. Accurately staging NAFLD in people with obesity who are scheduled for bariatric surgery is relevant as it would guide pre-operative investigations (for example determining whether a patient has portal hypertension, which would increase perioperative bleeding risk), decisions around the need for intra-operative liver biopsy (in those predicted to have advanced fibrotic NAFLD), and post-operative follow up (such as ultrasound surveillance of the liver, which would be indicated in patients identified as having cirrhosis).

Changes in NAFLD-related and metabolic health outcomes after conventional hepatology-led or bariatric surgical management of patients with NAFLD are also unclear. If bariatric surgery has similarly beneficial effects to hepatology-led management then cost-effectiveness analysis would be required, and if it has greater effects then further study and consideration of its inclusion in NAFLD treatment guidelines would be beneficial.

The relative contributions of routine preoperative caloric restriction and of subsequent bariatric surgery to improve NAFLD are incompletely defined as are the underlying mechanisms. Bariatric surgical studies in the literature have taken baseline measurements at different times relative to this preoperative diet, and the components and duration of the

diet are not always clearly defined. It would be useful to determine whether a routine preoperative low energy diet has a rapid impact on liver outcome measures including liver fat metabolism, since this could make the interpretation of studies in the literature more challenging and inform the design of future studies.

It is also unclear whether alternative bariatric operations have differential effects on NAFLD and the potential underlying mechanisms are unknown. For example, the changes that occur in liver fat metabolism pathways after sleeve gastrectomy or Roux-en-Y gastric bypass have not been determined. Identifying which pathways are modified as NAFLD improves is potentially helpful, since if may help provide focus for pharmaceutical research into targeting these candidate pathways. It is also unclear whether normalisation of BMI is required to restore liver outcome measures and liver fat metabolism to normal reference values. Addressing this question would be beneficial as many people undergoing bariatric surgery remain obese or overweight even after losing over 20% of their total body weight, a proportion that would rarely be achievable by non-operative approaches.

7.7.2 Bariatric surgery is an acceptable intervention to key stakeholders for the combined management of obesity and NAFLD

To test this hypothesis, I aim to:

- Develop, conduct and analyse parallel surveys for patients with obesity and NAFLD, hepatologists and bariatric surgeons (Chapter 9: Bariatric Surgery and NAFLD: Insights from Key Stakeholders)

7.7.3 NAFLD is common in bariatric surgical patients and there is a need for non-invasive NAFLD severity biomarker development

To test this hypothesis, I aim to:

- Determine whether non-invasive assessment of NAFLD severity forms part of existing clinical care pathways (Chapter 9: Bariatric Surgery and NAFLD: Insights from Key Stakeholders)

- Develop and analyse databases of patients undergoing bariatric surgery with routine intraoperative liver biopsy in two distinct healthcare settings (Chapter 10: NAFLD Epidemiology and its Prediction in Bariatric Surgical Patients)

- Determine the diagnostic accuracy of existing non-invasive biomarkers of NAFLD severity in patients with severe obesity, and assess the potential for improved predictive model development (Chapter 10: NAFLD Epidemiology and its Prediction in Bariatric Surgical Patients)

7.7.4 Bariatric surgery is an effective intervention for the combined management of obesity and NAFLD

To test this hypothesis, I aim to:

- Determine the views of healthcare professionals (hepatologists and bariatric surgeons) who treat patients with obesity and NAFLD (Chapter 9: Bariatric Surgery and NAFLD: Insights from Key Stakeholders)

- Develop databases of patients with obesity and NAFLD who have undergone either conventional hepatology-led management or bariatric surgical management then compare clinical outcomes following these interventions (Chapter 11: Bariatric Surgery as an Intervention for NAFLD)

- Design, perform and analyse a prospective mechanistic study to assess the effects of bariatric surgery on obesity and NAFLD (Chapters 12 and 13: Effects of Preoperative Low Energy Diet on NAFLD and Effects of Bariatric Surgery on NAFLD)

7.7.5 Caloric restriction and bariatric surgery both have beneficial metabolic effects, and the metabolic effects of sleeve gastrectomy and Roux-en-Y gastric bypass are similar at matched weight loss.

To test this hypothesis, I aim to:

- Design, perform and analyse a prospective mechanistic study to assess the effects of caloric restriction and sleeve gastrectomy or Roux-en-Y gastric bypass surgery on obesity and NAFLD (Chapters 12 and 13: Effects of Preoperative Low Energy Diet on NAFLD and Effects of Bariatric Surgery on NAFLD)

8 Statement of Contributions

8.1 Bariatric Surgery and NAFLD: Insights from Key Stakeholders

I (Dr Niall Dempster) designed this study and wrote the surveys with feedback from Professor Jeremy Tomlinson and Dr Jeremy Cobbold. The survey for bariatric surgeons incorporated additional feedback from Mr Bruno Sgromo and Mr Richard Gillies.

For the patient survey, I applied for and obtained approval from the Oxford University Hospitals NHS Foundation Trust to invite eligible NHS patients whilst attending hepatology clinics or if they had previously participated in research studies and given consent for future contact. I then distributed it to eligible patients attending Oxford's Metabolic Hepatology clinics (paper version) and contacted patients who had previously participated in research and provided consent for future contact (online version). I distributed the survey for bariatric surgeons at the International Bariatric Club's 2018 World Congress in Oxford (paper version) and through email and social media (online version). I also distributed the survey for hepatologists at the American Association for the Study of Liver Diseases' 2019 Liver Meeting (paper version) and through email and social media (online version). I planned and conducted all statistical analysis for this study.

8.2 NAFLD Epidemiology and its Prediction in Bariatric Surgical Patients

I designed this study with Professor Jeremy Tomlinson and Professor Leanne Hodson. The biobank ethical approval (Ref: 16/YH/0247) was already in place, but I obtained local approval to audit the histology of all Oxford University Hospitals NHS Foundation Trust (OUHFT) patients undergoing bariatric surgery with liver biopsy. I developed additional collaborations (Porto Alegre, Brazil and Derby, UK) that were required to strengthen this study and enable the robust development of new predictive models. I entered all data except for the Brazilian cases, which were entered by Dr Katia Souto. All statistical analysis was performed by me (Dr Niall Dempster) except for predictive model development and evaluation. This was performed by Dr Florian Klimm after we developed a new collaboration for this stage of the project, and he prepared the initial versions of Figure 10-4 and Figure 10-5 that I then edited for this chapter.

8.3 Bariatric Surgery as an Intervention for NAFLD

I designed this study with approval from Professor Jeremy Tomlinson and Professor Leanne Hodson. I obtained the necessary local approvals to audit and compare patient outcomes in the Bariatric Surgery (BS) and Metabolic Hepatology (MH) clinic services. I developed the BS database and updated an existing MH database with the support of Kenzo Motohashi and Iona Woodward (both medical students at the University of Oxford). I performed all statistical analysis for this project.

8.4 Effects of Preoperative Low Energy Diet on NAFLD and Effects of Bariatric Surgery on NAFLD

I designed these studies, wrote the document set and obtained ethical (Research Ethics Committee reference (17/WM/0130), HRA and OUHFT approvals with the vital support of Professor Jeremy Tomlinson, Professor Leanne Hodson and Dr Garry Tan. Numerous surgeons employed within OUHFT kindly identified potentially eligible patients for this study. I am particularly grateful to Mr Bruno Sgromo and Mr Richard Gillies, who performed all the bariatric operations. I recruited all participants and organised all research visits.

During research visits, I provided medical supervision and performed the Fibroscans, venous cannulation and the earlier subcutaneous fat biopsies before training a Clinical Research Unit (CRU) nurse to perform this procedure. MR scans were performed by trained Oxford Centre for Clinical Magnetic Resonance Research (OCMR) radiographers. CRU visits were conducted with the generous support of Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM)'s research nurses, particularly Michelle Haylock and Rachel Franklin.

Following research visits, I separated the lipoprotein fractions after initial training from Catriona Charlton. Dr Sion Parry and Tom Cornfield assisted with this and with iLab analysis for some research visits. SNP genotyping was performed by collaborators at Novo Nordisk Research Centre Oxford (NNRCO). AST and ALT measurements were provided by Oxford's NHS Biochemistry Laboratory in collaboration with Professor Tim James. Insulin ELISAs were performed by Dr Valentina Greto.

9 Bariatric Surgery and NAFLD: Insights from Key Stakeholders

9.1 Introduction

There is emerging evidence that bariatric surgery may be an effective intervention for the combined treatment of obesity and NAFLD[186] but whilst rare, paradoxical liver function deterioration has been reported to occur[182-185]. Perhaps because of this clinical uncertainty, bariatric surgery is not currently included in the UK's NICE guidelines for the management of NAFLD[143]. Similarly, NAFLD is not specified as a BMI eligibility threshold-reducing comorbidity in NICE guidelines for bariatric surgery[1].

There is a lack of high-quality studies demonstrating the safety and efficacy of bariatric surgery as an intervention for NAFLD. However, before undertaking expensive prospective randomised clinical trials, current practice should be defined and the acceptability of this intervention amongst key stakeholders should be confirmed. Currently, the views of patients with obesity and NAFLD are unknown despite an increasing emphasis on Patient and Public Involvement (PPI) in clinical pathway development. Insights gained from such PPI work can also be used to guide research strategy[187].

An understanding of current practice amongst the specialist hepatologists and bariatric surgeons responsible for managing patients with obesity and NAFLD would also be beneficial. Insights into their current medical practice may identify areas of clinical uncertainty that can be addressed through future research, as well as potential barriers to the future integration of bariatric surgery into care pathways for people with obesity and NAFLD.

9.1.1 Aims

The aim of this chapter is to generate insights from key stakeholders (bariatric surgeons, hepatologists and patients with obesity and NAFLD) into their views on current and potential NAFLD therapies. This will provide important context for the work that will be performed in subsequent chapters in this thesis and guide the design of future research studies in this area.

9.2 Subjects and Methods

9.2.1 Research strategy

In this chapter, patients with NAFLD, hepatologists and bariatric surgeons completed surveys to determine their views on NAFLD, and how it affects patient care in 'real world' clinical practice.

9.2.2 Study design

Individual surveys were designed for patients with NAFLD, bariatric surgeons and hepatologists. Surveys were designed to confirm participant eligibility and to identify areas of uncertainty in clinical practice. All surveys were designed and critically reviewed by consultant/attending clinician stakeholder panels before dissemination. It was agreed that detailed power calculations to determine sample sizes for this exploratory work would not be feasible – there was an absence of similar studies that addressed many of the questions asked to participants in the surgical literature. Pragmatically, it was agreed that ≥100 responses from surgeons and hepatologists should give a reasonable indication of the views of these specialties, particularly if individual responses to each question are fairly consistent. There was no requirement to meet a pre-specified annual bariatric surgical caseload in order for surgeons to be eligible to complete the survey, but only independent (consultant/attending) surgeons met our eligibility criteria. For the patient survey, an initial sample of approximately 50 responses was agreed as this would allow an initial analysis that may yield sufficient information whilst minimising patient burden. An unabridged copy of each survey is included in the Appendix section.

Approval was required for distribution of the patient survey. This was obtained from the Joint Research Office of Oxford University Hospitals NHS Foundation Trust (OUHFT). After review of the document set, it was determined that this work represented service improvement and PPI, and full ethical review was deemed unnecessary.

9.2.3 Study methods

9.2.3.1 Participant Eligibility

Patient Survey

Inclusion Criteria

- Previous attendance at the Metabolic Hepatology clinic in OUHFT
- Diagnosis of NAFLD
- BMI ≥35kg/m^2
- Verbal informed consent to participation or previous written consent for contact from OUHFT staff or research investigators

Exclusion Criteria

- Failure to meet the inclusion criteria
- Previous bariatric surgery
- Previous completion of the survey

Bariatric Surgeon Survey

Inclusion Criteria

- Consultant/attending bariatric surgeon

Exclusion Criteria

- Failure to meet the inclusion criteria
- Previous completion of the survey

Hepatologist Survey

Inclusion Criteria

- Consultant/attending hepatologist

Exclusion Criteria

- Failure to meet the inclusion criteria
- Previous completion of the survey

9.2.3.2 Survey Dissemination

Bariatric Surgeon Survey

Bariatric surgeons were invited to participate the survey in paper or online format subject to their eligibility. Paper copies of the survey were distributed to delegates at the International Bariatric Club's 2018 World Congress (Oxford, UK). Email invitations containing a secure weblink to an electronic version of the survey were also sent to bariatric surgeons in the study team's professional and personal networks. Finally, social media posts (Twitter, Facebook bariatric discussion groups) were used to invite additional potential respondents.

Hepatologist Survey

Hepatologists invited to participate in an additional bespoke survey in paper or online format subject to their eligibility. Paper copies of the survey were distributed to delegates at the American Association for the Study of Liver Diseases' 2019 Liver Meeting (Boston, USA). Email invitations containing a secure weblink to an electronic version of the survey were also sent to hepatologists in the study team's professional and personal networks. Finally, social media posts (Twitter, Facebook hepatology discussion groups) were used to invite additional potential respondents.

Patient Survey

Patients with obesity and NAFLD were invited to participate by their clinical care team whilst attending the Metabolic Hepatology clinic in OUHFT. Once verbal consent to participation was obtained, demographic and clinical details were recorded and participants completed a printed version of the questionnaire. After survey completion, all participants were given the opportunity to discuss the survey with a physician during their routine clinical appointment. OUHFT patients who had previously taken part in two NAFLD-related research studies and consented to future contact were also invited to take part subject to their eligibility. A secure weblink to enable anonymous survey completion was provided by email.

9.2.3.3 Statistical Analysis

The D'Agostino-Pearson test was used to assess the normality of the distribution of continuous variable responses. Normally distributed continuous variable responses are

presented as mean (standard deviation (SD)) values and non-normally distributed continuous variable responses are presented as median (interquartile range (IQR)). Questions with categorical response options are presented as the percentage that each option is selected by participants completing that question. Where respondents specified a range (for example the number of operations performed per year) this was recoded using the midpoint of the range for the purposes of analysis.

9.3 Results

9.3.1 Bariatric surgery and NAFLD: Insights from bariatric surgeons

The survey was distributed to consultant/attending bariatric surgeons generated 183 responses. 9 participants were determined not to meet the eligible criteria so 174 participants were included in subsequent analysis.

9.3.1.1 Geographical Region

All eligible respondents (n=174) answered the question "In which country do you work?". Their country of work, categorised by United Nations (UN) regional group, is shown in Figure 9-1

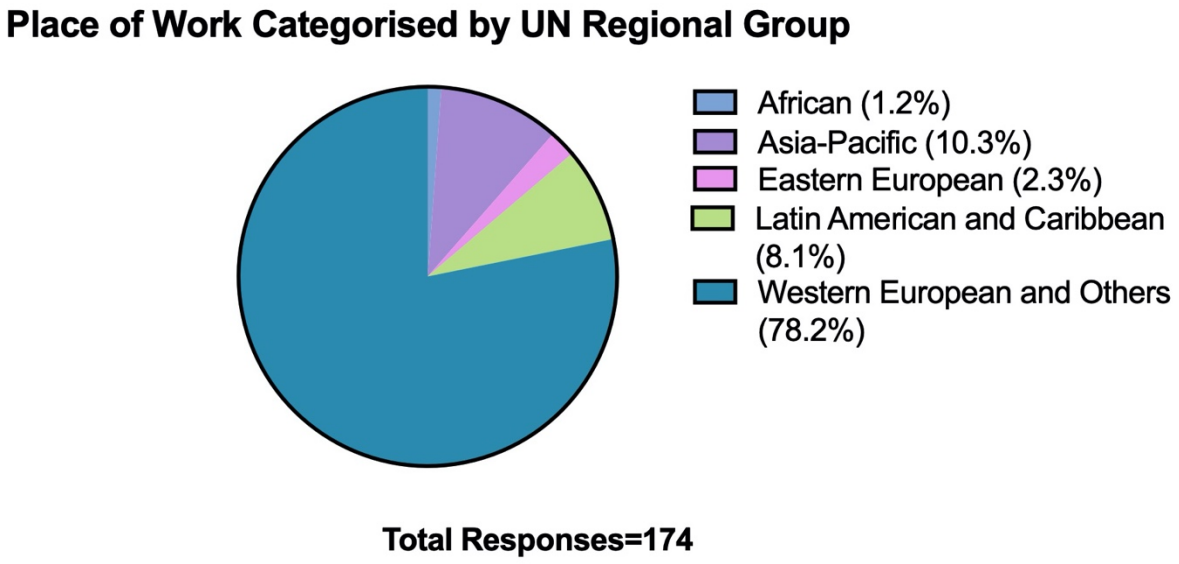


Figure 9-1 Pie chart of workplace of all participating surgeons (n=174) categorised by UN Regional Group

Figure 9-1 highlights that the bariatric surgeons completing this survey predominantly (n=136) worked in the Western European or politico-economically similar countries that

form the Western European and Others Group (WEOG). Of WEOG surgeons, 40 work in the UK (23.0% of all respondents) and 34 in the USA (19.5% of all respondents).

9.3.1.2 Type and Volume of Bariatric Surgical Operations

173 respondents (99.4%) answered the question: "Approximately how many of the following operations do you perform each year?" which included options for Gastric Banding (AGB), Sleeve Gastrectomy (SG), Roux-en-Y Gastric Bypass (RYGB), One-Anastomosis Gastric Bypass (OAGB) and a free textbox for Other operations. Normality testing using the D'Agostino-Pearson test demonstrated a non-normal distribution for the number of operations performed by respondents in each operation category.

The total number of bariatric operations performed annually by respondents was a median 135 cases (IQR 161), the breakdown of which is shown in violin plots in Figure 9-2A.

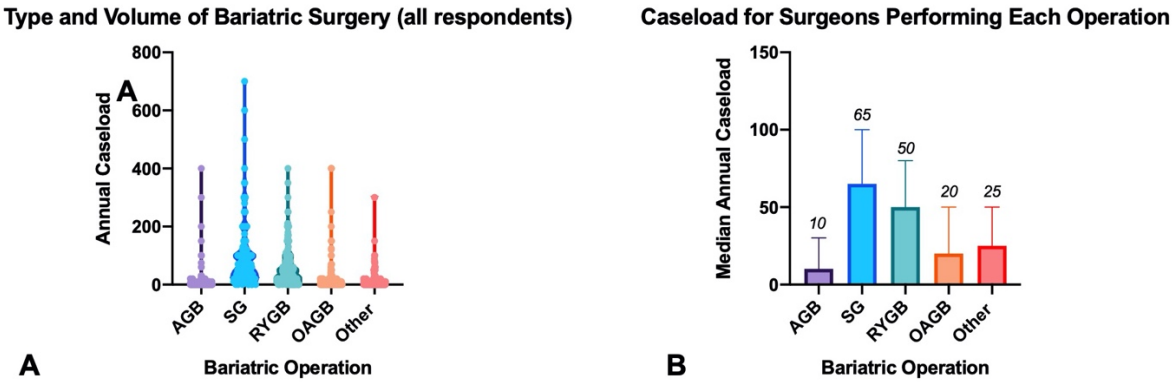


Figure 9-2 (A) Violin plot showing the number of each type of bariatric surgery performed annually by each respondent (n=173). (B) Bar chart of annual median number of operations performed by the surgeons undertaking each procedure. Error bars indicate the median value's IQR, numbers above error bars represent median values. AGB: adjustable gastric band, SG: sleeve gastrectomy, RYGB: Roux-en-Y gastric bypass, OAGB: one anastomosis gastric bypass.

Surgeons who responded in the 'Other' category most commonly specified a revisional caseload (n=32), followed by additional, and mostly more malabsorptive, bypass procedures (n=29) such as biliopancreatic diversion with duodenal switch (BPD-D) or single anastomosis duodenoileal bypass with sleeve gastrectomy (SADI-S). 10 respondents indicated that they also perform endoscopic bariatric procedures, 8 of whom specified intragastric balloon use.

Median annual caseload for surgeons performing operations in each category is demonstrated in Figure 9-2 B. Figure 9-2 confirms that SG is the most commonly performed bariatric operation amongst respondents followed by RYGB, which is consistent with published data[188].

9.3.1.3 Estimates of NAFLD Severity and Impact on Clinical Management

Estimates of the intraoperative histological severity of NAFLD were also provided by 151 (86.78%) of the bariatric surgeons (prevalence of NAFLD n=148, Advanced NAFLD n=133, Cirrhosis n=143, NASH n=132). Estimates were non-normally distributed and median (IQR) results are presented in Figure 9-3.

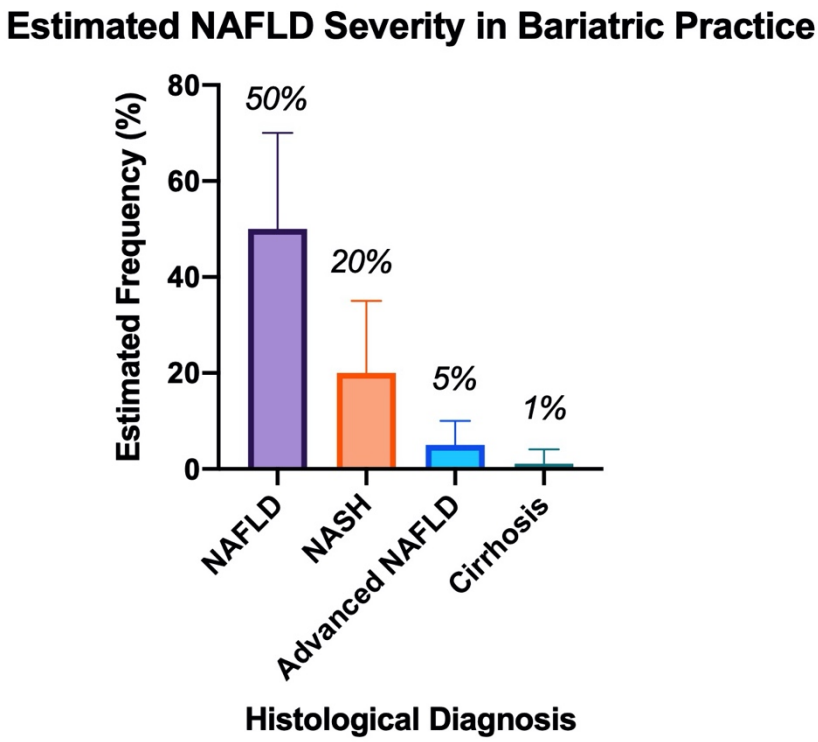


Figure 9-3 Bar chart of surgeons' estimates (n=151) of the prevalence of histological diagnoses across the spectrum of NAFLD in their own practice. Error bars correspond with the IQR of median values.

Figure 9-3 highlights that estimates of clinically-relevant pathology in NAFLD vary markedly between respondents. It is unclear whether this is due to regional variation in NAFLD severity or variability in the diagnosis of NAFLD.

9.3.1.4 Clinical Service Availability and Preoperative NAFLD Investigation

All (n=174) surgeons disclosed whether their bariatric department had a preoperative pathway or guideline for NAFLD investigation and treatment. Less than a third (31.0%) indicated that a local framework of this nature had been established.

Information on liver-related service availability was provided by 167 surgeons (96.0%). The percentage of respondents having local access to each service is shown in Figure 9-4.

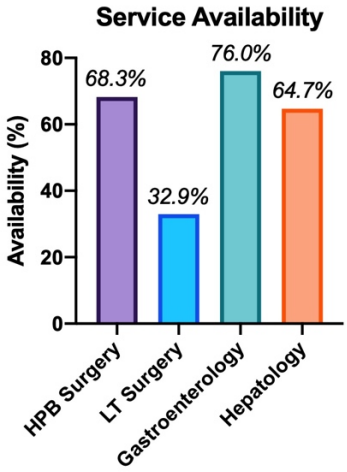


Figure 9-4 Bar chart showing the percentage of bariatric surgeons (n=167) with access to medical and surgical liver-related services. HPB: hepatobiliary, LT: liver transplant.

Preoperative NAFLD investigation details were provided by 173 surgeons (99.4%). Respondents were asked to record whether a range of blood tests, non-invasive biomarkers of NAFLD severity, and relevant medical history and imaging were performed.

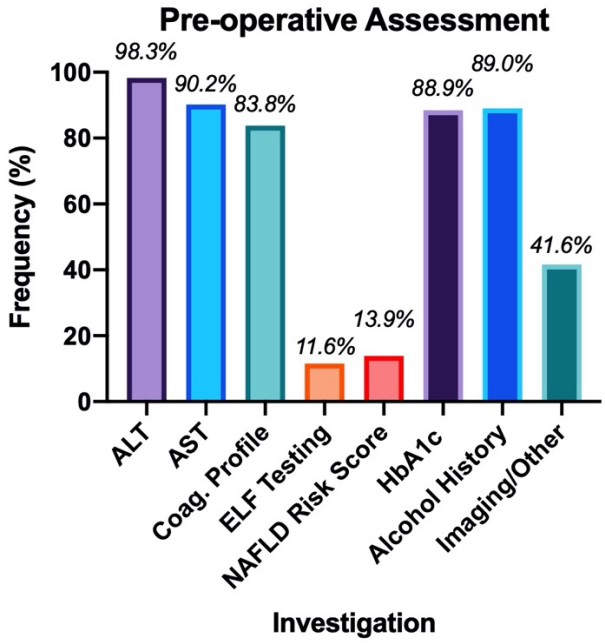


Figure 9-5: Bar chart showing the percentage of bariatric surgeons (n=173) requesting a range of NAFLD-related preoperative investigations. NAFLD risk scores are non-invasive biomarkers of NAFLD severity and are calculated from combinations of demographic, biochemical or imaging input variables. ALT: alanine transferase, AST: aspartate aminotransferase, Coag: coagulation, ELF: Enhanced Liver Fibrosis, HbA_1c: glycated haemoglobin.

Figure 9-5 summarises these results and shows that liver chemistry and glycated haemoglobin (HbA_1c) blood testing is usually undertaken, along with an assessment of alcohol intake history. However, non-invasive biomarkers that predict NAFLD severity are rarely incorporated into the preoperative assessment of individuals scheduled for bariatric surgery. Imaging investigations, when performed, consisted largely of abdominal ultrasonography (n=49), although cross-sectional imaging was requested in a few cases (MRI n=6, CT n=2). 14 surgeons specified that they routinely used hepatic elastography/Fibroscan (Echosens, Paris, France) for preoperative non-invasive liver fibrosis assessment.

9.3.1.5 Intraoperative NAFLD Assessment

Intraoperative liver biopsy practice and estimated ability to visually identify advanced fibrotic NAFLD was also assessed. 173 surgeons (99.4%) contributed by providing this information, which is presented in Figure 9-6.

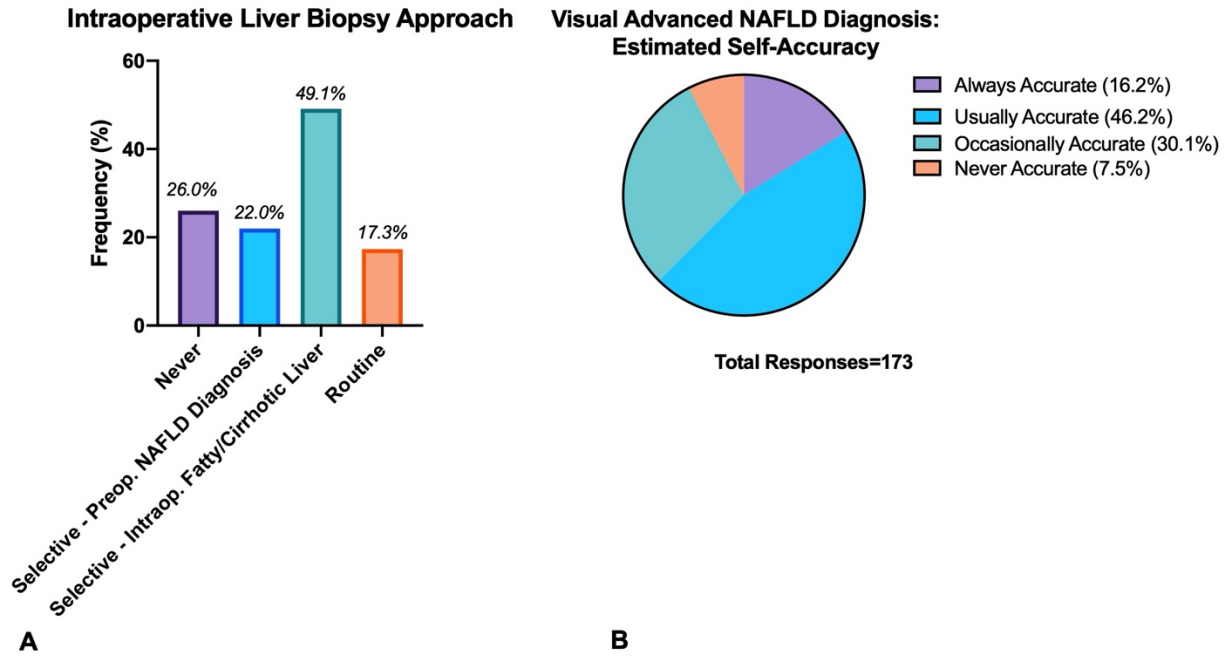


Figure 9-6 (A)Bar chart indicating the percentage of surgeons (n=173) employing a range of approaches to taking liver biopsies during bariatric surgery. (B) Pie chart of self-estimated ability of respondents (n=173) to visually identify advanced NAFLD during bariatric surgery.

It appears that a selective approach to performing liver biopsy for NAFLD staging is favoured by most respondents as shown in Figure 9-6 A. Approximately half (49.1%) performed biopsies based on the intraoperative appearance of the liver, with a further 22.0% of surgeons obtaining liver histology when there is a preoperative diagnosis of NAFLD. However, Figure 9-6 B highlights that few surgeons (16.2%), feel that they are always able to visually identify advanced NAFLD. Therefore, where routine intraoperative liver biopsies are not performed, the pre-operative identification of advanced NAFLD using non-invasive biomarkers would appear to be beneficial.

9.3.1.6 Postoperative NAFLD Outcomes and Clinical Care

Views on the effects of bariatric operations on people with advanced NAFLD were shared by 173 surgeons (99.4%). Figure 9-7 shows that almost a quarter of surgeons are of the opinion that there are insufficient data on postoperative outcomes in this patient group.

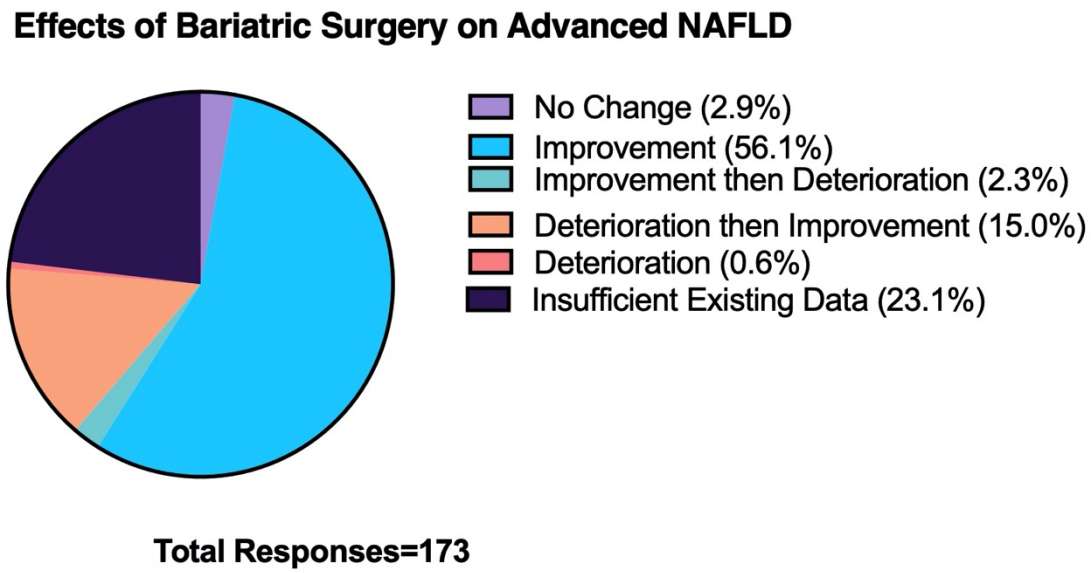


Figure 9-7: Pie chart characterising respondents' perceptions (n=173) of the usual effects of bariatric surgery on the liver function of patients with advanced NAFLD

However, the majority of respondents believed that the usual outcome is either improvement (56.1%), or acute deterioration followed by improvement (15.0%).

It is perhaps for this reason that 63.7% of participating surgeons (n=171) stated that patients diagnosed with NAFLD before or at the time of bariatric surgery receive standard postoperative clinical care (Figure 9-8). Almost half (48.5%) or respondents also specified that they refer some patients to hepatologists for specialist advice.

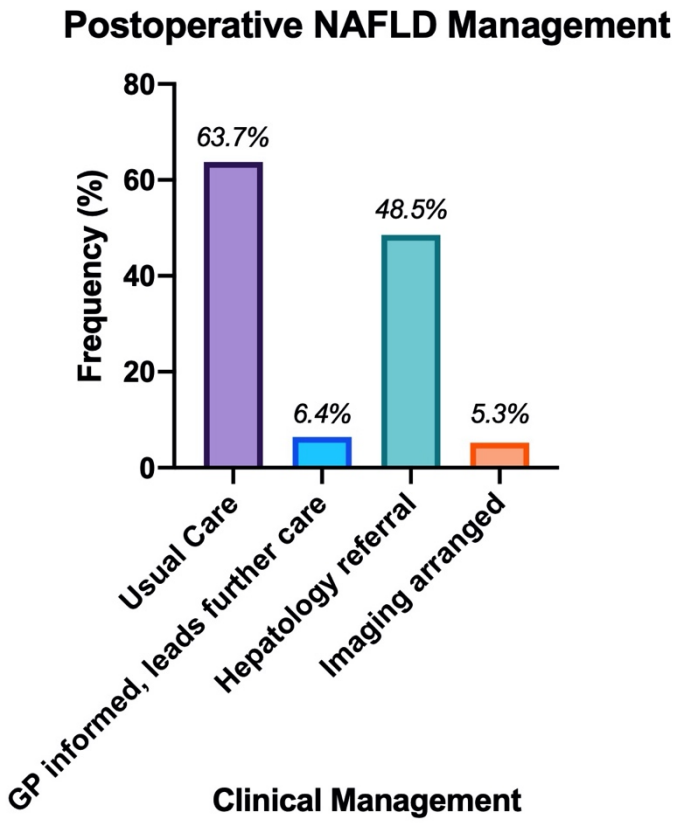


Figure 9-8 Bar chart showing the impact of a NAFLD diagnosis on clinical management after bariatric surgery amongst responding surgeons (n=171). GP: general practitioner (family physician).

45.0% of surgeons (n=77) indicated that they managed patients with NAFLD by usual care only, without informing the patient's general practitioner (GP) or hepatology services. 9 respondents specified that they arranged hepatic elastography, with 3 performing non-invasive NAFLD risk score calculations or Enhanced Liver Fibrosis (ELF) blood testing (Siemens/iQur Ltd, London, UK).

9.3.1.7 Advanced NAFLD-related Liver Deterioration after Bariatric Surgery

Whilst Figure 9-7 suggests that liver deterioration is a relatively rare postoperative event, 46/170 surgeons (27.1%) reported personal experiences where this had occurred. An additional 20.6% of respondents were unsure whether liver function had deteriorated after bariatric surgery due to advanced NAFLD, as illustrated in Figure 9-9.

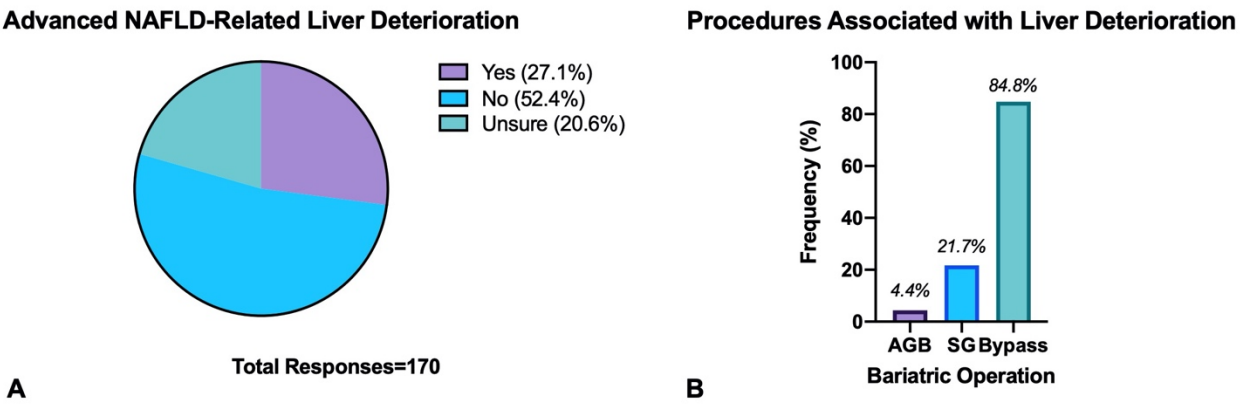


Figure 9-9 (A) Pie chart showing the proportion of respondents (n=170) who had managed patients with liver deterioration due to Advanced NAFLD after bariatric surgery. (B) Bar chart showing the proportion of responding bariatric surgeons (n=46) who reported cases of deteriorating liver function after adjustable gastric band (AGB), sleeve gastrectomy (SG) and Bypass surgery.

All surgeons reporting such cases, provided additional anonymised clinical details that contained no identifying data. Advanced NAFLD-related postoperative liver failure was mostly associated with bypass-type operations, as identified by 84.8% (n=39) of respondents (Figure 9-9 A). Operations with a greater malabsorptive component appeared overrepresented in Figure 9-9 B relative to their contribution to overall annual bariatric surgical caseloads in Figure 9-2, with one surgeon commenting that “the more distal the bypass the higher the chances that a liver complication occurs”.

The severity of reported liver function deterioration was variable and ranged from a transient elevation in liver chemistry and liver failure resulting in transplantation or death. Free-text responses were categorised and are summarised in Table 9-1:

Table 9-1 Table of the number of responding surgeons (n=46) specifying NAFLD-related complications after bariatric surgery in their clinical practice.

Clinical Event	**Reporting surgeons**
Blood test deterioration	31
Jaundice	14
Ascites	17
Encephalopathy	11
Liver failure	11
Coagulopathy	5
Hepatomegaly	1
Death	10

Surgeons with experience of liver function deterioration mostly referred to hepatologists for ongoing patient management (27/43 respondents). 10 surgeons highlighted a need for dietary and nutritional support, including two who specified feeding tube insertion. Five surgeons also described interventions to reverse bypass surgery or increase the common channel length and three referred to transplant surgical colleagues. Seven respondents reported that conservative approaches involving close monitoring had resulted in improved liver function. Treatment with ursodeoxycholic acid, endocrinologist referral and blood product administration were each detailed by one surgeon.

Alcohol was the most commonly specified additional factor contributing to liver deterioration after bariatric surgery (n=9), followed by malnutrition/malabsorption and viral hepatitis (n=5 each), and medication (n=3).

9.3.2 Bariatric surgery and NAFLD: Insights from hepatologists

The survey that was distributed to consultant/attending hepatologists generated 125 responses. 4 participants were determined not to meet the eligible criteria so 121 participants were included in subsequent analysis.

9.3.2.1 Geographical Region

111 respondents (91.74%) specified their country of work. These responses were categorised into UN Regional Groups, and are presented in Figure 9-10L

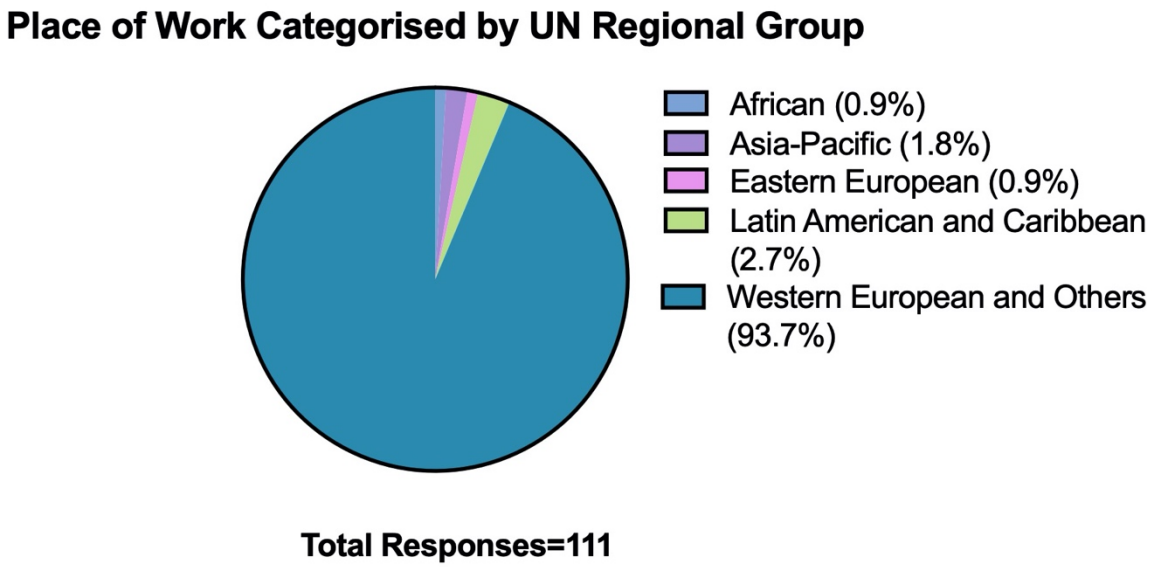


Figure 9-10: *Pie chart of the UN Regional Group where participating hepatologists (n=111) work*

Figure 9-10 highlights that the responses to this survey are dominated by WEOG attending/consultant hepatologists to a greater extent than in the bariatric surgeon survey (78.16% WEOG respondents). Within the WEOG, most (n=66) respondents worked in the UK (59.46% of all respondents), followed by the USA (n=18, 16.22% of all respondents).

9.3.2.2 Clinical Service Availability and Bariatric Surgery Referral Pathway

All (n=121) hepatologists provided data on the design of their local liver services, including the availability of specialist hepatologists and liver transplant services. Respondents also confirmed whether there was a local bariatric surgery service (Figure 9-11 A), and 119 (98.35%) gave details on local bariatric service referral protocols (Figure 9-11 B).

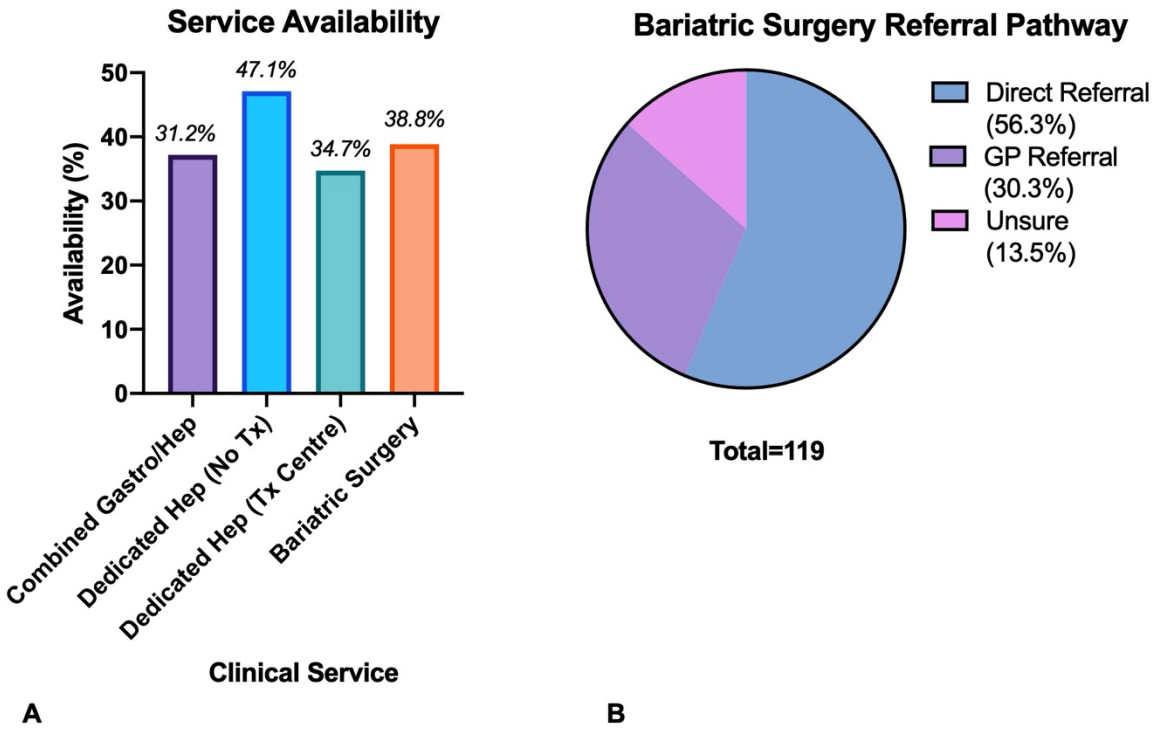


Figure 9-11 (A) Bar chart showing local liver and bariatric surgery service availability for respondents (n=121). (B) Pie Chart of local bariatric surgery referral pathways available to responding hepatologists (n=119). Gastro/Hep: gastrohepatology service, Hep: hepatology service, Tx: transplant.

Figure 9-11 A highlights that availability of bariatric surgical services is relatively poor amongst respondents, with only 38.8% reporting this service in their hospital or healthcare trust. Just over half of respondents were able to directly refer patients with obesity and NAFLD to their bariatric surgeon colleagues, with around a third stating that they needed to write to patients' GP/family doctor and request onward referral (Figure 9-11 B).

9.3.2.3 NAFLD-related Indications for Bariatric Surgery and Liver Biopsy Recommendations

All (n=121) respondents shared their views on NAFLD-related indications for bariatric surgery (Figure 9-12A), and 119 (98.4%) specified their policy for bariatric surgical intervention in individuals with severe obesity and decompensated NASH cirrhosis necessitating liver transplantation (Figure 9-12 B).

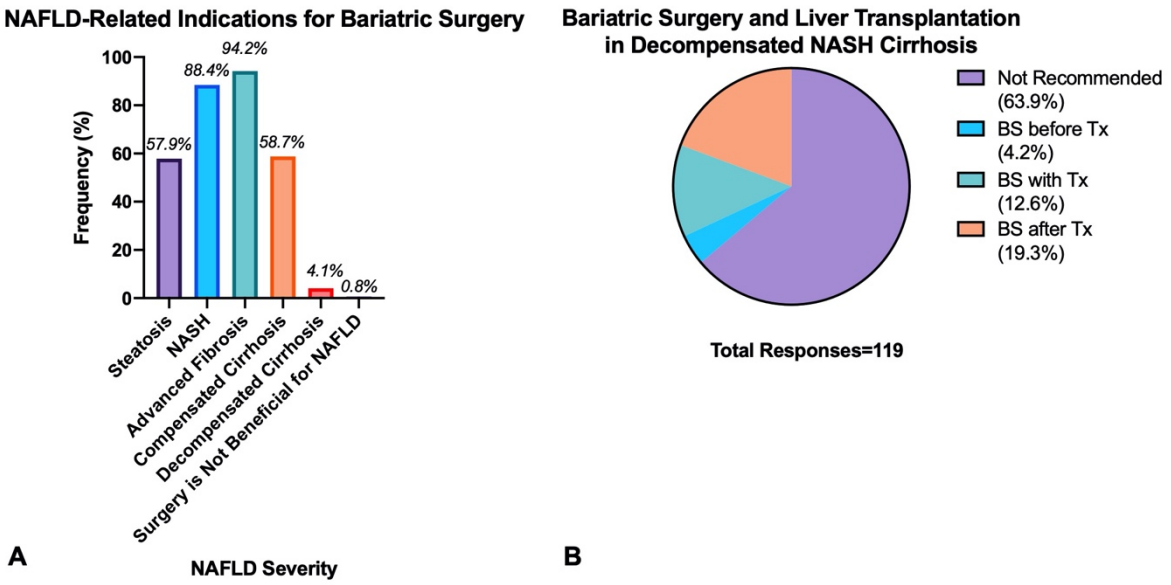


Figure 9-12 *(A) Bar chart of respondents' (n=121) views on NAFLD histology where bariatric surgery is likely to be beneficial. (B) Viewpoints (n=119) on the performance and timing of bariatric surgery in patients with obesity requiring liver transplantation for decompensated NASH cirrhosis. BS: bariatric surgery, Tx: transplant*

Figure 9-12 A shows that the majority of responding hepatologists believe that believe that patients with severe obesity and a wide range of NAFLD pathology would benefit from undergoing bariatric surgery. In particular, 94.2% believed that those with advanced fibrotic NAFLD, and 88.4% believed that those with NASH would derive benefit. Whilst the majority (58.7%) also believed that surgery was beneficial for individuals with compensated cirrhosis, few recommended in individuals with decompensated cirrhosis.

However, where liver transplantation was also scheduled, 19.3% recommended bariatric surgery after transplantation in patients with decompensated NASH cirrhosis (Figure 9-12 B), with a further 12.6% advocating combined liver transplant and bariatric surgery procedures. Only 4.2% would propose bariatric surgery before liver transplantation in this patient group.

9.3.2.4 NAFLD Staging and Monitoring in Bariatric Surgical Patients

The clinical investigations performed by responding hepatologists for NAFLD staging and monitoring before (Figure 9-13 A, n=116), during (Figure 9-13 B, n=119) and after (Figure 9-14, n=114) bariatric surgery were also determined.

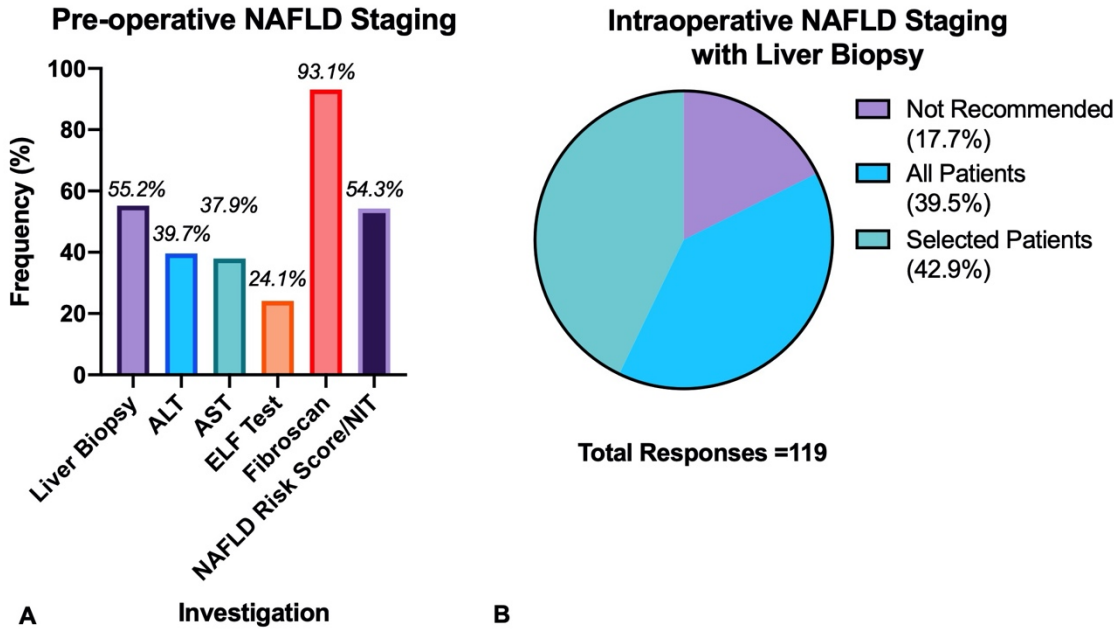


Figure 9-13 *(A) Bar chart of the proportion of hepatologists (n=116) requesting a range of investigations for NAFLD staging before bariatric surgery. (B) Recommendations on liver biopsy performance for NAFLD staging during bariatric surgery.* ***ALT: alanine transferase, AST: aspartate aminotransferase, Coag: coagulation, ELF: Enhanced Liver Fibrosis.***

Few responding hepatologists (n=119, 26.1%) reported the existence of a defined local pathway for NAFLD investigation and staging before bariatric surgery, which broadly parallels the surgeons' responses, 31.0% of whom reported NAFLD staging pathway availability. 80.6% of the hepatologists (n=25) working in hospitals offering preoperative NAFLD staging pathways reported a median 12.5 (IQR 26.5) patients accessing such services annually.

There was a marked contrast in the types of preoperative investigations used for NAFLD staging by hepatologists (Figure 9-13 A) and bariatric surgeons (
Figure 9-5). Whilst the bariatric surgeons largely reported that they used liver enzymes (such as AST or ALT) in isolation, non-invasive biomarker usage amongst hepatologists was

common. Despite reported technical limitations in individuals with severe obesity[189] due to the skin-liver distance, hepatic elastography (Fibroscan) was performed for NAFLD staging by 93.1% of responding hepatologists. The majority of hepatologists also reported using other non-invasive tests (NITs) in such patients and, somewhat surprisingly given that most felt that bariatric surgery is beneficial in non-decompensated patients with NAFLD, 55.17% also reported using percutaneous pre-operative liver biopsies for staging purposes.

56.9% of hepatologists recommending a selective approach to intraoperative liver biopsies during bariatric surgery (n=29) shared their selection criteria. These largely centred on indeterminate (n=12) preoperative non-invasive biomarker results, or for confirmation of results suggestive of NASH or advanced fibrotic NAFLD (n=10). Other respondents arranged biopsies in patients without previous liver histology (n=4), for research purposes (n=2) or if the liver appeared abnormal or cirrhotic during surgery (n=2). There is a striking contrast between the number of hepatologists making the latter recommendation and in the practice of bariatric surgeons, 49.1% of whom performed selective liver biopsies based on the intraoperative appearance of the liver (Figure 9-6 A).

After bariatric surgery, transient elastography (Fibroscan) was again most frequently recommended by responding hepatologists (Figure 9-14, n=114) for NAFLD monitoring.

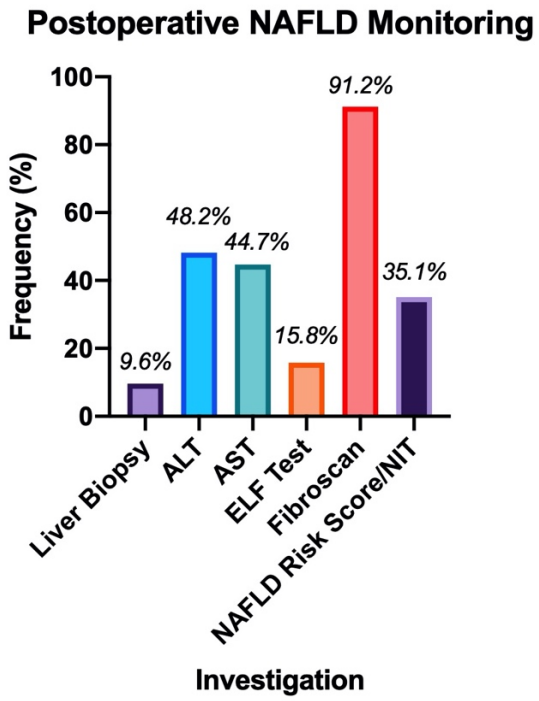


Figure 9-14: *Hepatologists' usage of a range of investigations for monitoring NAFLD after bariatric surgery (n=114).* ***ALT: alanine transferase, AST: aspartate aminotransferase, Coag: coagulation, ELF: Enhanced Liver Fibrosis. NIT: non-invasive test.***

Figure 9-14 shows continued support (91.2%) for hepatic elastography (Fibroscan) use after bariatric surgery, but decreased use of liver biopsies and serological non-invasive biomarkers compared to Figure 9-13 A. This is perhaps explained by the limited validation of these NITs after bariatric surgery, and because most hepatologists advocate liver biopsy before or during bariatric surgery and expect postoperative improvements in NAFLD except in patients with preoperative decompensation.

9.3.2.5 Advanced NAFLD-related Liver Deterioration after Bariatric Surgery

Approximately a quarter (24.4%) of responding hepatologists (n=119) reported definite experience managing patients with liver deterioration due to advanced NAFLD after bariatric surgery, as shown in Figure 9-15A:

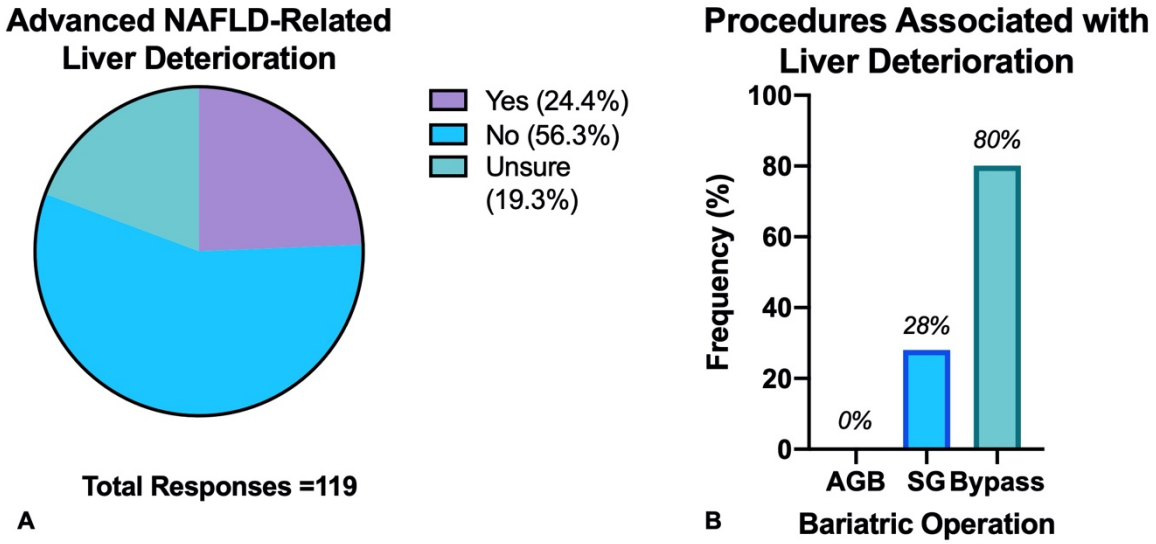


Figure 9-15 *(A) Proportion of hepatologists (n=119) with experience managing advanced NAFLD-related liver deterioration after bariatric surgery. (B) Bar chart showing the proportion of hepatologists reporting liver function deterioration after adjustable gastric band (AGB), sleeve gastrectomy (SG) and Bypass surgery.*

Hepatologists' experiences of liver dysfunction after bariatric surgery closely mirror those reported by the surgeons in Figure 9-9 A and B, despite likely differences in the case mix of these practitioners. These experiences were relatively rare, however, with a median 2 (IQR 2) cases per responding hepatologist.

Table 9-2 Table of the number of responding hepatologists (n=28) specifying NAFLD-related complications after bariatric surgery in their clinical practice

Clinical Event	**Reporting hepatologists**
Blood test deterioration	7
Jaundice	14
Ascites	14
Encephalopathy	6
Liver failure	9
Death	5

26 of these respondents supplied additional details on the clinical management of these cases. 10 reported that patients under their care had required liver transplantation, but other cases were usually managed supportively (n=9) and with dietary and nutritional supplementation (n=9). Medications such as antibiotics or lactulose (n=3) were occasionally specified, and reversal of the bariatric surgery was mentioned by one hepatologist.

Alcohol (n=10) and malnutrition (n=9) were frequently identified as additional contributory factors. Interestingly, 2 hepatologists cited unrecognised cirrhosis at the time of surgery as relevant factors in the development of severe postoperative liver dysfunction in patients they had managed.

9.3.2.6 Barriers to the Wider Implementation of Bariatric Surgery as a Treatment for NAFLD

Finally, we assessed the views of hepatologists (n=118) on whether there are existing barriers limiting the implementation of bariatric surgery as a more common treatment for NAFLD, the results of which are shown in Figure 9-16:

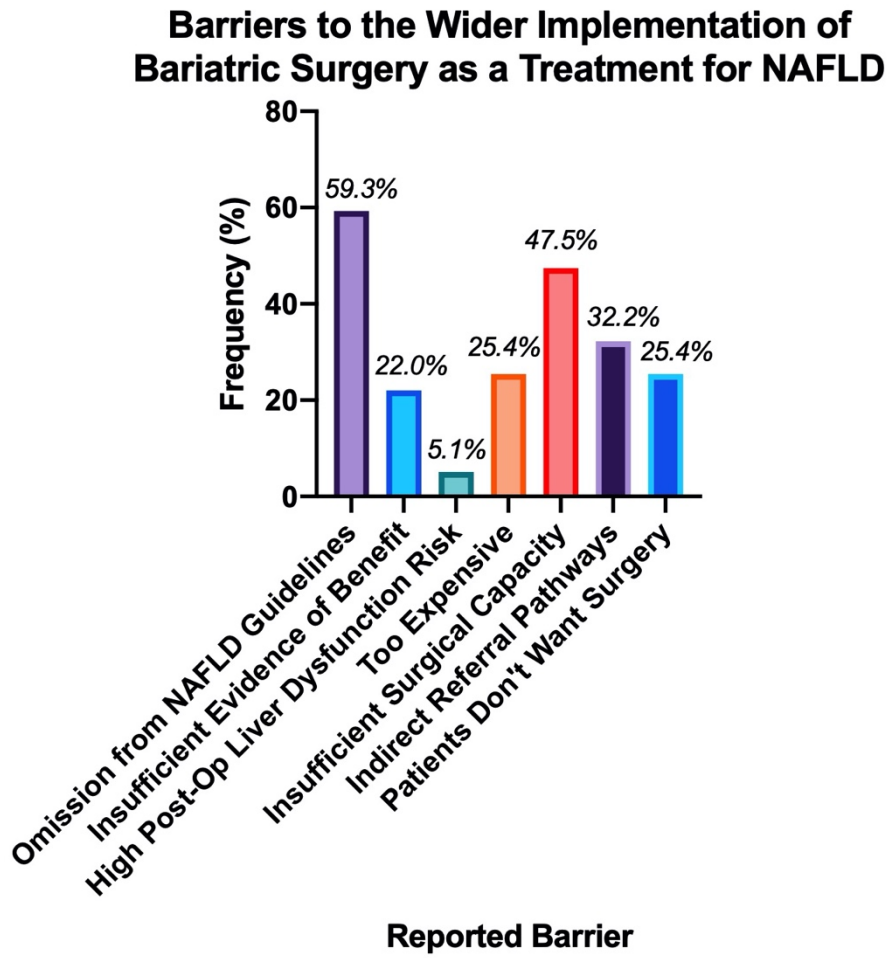


Figure 9-16 *Bar chart showing the frequency of reported barriers that limit the wider implementation of bariatric surgery as a treatment for NAFLD*

The lack of inclusion of bariatric surgery as a recommended intervention for NAFLD in clinical guidelines was the only barrier identified by the majority (59.3%) of respondents. Other commonly specified issues were structural, such as insufficient surgical capacity (47.5%) or due to an inability to directly refer patients to bariatric colleagues (32.2%). Whilst bariatric surgery has been reported to be a cost effective intervention for the management of obesity and NASH-cirrhosis[181], a quarter of hepatologists felt it was too expensive and a similar number stated that their patients are not interested in bariatric surgery as a treatment for obesity and NAFLD. Few (5.08%) specified concerns about the safety profile of bariatric surgery as a barrier to its wider implementation.

9.3.3 Bariatric surgery and NAFLD: Insights from patients

49 responses to the patient survey were received. 6 responses were excluded from this analysis due to a history of previous bariatric surgery, and 7 responses were excluded as that participant's BMI was below 35kg/m^2, the pre-specified minimum threshold for inclusion in this study. Data from 36 respondents was therefore included in subsequent analysis.

Table 9-3 Characteristics of individuals with NAFLD and severe obesity (BMI ≥35kg/m^2) completing the patient survey (n=36).

Characteristic	**Severe Obesity and NAFLD Patient Group**
Age (years)	57 (14.5)
Female Sex (%)	50%
BMI (kg/m^2)	38.74 (7.07)
T2D (%)	36.1%

Median (IQR) values displayed for non-normally distributed Age and BMI outcome measures.

Table 9-3 provides demographic and anthropometric data on the patients with obesity and NAFLD participating in this survey. More than a third of participants had co-existent T2D, which is lower than the proportion of patients attending this clinic as a whole[190].

The most commonly prescribed treatments for the patients participating in this study were weight loss by diet and exercise (Figure 9-17A), with respondents reporting that bariatric surgery was discussed infrequently. However, Figure 9-17B shows that weight management (by diet or exercise) was most commonly stopped due to a perception that it was ineffective amongst this patient group.

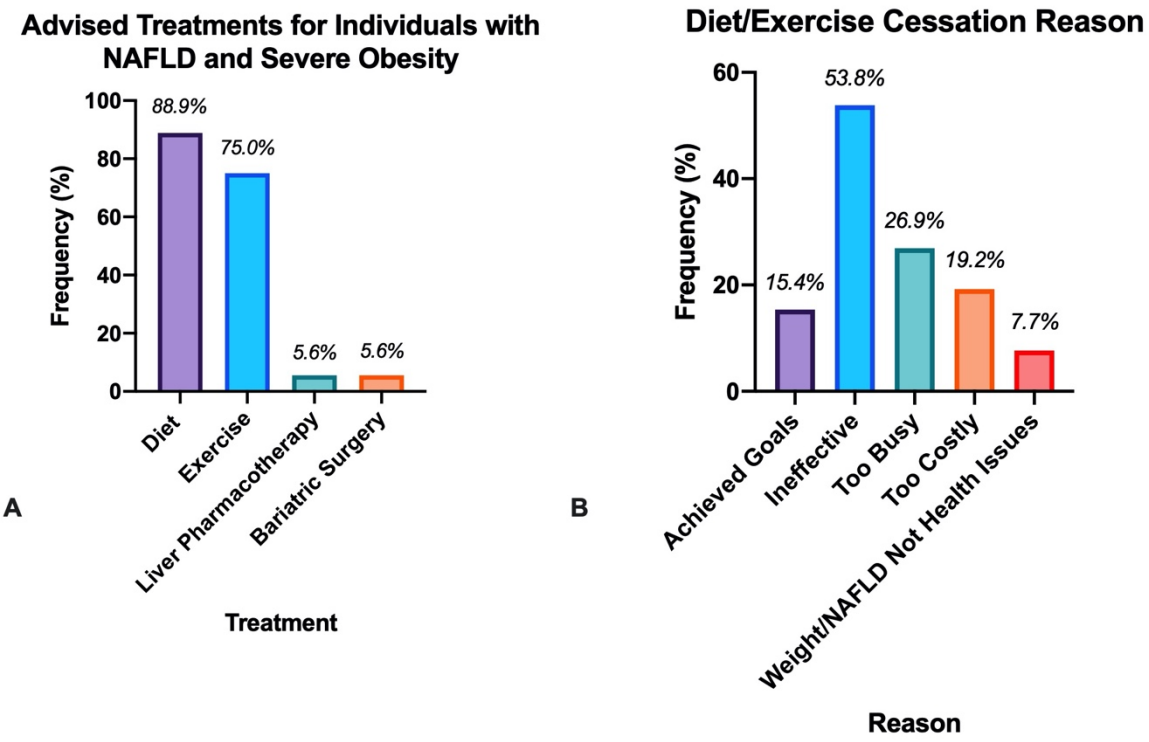


Figure 9-17 *(A) Prescribed interventions for obesity and NAFLD as reported by patient respondents. (B) Reasons for dietary or exercised-based weight management cessation.*

23 of the 36 eligible patients provided data on outcomes achieved through lifestyle (dietary or exercise) interventions. A median 6.35kg weight loss was reported, with subsequent weight regain often reported in line with the existing literature.

Since current NICE guidelines include bariatric surgery as a treatment option for individuals with Class 2 obesity and a comorbidity that may be improved following surgery, patients' perceptions of bariatric surgery were also explored. T2D, present in over a third of responding patients in this study, is highlighted as a comorbidity improved by bariatric surgery[1]. However, NAFLD is not currently specified in a similar way, which was reported by hepatologists as the greatest barrier to the increased utilisation of surgical intervention earlier in this chapter (Figure 9-16).

Figure 9-18 A shows that approximately 40% of patients with obesity and NAFLD reported that they had not previously heard of bariatric surgery. Amongst those that had, it appeared that the media was their most frequent source of information on this intervention, with 1 in 5 reporting that they had discussed surgery with a healthcare worker.

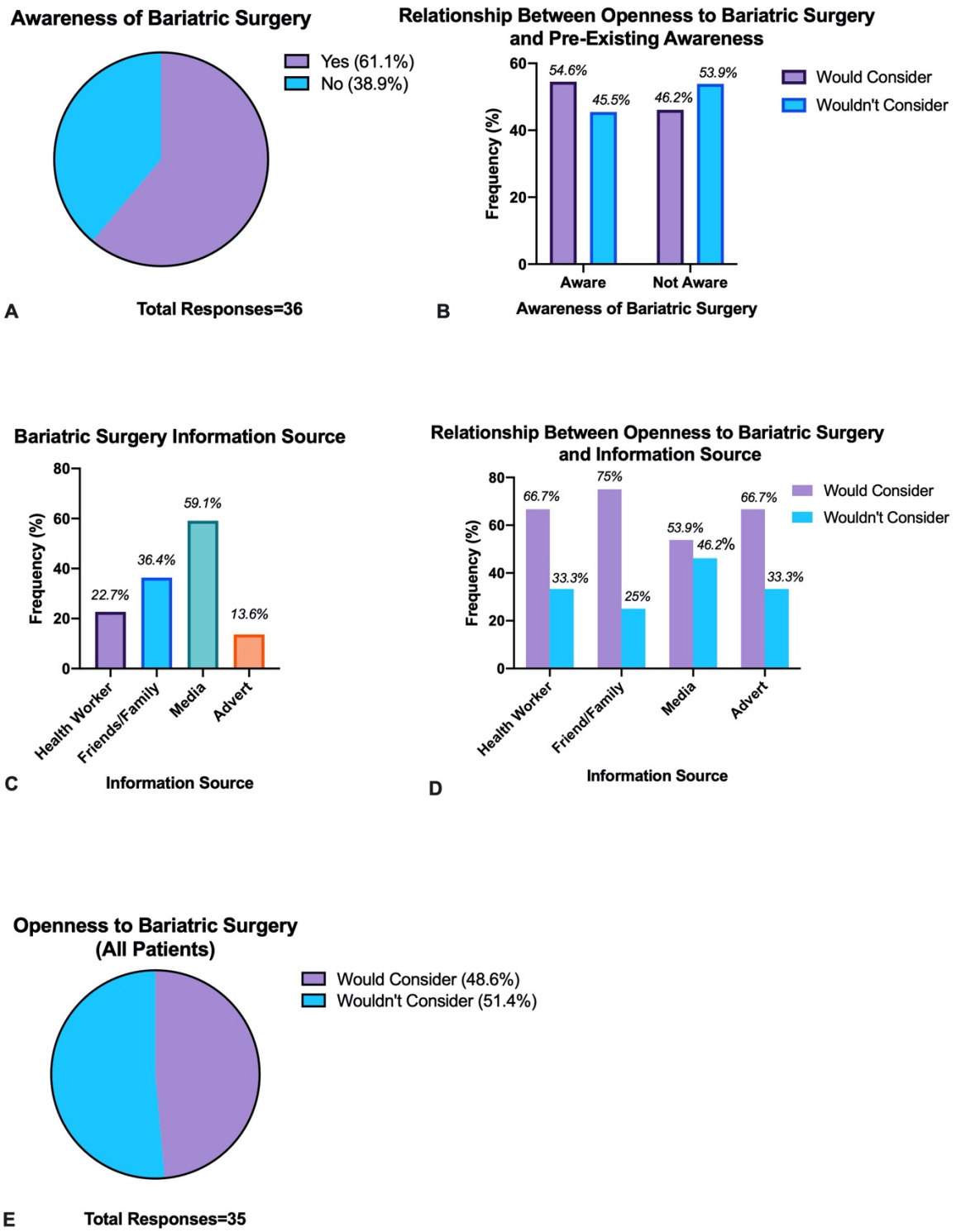


Figure 9-18 *(A) Pie chart indicating the percentage of patients with obesity and NAFLD (n=36) who were aware of bariatric surgery (B) Bar chart showing the relationship between pre-existing bariatric surgery knowledge and openness to surgical intervention (n=22) (C) Bar chart indicating bariatric surgery information source for people with pre-existing awareness (n=22) (D) Relationship between bariatric surgery information source and openness to surgery (n=22) (E) Overall openness to surgery amongst responding patients (n=35)*

Differences in attitudes towards bariatric surgery according to patient awareness (61.1% aware, 95% confidence interval (CI) 44.9%-75.2%) and sources of information were explored in a qualitative way in Figure 9-18 B and Figure 9-18 D. Whilst no significant differences in openness to bariatric surgery were found between groups and confidence intervals were wide (48.6% of all respondents would consider surgery, 95% CI 33.0%-64.4%) , this study was not powered for this exploratory analysis. 95% CIs were not calculated for binary responses on subgroups of patients for this reason, or when there were multiple response options. It does appear that there may have been a trend towards greater interest in bariatric surgical intervention after this had been discussed with a healthcare worker, or if participants had a personal connection with a recipient of bariatric surgery. Overall, approximately half of all responding patients would consider bariatric surgery as a potential combined treatment for their obesity and NAFLD (Figure 9-18 E) if recommended based on their current knowledge of the intervention.

Finally, patients' free-text responses were also reviewed in a qualitative way to develop further insights into the potential of bariatric surgical intervention. Participants who would consider bariatric surgery believed that it would be more effective than diet, which they found very difficult and was associated with weight regain, and improve health. Several others commented that physical impairments limited their ability to exercise. However, a fear of 'botched surgery' and 'liquid diet' was reported by one participant. Patients reluctant to consider bariatric surgery stated that weight loss and health gains should and could be achieved through diet alone. Some felt that "it's not right, raising concerns about its "drastic" nature, and that surgery can be "cheated, resulting in further health problems or weight gain again".

One participant's comments encapsulated many of these emerging themes:

"I have been overweight for a number of years now and had little help from my doctor. Even with the fatty liver disease and associated pain I get, I'm not taken seriously and have not been offered any meds or help…I would not be able to afford surgery, so have never really gone down this path. I feel that there is very little help on the NHS for obese patients.

I have tried many times to ask for help and advice, over time, but it has never been forthcoming. I feel the attitude is "you got this way, now do something about it". It is very difficult to be taken seriously or have some sympathy. I get depressed and hide myself away. Embarrassed. I struggle at work, as I now have painful feet and joints. If only there was a magic pill."

Regardless of personal views on bariatric surgery, there was a consensus amongst patients with Class 2 obesity that weight loss through non-surgical approaches is exceptionally challenging. Many felt that their healthcare professionals didn't "understand [their] struggle with diet and how emotionally destroying [their] weight is". There were also some misconceptions over the funding of bariatric surgery, with two participants commenting that they wouldn't be able to afford it.

9.4 Discussion

9.4.1 Principal findings

In this study, I aimed to develop insights into the views and practice of key stakeholders in NAFLD management in order to develop research questions for subsequent studies and to provide context for their results. Our key findings are summarised in Summary Table 9-4:

Table 9-4: Key Findings Summary Box

- **Limited and variable clinical NAFLD fibrosis stage assessment.** NAFLD staging pathways for bariatric surgical patients should be designed and evaluated in future studies.
- **Limited data on postoperative liver outcomes**. These should be studied in more detail in future studies, ideally through 'big data' approach using national registries in order to have statistical power to detect rare events.
- **Lifestyle advice for NAFLD is common but relatively ineffective in people with severe obesity.** There is therefore a need for additional NAFLD therapies, and patients consider bariatric surgery closely when it is discussed. Future studies should evaluate candidate NAFLD therapies, including bariatric surgery.
- **However, significant barriers to surgery exist**, including:
    - Omission from NAFLD treatment guidelines. There is scope to address this in future NICE guideline updates.
    - Limited local availability
- **Risk of bias in this exploratory study** – relatively small sample sizes and surgeons and hepatologists with an interest in NAFLD/bariatric surgery may be more likely to respond. Patients attending hepatology follow-up clinics may have more severe NAFLD or be less likely to have achieved effective non-surgical weight loss. They may therefore be more open to bariatric surgery than the wider community of patients with NAFLD. Relatively homogenous groups of respondents to each survey.

NAFLD and advanced fibrotic NAFLD was underestimated relative to published series, often relying on intraoperative visual inspection of the liver for disease staging. In contrast to hepatologists, use of non-invasive biomarkers of NAFLD severity was infrequent amongst surgeons. Bariatric surgery was reported to be beneficial for improving all stages of NAFLD except decompensated NASH-cirrhosis, and its omission from national clinical guidelines was the largest barrier to its wider implementation. Surgery was usually safe, but approximately a quarter of surgeons and hepatologists had experience in managing liver

dysfunction, mostly after bypass operations. Patients with severe obesity and NAFLD reported that diet and exercise were frequently prescribed but that these were often ineffective. Patients reported discussing bariatric surgery with health professionals infrequently, with the media their most common source of information. Although 40% of such patients reported that they had no previous awareness of bariatric surgery, half would nevertheless be open to surgical intervention if this was recommended by a healthcare professional.

9.4.2 Interpretation and comparison with previous studies

Few studies have previously assessed the views and clinical practice of bariatric surgeons in relation to NAFLD. They have largely focused on approaches to intraoperative liver biopsies. One abstract published in 2015 included responses from 30 UK surgeons and reported that approximately 90% of surgeons did not take liver biopsies during surgery. Surgeons believed that liver function tests inadequately diagnosed NAFLD and predicted progression to cirrhosis[191]. A manuscript published in 2017 included a survey of the liver biopsy practices of 22 French bariatric surgery units, and found that 10% of surgeons performed routine intraoperative liver biopsies, with 80% performing selective biopsies depending on liver enzymes, liver disease history and visual inspection. A 2019 published abstract included 124 bariatric surgeons working in the USA[192]. 30% of surgeons reported liver-related complications after bariatric surgery, which is a similar proportion to that reported in this chapter, but only 5% of surgeons routinely performed intra-operative liver biopsies. Visual detection of liver abnormalities was the most common reason for selective biopsy. All studies highlighted a need for improved diagnosis and monitoring of NAFLD in bariatric surgical patients, but it does not appear that practices have evolved over time to incorporate non-invasive biomarkers of NAFLD severity or standardise patient care.

Several previous studies have surveyed specialist hepatologists for their approach to NAFLD investigation and management, but none has generated data relating to bariatric surgery as a potential therapeutic intervention. However, one study assessed preferences for characteristics for future interventions to treat NASH[193]. Prevention of cirrhosis and improvements in liver disease severity, in glycaemic control, cholesterol and in body weight were identified as key priorities for NAFLD interventions. Another study surveyed Polish

physicians working in a range of specialties and confirmed body weight reduction through diet and exercise as the mainstays of treatment[194]. A 2017 UK study with 175 responses revealed a similar approach to NAFLD management, and additionally showed that transient elastography and straightforward non-invasive risk scores were most commonly used to stage NAFLD. A French study that included 352 gastroenterologists[195] also confirmed widespread use of such biomarkers, and use of non-overlapping biomarkers (e.g. serological and imaging-based) was more common than the isolated use of one methodology. Finally, a 2016 study compared NAFLD-related knowledge amongst USA-based primary care and specialist hepatologists. Specialists had increased familiarity with the spectrum of NAFLD pathology and used non-invasive biomarkers more frequently. They were more familiar with drugs undergoing evaluation for their potential as therapeutic agents to treat NAFLD, but their knowledge of bariatric surgery was not assessed[196].

Two industry-led studies from the same group have previously surveyed the opinions of patients with NAFLD. They included at total of 172 participants and focussed on patients' understanding of NASH along with the impact of this diagnosis on quality of life[197,198]. Improved quality of life and regression or stabilisation of disease severity were identified as key targets for future NASH treatments, aligning with this goal amongst hepatologists. The studies had a focus towards the potential development of novel pharmaceuticals, so the bariatric surgery was not discussed in either. However, some similar themes emerged in our study, including a perceived lack of support and need for additional education on NASH.

9.4.3 Strengths and limitations

One strength of this study is in its inclusion of key stakeholders in the management of people with severe obesity and NAFLD, namely hepatologists, bariatric surgeons and the patients themselves. It has therefore generated data that provides improved understanding of current non-surgical and surgical management of these metabolic diseases, identifying priorities for future research studies in this area. Actionable insights into barriers to the wider implementation of bariatric surgery as an intervention for their treatment have also been identified. Patient and Public Involvement (PPI) work through the patient survey has confirmed the local acceptability of bariatric surgical intervention amongst a reasonable proportion of patients with severe obesity and NAFLD.

A limitation of the present study is that respondents across all survey categories were largely based in western European environments or in the USA. It is therefore possible that results may not be representative of stakeholder views or medical practice in contrasting healthcare settings. Particular caution should be applied to the interpretation of the patient survey, which included patients from one UK region (Oxfordshire) and had a relatively low sample size. Surveys rather than interview-based approaches were implemented, and it is possible that more granular detail may have been obtained with the latter approach. Free-text response options were, however, included for all participants. Nevertheless, this overall study is the most comprehensive to have assessed the potential of bariatric surgical intervention for patients with NAFLD.

9.4.4 Conclusion

This study includes the most comprehensive evaluation of the views of key stakeholders on the potential of bariatric surgery as an intervention for NAFLD. Bariatric surgery appears to be an acceptable intervention for treating obesity and NAFLD for a reasonable proportion of eligible patients presenting to a tertiary metabolic hepatology outpatient clinic. The validation of non-invasive biomarkers of NAFLD severity in individuals with severe obesity and their subsequent inclusion in NAFLD staging pathways would be beneficial. Prospective studies comparing current medical therapies with outcomes after bariatric surgical intervention would improve information provision for patients, and these would also prove informative for the development of more comprehensive NAFLD clinical guidelines. There are additional needs to generate insights into the mechanisms through which bariatric surgery may represent an effective treatment for NAFLD, and to compare both the beneficial effects and safety profiles of current bariatric interventions. Given the potential bias associated with the design of these exploratory pilot surveys, there is a need to validate their findings in other geographical locations and with wider sampling of participant groups.

10 NAFLD Epidemiology and its Prediction in Bariatric Surgical Patients

10.1 Introduction

10.1.1 Background

10.1.1.1 NAFLD in bariatric surgical patients: Epidemiology

NAFLD is present in approximately 30% of the general population, and its prevalence is increased in people with obesity[199]. The prevalence and severity of NAFLD in individuals scheduled for bariatric surgery has been reported to range from less than 10% in the UK's National Bariatric Surgery Registry report[107] to 95% in other published series[108]. NAFLD severity has also been reported to vary in a similar way, and differences in NAFLD histology in different healthcare settings (state or independent sectors) within the same region have been described[191].

NAFLD is usually asymptomatic and isolated liver function tests are often normal even in patients with advanced fibrotic NAFLD[200]. Whilst patients undergoing bariatric surgery are more likely to have NAFLD than the general population, the previous results chapter demonstrated that intraoperative liver biopsies are not routine in many bariatric centres. Since there have been no previous reports of NAFLD severity in a series of patients undergoing bariatric surgery in the UK's National Health Service (NHS), NAFLD severity in this healthcare system is unknown.

10.1.1.2 Clinical Relevance of NAFLD severity in patients undergoing bariatric surgery

There have been several reports in the literature of liver dysfunction after bariatric surgery[182-185]. Postoperative complication risk in bariatric surgical patients has been associated with the severity of liver fibrosis at the time of surgery in one UK study[201].

The accurate preoperative prediction of NAFLD severity in patients undergoing bariatric surgery would therefore be clinically advantageous. It would enable the discussion of perioperative risk to be appropriately tailored in patients with severe NAFLD and promote preoperative referral to hepatologists where appropriate. Survey responses in the previous

results chapter suggested a preference amongst surgeons for performing sleeve gastrectomy rather than bypass operations in patients known to have advanced liver disease. Therefore, knowledge of liver fibrosis status may also inform procedure selection. At the time of surgery, risk stratification would also mean that liver biopsies may be selectively (if not routinely) performed where advanced disease is predicted. Postoperatively, patients with advanced fibrotic NAFLD may benefit from hepatologist care, including Ultrasound Scan (USS) surveillance for HCC development in individuals with cirrhosis.

10.1.1.3 NAFLD in bariatric surgical patients: Performance of existing non-invasive biomarkers

Several biomarkers have been developed to non-invasively predict NAFLD severity, but the previous chapter demonstrated that their use is infrequent amongst bariatric surgeons for NAFLD staging and monitoring. Existing biomarker predictive models incorporate blood tests and clinical variables such as BMI. However, the impact of phenotypic differences on biomarker calibration and accuracy in bariatric surgical patients is unclear.

10.1.1.4 Approaches to non-invasive biomarker development

Non-invasive biomarkers of NAFLD severity can be broadly categorised into imaging-based and serology-based, and their performance may be improved with the incorporation of risk-associated clinical variables such as BMI or diabetes status[146].

Machine learning is a form of artificial intelligence that may enhance biomarker accuracy when applied to diagnostic classification problems. However, some barriers exist that may limit its use in healthcare provision even once effective models have been established. For example, some machine learning algorithms are considered 'black box' as it is not clear how predictions are made[202], and patient-centred approaches to healthcare promote their involvement in clinical decision-making. Treatment 'explainability' is also important for legal reasons including compliance with the EU's General Data Protection Regulation (GDPR) legislation. It is unknown whether explainable machine learning could improve the prediction of liver histology in patients with obesity and NAFLD.

10.1.1.5 Aims

The aims of this chapter are to:

1) Determine the prevalence and histological severity of NAFLD in a population of bariatric surgical patients in the UK and compare this with another patient cohort from Brazil

2) Evaluate how a range of routinely-recorded preoperative bariatric surgical patient characteristics and serum measures vary across histological liver fibrosis stages in NAFLD

3) Calculate the diagnostic accuracy of existing NAFLD scoring systems at predicting liver biopsy histology in individuals with severe obesity

4) Evaluate the potential of machine learning for developing improved models for predicting advanced fibrotic NAFLD

10.2 Patients and Methods

10.2.1 Research strategy

In this chapter, the results of the first reported series of liver biopsies taken during bariatric surgery in the UK's NHS (Oxford patients) is reported. This sequential cohort was compared to a similar series of bariatric surgical patients who underwent intraoperative liver biopsies in Brazil. We then performed an evaluation of how these characteristics vary across NAFLD's severity spectrum, adding additional data from another collaborating UK centre (Derby) to increase statistical power. A range of existing non-invasive biomarkers of NAFLD severity is evaluated in this combined cohort. Finally, we assess whether a more accurate non-invasive biomarker of advanced fibrotic NAFLD can be developed using machine learning and novel predictor variable combinations.

10.2.2 Study design

10.2.2.1 Patient Cohorts

1) Oxford

Patients undergoing primary bariatric operations were eligible for recruitment into the Oxford bariatric surgery biobank study (Ref: 16/YH/0247). Written informed consent was obtained from all participants. The first research visit was on the morning of bariatric surgery, when clinical data (weight, height, medical history, current medication, family history, alcohol intake and smoking history) was recorded. Up to 20ml of blood was taken after an overnight fast just before surgery and serum was separated after centrifugation at 2500rpm for 15min at 4°C. Serum samples were stored at -80°C for subsequent ELF testing (iQur, London, UK).

In order to include Oxford patients undergoing bariatric surgery with intraoperative liver biopsy who were not bariatric biobank participants, a clinical audit was also registered (OUHFT ref: 191216-Surgery and Oncology-Gillies).

2) Derby

Additional cases for biomarker evaluation and development were obtained through a collaboration with another UK centre (Royal Derby Hospital). The results of the original study have previously been published, but additional data such as ELF testing results for participants with intraoperative liver biopsy histology are included in our analysis[203].

3) Brazil

Further data was obtained was obtained by establishing an additional collaboration with a bariatric centre in Puerto Alegre, Brazil. Their original dataset has been previously been published, but additional cases are included in the analysis in this chapter[108].

Liver biopsy techniques were consistent across all centres and the same variables (when available) were recorded for participants.

10.2.3 Study methods

A database incorporating the following variables was created using MS Excel v15.27 (Microsoft Corporation, Redmond, USA):

Demographic and clinical parameters (units)

- Sex (male/female)
- Age (years)
- BMI (kg/m^2)
- Total body weight (kg)
- Type 2 Diabetes (T2D) status (yes/no)

Blood tests (units)

- Liver function tests (AST (u/L), ALT (u/L), albumin (g/L))
- Ferritin (ng/ml)
- HbA1c (%)
- Lipid profile (Total cholesterol, HDL cholesterol, Low Density Lipoprotein (LDL) cholesterol and triglycerides, all millimole (mmol)/L)
- Haemoglobin (g/L)
- Platelet count (x10^9/L)
- Type III Procollagen Peptide (PIIINP, ng/mL)
- Tissue Inhibitor of Metalloproteinases-1 (TIMP-1, ng/mL)
- Hyaluronic acid (HA, ng/mL)

Liver histology

- Kleiner Fibrosis Score
- NAFLD Activity Score (NAS)

10.2.3.1 NAFLD Severity Biomarker Calculation

Non-invasive biomarkers of NAFLD severity were previously discussed in the Introduction chapter of this thesis. These incorporate variables such as liver function tests, extracellular

matrix proteins and clinical parameters such as diabetes status and BMI. The formulae for following biomarkers were entered into Microsoft Excel: AST/ALT ration, AST to Platelet Ratio Index (APRI), BARD Score, Fib-4 score, NAFLD Fibrosis Score (NFS), Enhanced Liver Fibrosis (ELF) Score. Each formula is detailed in the Introduction section to this thesis.

10.2.3.2 Evaluation of Existing Non-Invasive Biomarkers

For each existing non-invasive biomarker, the diagnostic accuracy for predicting advanced fibrotic (F3/F4) NAFLD and cirrhosis (F4) was assessed by determining the Area Under the Receiver Operating Characteristic curve (AUROC). For each biomarker, the point at which Youden's index was greatest (sensitivity+specificity-1) was determined, and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were established at this point. Graphpad Prism v8.4.1 (Graphpad Software, California, USA) and Microsoft Excel version 16.34 (Microsoft, Washington, USA) were used for this analysis.

The following established cut-off values that are used clinically were also used to evaluate the performance of the panel of existing non-invasive biomarkers:

AST/ALT Ratio: low cut-off of 0.8, high cut-off of 1.0[204]

AST to Platelet Ratio Index (APRI): low cut-off of 0.5, high cut-off of 1.5[205]

BARD Score: 2[149]

Fib-4 Score: low cut-off of 1.3, high cut-off of 2.67[205]

NAFLD Fibrosis Score (NFS): low cut-off of -1.455, high cut-off of 0.675[205]

Enhanced Liver Fibrosis (ELF) Score: manufacturer cut-off of 9.8[206], NICE guideline cut-off of 10.51[207].

10.2.3.3 Development of Novel Non-invasive Biomarker of Advanced Fibrotic NAFLD

All participants with liver biopsies were included in the biomarker development pathway. The prepared dataset was exported to Microsoft Excel v 16.34 (Microsoft, Washington, USA). In Microsoft Excel, the data was randomly partitioned in a 75:25 ratio into model training/validation and model testing datasets. An adaptive boosting machine learning algorithm (Stagewise Additive Modelling using a Multiclass Exponential loss function (SAMME) AdaBoost in sklearn library) using Python open-source software (Python Software Foundation, Delaware, United States) was implemented to train, validate and test a novel model for predicting advanced fibrotic (F3/F4) NAFLD. The AUROC and Youden's Index for predicting these histological diagnoses was determined. Feature importance was also used to quantify the relative contribution of each variable to the predictive model.

10.2.4 Statistical Analysis

Statistical analysis was undertaken on completion of the study, once all data had been entered and quality control had been performed. Normality testing using the D'Agostino and Pearson test was conducted to assess the distribution of all outcome measures before analysis. For unpaired analysis, unpaired t-tests were used for normally distributed variables and Mann-Whitney tests for non-normally distributed variables. Fisher's exact test was used to compare proportions in contingency tables. The software package Graphpad Prism for Mac v8.3.1 (GraphPad Software, California, USA) was used for all statistical analysis in this chapter.

In tables, normally distributed variables are presented as mean (SD) values, and non-normally distributed variables as median (IQR). ROC curves were produced using Graphpad Prism software, except for Figure 10-5, which was produced using Python software.

10.3 Results

10.3.1 The prevalence and severity of advanced fibrotic NAFLD is increased in Oxford's bariatric surgical patients in comparison to the Brazilian cohort

10.3.1.1 Phenotyping

A consecutive series of 268 participants undergoing primary bariatric surgery with intraoperative liver biopsy in a tertiary bariatric surgery centre (Oxford, UK) between 12th March 2014 and 19th December 2019 were recruited. 621 patients undergoing primary bariatric surgery in a Porto Alegre, Brazil[108] between 6th February 2002 and 8th January 2020 were also included. Biopsy technique (wedge biopsy from left lobe) was consistent between both groups. Table 10-1 compares clinical characteristics between these cohorts.

Table 10-1 Phenotyping and comparison of Oxford and Brazil bariatric surgical liver biopsy cohorts: Clinical Characteristics

		Bariatric Liver Biopsy Series			
		Total cohort (n=889)	Oxford (n=268)	Brazil (n=621)	Oxford *vs* Brazil Comparison
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Clinical Characteristic	BMI (kg/m^2)	49.0 (10.2)† *n=880*	46.7 (9.5)† *n=259*	50.3 (11.4)† *n=621*	<0.0001
	Age (years)	44.0 (16.0)† *n=888*	46.3 (10.4) *n=267*	42.0 (16.0)† *n=621*	<0.0001
	Female sex (%)	82.9% *n=889*	79.1% *n=268*	84.5% *n=621*	0.0524
	T2D (%)	33.2% *n=889*	32.5% *n=268*	33.5% *n=621*	0.8160

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented. Unpaired 2-tailed t-tests used to compare normally-distributed data and Mann-Whitney test used to compare non-normally distributed data. Fisher's exact test used for categorical variable comparisons.

Table 10-2: Phenotyping and comparison of Oxford and Brazil bariatric surgical liver biopsy cohorts: Biochemistry and Haematology

		Bariatric Liver Biopsy Series			
		Total cohort (n=889)	Oxford (n=268)	Brazil (n=621)	Oxford *vs* Brazil Comparison
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Biochemistry and Haematology	AST (IU/L)	20 (9)† *n=827*	22 (10)† *n=210*	20 (9)† *n=617*	<0.0001
	ALT (IU/L)	25 (19)† *n=885*	26 (16)† *n=267*	24 (19.5)† *n=618*	0.2965
	Albumin (g/L)	42 (6)† *n=848*	39 (5)† *n=251*	43 (5)† *n=597*	<0.0001
	Ferritin (ng/mL)	118.8 (145.9)† *n=841*	88.7 (93.5)† *n=257*	134.1 (169.6)† *n=584*	<0.0001
	Platelet count (10^9/L)	277.0 (92.3)† *n=886*	279.5 (92.8)† *n=268*	275.0 (91.8)† *n=618*	0.6437
	Haemoglobin (g/L)	135.0 (14)† *n=885*	139.0 (15)† *n=268*	133.7 (11.17) *n=617*	<0.0001
	Total cholesterol (mmol/L)	4.9 (1.4)† *n=876*	4.4 (1.4)† *n=261*	5.07 (1.4)† *n=615*	<0.0001
	HDL cholesterol (mmol/L)	1.1 (0.3)† *n=875*	1.1 (0.3)† *n=260*	1.2 (0.4)† *n=615*	<0.0001
	Total cholesterol/HDL ratio	4.2 (1.5)† *n=875*	4.1 (1.5)† *n=260*	4.3 (1.6)† *n=615*	0.0354
	HbA_1c (%)	5.9 (1.10)† *n=879*	5.6 (1.0)† *n=263*	6.0 (1.0)† *n=616*	<0.0001

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented. Unpaired 2-tailed t-tests used to compare normally-distributed data and Mann-Whitney test used to compare non-normally distributed data. Fisher's exact test used for categorical variable comparisons.

Table 10-3: Phenotyping and comparison of Oxford and Brazil bariatric surgical liver biopsy cohorts: Non-Invasive Biomarkers of NAFLD Severity and Liver Histology

		Bariatric Liver Biopsy Series			
		Total cohort (n=889)	Oxford (n=268)	Brazil (n=621)	Oxford *vs* Brazil Comparison
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Non-Invasive NAFLD Severity Biomarker Panel	AST/ALT Ratio	0.80 (0.30)† *n=800*	0.88 (0.37)† *n=210*	0.83 (0.37)† *n=590*	0.0107
	APRI	0.18 (0.11)† *n=800*	0.19 (0.17)† *n=210*	0.17 (0.11)† *n=590*	0.0036
	BARD	3 (2)† *n=800*	3 (2)† *n=210*	3 (2)† *n=590*	0.3191
	Fib-4	0.63 (0.41)† *n=800*	0.71 (0.49)† *n=210*	0.60 (0.39)† *n=590*	<0.0001
	NAFLD Fibrosis Score	-0.51 (2.1)† *n=800*	-0.51 (1.8)† *n=210*	-0.51 (2.2)† *n=590*	0.8205
Liver Histology	Kleiner Fibrosis Stage	1 (2)† *n=889*	2 (1)† *n=268*	1 (1)† *n=621*	<0.0001
	Kleiner F0	33.9% *n=301*	2.2% *n=6*	47.5% *n=295*	0.0001
	Kleiner F1	29.8% *n=265*	29.9% *n=80*	29.8% *n=185*	1.0000
	Kleiner F2	25.7% *n=228*	47.8% *n=128*	16.1% *n=100*	0.0001
	Kleiner F3	7.9% *n=70*	16.0% *n=43*	4.3% *n=27*	0.0001
	Kleiner F4	2.8% *n=25*	4.1% *n=11*	2.3% *n=14*	0.1279
	NAFLD Activity Score	2 (2)† *n=760*	2 (3)† *n=267*	2 (2)† *n=493*	0.4915

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented. Unpaired 2-tailed t-tests used to compare normally-distributed data and Mann-Whitney test used to compare non-normally distributed data. Fisher's exact test used for categorical variable comparisons.

Table 10-1 , Table 10-2 and Table 10-3 highlight phenotypic differences between the Oxford and Brazilian bariatric cohorts. Several non-invasive NAFLD severity biomarker values were increased in the Oxford group compared to the Brazilian cohort, and this observation is supported by greater histological NAFLD severity in the Oxford patients. A greater proportion of Oxford patients had advanced fibrotic NAFLD (F3 or F4) than those in the Brazil series (p=0.0001).

10.3.2 Evaluation of preoperative characteristics in bariatric surgical patients across NAFLD's fibrosis spectrum

The Oxford and Brazil cohorts were then combined and supplemented with data from an additional study conducted in an another UK centre (Derby[203]) to increase the statistical power for addressing our subsequent aims. The Derby biopsies were selective rather than sequential so could not be included in the prior analysis detailed in Table 10-1. All participants were stratified according to their histological fibrosis stage. Clinical parameters were then compared (Table 10-4) to determine whether there were distinct preoperative clinical characteristics in patients with advanced fibrotic NAFLD (F3/4) and cirrhosis (F4) compared to controls without these histological diagnoses.

Table 10-4 Clinical parameters in bariatric surgical patients with advanced fibrotic NAFLD (F3/4) or cirrhosis (F4) compared to control participants.

		Histological Liver Fibrosis Stage (Kleiner Classification)					
		F0-F2	F3/F4	F0-F2 *vs* F3/F4	F0-3	F4	F0-3 *vs* F4
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Clinical Characteristic	BMI (kg/m^2)	49.0 (10.3)† *n=853*	48.3 (8.3)† *n=99*	0.3541	48.9 (10.2)† *n=924*	49.8 (6.1) *n=28*	0.7871
	Age (years)	44.0 (16.0)† *n=860*	49.7 (9.0) *n=101*	0.0011	44.0 (15.0)† *n=933*	46.1 (10.1) *n=28*	0.2320
	Female sex (%)	85.0% *n=860*	64.7% *n=102*	0.0001	83.2% *n=933*	72.4% *n=29*	0.1352
	T2D (%)	*28.1% n=860*	69.6% *n=102*	0.0001	31.2% *n=933*	75.9% *n=29*	0.0001

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented. Unpaired 2-tailed t-tests used to compare normally-distributed data and Mann-Whitney test used to compare non-normally distributed data. Fisher's exact test used for categorical variable comparisons.

Table 10-5: Biochemical parameters in bariatric surgical patients with advanced fibrotic NAFLD (F3/4) or cirrhosis (F4) compared to control participants.

		Histological Liver Fibrosis Stage (Kleiner Classification)					
		F0-F2	F3/F4	F0-F2 *vs* F3/F4	F0-3	F4	F0-3 *vs* F4
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Clinical Biochemistry and Haematology	AST (IU/L)	20.0 (8.0)† *n=795*	29.0 (15.0)† *n=87*	<0.0001	20.0 (8.3)† *n=856*	36.8 (13.29) *n=26*	<0.0001
	ALT (IU/L)	24.0 (18.0)† *n=852*	32.0 (20.25)† *n=102*	<0.0001	24.0 (18.0)† *n=925*	39.5 (19.42) *n=29*	0.0008
	Albumin (g/L)	42.0 (6.0)† *n=820*	41.0 (7.0)† *n=97*	0.1238	42.0 (6.0)† *n=889*	40.8 (4.52) *n=28*	0.4399
	Ferritin (ng/mL)	113.8 (140.6)† *n=757*	140.4 (160.3)† *n=90*	0.2573	118.8 (145.3)† *n=823*	94.6 (176.3)† *n=24*	0.3357
	Platelet count (10^9/L)	279.0 (87.0)† *n=854*	256.1 (71.9) *n=101*	<0.0001	279.0 (88.0)† *n=926*	225.0 (81.0)† *n=29*	<0.0001
	Total cholesterol (mmol/L)	4.9 (1.4)† *n=792*	4.5 (1.1) *n=97*	0.0005	4.9 (1.4)† *n=862*	4.6 (1.3) *n=27*	0.0493
	HDL cholesterol (mmol/L)	1.1 (0.35)† *n=791*	1.0 (0.4)† *n=97*	<0.0001	1.1 (0.3)† *n=861*	1.0 (0.2) *n=27*	<0.0001
	Total cholesterol/HDL ratio	4.2 (1.5)† *n=791*	4.6 (1.2) *n=97*	0.0193	4.2 (1.5)† *n=861*	4.8 (1.6) *n=27*	0.2101
	HbA_1c (%)	5.8 (1.0)† *n=795*	6.5 (1.0)† *n=98*	<0.0001	5.9 (1.0)† *n=866*	7.3 (1.8) *n=27*	0.0002

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented. Unpaired 2-tailed t-tests used to compare normally-distributed data and Mann-Whitney test used to compare non-normally distributed data. Fisher's exact test used for categorical variable comparisons.

Table 10-6 Non-Invasive biomarkers of NAFLD severity in bariatric surgical patients with advanced fibrotic NAFLD (F3/4) or cirrhosis (F4) compared to control participants.

		Histological Liver Fibrosis Stage (Kleiner Classification)					
		F0-F2	F3/F4	F0-F2 *vs* F3/F4	F0-3	F4	F0-3 *vs* F4
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Non-Invasive NAFLD Severity Biomarker Panel	AST/ALT Ratio	0.84 (0.39)† *n=728*	0.87 (0.35)† *n=70*	0.2100	0.84 (0.39)† *n=774*	1.08 (0.45) *n=24*	0.0243
	APRI	0.17 (0.10)† *n=728*	0.26 (0.27)† *n=70*	<0.0001	0.17 (0.11)† *n=774*	0.36 (0.26)† *n=24*	<0.0001
	BARD	3 (2)† *n=728*	3 (2)† *n=70*	<0.0001	3 (2)† *n=774*	3.5 (2)† *n=24*	0.0058
	Fib-4	0.61 (0.39)† *n=728*	1.00 (0.83)† *n=70*	<0.0001	0.63 (0.40)† *n=774*	1.37 (0.87)† *n=24*	<0.0001
	NAFLD Fibrosis Score	-0.50 (2.06)† *n=728*	0.63 (1.71) *n=70*	<0.0001	-0.46 (2.07)† *n=774*	1.19 (1.66) *n=24*	<0.0001
	ELF Score	8.19 (0.92) *n=180*	8.38 (1.07))† *n=35*	0.2410	*8.19 (0.90)* *n=*204	8.72 (1.3)† *n=11*	0.0362
	NAFLD Activity Score	2 (2)† *n=732*	3.2 (1.5) *n=100*	<0.0001	2 (2)† *n=803*	3.2 (1.7) *n=29*	0.0012

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented. Unpaired 2-tailed t-tests used to compare normally-distributed data and Mann-Whitney test used to compare non-normally distributed data. Fisher's exact test used for categorical variable comparisons.

Table 10-4, Table 10-5 and Table 10-6 highlight that multiple clinical and biochemical parameters vary according to histological NAFLD severity. This finding confirms the potential utility of including these routinely recorded variables in non-invasive biomarker algorithms designed to predict advanced NAFLD (F3/F4). Whilst the median values of all biomarkers increased in advanced liver fibrosis, the observed differences were not significant when comparing AST/ALT ratio and ELF values in patients with advanced fibrotic NAFLD to those with less severe fibrosis.

10.3.3 Evaluation of existing non-invasive biomarkers of NAFLD severity in bariatric surgical patients

The performance of each biomarker was next evaluated in more detail using established cut-off values to determine their diagnostic accuracy in individuals with severe obesity undergoing bariatric surgery (Table 10-7). Only patients with a complete set of biomarker values were included for consistency, and for UK patients this included the ELF test. The performance of each biomarker at established cut-offs was studied. For the ELF test, there was a discrepancy in cut-off value recommended in the UK's NICE guidelines for NAFLD staging and the value suggested by the manufacturer. Both cut-offs were evaluated to determine which was more appropriate in the patient population included in this study.

Table 10-7 Evaluation of non-invasive NAFLD severity biomarker panel for their accuracy in the prediction of advanced fibrotic NAFLD and cirrhosis.

		Non-invasive Biomarker (Total Cohort)										
		APRI (n=798)		Fib-4 (n=798)		NFS (n=798)		BARD (n=798)	AST/ALT (n=798)		ELF (UK only, n=208)	
	Test Characteristic	*Low Cut-Off (0.5)*	*High Cut-Off (1.5)*	*Low Cut-Off (1.3)*	*High Cut-Off (2.67)*	*Low Cut-Off (-1.455)*	*High Cut-Off (0.675)*	*Standard Cut-Off (2)*	*Low Cut-Off (0.8)*	*High Cut-Off (1.0)*	*Manufacturer Cut-Off (9.8)*	*NICE Cut-Off (10.51)*
Advanced Fibrosis (F3/4) Prediction	Sensitivity	18.6%	1.4%	34.3%	4.3%	88.6%	50.0%	85.7%	61.4%	32.9%	3.0%	3.0%
	Specificity	96.6%	100%	92.6%	99.9%	26.0%	78.2%	33.9%	43.4%	67.0%	95.4%	96.0%
	PPV	36.1%	100%	31.2%	66.7%	10.3%	18.1%	11.1%	9.6%	9.1%	26.4%	29.2%
	NPV	92.5%	91.3%	93.6%	91.5%	95.9%	94.2%	96.1%	92.3%	91.4%	87.7%	88.1%
Cirrhosis (F4) Prediction	Sensitivity	29.2%	0%	58.3%	8.3%	87.5%	75.0%	83.3%	66.7%	54.2%	9.1%	9.1%
	Specificity	96.3%	99.9%	91.7%	99.7%	25.1%	77.3%	32.7%	43.3%	67.7%	95.4%	96.5%
	PPV	19.4%	0%	18.2%	66.7%	3.5%	9.3%	3.7%	3.6%	5.5%	9.4%	10.4%
	NPV	97.8%	97.0%	98.6%	97.2%	98.5%	99.0%	98.4%	97.7%	97.9%	96.1%	96.3%

Abbreviations: Positive Predictive Value (PPV), Negative Predictive Value (NPV)

Table 10-7 shows that the studied biomarkers are generally more sensitive when used to predict cirrhosis in isolation than when predicting advanced fibrotic NAFLD. NPV was also usually higher than PPV at these cut-offs, suggesting that they may be more useful in excluding advanced disease than in identifying patients with clinically-relevant NAFLD. However, since these biomarkers were not developed or validated in patients with severe obesity, such as those undergoing bariatric surgery, it may possible to improve biomarker performance by optimising cut-off values for this patient population.

Therefore, the overall performance of these biomarkers was further assessed across their full range of values using ROC curve analysis in the whole cohort (Figure 10-1).

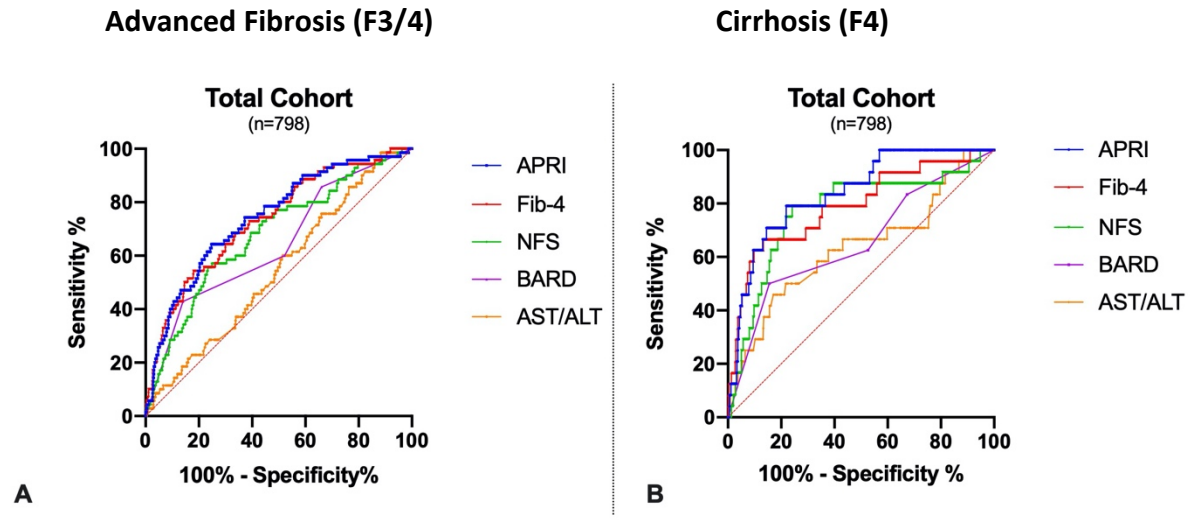


Figure 10-1: ROC curves for the panel of existing biomarkers of NAFLD severity (APRI, Fib-4, NFS, BARD, AST/ALT ratio) summarising their overall performance for predicting advanced fibrotic NAFLD (Kleiner F3/F4) and cirrhosis (F4) in the overall combined cohort.

Figure 10-1 suggests that the evaluated biomarkers perform suboptimally in participants with severe obesity undergoing bariatric surgery. In addition to evaluating biomarkers in the overall cohort, specific analysis of their performance in the UK and Brazil participant groups was performed to determine whether diagnostic accuracy varied in these distinct patient populations (Figure 10-2)

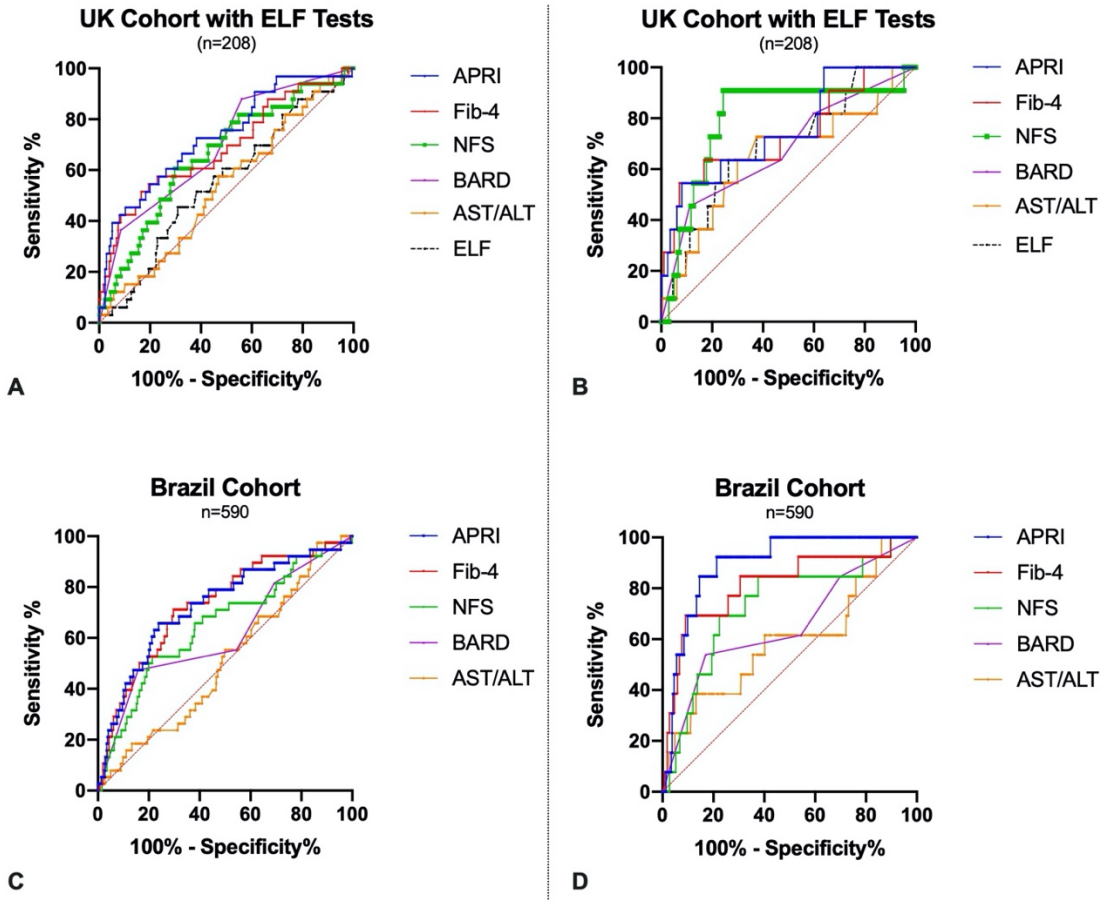


Figure 10-2: ROC curves for the panel of existing biomarkers of NAFLD severity (APRI, Fib-4, NFS, BARD, AST/ALT ratio) summarising their overall performance for predicting advanced fibrotic NAFLD (Kleiner F3/F4) and cirrhosis (F4) separately in the UK and Brazilian cohorts. Additionally, the ELF test was evaluated in the UK cohort in (A) and (B).

Relative biomarker performance appeared broadly consistent between centres for both histological diagnoses, although the NFS appeared to be more effective for the prediction of cirrhosis in the UK cohort than in the Brazilian cohort. Overall performance for BARD, AST/ALT ratio and ELF appeared consistently poor. This is of relevance as the ELF test is a proprietary biomarker that is recommended by the UK's NICE in their guidelines for NAFLD management. Its use therefore has cost implications for NHS healthcare trusts. No other non-invasive biomarkers are discussed in the NICE guideline recommendations[145].

A more detailed performance evaluation was then conducted for each biomarker. The area under the ROC curve for each curve and Youden's Index was calculated to determine whether adjusted cut-offs may improve biomarker performance, as shown in Table 10-8: (Total Cohort),

Table 10-9: (UK Cohort) and Table 10-10: (Brazil Cohort).

Table 10-8: Non-invasive Biomarker Performance for Advanced Fibrosis and Cirrhosis Prediction in the Total Cohort

		Non-invasive Biomarker (Total Cohort)				
	Test Characteristic	APRI	Fib-4	NFS	BARD	AST/ALT
Advanced Fibrosis (F3/4) Prediction	Area Under the Curve (AUC) (SE)	0.74 (0.03)	0.73 (0.03)	0.68 (0.03)	0.64 (0.04)	0.55 (0.03)
	AUC 95% CI	0.68 to 0.80	0.67 to 0.80	0.61 to 0.75	0.57 to 0.71	0.48 to 0.61
	Biomarker *vs* P=0.5: p value	<0.0001	<0.0001	<0.0001	<0.0001	0.2095
	Youden's Index (YI)	0.40	0.36	0.32	0.29	0.10
	Cut-Off Value at YI	>0.2293	>0.9456	>0.6079	>3.500	>0.5298
	Sensitivity at YI	64.3%	54.3%	55.7%	42.9%	98.6%
	Specificity at YI	72.0%	82.0%	76.7%	86.0%	11.7%
	PPV at YI	20.0%	22.5%	18.7%	22.7%	9.7%
	NPV at YI	95.6%	94.9%	94.7%	94.0%	98.8%
Cirrhosis (F4) Prediction	AUC	0.84 (0.04)	0.79 (0.05)	0.77 (0.06)	0.65 (0.06)	0.63 (0.07)
	AUC 95% CI	0.77 to 0.91	0.69 to 0.90	0.66 to 0.88	0.53 to 0.78	0.51 to 0.76
	Biomarker *vs* P=0.5: p value	<0.0001	<0.0001	<0.0001	0.0099	0.0250
	Youden's Index (YI)	0.57	0.53	0.55	0.35	0.29
	Cut-Off Value at YI	>0.2445	>1.086	>0.6222	>3.500	>1.123
	Sensitivity at YI	79.2%	66.7%	79.2%	50.0%	45.8%
	Specificity at YI	78.2%	84.1%	75.8%	81.8%	83.0%
	PPV at YI	10.1%	13.5%	9.2%	9.1%	8.3%
	NPV at YI	99.2%	98.8%	99.2%	98.2%	98.2%

Table 10-9: Non-invasive Biomarker Performance for Advanced Fibrosis and Cirrhosis Prediction in the UK Cohort

		Non-invasive Biomarker (UK Cohort)					
	Test Characteristic	APRI	Fib-4	NFS	BARD	AST/ALT	ELF
Advanced Fibrosis (F3/4) Prediction	AUC	0.73 (0.05)	0.69 (0.06)	0.66 (0.05)	0.69 (0.05)	0.53 (0.05)	0.55 (0.05)
	AUC 95% CI	0.64 to 0.83	0.58 to 0.80	0.56 to 0.76	0.60 to 0.79	0.43 to 0.64	0.45 to 0.66
	Biomarker *vs* P=0.5: p value	<0.0001	0.0007	0.0037	0.0004	0.5779	0.3523
	Youden's Index (YI)	0.35	0.35	0.31	0.32	0.11	0.15
	Cut-Off Value at YI	>0.2970	>0.9898	>0.5377	>1.500	>0.9160	>8.515
	Sensitivity at YI	45.5%	54.6%	60.6%	87.9%	57.6%	45.5%
	Specificity at YI	89.7%	80.6%	70.3%	44.0%	53.1%	69.1%
	PPV at YI	45.5%	34.6%	26.8%	22.8%	18.8%	24.0%
	NPV at YI	89.7%	90.4%	89.8%	95.0%	86.9%	92.3%
Cirrhosis (F4) Prediction	AUC	0.75 (0.08)	0.74 (0.09)	0.79 (0.08)	0.68 (0.09)	0.65 (0.09)	0.69 (0.08)
	AUC 95% CI	0.60 to 0.91	0.56 to 0.91	0.64 to 0.94	0.50 to 0.96	0.47 to 0.83	0.54 to 0.85
	Biomarker *vs* P=0.5: p value	0.0048	0.0086	0.0010	0.0461	0.0949	0.0298
	Youden's Index (YI)	0.46	0.47	0.67	0.34	0.35	0.37
	Cut-Off Value at YI	>0.3480	>1.464	>0.9090	>3.500	>0.9969	>0.8605
	Sensitivity at YI	54.6%	54.6%	90.9%	45.5%	72.7%	63.6%
	Specificity at YI	91.9%	92.9%	75.6%	88.8%	62.4%	73.6%
	PPV at YI	27.3%	30.0%	17.2%	18.5%	9.8%	9.5%
	NPV at YI	97.3%	97.3%	99.3%	96.7%	97.6%	98.2%

Table 10-10: Non-invasive Biomarker Performance for Advanced Fibrosis and Cirrhosis Prediction in the Brazil Cohort

		Non-invasive Biomarker (Brazil Cohort)				
	Test Characteristic	APRI	Fib-4	NFS	BARD	AST/ALT
Advanced Fibrosis (F3/4) Prediction	AUC	0.73 (0.05)	0.73 (0.04)	0.65 (0.05)	0.62 (0.05)	0.51 (0.05)
	AUC 95% CI	0.64 to 0.82	0.64 to 0.82	0.55 to 0.75	0.51 to 0.72	0.42 to 0.60
	Biomarker *vs* P=0.5: p value	<0.0001	<0.0001	0.0018	0.0168	0.8792
	Youden's Index (YI)	0.42	0.41	0.31	0.14	0.11
	Cut-Off Value at YI	>0.2303	>0.7518	>0.6202	>3.500	>0.5298
	Sensitivity at YI	65.8%	71.1%	52.6%	47.4%	97.4%
	Specificity at YI	76.1%	70.3%	78.6%	84.2%	13.8%
	PPV at YI	15.9%	14.1%	14.5%	17.1%	7.2%
	NPV at YI	97.2%	97.5%	96.2%	95.9%	100%
Cirrhosis (F4) Prediction	AUC	0.89 (0.03)	0.82 (0.07)	0.73 (0.07)	0.65 (0.09)	0.59 (0.09)
	AUC 95% CI	0.83 to 0.96	0.68 to 0.95	0.59 to 0.88	0.48 to 0.82	0.42 to 0.77
	Biomarker *vs* P=0.5: p value	<0.0001	0.0001	0.0045	0.0594	0.2573
	Youden's Index (YI)	0.71	0.60	0.47	0.37	0.25
	Cut-Off Value at YI	>0.2447	>1.210	-0.05009	>3.500	>1.136
	Sensitivity at YI	92.3%	69.2%	84.6%	53.9%	38.5%
	Specificity at YI	78.7%	91.0%	62.4%	83.0%	86.8%
	PPV at YI	8.9%	14.8%	4.8%	6.7%	5.4%
	NPV at YI	99.8%	99.2%	99.5%	98.8%	98.4%

The above tables (Table 10-9 and Table 10-10) confirm that recalibration at Youden's Index improves performance of existing biomarkers across a range of test characteristics, although NPV remained higher than PPV at this point. The relatively strong performance of APRI is somewhat unexpected, as it was developed for use in patients with Hepatitis C and is used less frequently in patients with NAFLD than biomarkers such as the NFS and Fib-4.

In our study of individuals with severe obesity, some variables such as BMI had less discriminative benefit when predicting liver fibrosis severity than in the populations in which the existing biomarkers were developed. For example, every patient in this study had a BMI >28, for which 1 point is given in the BARD score. The inclusion of this variable therefore provided no benefit in our cohorts, and the optimal cut-off BARD score was increased in our bariatric patient cohort. It appears biomarkers that incorporate platelet count (APRI, Fib-4 and NFS) had a higher AUC than tests that did not (BARD, AST/ALT, ELF). This is consistent with Table 10-4, which showed that this parameter decreases in advanced fibrotic NAFLD.

Table 10-4 also revealed several additional clinical variables that could potentially improve predictive model performance, including using HbA_1c as a continuous variable reflective of glycaemic control rather than indicating T2D presence in a binary fashion (as with Fib-4, NFS and BARD). Several lipoprotein cholesterol-related parameters, which are known to be surrogate markers of metabolic health, were also altered in patients with advanced fibrosis and cirrhosis (Table 10-4) compared to controls. No existing non-invasive biomarkers of NAFLD severity incorporate these variables.

10.3.4 Development of novel non-invasive biomarker of advanced fibrotic NAFLD

Finally, we explored whether we could develop a more accurate biomarker of advanced fibrotic NAFLD that is calibrated for people with obesity undergoing bariatric surgery. Insights developed from the previous evaluation of existing non-invasive biomarkers and candidate input variables were combined with a machine learning algorithm (AdaBoost classifier) to develop a novel predictive model. It was decided that a model that incorporated routinely recorded variables rather than additional or expensive assays would more likely be adopted in clinical practice. Figure 10-3 provides an outline of this process, which utilised all cases with intraoperative liver biopsy histology.

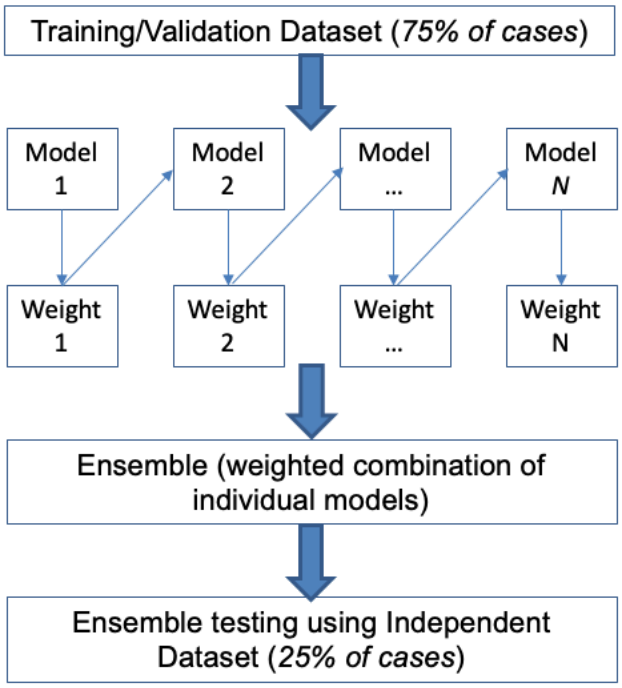


Figure 10-3: Schematic representation of advanced fibrotic NAFLD predictive model development pathway

Figure 10-3 illustrates how multiple individual models are incorporated into an ensemble model. There is increased weighting of incorrectly-classified cases in each successive individual model to increase the ensemble model's diagnostic accuracy.

We quantified the importance of the variables used in our novel predictive model designed to predict advanced fibrotic NAFLD (Figure 10-4):

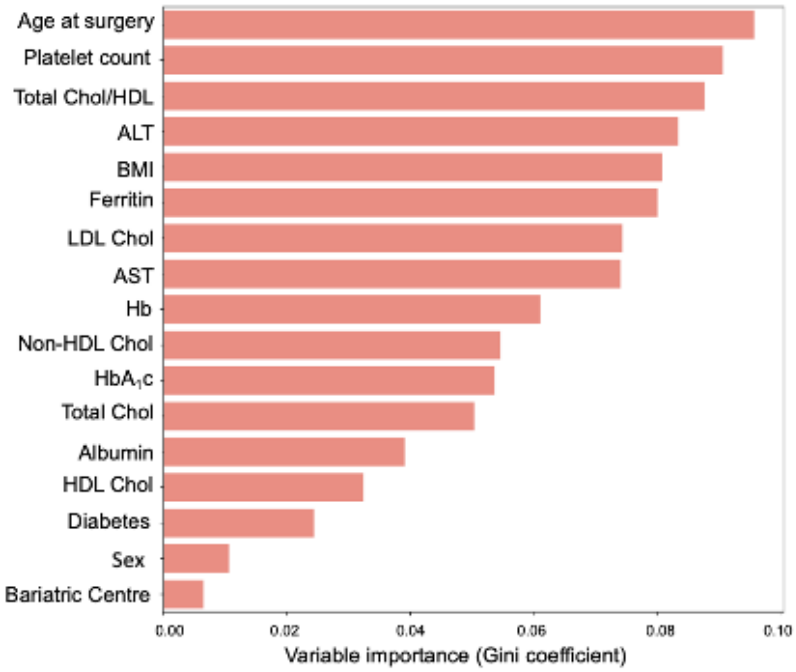


Figure 10-4: Relative importance (Gini coefficient) of the variables included in novel model for predicting advanced fibrotic (F3/4) NAFLD

Figure 10-4 confirms that HbA_1c as a continuous measure of glycaemic control (HbA_1c) provides a greater contribution to our novel model than the binary classification of T2D status used in previous non-invasive biomarkers of advanced fibrotic NAFLD. Additionally, routinely recorded variables such as cholesterol-related parameters and ferritin levels provide discriminative power. It also appears that the model can be applied in different bariatric surgical units (UK and Brazil) and that the model is not overfitted to either healthcare system, since the bariatric centre coefficient contributed minimally to the ensemble model.

The diagnostic accuracy of our novel non-invasive biomarker for predicting advanced fibrotic NAFLD in the independent testing cohort was then evaluated, with its performance summarised in Figure 10-5:

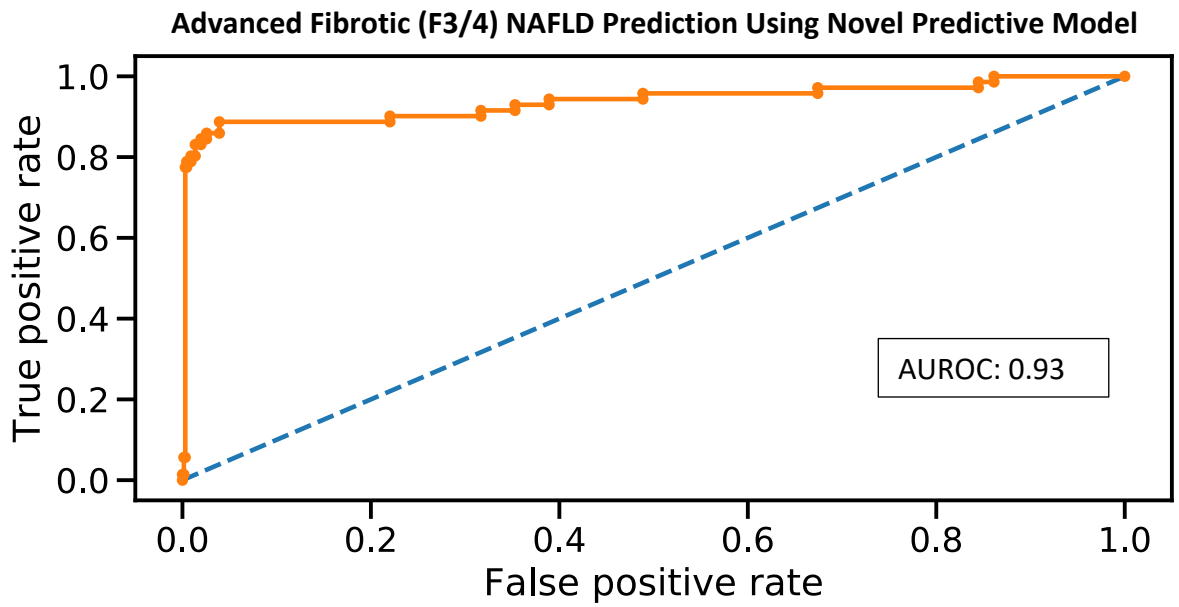


Figure 10-5: ROC curve summarising the overall performance of our novel predictive model (AUROC: 0.93) for predicting advanced fibrotic NAFLD (Kleiner F3/F4)

This result suggests that the machine learning approach we implemented has potential clinical utility if its performance can be maintained in further external independent testing cohorts. Table 10-11 shows that it performed favourably relative to the best-performing existing non-invasive biomarker in our datasets. This is particularly striking as Table 10-11 compares our model against an APRI Youden's Index optimised for our cohort of people with severe obesity, rather than against its (less accurate) performance at the cut-off scores currently used in clinical practice.

Table 10-11: Comparison of our novel model and the best-performing existing model (APRI) at predicting advanced fibrotic NAFLD

		Novel Predictive Model Performance Evaluation	
	Test Characteristic	Novel predictive model (Independent Testing Cohort)	Best-performing Existing Biomarker (APRI)
Advanced Fibrosis (F3/4) Prediction	AUC	0.93	0.74
	Youden's Index (YI)	0.90	0.40

10.4 Discussion

10.4.1 Principal findings

This study includes the first reported series of liver biopsies taken in patients with obesity undergoing bariatric surgery in the UK. Greater rates of advanced fibrotic NAFLD were observed in UK patients compared to those undergoing bariatric surgery in Brazil. Whilst the performance of existing non-invasive biomarkers of NAFLD severity was improved by developing modified cut-offs that improved calibration in patients with severe obesity and NAFLD, their diagnostic accuracy remained suboptimal. No advantages to using the proprietary ELF test rather than existing biomarkers that incorporated routine clinical tests were found. The novel non-invasive biomarker of advanced fibrotic NAFLD that we developed using a machine learning approach had better diagnostic accuracy than existing biomarkers.

10.4.2 Interpretation and comparison with previous studies

This study demonstrates that advanced fibrotic NAFLD is common in a series of individuals with morbid obesity undergoing bariatric surgery in the UK's NHS, with higher non-invasive biomarker scores and histological severity than in the Brazilian comparator patients. The intraoperative prevalence of cirrhosis was 4.1%, which is higher than in several previous studies from other centres, contrasting sharply with a French series of 362 intraoperative liver biopsies in which no cases of cirrhosis were recorded[208]. Access to bariatric surgery is known to vary across healthcare systems. The UK has the second highest obesity rate in Europe but its annual number of bariatric operations per head of population is particularly low and has decreased in recent years[100]. It is plausible that barriers to bariatric surgery in the UK permit access to only the most metabolically sick individuals, which may explain the relatively high proportion of advanced fibrotic NAFLD and cirrhosis seen in our study.

An evaluation of the ELF test was included in UK patients in this study since it is the non-invasive biomarker recommended in the UK's national clinical guidelines for staging NAFLD[145]. The score is an incorporates the serum concentration of three fibrosis markers (TIMP-1, PIIINP and HA). It is a proprietary test with a cost to the NHS of approximately £42 per sample[153,209]. One study has previously assessed the diagnostic accuracy of ELF testing in

predicting liver biopsy histology in individuals with severe obesity. It included a cohort of 41 bariatric patients, 40 of whom did not have significant liver fibrosis (0% F0, 98% F1, 0% F2, 2% F3, 0% F4)[210]. Since ELF testing's performance had previously only been assessed over this limited (and less clinically relevant) range of NAFLD's severity spectrum in bariatric surgical patients, our finding that it has no clear advantage over other non-invasive biomarkers in the diagnosis of advanced fibrotic NAFLD and cirrhosis is clinically informative.

It also appears that existing biomarkers of NAFLD severity perform relatively poorly in individuals with severe obesity scheduled for bariatric surgery. One explanation for this may be that these biomarkers have often been developed and validated for use in different disease processes and in individuals with markedly different obesity and metabolic disease phenotype than that of bariatric surgical patients. For examples, the Fib-4 biomarker was developed in a population with mean BMI of 25kg/m^2 and Human Immunodeficiency Virus (HIV)/ Hepatitis C Virus (HCV) coinfection rather than NAFLD[150].

The accurate prediction of advanced fibrosis and cirrhosis in patients scheduled for bariatric surgery is, however, clinically important. Cirrhosis has been reported to worsen postoperative outcomes[211] and hepatocellular carcinoma surveillance should also be offered to these patients. Cirrhosis should therefore ideally be diagnosed prior to surgery to improve risk stratification, patient counselling and inform the choice of surgical intervention.

Whilst the existing non-invasive biomarkers performed relatively poorly, improved performance at predicting advanced fibrotic NAFLD was seen after using machine learning to develop our predictive model. Machine learning offers some additional potential advantages to traditional static algorithms, as predictive models can be uploaded to an online computer server for deployment, with new cases used for ongoing model refinement. Software packages such as Python make these processes accessible for non-specialists, and the model development pathway described in this chapter could be freely applied to many clinical scenarios with binary outcomes using standard computer hardware.

10.4.3 Strengths and limitations

The strengths of this study include that it is the first to have assessed the prevalence and severity of NAFLD in a series of patients undergoing bariatric surgery in the UK's NHS. It also represents the most comprehensive assessment of existing biomarkers of NAFLD severity, including the ELF test, in individuals with severe obesity. Liver fibrosis and serum samples were measured in different labs (in the UK and in Brazil), and this did not appear impair the performance of our novel model for predicting advanced fibrotic NAFLD. Another strength of this model is that it uses routinely-recorded clinical variables, so advanced fibrosis could be predicted using preoperative assessment clinic blood tests before surgery.

It is also possible that some differences between cohorts may reflect different public healthcare systems in Brazil and in the UK's NHS. For example, the Brazilian patients largely underwent open surgery for funding reasons whilst laparoscopic surgery is standard in Oxford. Samples were assessed in different labs and methodologies may have varied. Serum measurements were taken during preoperative assessment clinic visits, which may occur at a variable time before surgery for pragmatic reasons. It is possible that if blood was taken at a standardised optimal time before surgery then the diagnostic accuracy of our model may have improved further. Additionally, there may be socioeconomic and genetic differences between these cohorts and differences in preoperative low energy diets may affect some outcome biochemical outcome measures and intraoperative liver steatosis and NAS. However, the focus of this study is liver fibrosis, which changes very slowly[212] and is unlikely to be affected by short-term dietary changes. We did not develop a NASH predictive model for this reason. The diverse nature of the cohorts could also be considered a strength of this study, since biomarkers were robustly evaluated in distinct population, improving the generalisability of its findings.

Whilst liver biopsy histology represents the 'gold standard' for staging NAFLD, it is recognised as being only semi-quantitative. The assessment of liver fibrosis can be confounded by inter-observer variability and biopsy technique, and therefore it is a challenging variable for a biomarker to accurately predict (compared to 'hard' outcomes such as mortality) since it is itself imperfect. In this study, experienced histopathologists

graded fibrosis severity, and data from bariatric liver biopsy series was used to determine whether findings were consistent across these distinct bariatric surgical populations.

Whilst data from nearly 1000 intraoperative liver biopsies was included in this study and used for biomarker development, liver cirrhosis was relatively rare and prohibited the development of a model designed specifically to predict cirrhosis. Future collaborative work using larger datasets would allow this to be generated. Although our advanced fibrosis biomarker development pathway was designed to be robust and incorporated training, validation and independent testing stages, further external independent validation would be beneficial.

10.4.4 Conclusion

Advanced fibrotic NAFLD was common in geographically and socioeconomically distinct bariatric patient cohorts so NAFLD staging in bariatric surgical patients is of clinical value. Intraoperative liver histology was suboptimally predicted by existing non-invasive biomarkers, but APRI and Fib-4 were most reliable, and it is suggested that they are incorporated into preoperative bariatric surgical care pathways. Dedicated cut-off values for individuals with obesity may improve their clinical utility, but machine learning offers the potential for the development of dynamic biomarkers of advanced fibrotic NAFLD with improved diagnostic accuracy.

11 Bariatric Surgery as an Intervention for NAFLD

11.1 Introduction

11.1.1 Background

Non-Alcoholic Fatty Liver Disease (NAFLD) is rapidly becoming the leading cause of cirrhosis worldwide and, even in the most advanced stages, liver chemistry values are often within normal limits[213]. No pharmaceutical agents are currently licensed to treat NAFLD; current medical treatment is focused on weight loss (via reduced energy intake or increased energy expenditure) and addressing metabolic comorbidities and liver disease risk factors[102].

Bariatric surgery is recognised as the most effective and durable treatment for weight loss. Its efficacy relative to lifestyle interventions and intensive medical care has now also been established in Type 2 Diabetes (T2D). T2D and NAFLD are closely associated components of the metabolic syndrome and have similar underlying pathophysiology[214]. However, despite emerging reports that bariatric surgery improves liver histology[186] and an absence of effective pharmacotherapies that treat NAFLD, surgery is not recommended in the UK's NICE guidelines for NAFLD management[215].

There is a therefore a need for studies comparing NAFLD-related outcomes after conventional hepatology-led clinical management to those achieved after bariatric surgery. The hypothesis of this study was that bariatric surgery is associated with greater metabolic health benefits than conventional metabolic hepatology clinic management in patients with obesity and NAFLD, and that these would result in improved survival in the bariatric patient group.

11.1.2 Aims

The aims of this chapter are to:

1) Describe and compare the baseline characteristics of patients accessing bariatric surgery (BS) and metabolic hepatology (MH) services

2) To compare mortality over time in patients with NAFLD undergoing MH or BS management

3) To compare longitudinal changes in key clinical targets of NAFLD management in MH and BS patient groups

4) To describe and compare baseline characteristics and metabolic health outcomes in patients undergoing SG or RYGB surgery

11.2 Patients and Methods

11.2.1 Research strategy

In this chapter, I will evaluate the potential of bariatric surgery as an intervention for NAFLD by comparing outcomes after bariatric surgery to those following 'gold standard' metabolic hepatology clinic management.

11.2.2 Study design

This is a single centre retrospective cohort study that included patients undergoing BS or MH clinic management in Oxford.

11.2.2.1 Bariatric Surgical Service

Oxford's bariatric surgical unit provides a regional/tertiary clinical service for individuals with obesity. Patients enter the service after GP referral and subject to meeting the eligibility criteria specified in the UK's national clinical guidelines for bariatric surgery[1]. These include engagement with a structured weight loss programme and a BMI of ≥40, or a BMI≥35 with a comorbidity (such as T2D), which could be improved by bariatric surgery.

The preoperative assessment process includes comprehensive medical and psychological evaluation. Specialised dietary, lifestyle and medical approaches to the improvement of obesity and the metabolic syndrome are provided. The choice of bariatric operation is a result of a multidisciplinary shared decision-making process involving patients and health professionals that is informed by preoperative investigations (such as oesophago-gastro-duodendoscopy) and co-morbidities.

Following surgery, patients routinely undergo follow-up with health professionals (including dieticians, specialist nurses and surgeons) in the hospital clinic setting for 2 years, after which time ongoing care is provided by the GP.

11.2.2.2 Metabolic Hepatology service

Oxford's Metabolic Hepatology clinic provides a regional/tertiary service for people with NAFLD. Patients are referred to the clinic by their GP after risk stratification, with non-invasive biomarkers used to predict liver fibrosis severity. However, access is not refused to patients where the referral does not include risk stratification data.

The multidisciplinary MH clinic includes access not only to dedicated hepatologists, but to endocrinologists who aim to optimise co-existent T2D management. Specialist nurses perform hepatic elastography (Fibroscan) for non-invasive fibrosis stage prediction. Dietary and lifestyle advice (including exercise and excess alcohol reduction) is also provided by specialist practitioners.

Subsequent clinic assessment is arranged to repeat non-invasive risk stratification, screen for evidence of NAFLD progression and to optimise clinical management.

11.2.2.3 Approvals

Permission was obtained to audit longitudinal outcomes after bariatric surgery in Oxford's bariatric service (audit reference: 191216-Surgery and Oncology-Gillies (Upper GI Surgery CSU)). Permission was also obtained to audit longitudinal outcomes after MH clinic management and to compare these to those in the bariatric service (audit reference: 19.9.18-S&O-Dempster).

11.2.2.4 Database development

A database incorporating the following variables was created using MS Excel v15.27 (Microsoft Corporation, Redmond, USA):

Baseline Demographic and clinical parameters (units)

- Intervention (BS or MH clinic management, with bariatric surgery further classified into SG or RYGB operations)

- Sex (male/female)
- Age (years)
- BMI (kg/m^2)
- Type 2 Diabetes (T2D) status (yes/no)

Baseline blood tests (units)

- Liver function tests (AST (u/L), ALT (u/L)
- HbA1c (mmol/mol)
- Lipid profile (Total cholesterol and HDL cholesterol in mmol/L)

Longitudinal outcome measures (units)

- BMI (kg/m^2)
- Liver function tests (AST (u/L), ALT (u/L)
- HbA1c (mmol/mol)
- Lipid profile (Total cholesterol and HDL cholesterol in mmol/L)

11.2.2.5 Statistical analysis

Normality testing was performed with the D'Agostino and Pearson test in GraphPad Prism for Macintosh Version 8.4.1 (GraphPad Software, California, USA) for each of the studied variables. Baseline characteristics were then compared between groups, with 2-tailed unpaired t-tests used to compare normally distributed data and Mann-Whitney tests used to compare non-normally distributed data.

Survival after MH or BS management was specified as the primary outcome measure and was evaluated using SPSS Statistics for Macintosh Version 26.0 (IBM Corp., NY, USA). Kaplan-Meier survival analysis was used for unadjusted comparisons and Cox proportional hazards regression model for adjusted comparisons. Adjustments were made for age, sex and a panel of metabolic health makers (BMI, T2D status and ALT) at baseline in the 1058 participants for whom each these parameters were recorded (96.98% of whole cohort).

Comparisons of changes in longitudinal outcome measures were then performed in SPSS using mixed models. The intervention (e.g. BS or MH management) was specified as a between-subject factor and time as a within-subject factor.

11.3 Results

11.3.1 Baseline characteristics

The baseline characteristics of patients undergoing BS or MH clinic management are described and compared in Table 11-1:

Table 11-1: Baseline Characteristics of the BS and MH patient groups

		Baseline Participant Characteristics		
		Bariatric Series	Metabolic Hepatology Series	BS *vs* MH Comparison
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Clinical Characteristic	BMI (kg/m^2)	47.5 (10.2)† *n=365*	33.0 (9.1)† *n=702*	<0.0001
	Age (years)	*47 (13)* † *n=382*	54 (22)† *n=649*	<0.0001
	Female sex (%)	*78.0% n=382*	*38.2% n=707*	<0.0001
	T2D (%)	*37.7% n=382*	*43.6% n=707*	0.0618
	Index of Multiple Deprivation Decile	7 (4)† *n=382*	8 (3)† *n=707*	0.0233

		Baseline Participant Characteristics		
		Bariatric Series	Metabolic Hepatology Series	BS *vs* MH Comparison
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Clinical Biochemistry and Haematology	ALT (IU/L)	27 (17)† *n=382*	51 (44)† *n=705*	<0.0001
	AST (IU/L)	22 (10)† *n=219*	39 (28)† *n=541*	<0.0001
	Total cholesterol (mmol/L)	4.4 (1.5)† *n=367*	*4.7 (1.7)*† *n=566*	<0.0001
	HDL cholesterol (mmol/L)	1.0 (0.3)† *n=366*	*1.1 (0.4)*† *n=567*	0.0210
	HbA_1c (mmol/mol)	38 (15)† *n=362*	*41 (20)*† *n=605*	0.0013

Two-tailed unpaired t-tests used to compare normally distributed, and Mann-Whitney test used to compare non-normally distributed, baseline characteristics between BS and MH groups. † indicates non-normally distributed data.

Table 11-1 highlights that there were several differences in the baseline characteristics of participants undergoing either BS or MH clinic management. BMI was higher in the bariatric group but there were indicators of relatively impaired metabolic health, such as higher liver enzyme, HbA_1c and cholesterol levels in the MH group. The MH group was also older and had a higher proportion of male patients. These differences may limit the validity of comparisons between groups, and these should be interpreted with caution in this exploratory study.

11.3.2 Survival Analysis of BS and MH Patient Groups

All participants (n=1091) were included in a 5-year Kaplan-Meier survival analysis, with the endpoint for each participant defined as the earliest of the following dates: date of death, 5-years post-intervention or 24th February 2020 (the date on which mortality data was extracted from Office of National Statistics (ONS) records). The survival curve is shown below in Figure 11-1:

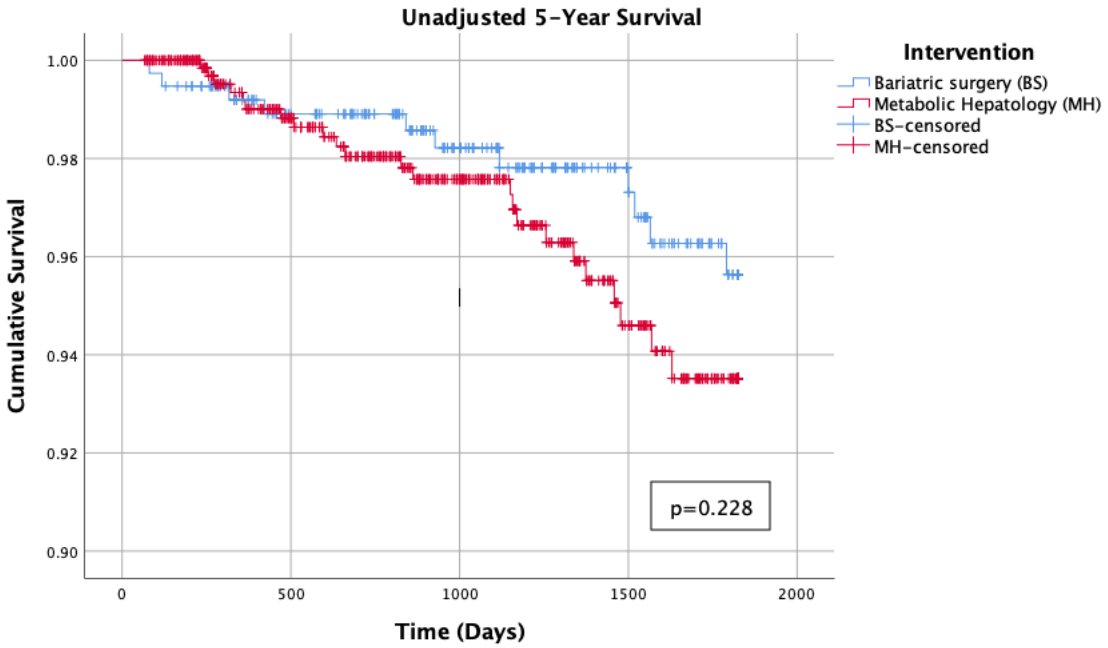


Figure 11-1: Unadjusted Kaplan-Meier 5-year survival curve for BS and MH Groups

There was no significant difference in unadjusted 5-year all-cause mortality between the BS and MH clinic groups in Figure 11-1 when compared using the Log Rank test (p=0.228). However, there is a suggestion of a non-significant initial relative decrease in survival associated with bariatric surgical compared to metabolic hepatology intervention that is offset by a reduced mortality rate in later years.

Since Figure 11-1 does not account for differences in baseline demographic and metabolic health characteristics in the BS and MH groups (
Table 11-1) that may impair survival and confound the interpretation of unadjusted comparisons, Cox regression was also performed. An adjusted survival curve was generated using the average covariate method and is shown in Figure 11-2:

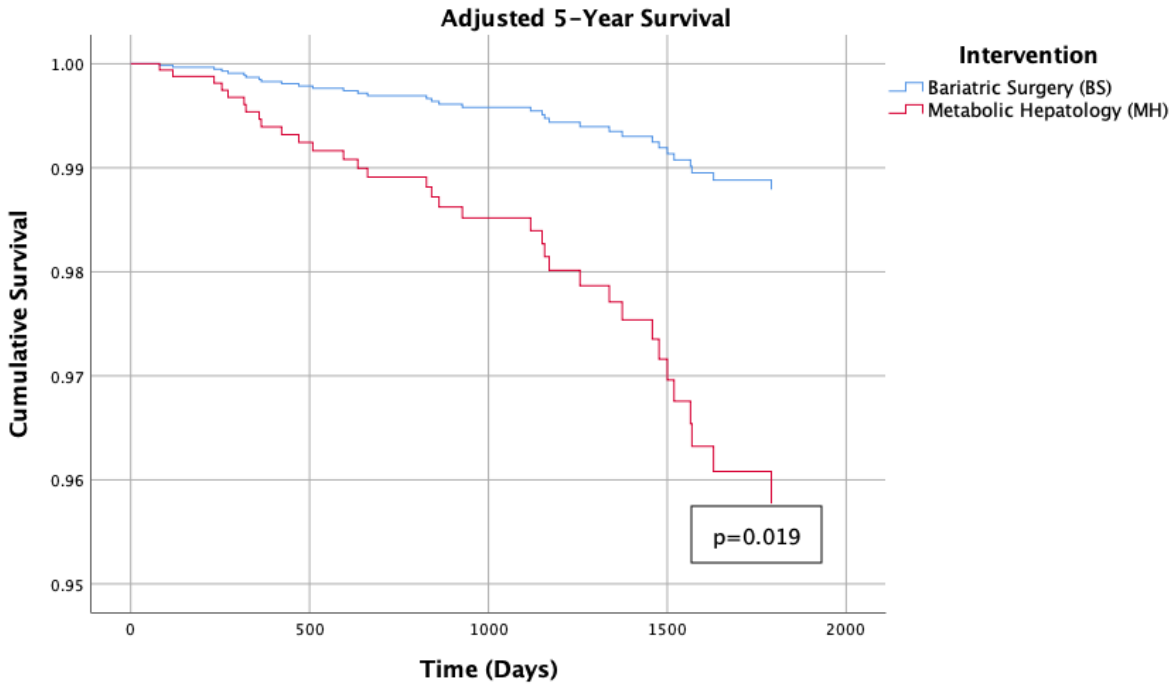


Figure 11-2: Adjusted Cox proportional hazards survival curve for BS and MH Groups

Figure 11-2 shows that 5-year survival was significantly higher in the bariatric surgical group relative to the metabolic hepatology clinic group after adjustment for age, sex and metabolic health markers (BMI, T2D status and ALT). This is reflected in the reduced Hazard Ratio (HR) for 5-year mortality associated with bariatric surgical intervention in Table 11-2, which also includes the HRs for the other covariates in the Cox proportional hazards model.

Table 11-2: 5-Year Mortality Hazard Ratios for Covariates included in Cox Regression Model

Cox Regression Covariate	**Significance (p value)**	**Hazard Ratio (5-Year Mortality)**	**95% CI for HR (Lower)**	**95% CI for HR (Upper)**
Intervention (MH=0, BS=1)	0.019	0.28	0.10	0.81
Age (years)	0.003	1.06	1.02	1.10
Sex (F=0, M=1)	0.633	1.19	0.58	2.46
BMI (kg/m^2)	0.003	1.07	1.02	1.12
T2D (T2D absent=0, present=1)	0.011	2.87	1.28	6.44
ALT (u/L)	0.010	0.98	0.96	0.995

In addition to showing that bariatric surgical intervention was associated with a reduced HR for 5-year mortality, Table 11-2 confirms that increasing age, increasing BMI and the presence of T2D were also associated with a greater HR. Interestingly, increasing ALT was associated with a slight reduction in this HR, which perhaps reflects that it is a relatively poor biomarker of liver disease severity in NAFLD. No significant association between sex and HR was observed. Despite adjustment, the observed difference in 5-year survival may reflect residual differences in group characteristics and should be interpreted with caution.

11.3.3 Longitudinal Analysis of Metabolic Health in BS and MH Groups

The finding that bariatric surgery was associated with improved 5-year survival compared to metabolic hepatology intervention was interrogated to determine whether there may be plausible biological explanations for this observation. Changes after bariatric surgical intervention in a range of metabolic health outcome measures were compared to those after metabolic hepatology clinic management to determine whether their directionality supported the association between bariatric surgical intervention and improved adjusted 5-year survival observed in Figure 11-2. These are summarised in Figure 11-3:

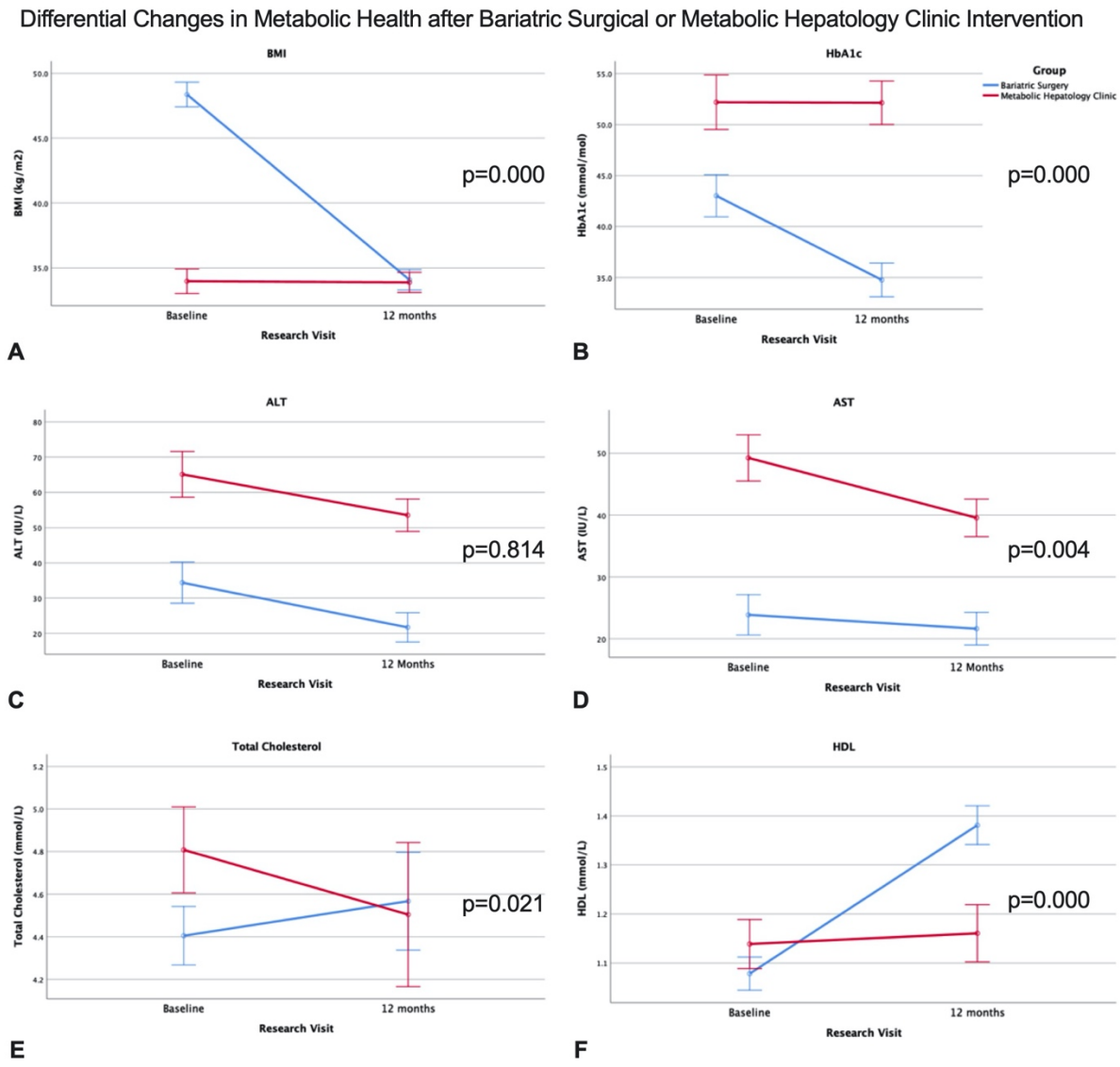


Figure 11-3: Graph demonstrating 12-month changes in a range of markers of metabolic health after bariatric surgical or metabolic hepatology clinic management: (A) BMI, (B) HbA_1c, (C) ALT, (D) AST, (E) Total Cholesterol, (F) HDL Cholesterol. Blue lines indicate changes in the bariatric surgery group and pink lines indicate changes in the metabolic hepatology clinic group. P values are reported for the interaction between research visit (12-month change) and intervention (BS or MH) in two-way mixed Analysis of Variance (ANOVA) analysis.

Figure 11-3 shows that BMI decreased after bariatric surgical intervention to a similar level of that of the MH group, in which there was no significant change in BMI. Similarly beneficial

relative changes were seen after bariatric surgery in HbA_1c and HDL cholesterol levels. Both interventions were associated with an improvement in liver enzymes, with higher baseline values observed in the MH group, but there was a relatively greater decrease in AST at 12-months in AST in patients undergoing MH clinic management.

Mean baseline and 12-month metabolic health marker values for the BS and MH groups are reported in more detail in Table 11-3:

Table 11-3: Mean baseline and 12-month metabolic health marker values for the BS and MH groups

Outcome measure	Intervention	Time	Mean	Std Deviation
BMI	MH Clinic	Baseline	34.0	6.3
		12 Months	33.9	6.1
	Bariatric Surgery	Baseline	48.4	8.7
		12 Months	34.1	6.3
HbA_1c	MH Clinic (n=154)	Baseline	52.2	21.3
		12 Months	52.1	19.8
	Bariatric Surgery (n=256)	Baseline	43.0	13.5
		12 Months	34.8	7.4
ALT	MH Clinic (n=232)	Baseline	65.1	41.9
		12 Months	53.5	50.5
		Baseline	34.9	56.1

Outcome measure	Intervention	Time	Mean	Std Deviation
	Bariatric Surgery (n=284)	12 Months	21.7	15.0
AST	MH Clinic (n=103)	Baseline	49.2	27.5
		12 Months	39.6	20.1
	Bariatric Surgery (n=135)	Baseline	23.9	8.9
		12 Months	21.6	11.0
Total Chol.	MH Clinic (n=119)	Baseline	4.8	1.2
		12 Months	4.5	1.2
	Bariatric Surgery (n=258)	Baseline	4.4	1.1
		12 Months	4.6	2.1
HDL Chol.	MH Clinic (n=119)	Baseline	1.1	0.3
		12 Months	1.2	0.3
	Bariatric Surgery (n=258)	Baseline	1.1	0.3
		12 Months	1.4	0.3

Table 11-3 shows that the apparent 12-month increase in total cholesterol levels in the BS group in Figure 11-3 was largely driven by an increase in HDL cholesterol. This improvement in total cholesterol:HDL cholesterol ratio provides a more sensitive biomarker of cardiovascular disease risk reduction than using isolated lipoprotein fractions[216].

The significance of the association between research visit (time), BS or MH clinic management (intervention), and their interaction on each metabolic health marker outcome measure was then studied. This is reported in Table 11-4 along with degrees of freedom, mean square values and F-ratios:

Table 11-4 Two-way Mixed ANOVA Summary Table for Metabolic Health Marker Changes after 1 Year in MH and BS Groups

Outcome measure	Variable	Degrees of freedom	Mean square	F-Ratio	Significance (p=)
BMI	Time	1	12657.8	588.4	0.000
	Intervention	1	13064.3	175.6	0.000
	Time*Intervention	1	12351.9	574.2	0.000
HbA1c	Time	1	3315.9	34.1	0.000
	Intervention	1	33885.1	92.2	0.000
	Time*Intervention	1	3233.4	33.3	0.000
ALT	Time	1	37627.0	26.0	0.000
	Intervention	1	250069.7	106.6	0.000
	Time*Intervention	1	80.3	0.1	0.814
AST	Time	1	4138.5	21.1	0.000
	Intervention	1	54705.3	130.9	0.000
	Time*Intervention	1	1624.2	8.3	0.004
Total Chol.	Time	1	0.8	0.5	0.481
	Intervention	1	4.7	1.5	0.221
	Time*Intervention	1	8.8	5.4	0.021
HDL Chol.	Time	1	4.3	90.4	0.000
	Intervention	1	1.0	7.8	0.000
	Time*Intervention	1	3.2	67.6	0.005

Table 11-4 shows that whilst there were significant changes between baseline and 12-months (within subjects 'time' effect) in each metabolic health marker studied except for in total cholesterol. However, there were also significant differences in the degree of change in these outcome measures in the BS and MH groups over this time period (within subjects 'time*intervention' interaction effect) and there was an association between BS or MH management and differences in baseline or 12-month values (between subjects 'intervention' effect).

11.3.4 Baseline Characteristics and Longitudinal Analysis of Metabolic Health in SG and RYGB Groups

The baseline characteristics of patients undergoing SG or RYGB surgery were compared to determine whether these varied in this observational 'real world' case series.

Table 11-5: Baseline Characteristics of the SG and RYGB patient groups

		Baseline Participant Characteristics		
		SG	RYGB	SG *vs* RYGB Comparison
	Parameter	*Mean (SD) / Median (IQR)*	*Mean (SD) / Median (IQR)*	*p value*
Clinical Characteristic	BMI (kg/m^2)	48.7 (10.0)† *n=174*	46.4 (10.1)† *n=177*	0.0541
	Age (years)	45.8 (10.7) *n=180*	47.7 (9.6) *n=196*	0.0807
	Female sex (%)	*77.2%* *n=180*	79.1% *n=196*	0.7083
	T2D (%)	*26.7%* *n=180*	48.5% *n=196*	<0.0001
	Index of Multiple Deprivation Decile	7 (4.8)† *n=180*	7 (4)† *n=196*	0.7229
Clinical Biochemistry and Haematology	ALT (IU/L)	25.5 (16.8)† *n=180*	27.5 (17.0)† *n=196*	0.0226
	AST (IU/L)	22 (10)† *n=139*	22 (8)† *n=75*	0.9314
	Total cholesterol (mmol/L)	4.4 (1.3)† *n=174*	4.4 (1.7)† *n=188*	0.9623
	HDL cholesterol (mmol/L)	1.1 (0.3)† *n=174*	1.0 (0.3)† *n=187*	0.4679
	HbA_1c (mmol/mol)	36.5 (8.0)† *n=172*	43.0 (19.0)† *n=186*	<0.0001

Two-tailed unpaired t-tests used to compare normally distributed, and Mann-Whitney test used to compare non-normally distributed, baseline characteristics between BS and MH groups. † indicates non-normally distributed data.

Table 11-5 highlights that there were more similarities in the baseline characteristics of patients undergoing SG or RYGB than there were when those undergoing hepatology clinic or bariatric surgical management were compared. However, HbA_1c and T2D prevalence were higher in the RYGB group in this non-randomised study. This may reflect a degree of selection bias due to evidence that RYGB results in greater remission rates in T2D than SG[217]. This evidence may have influenced the decision-making of the surgical team and their patients. ALT was also significantly higher in the RYGB group, which may relate to increased baseline NAFLD prevalence or severity due to T2D.

Changes in metabolic health markers after SG or RYGB were also compared to determine whether these interventions were associated with differential effects. These results are summarised in Figure 11-4:

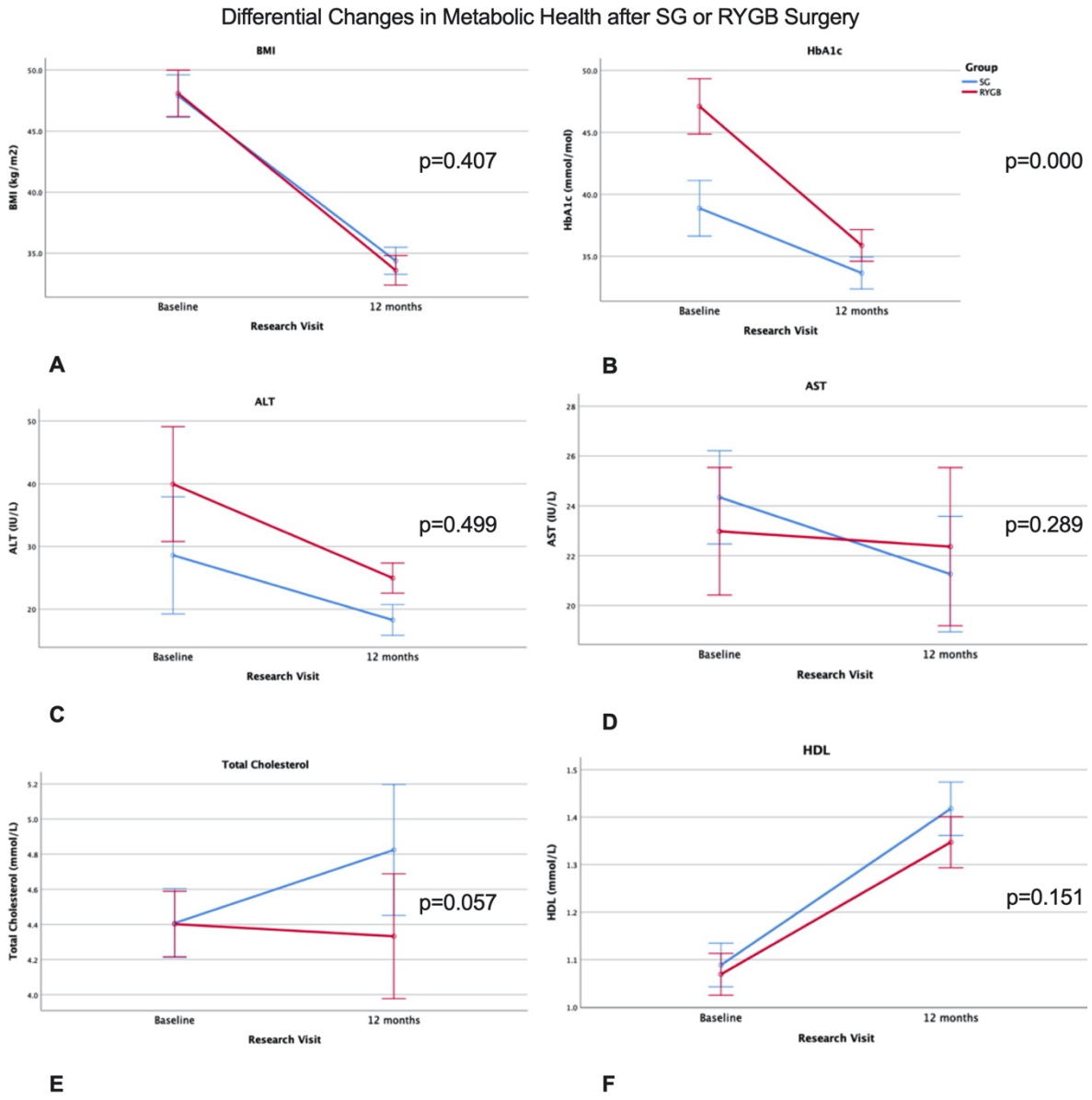


Figure 11-4: Graph demonstrating 12-month changes in a range of markers of metabolic health after SG or RYGB Surgery: (A) BMI, (B) HbA_1c, (C) ALT, (D) AST, (E) Total Cholesterol, (F) HDL Cholesterol. Blue lines indicate changes in the SG group and pink lines indicate changes in the RYGB clinic group. P values are reported for the interaction between research visit (12-month change) and surgical intervention group (SG or RYGB) in two-way mixed ANOVA analysis.

These results suggest a broadly similar impact of SG and RYGB on this panel of metabolic health markers except for HbA_1c, where greater changes were observed after RYGB. This supports existing evidence that RYGB may represent a more effective treatment for T2D in

the first postoperative year, although causation cannot be determined with this observational study design. An alternative explanation could be that there is a 'regression to the mean' effect[218], since baseline HbA_1c and T2D prevalence were higher in the RYGB group.

Table 11-6 describes baseline and 12-month follow-up values of the range of metabolic health markers in the SG and RYGB groups:

Table 11-6: Mean baseline and 12-month metabolic health marker values for the SG and RYGB groups

Outcome measure	Intervention	Time	Mean	Std Deviation
BMI	SG	Baseline	47.9	11.4
		12 Months	34.4	6.7
	RYGB	Baseline	48.1	7.6
		12 Months	33.6	5.7
HbA_1c	SG	Baseline	38.9	8.7
		12 Months	33.6	5.7
	RYGB	Baseline	47.1	15.9
		12 Months	35.9	8.7
ALT	SG	Baseline	28.6	14.9
		12 Months	18.3	13.7
	RYGB	Baseline	39.9	76.9
		12 Months	25.0	15.5

AST	SG	Baseline	24.3	10.0
		12 Months	21.3	12.1
	RYGB	Baseline	22.3	6.4
		12 Months	22.4	8.5
Total Chol.	SG	Baseline	4.4	1.0
		12 Months	4.8	1.1
	RYGB	Baseline	4.4	1.2
		12 Months	4.3	2.7
HDL Chol.	SG	Baseline	1.1	0.3
		12 Months	1.4	0.3
	RYGB	Baseline	1.1	0.2
		12 Months	1.3	0.2

This table suggests that both SG and RYGB were both associated with an improvement in a range of markers of metabolic health. Baseline BMI was very similar but baseline HbA_1c differed, which may suggest that RYGB was selected for its perceived metabolic health benefits rather than to promote greater weight loss[219].

Finally, the significance of these observations was formally assessed using two-way mixed Analysis of Variance (ANOVA). The results of this analysis are reported in Table 11-7:

Table 11-7: Two-way Mixed ANOVA Summary Table for Metabolic Health Marker Changes in the first postoperative year in SG and RYGB Groups

Outcome measure	Variable	Degrees of Freedom	Mean Square	F-Ratio	Significance (p=)
BMI	Time	1	22433	543.5	0.000
	Intervention	1	9.2	0.1	0.757
	Time*Intervention	1	28.5	0.7	0.407
HbA1c	Time	1	8667.8	164.2	0.000
	Intervention	1	3490.2	20.9	0.000
	Time*Intervention	1	1152.1	21.8	0.000
ALT	Time	1	22711.4	13.4	0.000
	Intervention	1	11538.0	7.0	0.024
	Time*Intervention	1	775.9	0.5	0.499
AST	Time	1	209.5	2.6	0.113
	Intervention	1	1.1	0.0	0.925
	Time*Intervention	1	93.0	1.1	0.289
Total Chol.	Time	1	3.9	1.9	0.174
	Intervention	1	8.0	2.2	0.135
	Time*Intervention	1	7.6	3.7	0.057
HDL Chol.	Time	1	11.9	291.3	0.000
	Intervention	1	0.3	2.1	0.152
	Time*Intervention	1	0.1	2.1	0.151

These results endorse the statement that both operations were associated with an improvement in metabolic health markers (significant within subjects 'time' effect), but that the operations were associated with relatively similar changes in these outcome measures (non-significant 'intervention' and time*intervention' interaction effects). HbA_1c is the exception to this observation, since greater improvements were seen after RYGB surgery. Similar reductions in ALT levels were seen after SG or RYGB surgery (non-significant time*intervention effect), but there was a difference in baseline and 12-month values that resulted in a significant intervention effect for this outcome measure.

11.4 Discussion

11.4.1 Principal findings

In this chapter, my primary aim was to measure survival and changes in markers of metabolic health in patients undergoing BS or MH clinic management and perform exploratory comparisons of these patient groups. Bariatric surgical intervention was associated with higher adjusted 5-year survival compared to metabolic hepatology clinic management, although this may be due to residual differences between the groups. Bariatric surgery was also associated with greater improvements in a range of metabolic health markers that represent key targets in the clinical management of NAFLD, and this gives a plausible biological reason for surgery potentially having greater effects on NAFLD and metabolic health. A greater reduction in HbA_1c level was observed after RYGB surgery than after SG, but postoperative changes in metabolic health markers were otherwise similar.

11.4.2 Interpretation and comparison with previous studies

Specialist metabolic hepatology clinics that adopt a multidisciplinary approach have been associated with favourable outcomes in NAFLD[190]. Bariatric surgery has also been suggested as a potential treatment for NAFLD, with encouraging results reported in several bariatric case series[186,170,220]. However, there is a lack of data in the literature on comparative NAFLD-related outcomes after bariatric surgery on metabolic hepatology interventions. This is required in order to develop hypotheses that can be addressed in future prospective clinical trials. This work has previously been done in order to evaluate bariatric surgery as an intervention for T2D[44], and a similar approach for NAFLD may generate the evidence necessary to support its wider integration into NAFLD clinical care pathways.

The present study highlights differences in the baseline characteristics of MH and BS clinic patients which may confound the interpretation of its results. Identifying matched pairs for comparison was attempted, however this was not possible due to the magnitude of between-groups differences in relevant variables such as BMI and resulted in a sample size that precluded subsequent statistical testing. It is therefore possible that the present analysis does not provide a fair comparison of the MH and BS interventions a subsequent randomised study will be required to remove selection bias and enable this. Comparisons between these groups in this observational work are considered exploratory; this study's results indicate that a prospective randomised study may be beneficial for more robust comparison. Additionally, the observed differences in T2D prevalence, HbA_1c and ALT in patients undergoing SG or RYGB suggest that randomisation to these interventions would

be important in order to fairly compare their effects on NAFLD and to study their underlying mechanisms.

It will also be important to design bariatric surgical studies that assess NAFLD outcomes in patients with different baseline fibrosis severity. There have been reports that bariatric surgery is less safe in patients with cirrhosis[201,221] , although this risk may be acceptable if clear benefits can be established, or if candidacy for liver transplantation is improved.

11.4.3 Strengths and limitations

The strengths of this study include that it is the first to measure and compare traditional hepatology-led management of NAFLD with bariatric surgical intervention. The study's sample size was sufficient to detect differences in the primary outcome measure (adjusted 5-year mortality), and national (ONS) rather than local records were used to extract mortality data to increase the reliability of primary outcome measure analysis.

However, it is possible that the 3-week preoperative diet that is routinely undertaken before bariatric surgery (900kcal, maximum 100g carbohydrate) may have already resulted in NAFLD improvement, leading to an underestimation of the overall effects of these co-interventions. A longitudinal study quantifying the relative effects of the preoperative diet and subsequent bariatric surgery would be beneficial.

One limitation of this study is that it only includes patients attending metabolic hepatology or bariatric surgical services in a single healthcare trust (Oxford). Results may, therefore, not be representative of those achieved in other geographical locations or healthcare systems.

In common with many other studies, whilst data was cross-checked for accuracy, typographical errors may occur and there is missing data that exists in part due to the study's retrospective observational design.

Whilst efforts have been made to include relevant covariates in adjusted survival analysis, unmeasured confounders may still exist and lead to residual confounding. One previous study found that age and T2D were associated with increased mortality in NAFLD[222]. Selection bias may also have influenced outcomes, and causation cannot be implied due to the observational study design. Unfortunately, matched pairs analysis was not feasible, but propensity score analysis (which combines multiple variables into a propensity score that is then used for case matching or weighting) will be explored in future work.

11.4.4 Conclusion

Bariatric surgery appears to represent a promising intervention for patients with obesity and NAFLD, but it is not possible to conclude from this observational study design that it is more effective than MH management. Comparison of BS and MH clinic groups may be limited by marked differences in their baseline characteristics and these results should be considered exploratory and hypothesis-generating. SG and RYGB operations were both associated with improvements in a range of clinical targets in NAFLD management, although the underlying mechanisms need to be established. This observational study's findings should be prospectively confirmed by a prospective multicentre randomised clinical trial that compares outcomes after bariatric surgical intervention and conventional hepatology-led medical management.

12 Effects of Preoperative Low Energy Diet on NAFLD

12.1 Introduction

12.1.1 Background

12.1.1.1 Low energy diets as interventions for NAFLD

There is existing evidence that low energy and carbohydrate-restricted diets improve glycaemic control and can result in the remission of T2D[63] and habitual diet is also known to be important both in the development and treatment of NAFLD[223]. It is generally accepted that energy balance is the most important factor regulating liver steatosis, but the contributions that different food groups make to total energy intake and of individual nutrients within food groups (for fructose or sucrose intake) have also been shown to have more modest effects[224].

However, previous dietary intervention studies have largely recruited individuals with overweight or with less severe obesity than those scheduled to undergo bariatric surgery. It is therefore unclear whether metabolic responses will be similar in bariatric surgical patients and such a study would be beneficial since severe obesity is increasingly common[225].

12.1.1.2 Low energy diets prior to bariatric surgery

Laparoscopic bariatric surgery requires liver retraction to enable visualisation and access to the oesophageal hiatus. Enlarged and fatty, friable livers can therefore present technical challenges to the bariatric surgeon[226]. For this reason, low energy diets are often prescribed for patients scheduled to undergo bariatric surgery with the aim of reducing the volume and rigidity of the liver[227].

These hypocaloric, and usually carbohydrate-restricted, diets result in hepatic glycogenolysis and reduction of liver volume and mass[177], since each gram of glycogen has been reported to bind up to 4 grams of water[58]. A recent systematic review suggested that the greatest reductions in hepatic volume occur between 2 and 4 weeks after diet initiation[228] and they are usually prescribed for a similar period for this reason. Previous imaging-focused studies have also reported reductions in liver fat percentage following such diets and a reduction in

blood loss during hepatobiliary surgery has been shown[229]. However, a recent study has found that preoperative low energy diets reduce collagen deposition in surgical wounds after bariatric surgery. It was suggested that this may lead to impaired wound healing, but this finding was not associated with the actual development of adverse clinical outcomes[230].

Low energy diets are frequently used before bariatric surgery, but this co-intervention is infrequently described in detail when bariatric surgical studies are reported. Additionally, baseline liver outcome measures have often either been recorded before initiation of any preoperative diet with follow-up measurements after surgery (so its contribution to metabolic and liver health cannot be determined)[231,232], after the preoperative diet[233] (when diet-induced changes may have already occurred), or the timing of baseline samples in relation to the preoperative diet is not specified in the trial protocol or manuscript[234,235]. Low energy diets may in themselves reduce liver fat and hepatic insulin resistance, and therefore changes in liver-focused outcome measures reported in bariatric surgical studies may not be due to the surgery itself depending on the timing of baseline measurements in relation to preoperative diet. This may in some cases explain discordant outcomes reported in the bariatric surgical literature. Previous surgical studies have been histology[233], serology[236] or MR imaging-focused and none have defined the relative effects of the preoperative diet and bariatric surgery on liver fat metabolism[173,237,238]. There is therefore a need for a longitudinal study that defines the relative physiological effects, particularly in the postprandial state, of these dietary and bariatric surgical co-interventions that are commonly used in combination to treat individuals with severe obesity.

12.1.2 Aims

The key aims of this chapter are:

1) To define the effects of a routinely prescribed low energy carbohydrate-restricted preoperative diet on liver steatosis in bariatric surgical patients

2) define the effects of the low energy diet on fasting and postprandial plasma metabolic substrates (such as glucose and fatty acids), metabolites (such as lactate and 3-hydroxybutyrate) and hepatic and circulating triglycerides

3) To define the changes in body composition (total and regional muscle and fat mass) and grip strength after the low energy diet

4) To define differences in the above outcome measures for participants with obesity before and after dietary weight loss, and 'normal' results, as recorded from non-obese control participants

12.2 Patients and Methods

12.2.1 Research strategy

In this chapter, a prospective clinical study was performed to define the effects of routine preoperative low energy diet and subsequent bariatric surgery on NAFLD. Outcomes achieved after these interventions were compared to measurements from a non-obese control (NOC) group.

12.2.2 Study design

12.2.2.1 Summary

In brief, the plan of investigation was as follows: 24 individuals with severe obesity were identified from Oxford's clinical bariatric surgery service. They were recruited into the Effects of Dietary Intervention and Surgery on NAFLD (EDISON) study and commenced on a very low-calorie diet before their operation.

The preoperative diet was a 3-week 900kcal dietary programme. Carbohydrate intake was limited to a maximum of 100g per day with no restriction on protein or fat macronutrient proportions, although balanced intake was promoted. A sample whole food diet was provided, but participants could also substitute some or all meals with a suitable liquid meal replacement product according to their preferences. Meals were purchased by the participants rather than provided by the research team. Participants were also encouraged to drink at least 2 litres of fluid per day, avoid alcohol and to take a multivitamin and mineral supplement tablet.

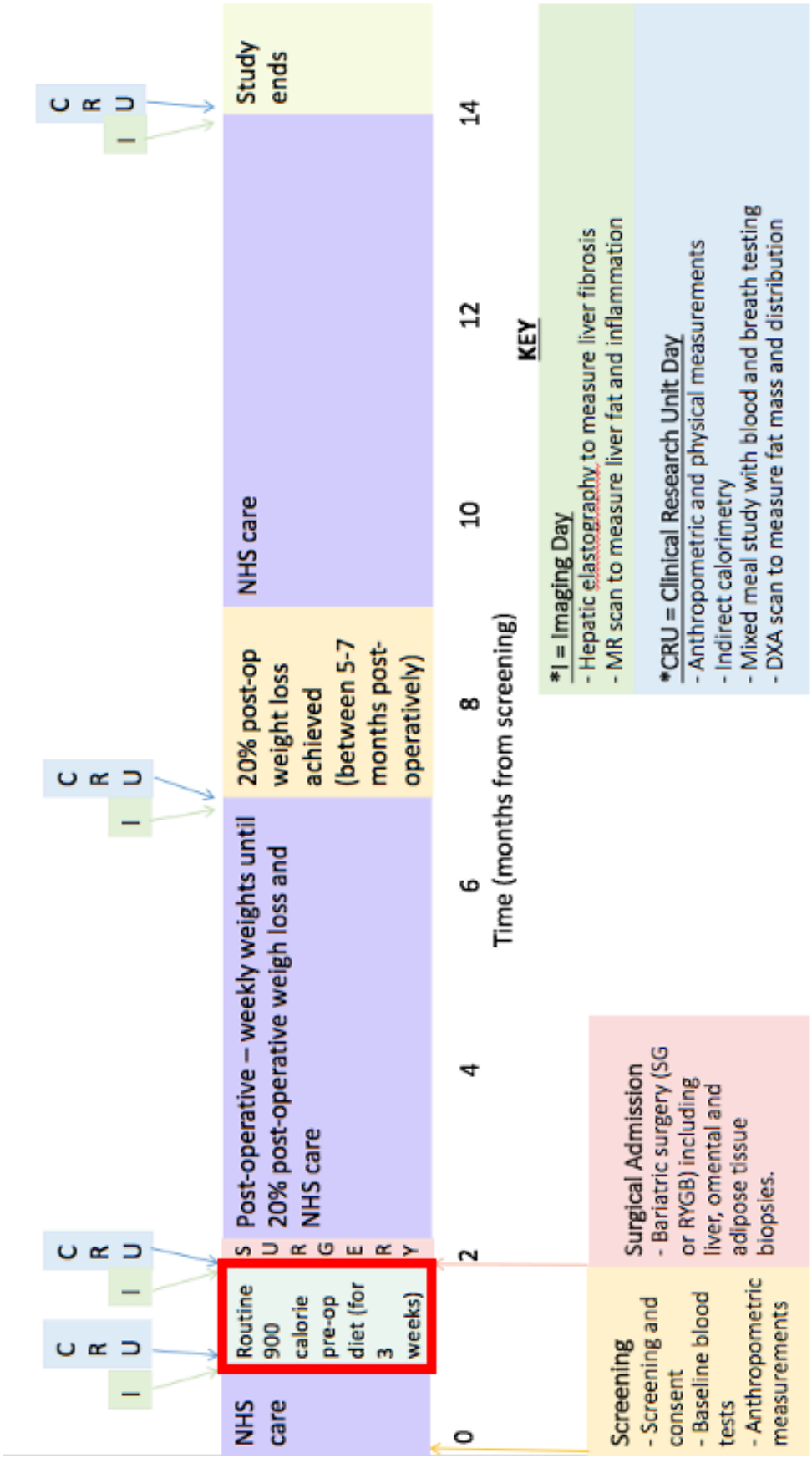


Figure 12-1: Schematic representation of overall EDISON study design. Study phases analysed and discussed in this chapter are contained within red outline.

After completion of the post-diet visit, bariatric surgery was performed and additional research visits were conducted after 20% postoperative total body weight loss and 1 year after surgery. This chapter focuses on the effects of the preoperative diet by comparing measurements taken before commencing this diet (Pre-Diet Visit/Research Visit 1) to those taken at the end of the low-calorie diet (Post-Diet Visit/Research Visit 2).

Additionally, a group of 16 non-obese individuals scheduled to undergo abdominal surgery for clinical reasons (for example laparoscopic gallbladder removal for biliary colic, laparoscopic hernia repair or laparoscopic fundoplication surgery) were recruited as control participants; they attended for a single research visit (Research Visit 1) just before their operation.

The same research investigations were performed at each Research Visit in both groups.

12.2.2.2 Study sponsorship, ethical and healthcare trust approvals

The EDISON study protocol and document set was approved by the University of Oxford's Clinical Trials and Research Governance (CTRG) department and the University agreed to sponsor the study. Ethical approval was obtained from the West Midlands – Black Country Research Ethics Committee and from the Health Research Authority (REC reference: 17/WM/0130). Oxford University Hospitals NHS Foundation Trust agreed for participants to be identified from its clinical services and to perform the surgical operations and clinical care for each participant.

12.2.2.3 Study registration

The EDISON study was registered prospectively on the ClinicalTrials.gov database before the first participant was recruited (ClinicalTrials Indentifier.gov: NCT03186859).

12.2.2.4 Participant eligibility criteria

Participants with Obesity

Female patients on the NHS waiting list for bariatric surgery with no medical condition or relevant drug therapy that affects liver metabolism, no contra-indication to undergoing either SG or RYGB, with a BMI ≥35 and ≤60 kg/m^2 and aged ≥18 or ≤75 years were studied. Females were recruited as they represent approximately 80% of patients undergoing bariatric surgery[239] and because sex-specific differences in NAFLD prevalence and liver fat metabolism are known to exist[240,241].

Inclusion Criteria

- Bariatric surgery was already planned for the participant
- Participant was willing and able to give informed consent for participation in the study.
- Aged ≥18 or ≤75 years.
- Body Mass Index ≥35 ≤60 kg/m^2

Exclusion Criteria

- Contraindication to MRI
- Prior or current participation in a Controlled Trial of Investigational Medicinal Product (CTIMP) that could affect study results
- Type 2 Diabetes
- History of alcoholism or a greater than recommended weekly alcohol intake (14 units per week)
- Anticoagulant treatment
- Insulin, glitazone or GLP-1 analogue treatment
- Pregnant or nursing mothers
- A liver disease other than NAFLD
- Histological confirmation of lack of NAFLD on liver biopsy
- Large hiatus hernia
- Active gastrooesophageal reflux disease
- Active malabsorptive intestinal disease

Non-Obese Control Participants

Female participants on the NHS waiting list for elective (planned) abdominal surgery with no medical condition or relevant drug therapy that affects liver metabolism, with a BMI ≥18.5 ≤29.9kg/m^2 and aged ≥18 or ≤75 years were studied.

Inclusion Criteria

- Elective abdominal surgery already planned for the participant
- Participant willing and able to give informed consent for participation in the study.
- Aged ≥18 or ≤75 years.
- Body Mass Index ≥18.5 ≤29.9 kg/m^2

Exclusion Criteria

- Contraindication to MRI
- Prior or current participation in a CTIMP that could affect study results
- History of alcoholism or a greater than recommended weekly alcohol intake (14 units per week)
- Anticoagulant treatment
- Type 2 Diabetes
- Pregnant or nursing mothers
- A liver disease other than NAFLD
- Active inflammatory or malignant disease
- Active malabsorptive intestinal disease

12.2.2.5 Participant screening, consent and withdrawal from study

The Participant Information leaflet, Participant Consent Form and Protocol for this study are reproduced in the Appendix section of this thesis.

Screening

A full medical history was taken. Pre- and peri-menopausal women were asked to sign a statement confirming that they were not pregnant and in cases of uncertainty were offered

a pregnancy test or withdrawal from the study. After written consent had been obtained from the volunteer, a blood sample was taken to measure any biochemical parameters required to confirm participant eligibility (e.g. HbA1c or Follicle-Stimulating Hormone (FSH) if menopausal status was unclear) and measurements (height and weight) that were required for subsequent stratified randomisation were made.

Consent

Potentially interested individuals were identified by their clinical care teams and their consent was sought at the screening visit. The participant signed and dated the latest approved version of the Informed Consent form before any study specific procedures were performed. An additional appointment was scheduled to re-discuss the study prior to taking consent if needed.

Written and verbal versions of the Participant Information and Informed Consent were presented to the participants detailing: the exact nature of the study; what it involved for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It was clearly stated that the participant was free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal. Although consent was sought at the screening visit, the participant was allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they would participate in the study.

Written Informed Consent was then obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. A copy of the signed Informed Consent was given to the participant. The original signed form was retained at the study site. A letter was sent to the GP of volunteers to inform them of their participation and provide information on the study. There was no maximum time allowed between screening and participation. As long as participants fulfilled the inclusion criteria, they were considered eligible.

Withdrawal of participants from the study

Each participant had the right to withdraw from the study at any time. In addition, the Investigator could discontinue a participant from the study at any time if the Investigator considered it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- Withdrawal of Consent
- Loss to follow up

The reason for withdrawal was recorded in the CRF. Withdrawal from the study did not necessarily result in exclusion of the data or samples for that participant from analysis.

12.2.2.6 Randomisation and blinding protocols

Randomisation

The randomisation strategy (which was applied to patients with obesity) was planned with the specialist consultant bariatric surgeons responsible for the surgical management of the patients who volunteer for this study. It was established that, with the inclusion and exclusion criteria detailed above, there was no evidence as to whether SG or RYGB should be preferentially offered to our participants. There was therefore equipoise and support for patient randomisation with the aim of reducing selection bias and ensuring similar baseline characteristics in both groups.

The routine standardised dietary intervention was the same for all bariatric surgical patients but they underwent stratified randomisation in a 1:1 ratio to either SG or RYGB after Research Visit 2. Randomisation took place within one week of consent. The following strata were constructed:

- BMI ≥35 <45 kg/m^2, pre or peri-menopausal
- BMI ≥35 <45 kg/m^2, menopausal
- BMI ≥45 ≤60 kg/m^2, pre or peri-menopausal
- BMI ≥45 ≤60 kg/m^2, menopausal

A secure online computer-based randomisation system (www.sealedenvelope.com) was used to ensure allocation concealment. The allocation was not revealed until participant details were entered into the randomisation system. Analysis was planned on an intention-to-treat basis.

Blinding

For pragmatic reasons, it was considered that participant and surgeon blinding would reduce the feasibility of this study. Subjects were therefore informed about their assigned surgical treatment as soon as possible to allow them to make plans around their hospital admission. Surgeons were also informed as early as possible to allow them to efficiently plan their operating lists (SG and RYGB require different lengths of operating time). Where possible, data was analysed with blinding to the surgical intervention.

12.2.2.7 Timing of Research Visits

In the EDISON study, participants with obesity attended for Research Visits just before (within 1-week of) commencing a routine pre-operative low energy diet (Research Visit 1) and at its completion (Research Visit 2). They then underwent bariatric surgery during which tissue samples (liver and visceral adipose tissue) were collected. The effects of the bariatric surgical interventions were assessed when participants reached 20% postoperative total body weight loss (Research Visit 3) and 1 year after surgery (Research Visit 4). Participants without obesity did not undergo a dietary intervention and were studied on a single occasion before benign elective surgery. Liver and visceral adipose tissue was collected during these operations. The timing and format of Research Visits in this study was outlined in Figure 12-1 for participants with obesity and is outlined in Figure 12-2 below for the Non-Obese Control (NOC) participant group.

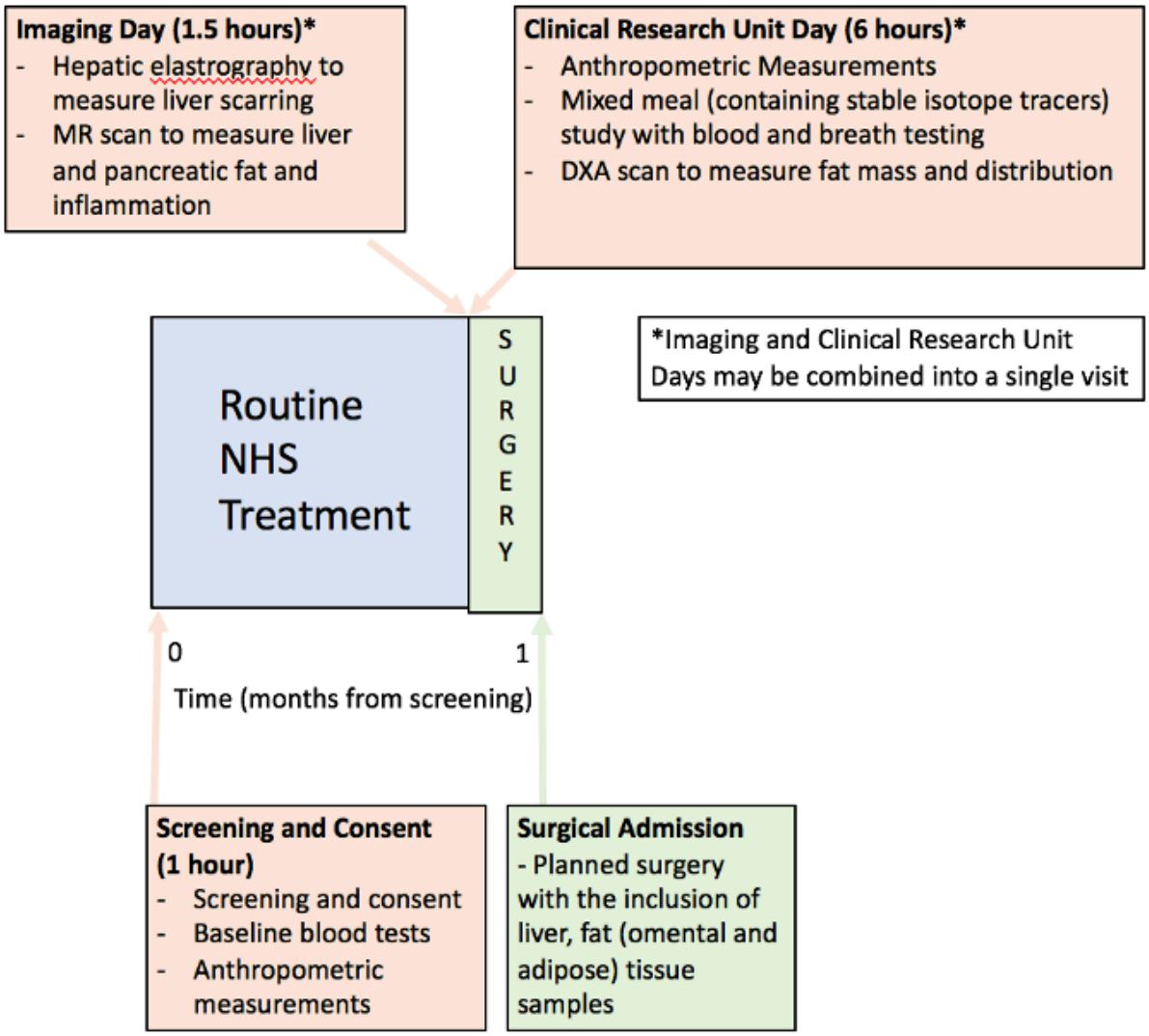


Figure 12-2 Schematic of EDISON study protocol for non-obese control participants

12.2.2.8 Format of Research Visits

Participants underwent the same protocol at each Research Visit. Each visit consisted of an Imaging Day and a Clinical Research Unit (CRU) Day. These visits could be on separate days (within the same week) or combined into a single day if preferred by the participant.

Imaging Day

All liver imaging investigations were performed at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). Liver fat was non-invasively measured using MR Spectroscopy, liver inflammation using multiparametric MRI, and liver fibrosis using hepatic elastography (Fibroscan).

CRU Day

The CRU component was performed at the Clinical Research Unit (CRU) in the Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM). Participants attended after an overnight fast but medications were taken as normal. Volunteers were asked to avoid excessive exercise and alcohol for 24 hours prior to this study day.

On arrival, participants were given an opportunity to relax, then the following physical measurements were taken: height, weight, waist circumference, blood pressure, heart rate, grip strength. Medications (changes) were recorded. Indirect calorimetry was performed to measure energy expenditure and respiratory quotient then a venous cannula was inserted into an upper limb vein to enable repeated blood samples to be taken throughout a 6-hour postprandial period. Saline was run through this cannula at a slow rate between blood samples to maintain patency. After stopping the saline drip, 5ml of blood was aspirated and discarded before sample collection at each time point.

After fasting samples were taken, participants consumed a test meal whilst reclining at a 45-degree angle. The meal consisted of a chocolate drink (40g olive oil, 30g glucose, 35g boiled deuterated water, 2.4g cocoa powder, 400mg Myverol 18-04k emulsifier) labelled with 200mg of 13C-palmitate (CK Isotopes Limited, Leicester, UK). Its preparation is described in the Appendix. Participants did not consume any more food for the remainder of the Research Visit but were allowed to drink water. Blood and breath samples were taken initially every 15 minutes for the first hour and then every 30 minutes up until the second hour and thereafter hourly until the end of the 6 hours. Indirect calorimetry was repeated 2 hours after the test meal and a DEXA scan was performed in the final hour to assess body composition.

12.2.2.9 Description of bariatric surgical procedures

Roux-en-Y Gastric Bypass

A stapling device was used to divide the stomach, creating a 30cm gastric pouch. The small bowel (jejunum) was divided and the distal end was anastomosed to the gastric pouch in an

ante-colic ante-gastric fashion using a stapling device with suture reinforcement of the staple line. The proximal end was anastomosed to the jejunum in a similar fashion, creating a 100cm Roux limb. Anastomoses were tested for air leaks. Mesenteric windows were closed to prevent internal herniae. Wounds were closed with sutures to conclude the operation and the anaesthetic agents were reversed. The patient was transferred to a recovery area then clinical ward, where they received standard NHS post-operative care.

Sleeve gastrectomy

A size 40 French bougie was inserted then the stomach was divided using a stapling device along its greater curve so that the portion in continuity with the oesophagus and duodenum (into which the protecting bougie had been inserted) was approximately 20% of its original size. The excluded stomach portion was removed and the staple line was tested to detect the presence of a leak. Wounds were closed with sutures to conclude the operation and the anaesthetic agents were reversed. The patient was transferred to a recovery area then clinical ward, where they received standard NHS post-operative care.

12.2.3 Study methods

12.2.3.1 Imaging

Non-invasive measurement of liver steatosis

Liver steatosis was measured with magnetic resonance (MR) spectroscopy using a 3.0 Tesla Siemens Magnetom Prisma scanner (Siemens Medical Solutions, Erlangen, Germany). Established protocols were used for imaging acquisition, with referral to previous scans to ensure consistent voxel placement in the right lobe across the study, avoiding blood vessels and bile ducts. Unsuppressed and water-supressed sequences were performed. In-house software was used to identify lipid peaks according to their frequency and measure the percentage of lipid within the voxel. Less than 5.5% liver steatosis was defined as normal.

Non-invasive measurement of liver inflammation

Liver inflammation was measured with multiparametric MR imaging using a 3.0 Tesla Siemens Magnetom Prisma scanner (Siemens Medical Solutions, Erlangen, Germany). It has been established that the liver's T1 relaxation time, after correction for iron concentration,

correlates with the severity of hepatic inflammation. Modified Look-Locker Inversion Recovery (MOLLI), T2* Dixon and Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation (IDEAL) sequences were performed during image acquisition. Image post processing was performed using proprietary software (Perspectum Diagnostics, Oxford, United Kingdom).

Non-invasive measurement of liver fibrosis

Liver fibrosis was measured with transient elastography using a Fibroscan device (EchoSens, Paris, France). This device measures shear wave velocity, which has been reported to correlate with liver fibrosis. Standard operating procedures used by clinical hepatology staff in Oxford University Hospitals Foundation Trust (OUHFT) were used for imaging acquisition. In brief, this involved a period of training by an experienced scan operator and use of the median value from 10 valid readings at each research visit to improve reliability. An interquartile range of ≤30% of this median value was required for quality control.

Non-invasive measurement of body composition

Body composition was measured with a single GE Lunar iDXA Dual Energy X-Ray Absorptiometry (DXA) scanner (GE Healthcare, Buckinghamshire, UK) and regions of interest were defined by its associated Encore software. Urine pregnancy tests were performed at each visit before scanning pre-menopausal participants and the scanner was calibrated daily. The scan was performed according to the CRU's standard operating procedure, and half the participant was scanned in the coronal plane, with obtained values doubled, in the event that the participant was unable to lie supine within the field of the scanner.

12.2.3.2 Physical anthropometric measurements

Anthropometric measurements were taken in the CRU of OCDEM at the beginning of each research visit after an overnight fast. Confirmation of fasting status, side effects from interventions or research investigations and medication changes were recorded at each visit.

Measurement of anthropometry

Body weight was measured in kilograms (kg) with calibrated scales after the removal of shoes, coats and other heavier clothing. A calibrated height measure was then used. Body Mass Index (BMI) was calculated using the formula: BMI = weight (kg)/ [height (m)]^2. Waist circumference was measured in centimetres with a standard tape measure.

Measurement of physiology

Blood pressure was measured with a calibrated sphygmomanometer and appropriate blood pressure cuff for the participant's arm circumference. Heart rate was measured in beats per minute using the same device, or manually in the event of clinical uncertainty (for example in participants with atrial fibrillation).

Measurement of hand grip strength

Grip strength was measured using a calibrated Jamar Smart hand dynamometer (Performance Health, Chicago, USA). Participants were seated with their feet on the ground and forearm flexed at 90 degrees on the armrest of a chair selected for this purpose. The hand bar of the dynamometer was adjusted to a comfortable position, then 6 alternating measurements were taken from the left and right hands. Results were recorded in kilogram force units and encouragement to reach maximal exertion was provided. Hand dominance was recorded, as were any injuries that may limit grip strength.

12.2.3.3 Indirect calorimetry

Indirect calorimetry was performed using the GEM open circuit indirect calorimeter (GEMNutrition Ltd, Cheshire, UK). The device was calibrated then gas exchange was measured in the fasting state using a transparent hood for 23 minutes to determine resting energy expenditure and respiratory quotient. Participants were instructed to remain still and awake during this period and were observed throughout. This process was repeated 120 minutes after consumption of the standardised mixed meal.

12.2.3.4 Chylomicron and VLDL separation from plasma

Established protocols were used to separate chylomicron and VLDL lipoprotein fractions from plasma at each study visit using density gradient ultra-centrifugation. The amended Standard Operating Procedure (SOP) is detailed below:

3ml of plasma was obtained for subsequent lipoprotein separation during each study visit at 0min (fasting), then at 120, 180, 240, 300 and 360min following the mixed meal. During the study visit, LP4 glass tubes were prepared with the following preservatives: 6µl Ethylenediaminetetraacetic acid (EDTA) (0.5 Mol l^{-1}, pH 7.4), 3µl Phenylmethylsulfonyl fluoride (PMSF) (10 mmol l^{-1} in propan-2-ol), 15µl Trasylol (10,000 KIE/ml) and the plasma was then added.

Following the study visit, 1.5ml of sodium bromide density 1.42 kgl^{-1} was added to each tube and mixed by inversion. 4ml of this solution was pipetted into PVA-coated ultracentrifuge tubes.

Sodium chloride of progressively lower densities was then added to each ultracentrifuge tube through apparatus developed in-house to limit the mixing of solutions of different densities. 3ml of sodium chloride density 1.063 kgl^{-1}, 3ml of sodium chloride density 1.02 kgl^{-1} then 2.8ml sodium chloride density 1.006 kgl^{-1} was added. It was ensured that tube volumes were equal then these were transferred into ultracentrifuge tube holders.

Samples underwent ultracentrifugation at 40,000rpm for 32 minutes at 15^{o}C for the isolation of the $S_f > 400$ (chylomicron) fraction, and approximately 0.5ml of this was aspirated into pre-weighed 2ml plastic tubes, with sodium chloride density 1.006 kgl^{-1} used to rinse out any residual sample within the system between each tube. After lipoprotein samples were added to the tubes, the tubes were reweighed and stored at 4^{o}C pending the measurement of triglycerides and Apolipoprotein B in each sample on the iLab clinical chemistry analyser.

The ultracentrifuge tubes were then topped up with sodium chloride density 1.006 kgl^{-1} and returned to the ultracentrifuge, which was run for 16-17 hours at 40,000rpm, 15^{o}C. At this

time, the S_f 20-400 (VLDL) fraction was separated into pre-weighed tubes in the same manner as for the chylomicron fraction.

The reagents used in the above method were prepared in batches according to established lab SOPs and are described in the Appendix.

12.2.3.5 DNA extraction

DNA was extracted from 9mL whole blood samples using the Maxwell 16 Instrument and Maxwell 16 blood DNA purification kits (Promega Corporation, Madison, USA). Blood samples were thawed then 500uL of blood and 500uL of elution buffer were added to wells within the otherwise pre-filled cartridge. The Maxwell 16 Instrument was then used to mix the samples, bind DNA to magnetic beads and undergo a series of wash steps to remove contaminants. The extracted DNA was then separated from the magnetic beads in the elution buffer. DNA quantification and quality control (230/260 and 260/280 ratio assessment) was performed on a NanoDrop 1000 (ThermoScientific, Wilmington, USA) before TaqMan SNP genotyping (Applied Biosystems, California, USA) for PNPLA3, TM6SF2 and MBOAT7 genes was performed by collaborators.

12.2.3.6 Plasma and lipoprotein analysis

The iLAB Clinical Chemistry 600 and 650 systems (Instrumentation Laboratory, Milan, Italy) were used for the clinical chemistry analysis of plasma and lipoprotein (chylomicron and VLDL) fractions. These devices use a photometric method to detect colour changes within samples at defined wavelengths. Calibration and quality control were performed before sample analysis. Results within 10% of mean in-house values were accepted. 500µL serum (AST, ALT and ELF testing) or plasma (all other assays) were analysed in 2mL iLAB-compatible containers. Established lab standard operating procedures were used for each assay and are described in the Appendix.

Measurement of insulin concentration

The Mercodia Insulin ELISA kit (10-1113-10, Mercodia AB, Uppsala, Sweden) was used to measure insulin concentration in plasma samples. This is a solid phase two-site enzyme

immunoassay, with 2 monoclonal antibodies used to bind antigens on the insulin molecule. The plate was incubated to promote binding then washed to remove unbound anti-insulin antibodies. The bound conjugate was detected by reaction with 3,3',5,5'-tetramethylbenzidine and the reaction was stopped by adding acid, which creating a colorimetric endpoint that was read with spectrophotometry. Each plate included wells for calibration and quality control as recommended by the manufacturer. All samples were run in duplicate and mean values were used.

Measurement of ELF score, AST and ALT concentration

All serological measurements reported in this chapter (ELF score, AST and ALT results) were performed by collaborators. The ELF score is a serological biomarker of liver fibrosis and the testing was performed in the company's own accredited laboratory (iQur, London, UK). AST and ALT testing was performed using NHS clinical chemistry analysers in the John Radcliffe Hospital, Oxford in collaboration with Professor Tim James.

12.2.3.7 Statistics and analysis

Statistical analysis was undertaken on the completion of the study, once all data had been analysed. Normality testing using the D'Agostino and Pearson test was conducted to assess the distribution of all outcome measures before analysis. For paired analysis, paired two-way t tests were used for normally distributed variables and Wilcoxon matched-pair tests were used for non-normally distributed variables. For unpaired analysis, unpaired t-tests were used for normally distributed variables and Mann-Whitney tests for non-normally distributed variables. Repeated-measures two-way analysis of variance (ANOVA) was used in the analysis of postprandial changes following consumption of the test meal. Total Area Under the Curve (AUC) was calculated for variables measured in the postprandial period using the trapezoidal method. The software package Graphpad Prism for Mac v8.3.1 (GraphPad Software, California, USA) was used for all statistical analysis in this chapter.

In tables, normally distributed variables are presented as mean (SD) values, and non-normally distributed variables as median (IQR). Mean (Standard Error of Mean (SEM)) values are used for all figures, which were also produced using Graphpad Prism software.

The primary endpoint was change (delta) in liver fat by very low-calorie diet and surgical intervention. Changes in non-parametric outcome measures were analysed using Wilcoxon matched-pair test. The accepted level of significance was pre-defined as 5% and an intention-to-treat analysis was adhered to when analysing changes in outcome measures after surgical interventions.

Data for all participants who completed the study or had at least baseline and end of phase data from the dietary intervention was included in the analysis. After withdrawal from the study, data collected was not destroyed and was used in the analysis unless destruction was requested by the individual.

The number of participants

24 people with obesity were recruited to this study. This was the number required to detect a predicted change in liver fat on MRI/S at 6 months (the approximate time when 20% weight loss was expected to be achieved after bariatric surgery[242]). It was known from previous studies that continued weight loss would occur during the first post-operative year, so this number of participants was also expected to be sufficient to detect significant changes in liver fat at 12 months post-operatively. This power calculation was based (for non-parametric tests) on an average baseline liver fat content of 10.3 (8)% (mean (SD)), which was derived from a published study in 14 non-diabetic patients undergoing bariatric surgery (SG or RYGB)[243]. If similar liver fat reductions were present in our study (reduction to 2.5 (2.1)% (mean (SD)) liver fat content at 6 months), it was determined that we would need to study 12 individuals in each surgical intervention arm (power 0.85 at α of 0.05) to detect these differences. Additionally, we aimed to recruit 16 non-obese control participants whose data was compared to that of the individuals with obesity.

12.3 Results

12.3.1 Characterisation of participants in the EDISON study at screening visit

16 participants were recruited to the Non-Obese Control (NOC) Group, and 24 to the Bariatric Surgery (BS) Group. At screening, the participants with obesity in the BS Group

underwent further stratification to SG or RYGB surgery (n=12 in each group). All participant characteristics were determined to be normally distributed at the screening visit using the D'Agostino-Pearson test and mean (SD) values are presented in

Table 12-1.

Table 12-1 Comparison of EDISON study participant group characteristics at screening visit.

	Participant Group					
Screening characteristics	**NOC (n=16)**	**All BS (n=24)**	**NOC *vs* BS**	**SG (n=12)**	**RYGB (n=12)**	**SG *vs* RYGB**
	Mean (SD) / Median (IQR)	Mean (SD) / Median (IQR)	p value	Mean(SD) / Median (IQR)	Mean (SD)	p value
Age (Years)	46.56 (12.79)	44.83 (11.35)	0.6562	44.00 (10.16)	45.67 (23.83)	0.7276
Total body weight (kg)	67.85 (8.61)	127.0 (12.79)	<0.0001	129.3 (9.61)	124.7 (15.42)	0.3848
BMI (kg/m^2)	25.29 (3.07)	46.80 (4.38)	<0.0001	47.95 (3.31)	45.65 (5.13)	0.2050
ALT (IU/L)	15.50 (7.25)†	23.00 (10.75)†	<0.0081	25.50 (9.75)†	22.75 (6.9)	0.4169

Two-tailed unpaired t-tests used to compare normally distributed, and Mann-Whitney test used to compare non-normally distributed, screening characteristics between NOC and BS groups, and SG and RYGB groups. † indicates non-normally distributed data.

Participants in the NOC Group were well matched by age and sex to those in the BS Group (Table 12-1). The expected differences in BMI and total body weight were observed along with a significant difference in ALT levels between NOC and BS groups. Since obesity is a known risk factor for NAFLD and other causes of liver disease (such as excessive alcohol intake) had been excluded, this is likely to reflect an increased prevalence and severity of NAFLD in the BS group.

No baseline differences in screening characteristics were observed when comparing participants scheduled to undergo SG or RYGB surgery, suggesting the stratified randomisation protocol was effective.

One participant withdrew from the study before undertaking a research visit after expressing a preference for one of the surgical interventions following randomisation. An additional participant attended the Pre-Diet research visit but did not complete the preoperative low-calorie dietary intervention and Post-Diet visit, as they decided not to proceed with subsequent bariatric surgery. This participant was therefore excluded from all paired analyses. All other participants (n=22) completed the dietary intervention component of the study and Post-Diet research visit, although this was terminated early (at 2 hours) for one participant due to challenging venous access. Since surgery was scheduled the following day, it was not felt appropriate to continue when access was lost. For this participant, postprandial data was excluded since the majority of the 360-minute postprandial period was not studied, all other outcome measures were obtained and included.

Whole blood samples were collected at the first research visit for subsequent DNA extraction and genotyping. NAFLD-related genotypes for each participant group are presented in Table 12-2.

Table 12-2 Table of NAFLD-related genotype frequencies across participant groups (PNPLA3, TM6SF2, MBOAT7).

		Participant Group			
		NOC (n=16)	**All BS (n=23)**	**SG (n=12)**	**RYGB (n=11)**
Gene	Genotype	Number (n=), Frequency (%)			
PNPLA3	Wild-type	n=8, 50%	n=16, 69.56%	n=8, 66.67%	n=8, 72.73%
	Heterozygote	n=8, 50%	n=7, 30.43%	n=4, 33.33%*	n=3, 27.27%
	Common variant (I148M)	n=0, 0%	n=0, 0%	n=0, 0%	n=0, 0%
TM6SF2	Wild-type	n=14, 87.5%	n=20, 86.96%	n=10, 83.33%	n=10, 90.90%
	Heterozygote	n=2, 12.5%	n=3, 13.04%	n=2, 16.67%	n=1, 9.09%
	Common variant (E167K)	n=0, 0%	n=0, 0%	n=0, 0%	n=0, 0%
MBOAT7	Wild-type	n=7, 43.75%	n=8, 34.78%	n=5, 41.67%	n=3, 27.27%
	Heterozygote	n=7, 43.75%	n=10, 43.48%	n=2, 16.67%	n=8, 72.72%
	Common variant (rs641738)	n=2, 12.5%	n=5, 21.74%	n=5, 41.67%	n=0, 0%

**1 participant did not complete post-diet visit, so PNPLA3 genotypes balanced between surgical groups for subsequent paired analyses in this chapter.*

12.3.2 Effects of preoperative low energy diet: anthropometric and physiological measures

A range of anthropometric and physiological measures were recorded at each research visit. Trained research nurses recorded these data to ensure methodological consistency and

calibrated instruments were used throughout. The effects of the preoperative low energy diet on these outcome measures are reported in Table 12-3. Participants in the BS group were compared to NOC participants on completion of this dietary intervention.

Table 12-3 Table of anthropometric and physiological measures before (V1) and after (V2) preoperative low energy diet in participants scheduled for bariatric surgery

	Participant Group						
	BS					**NOC**	
Outcome measure	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V2 *vs* NOC**
	Mean (SD)	*Mean (SD) / Median (IQR)*	*Mean/Median (SD)*			*Mean (SD)*	*p value*
Total Body Weight (TBW) (kg)	128.9 (12.96)	123.2 (11.51)	-5.65 (2.36)	-6.70 to -4.60	<0.0001	67.23 (8.56)	<0.0001
BMI (kg/m^2)	47.56 (4.56)	45.5 (4.24)	-2.07 (0.83)	-2.43 to -1.70	<0.0001	25.06 (3.10)	<0.0001
Waist circumference (cm)	133.4 (9.88)	125.2 (11.49)	-8.21 (6.28)	-10.99 to -5.42	<0.0001	85.28 (9.51)	<0.0001
Systolic Blood Pressure (BP) (mmHg)	130.6 (11.36)	127.5 (13.3)†	-5	N/A	0.2247	130.9 (18.69)	0.5668

	Participant Group						
	BS					NOC	
Outcome measure	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V2 *vs* NOC**
	Mean (SD)	*Mean (SD) / Median (IQR)*	*Mean/Median (SD)*			*Mean (SD)*	*p value*
Diastolic BP (mmHg)	81.95 (7.0)	80.27 (7.79)	-1.68 (9.05)	-5.69 to 2.33	0.3931	76.63 (8.07)	0.1690
Heart rate (bpm)	72.45 (8.57)	65.18 (8.10)	-7.27 (8.06)	-10.85 to -3.70	0.0004	68.69 (9.71)	0.2336
Max. dominant hand grip strength (kg)	32.85 (6.93)	33.47 (7.17)	0.62 (3.43)	-0.90 to 2.14	0.4079	31.17 (7.14)	0.3346
Max. non-dominant hand grip strength (kg)	32.62 (6.43)	32.95 (7.06)	0.33 (2.21)	-0.65 to 1.31	0.4946	28.77 (7.19)	0.0882

BS Group, n=22 paired values for each outcome measure. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to post-diet (V2) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of the preoperative diet on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V2 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

Significant improvements in a range of anthropometric measures such as BMI and waist circumference were observed, as shown in Table 12-3. The systolic blood pressure of NOC participants was noted to be elevated relative to published normal ranges. Whilst there was also a significant decrease in total lean mass on DXA (Table 12-5), this was not associated with a functional decrease in upper limb grip strength, which increased non-significantly in dominant and non-dominant hands (Table 12-3). A training effect, with increased participant familiarity with the hand dynamometer and study protocol on the Post-Diet visit may provide a partial explanation for this finding.

12.3.3 Effects of preoperative low energy diet: Imaging Investigations

Additionally, a range of imaging investigations were performed to characterise organ-specific effects of our interventions. MR Spectroscopy was used to measure liver steatosis percentage (primary outcome measure for the study), multiparametric MRI to assess liver fibro-inflammation (T1 relaxation time corrected for iron content, cT1) and hepatic elastography was used as an imaging biomarker of liver fibrosis. Additionally, pancreatic steatosis was measured by finding the average MRI-Proton Density Fat Fraction (PDFF) across three regions of interest (head, body and tail). Changes in these outcome measures are recorded in Table 12-4 and Post-Diet values were also compared to those in the NOC group.

Table 12-4 Table of imaging outcome measures before (V1) and after (V2) preoperative low energy diet in participants scheduled for bariatric surgery

	Participant Group						
	BS					**NOC**	
Outcome measure	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V2 vs NOC**
	Mean (SD)/ Median (IQR)	*Mean (SD)/ Median (IQR)*				*Mean (SD)/Median (IQR)*	*p value*
Liver steatosis (%)	13.02 (10.28)	6.90 (10.89)†	-2.15	N/A	0.0003	3.21 (4.25)†	0.0093
Whole liver cT1 relaxation time (ms) *n=19 pairs*	794 (112)†	797.4 (86.33)	-9.00	N/A	0.2535	758.1 (85.92)	0.199
Liver elasticity (kPa)	10.85 (10.89)†	8.00 (6.83)†	-0.95	N/A	0.0622	3.81 (0.67)	<0.0001
Pancreatic steatosis (%) *n=19 pairs*	5.14 (4.38)†	5.83 (2.97)	-0.18	N/A	0.9217	*n=15:* 2.49 (2.74) †	0.0076

BS Group, n=22 paired values for each outcome measure unless otherwise specified. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to post-diet (V2) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of the preoperative diet on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V2 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

Table 12-4 shows that there was a marked decrease in hepatic steatosis during the preoperative diet but that liver fat remained elevated compared to NOC participants. The preoperative diet did not lead to a statistically significant change in any of our other imaging outcome measures.

DXA scanning was also performed as this is considered the 'gold standard' technique for body composition analysis. Changes in body composition due to the low calorie diet are reported in Table 12-5 and post-dietary values are compared to those observed in the NOC group.

Table 12-5 Table of DXA scan-derived anthropometric outcome measures before (V1) and after (V2) preoperative low energy diet in participants scheduled for bariatric surgery

	Participant Group						
	BS					**NOC**	
Outcome measure	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit *(n=16)***	**BS V2 vs NOC**
	Mean (SD)	*Mean (SD) / Median (IQR)*				*Mean (SD)*	*p value*
Visceral fat mass (kg) *n=16**	2.53 (0.83)	2.22 (0.59)	-0.30	-0.59 to -0.009	0.0443	0.64 (0.44)	<0.0001
Android fat mass (kg)	6.91 (1.34)	6.51 (1.14)†	-0.36	N/A	0.0391	1.96 (7.38)	<0.0001
Android fat (%)	63.39 (3.56)	63.89 (4.21)	0.50 (2.45)	-0.59 to 1.58	0.3542	40.05 (10.43)	<0.0001
Gynoid fat mass (kg)	10.65 (1.69)	9.77 (2.34)†	-0.61	N/A	0.0004	4.17 (0.85)	<0.0001
Gynoid fat (%)	55.1 (3.89)	56.2 (7.15)†	-0.60	N/A	0.7805	40.56 (4.71)	<0.0001

	Participant Group						
	BS					NOC	
Outcome measure	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit *(n=16)***	**BS V2 vs NOC**
	Mean (SD)	*Mean (SD) / Median (IQR)*				*Mean (SD)*	*p value*
Android/Gynoid fat ratio	0.65 (0.11)	0.65 (0.10)†	0.01	N/A	0.5661	0.47 (0.16)	<0.0001
Total fat mass (kg)	68.23 (8.46)	64.63 (8.71)	-3.63	-5.32 to -1.95	0.0002	24.80 (5.94)	<0.0001
Total tissue fat (%)	55.31 (2.88)	55.36 (2.83)	0.05	-0.77 to 0.87	0.9003	38.25 (6.11)	<0.0001
Total arms lean mass (kg)	5.13 (1.14)	4.99 (1.35)	-0.14	-0.93 to 0.65	0.7153	4.24 (0.76)	0.0515
Total lean mass(kg)	54.88 (5.00)	51.86 (5.09)	-3.02	-5.27 to 0.77	0.0109	39.54 (4.98)	<0.0001

*BS Group, n=22 paired values for each outcome measure unless otherwise specified. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to post-diet (V2) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of the preoperative diet on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V2 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data. *n=16 for paired visceral fat measurements as these are only obtained when whole patient (rather than half) is contained within the DXA scanning field.*

Table 12-5 shows that reductions in both lean and fat mass were observed after the preoperative dietary intervention and that there was therefore no change in percentage fat mass. Increased baseline lean mass in participants with obesity was confirmed. Lean mass

preservation throughout the study would be beneficial for reducing peripheral insulin resistance and maximising basal metabolic rate to promote body weight loss.

12.3.4 Effects of preoperative low energy diet: indirect calorimetry

Indirect calorimetry was performed at each research visit after an overnight fast (T0) and repeated 120min after consumption of the test meal (T120). Changes in energy expenditure and respiratory quotient due to the preoperative diet are summarised in Table 12-6 and Post-Diet changes are compared to NOC values.

Table 12-6 Table of indirect calorimetry outcome measures before (V1) and after (V2) preoperative low energy diet in participants scheduled for bariatric surgery

	Participant Group						
	BS					**NOC**	
Outcome measure	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit *(n=16)***	**BS V2 vs NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
T0* Energy Expenditure (kcal/24hrs)	1704 (278.2)	1577 (221.0)	-126.9	-255.0 to 1.27	0.0521	1243 (204.7)	<0.0001
T120* Energy Expenditure (kcal/24hrs)	1688 (303.7)	1641 (325.4)	-46.63	-203.2 to 110.0	0.5415	1254 (217.9)	0.0002
T0* Respiratory Quotient	0.79 (0.10)†	0.73 (0.10)	-0.03	N/A	0.0144	0.86 (0.17)†	0.0142
T120* Respiratory Quotient	0.76 (0.11)†	0.73 (0.12)†	-0.03	N/A	0.1698	0.85 (0.08)	0.0060

BS Group, n=21 paired values for each outcome measure. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to post-diet (V2) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of the preoperative diet on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V2 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.T0=fasting, (immediately before chocolate drink consumption), T120=postprandial (120min after chocolate drink consumption)

Table 12-6 shows that fasting respiratory quotient (RQ) decreased on completion of the preoperative diet, which is suggestive of a metabolic switch from carbohydrate to fat substrate oxidation.

12.3.5 Effects of preoperative low energy diet: fasting biochemical measures

A range of biochemical parameters were measured after an overnight fast and the effects of the preoperative dietary intervention on these are shown in Table 12-7.

Table 12-7 Table of fasting biochemical outcome measures before (V1) and after (V2) preoperative low energy diet in participants scheduled for bariatric surgery

	Participant Group						
	BS					**NOC**	**BS vs NOC comparisons**
Fasting clinical chemistry (plasma).	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V2 *vs* NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
ALT (IU/L)	18.15 (13.6) †	24.20 (15.85) †	4.450	N/A	0.0553	17.00 (11.5)†	0.0588
AST (IU/L)	16.85 (6.1)†	21.35 (11.75) †	4.35	N/A	0.0172	23.45 (9.00)	0.8208
ELF test *n=19 pairs*	8.12 (0.87)	7.89 (0.73)	-0.23	-0.49 to 0.03	0.0773	7.99 (0.74)	0.4688
Glucose (mmol/L)	5.94 (1.33)†	5.56 (0.52)	-0.44	N/A	<0.0001	5.66 (1.23)†	0.4965
Insulin (mU/L)	12.84 (13.89)†	13.23 (14.62)†	-0.92	N/A	0.7024	5.53 (5.38)†	<0.0001

		Participant Group					
	BS					**NOC**	**BS vs NOC comparisons**
Fasting clinical chemistry (plasma).	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V2 *vs* NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
Beta cell Function: Homeostatic Model Assessment 2 (HOMA2) %B	111.9 (57.5)	137.7 (70.2)	25.8	1.2 to 52.9	0.0601	59.15 (31.5)†	0.0001
Insulin sensitivity: HOMA2 %S	58.9 (31.6)	56.5 (25.4)	-2.3	-14.8 to 10.2	0.7028	135.4 (123.0)†	<0.0001
Non-Esterified Fatty Acids (NEFA) (µmol/L)	795.8 (248.8)	1017.0 (278.4)	221.6	104.4 to 338.8	0.0008	801.2 (243.1)	0.0176
TAG (µmol/L)	1184 (1470.7)†	983.1 (866.5)†	-212.4	N/A	0.0542	1267 (546.4)	0.3560
Lactate (mmol/L)	0.96 (0.34)	0.77 (0.20)	-0.19	-0.32 to -0.05	0.0092	0.82 (0.19)	0.4948
Hydroxybutyrate (OHB) (µmol/L)	93.7 (84.3)†	249.0 (306.6)†	162.4	N/A	0.0002	64.05 (114.75)†	0.0001
Total chol. (mmol/L)	5.00 (1.09)	4.21 (1.02)	-0.79	-1.11 to -0.48	<0.0001	4.95 (1.31)	0.0608
HDL chol. (mmol/L)	1.21 (0.22)	1.03 (0.20)	-0.18	-0.23 to -0.13	<0.0001	1.31 (0.33)	0.0021

	Participant Group						
	BS					**NOC**	**BS vs NOC comparisons**
Fasting clinical chemistry (plasma).	**Pre-diet visit (V1)**	**Post-diet visit (V2)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V2 *vs* NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
Total chol./HDL ratio	4.28 (1.23)	4.18 (0.97)	-0.95	-0.43 to 0.24	0.5677	3.96 (1.3)	0.5491
Apolipoprotein B (ApoB) (g/L)	0.94 (0.29)	0.84 (0.24)	-0.10	-0.18 to -0.02	0.0202	0.89 (0.29)	0.5261
VLDL ApoB (g/L)	0.12 (0.18)†	0.13 (0.11)†	0.03	N/A	0.4482	0.16 (0.06)	0.5936
Chylomicron TAG (µmol/L)	20.96 (62.19)†	11.42 (25.00)†	-10.61	N/A	0.0008	8.24 (24.35)†	0.2490
VLDL TAG (µmol/L)	558.0 (815.3)†	468.7 (577.8)†	-131.3	N/A	0.1207	609.9 (434.2)	0.4918

BS Group, n=22 paired values for each outcome measure unless otherwise specified. Fasting values for NOC participants (n=16 unless otherwise specified) also shown and compared to post-diet (V2) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of the preoperative diet on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V2 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

Table 12-7 shows changes in a range of fasting parameters that taken together are suggestive of a metabolic shift towards increased fat rather than carbohydrate metabolism and improvement in a range of metabolic health markers. There is a somewhat paradoxical increase in liver enzyme levels that has also been reported in other studies of low calorie dietary interventions in individuals without severe obesity[244,245]. This perhaps reflects

transient metabolic stress as hepatic fat is mobilised and oxidised. Interestingly, one previous study reported a sexually dimorphic liver enzyme response to low calorie diet, with increased levels observed in females but not in males[246]. More research will be required to determine the clinical significance and underlying aetiology of these changes.

12.3.6 Effects of preoperative low energy diet: postprandial biochemical measures

In addition to fasting values, postprandial responses were characterised for outcome measures known to vary in response to feeding. These biochemical metabolites were measured repeatedly over the 6-hour postprandial period. Total, Chylomicron and VLDL TAG responses to the standardised test meal before and after the preoperative low energy diet are shown in Figure 12-3.

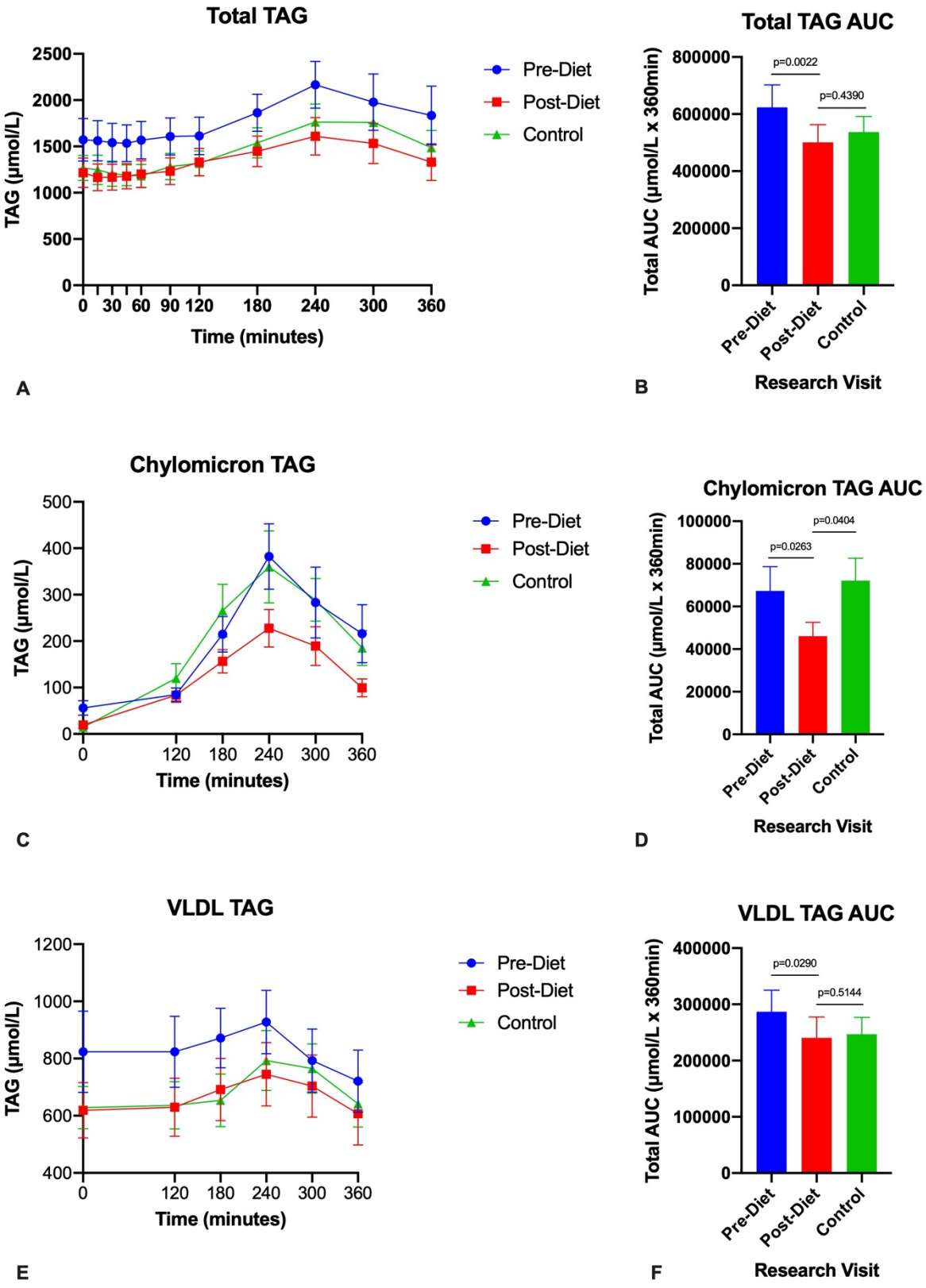


Figure 12-3: Postprandial peripheral venous (A) total triacylglycerol (TAG) level and (B) total TAG Area Under the Curve (AUC) in response to standardised test meal consumed before and after routine preoperative low energy diet. First samples taken after overnight fast, timing for subsequent samples initiated after complete

ingestion of test meal. (C) Postprandial chylomicron TAG changes, (D) chylomicron TAG AUC, (E) VLDL TAG changes and VLDL TAG AUC (F) also recorded. Data plotted are mean ± SEM, n=21 for bariatric group (Pre-diet and Post-diet), n=16 for control group. AUCs compared in (B), (D) and (F) with paired analysis for Pre-Diet and Post-Diet visits, and unpaired analysis to compare Post-Diet and Control AUCs. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 12-8: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison					
	Pre-Diet (V1) vs Post-Diet (V2)			Post-Diet (V2) vs Control		
Variable	Total TAG	Chylo TAG	VLDL TAG	Total TAG	Chylo TAG	VLDL TAG
Variation Source	p value			p value		
Participant Group/Research Visit	0.0237	0.0184	0.0611	0.7373	0.0258	0.8838
Postprandial Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	0.1258	0.0889	0.4550	0.4516	0.2677	0.6003

Figure 12-3 and Table 12-8 show that the preoperative diet was sufficient to restore total, VLDL and chylomicron TAG concentrations to levels similar to or below those seen in NOCs despite ongoing obesity. The shape of the postprandial response curves remained similar, so changes in AUCs were largely driven by reductions in fasting TAG concentrations rather than in the incremental response to the test meal.

Plasma concentrations of several other relevant biochemical parameters were also measured during the postprandial period. The results are shown in Figure 12-4:

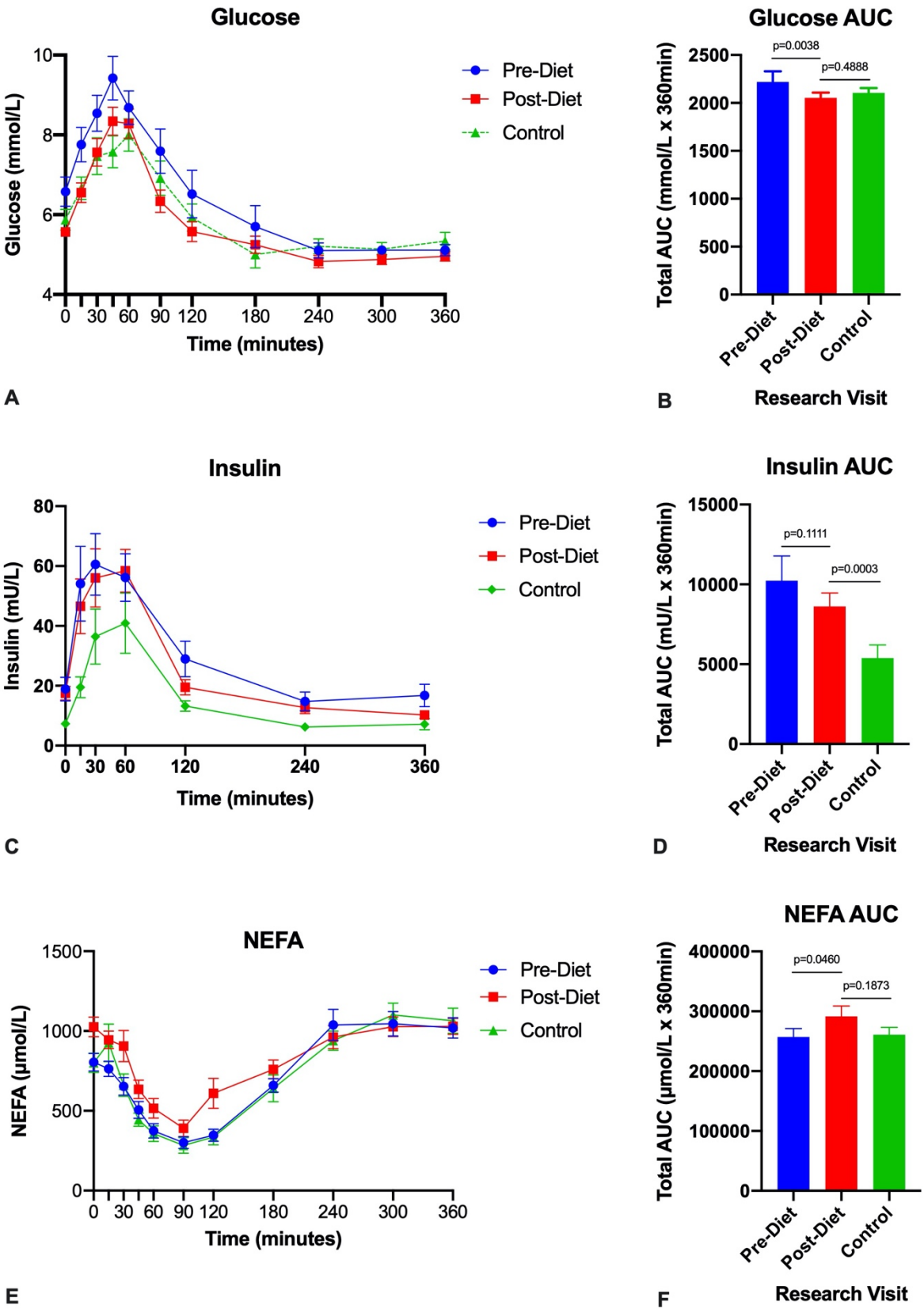


Figure 12-4: Postprandial peripheral venous (A) Glucose, (C) Insulin and (E) NEFA levels in response to standardised test meal. First samples taken after overnight fast, timing for subsequent samples initiated after complete ingestion of test meal. AUCs for Glucose (B), Insulin (D) and NEFA (E) also calculated and

compared. Data plotted are mean ± SEM, n=21 for bariatric group (Pre-diet and Post-diet), n=16 for control group. AUCs compared in (B), (D) and (E) using paired analysis for Pre-Diet and Post-Diet visits, and unpaired analysis to compare Post-Diet and Control AUCs. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 12-9: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison					
	Pre-Diet (V1) vs Post-Diet (V2)			Post-Diet (V2) vs Control		
Variable	Glucose	Insulin	NEFA	Glucose	Insulin	NEFA
Variation Source	p value			p value		
Participant Group/Research Visit	0.0327	0.2104	0.0017	0.7112	0.0182	0.0819
Postprandial Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	0.0492	0.8484	<0.0001	0.2305	0.1675	0.0168

Figure 12-4 and Table 12-9 show a significant decrease in glucose AUC in our non-diabetic group of participants with obesity on diet completion that sufficient to normalise this outcome measure compared to NOCs. There was a corresponding increase in NEFA, which may be due to a negative energy balance leading to increased lipolysis of stored fat in order to release NEFA as a substrate for oxidation. The observed reduction in insulin AUC did not reach statistical significance.

Additionally, lactate was measured since it is a product of carbohydrate metabolism, and 3-hydroxybutyrate as a marker of hepatic fatty acid oxidation. The stable isotope ^{13}C-palmitate was included in the test meal and $^{13}CO_2$ was collected by breath sampling during the post-prandial period. Expired $^{13}CO_2$ was adjusted for lean mass to determine whole-body fatty acid oxidation of the test meal.

Figure 12-5 shows diet-induced changes in these parameters across the postprandial period.

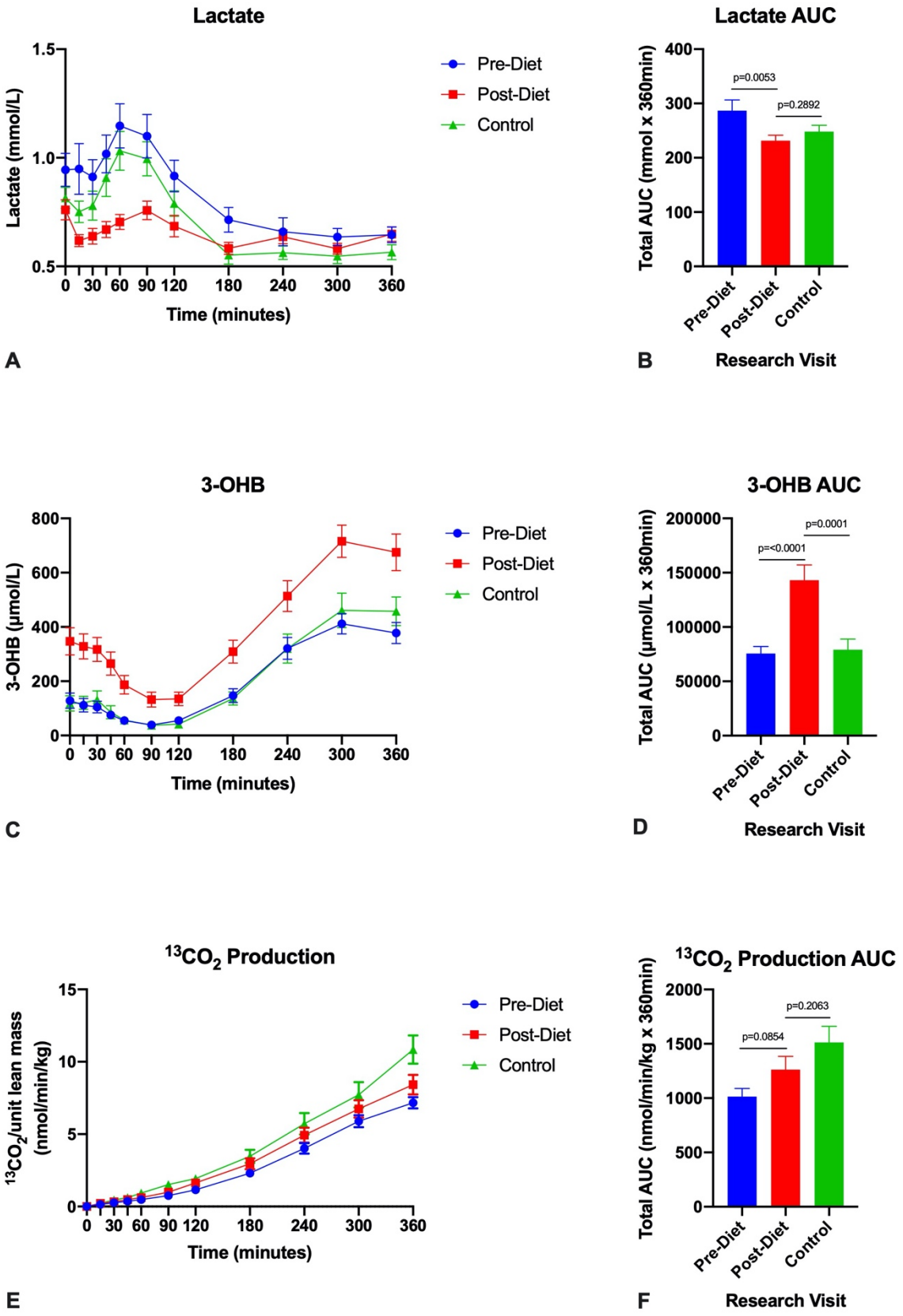


Figure 12-5 Postprandial peripheral venous (A) Lactate, (C) 3-OHB and (E) lean mass adjusted $^{13}CO_2$ production levels in response to standardised test meal. First samples taken after overnight fast, timing for

subsequent samples initiated after complete ingestion of test meal. AUCs for Lactate (B), 3-OHB (D) and $^{13}CO_2$ production (F) also calculated and compared. Data plotted are mean ± SEM, n=21 for bariatric group (Pre-diet and Post-diet), n=16 for control group, except for $^{13}CO_2$ production (n=20 for Pre-diet and Post-Diet visits, n=12 for Control group). AUCs compared in (B), (D) and (E) using paired analysis for Pre-Diet and Post-Diet visits, and unpaired analysis to compare Post-Diet and Control AUCs. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 12-10: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison					
	Pre-Diet (V1) vs Post-Diet (V2)			Post-Diet (V2) vs Control		
Variable	Lactate	3-OHB	$^{13}CO_2$ Production	Lactate	3-OHB	$^{13}CO_2$ Production
Variation Source	p value			p value		
Participant Group / Research Visit	0.0021	0.0002	0.0731	0.0599	0.0009	0.1406
Postprandial Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	<0.0001	<0.0001	0.0181	<0.0001	0.0694	0.0248

Figure 12-5 and Table 12-10 show a decrease in lactate and increase in 3-OHB after the preoperative diet. These are, respectively, products of carbohydrate and fat oxidation so these findings are consistent with changes in labelled palmitic acid oxidation and in other outcome measures that suggest an increased reliance on fat oxidation to meet energy demands.

12.4 Discussion

12.4.1 Principal findings

In this chapter I aimed to study the effects of a routine 3-week 900kcal carbohydrate-restricted preoperative diet in bariatric surgical patients on liver steatosis, a range of fasting

and postprandial plasma metabolites and anthropometric measures. This chapter's results demonstrate that this diet leads to a significant reduction in liver steatosis and significant improvements in a range of metabolic health markers, such as plasma glucose, in the fasting and postprandial states. Metabolic effects of the preoperative diet were also seen across the postprandial period and it appears that the preoperative diet promotes a shift from carbohydrate substrate utilisation towards fatty acid oxidation, as evidenced by increased 3-OHB, $^{13}CO_2$ production and decreased lactate levels. However, increased ALT and AST levels were also observed, which may reflect transient hepatic injury or stress.

12.4.2 Interpretation and comparison with previous studies

In this chapter, liver steatosis significantly reduced from baseline levels following the routine preoperative diet (Table 12-4). This result is supported by previous MR-focused studies that measured liver fat percentage before and after similar dietary interventions in individuals with severe obesity[247-249]. This is likely because an achieved negative energy balance decreased hepatic triglyceride deposition and increased hepatic fat oxidation and mobilisation. Non-significant reductions in liver cT1 relaxation time (a biomarker of fibro-inflammation), ELF score and liver elasticity (biomarkers of fibrosis) were also observed in the present study. It has previously been reported that diet-induced T2D remission requires reductions in both hepatic and pancreatic steatosis[158]. In our non-diabetic subjects with obesity, we observed increased baseline hepatic and pancreatic steatosis relative to non-obese controls and the preoperative diet improved glucose AUC. Whilst hepatic steatosis reduced, which is likely to have decreased hepatic insulin resistance, there was no change in pancreatic steatosis after our dietary intervention. Our diet also reduced VLDL-triglyceride AUC, reflecting reduced liver fat export, in accordance with another study[250]. Taken together, it appears that reduced hepatic steatosis made a greater contribution to improved glucose homeostasis than changes in pancreatic steatosis, and that reversal of pancreatic steatosis may be unnecessary to normalise glycaemic control in our non-diabetic participants.

However, significant increases in AST, and non-significant increases ALT levels were seen following the dietary intervention (Table 12-7) in accordance with previously reported similar findings after preoperative low energy diet[249]. These results may reflect rapid

lipolysis in visceral fat (which decreased on DXA body composition analysis in Table 12-5) causing a degree of liver injury, as fatty acids and proinflammatory cytokines are delivered directly to the liver via the hepatic portal vein, and whilst increased hepatic fatty acid oxidation may lead to an altered hepatocellular redox state[251].

One previous study has suggested that the PNPLA3 p.I148M allele, known to be associated with hepatic steatosis, independently predicts a greater reduction in liver fat after bariatric surgery[252]. The same study found no relationship between TM6SF2 or MBOAT7 variant alleles and the presence of hepatic steatosis or the response to surgery. The present study's randomisation protocol was designed to result in similar characteristics between participant groups, and Table 12-2 confirms that PNPLA3 genotypes were balanced between SG and RYGB participants. We did not aim to assess genotype-phenotype interactions as this would have required a much larger sample size.

There were significant reductions in chylomicron, VLDL and plasma TAG AUCs during the 360 minute period measured after consumption of the test meal (on completion of the preoperative diet. Correspondingly, atherogenic Apolipoprotein B was also found to have decreased in plasma. Fasting glucose and glucose AUC were reduced, with increased lipolysis reflected in elevated fasting and postprandial plasma NEFA. The magnitude of this effect was sufficient to normalise several outcome measures relative to NOCs despite relatively modest weight loss over this 3-week diet. This suggests that BMI normalisation may not be required to address impaired metabolic health in individuals with severe obesity.

In accordance with a previous study assessing the effects of a short term carbohydrate-restricted diet[253], the dietary intervention in this study was associated with a reduction in fasting respiratory quotient suggestive of increased lipid oxidation as shown in Table 12-6. This is supported by increased fasting and postprandial plasma 3-OHB, and reduced fasting and postprandial lactate (Figure 12-5). These observations are likely due a negative energy balance, decreased hepatic *de novo* lipogenesis due to carbohydrate restriction, and increased NEFA availability due to lipolysis. Additionally, Figure 12-5 reveals an interaction effect between Pre-Diet and Post-Diet $^{13}CO_2$ production and elapsed time during the

postprandial study visit that reflects increased test meal fatty acid oxidation its latter stages. Indeed, CO_2 production due to FA may be underestimated, as increased $^{12}CO_2$ production (due to non-test meal FA oxidation during the postprandial visit) would dilute the tracer to tracee ratio (TTR: $^{13}CO_2/^{12}CO_2$) upon which these oxidation calculations are based. However, this effect may be balanced by the observed decrease in participant lean mass, which $^{13}CO_2$ production is corrected against. As expected, fasting and postprandial energy expenditure were increased in the participants with obesity in the intervention group compared to non-obese controls and there were non-significant energy expenditure decreases towards control group values after caloric restriction.

Since this routine preoperative dietary intervention has been shown to have effects on NAFLD and on fasting and postprandial metabolism, these diets should be described in studies in the bariatric surgical literature along with the timing of baseline measurements in relation to them. It may be preferable for participants in future studies that aim to determine the specific effects of bariatric surgery on NAFLD to omit preoperative very low calorie diet if considered safe from a clinical and ethical perspective. Additionally, if longitudinal liver histology is to be obtained then this may ideally be taken before any dietary intervention if measurement of changes in steatosis is planned. If the focus of longitudinal histological analysis of liver tissue is liver fibrosis measurements, then the preoperative diet may be less relevant because changes in fibrosis are known to be less rapid. An alternative methodology for defining surgery-specific effects in future studies could be the inclusion of a control group that undergoes only the preoperative diet, and this group that can be compared to a surgical participants (with similar baseline characteristics) who undergo the same preoperative diet followed by bariatric surgery.

12.4.3 Strengths and limitations

As shown in Table 12-1, the strengths of this study include that the bariatric and NOC participant groups were well matched for sex, age and diabetes status. Additionally, the stratified randomisation protocol was effective, and participants scheduled to undergo SG or RYGB were well matched for these variables and for BMI, ALT and NAFLD-related genotype. The number of participants dropping out from the study was also lower than expected, suggesting that studies with this design are feasible in this participant population.

In this study, both fasting and postprandial chylomicron and VLDL lipoprotein fractions were obtained by density gradient ultracentrifugation with adherence to established protocols. Hyperlipidaemia is associated with cardiovascular disease risk and lipid levels are often measured whilst fasting. However, it is increasingly recognised that people with obesity are usually in a physiologically postprandial state, and a 12-hour fast may be required for plasma triglycerides to return to fasting levels[254]. This chapter demonstrates that this dietary intervention alone is sufficient to restore total, chylomicron and VLDL triglycerides to similar or even lower levels than those observed in non-obese control participants, largely by reducing fasting baseline concentrations. It is therefore important that future bariatric surgical studies that measure lipoprotein metabolism define the timing of their baseline measurements and describe whether a preoperative diet was prescribed. Whilst triglyceride AUCs decreased following low calorie diet, the shape of the postprandial response remained similar, and it is possible that anatomical manipulations during bariatric surgery alter kinetics more markedly.

Whilst this study design reduces the number of potential confounding factors in subsequent analyses, it is acknowledged that more advanced NAFLD is often seen in people with co-existent diabetes and we were not able to include the full spectrum of NAFLD severity. Additionally, whilst a recent national registry analysis reported that the sex of approximately 80% patients undergoing bariatric surgery in the UK was female[255], it is uncertain to what extent our findings from this will be applicable to male patients as sex-related differences in metabolism may exist.

A pragmatic approach to this low calorie carbohydrate-restricted dietary intervention was adopted. This diet is routinely used as part of NHS clinical care, and therefore provides 'real world' data, but food was not provided for participants. Instead, patient education sessions were provided by specialist dieticians along with printed information sheets. Dietary questionnaires were completed at research visits instead of comprehensive food diaries to reduce the burden on participants and since the reliability of self-reported food intake is uncertain. This chapter's results, which include weight loss, reduced lactate levels and increased fatty acid oxidation, are suggestive of adherence to the low energy carbohydrate-

restricted dietary intervention. Broadly comparable weight loss[203,256] and hepatic steatosis reduction[257] rates were achieved to those previously reported, although low calorie diet composition and duration vary quite markedly across studies.

Changes in the liver were assessed non-invasively at study visits rather than using 'gold standard' liver biopsy histology. The reason for this is that obtaining liver biopsy tissue is associated with some clinical risk, and it was felt unethical to expose participants to this on four occasions in a period of just over 1 year. However, we have used a range of imaging and serological biomarkers to characterise liver phenotype, and our primary outcome was measured using MR spectroscopy, which has been well validated in previous studies. Some of the other approaches, such as Fibroscan, are technically challenging may be less reliable in people with severe obesity, but a trained operator performed all scans.

There were some additional challenges associated with studying individuals with severe obesity scheduled for bariatric surgery. It was necessary to restrict the maximum BMI and body weight of participants in the study in an effort to ensure that MR scanning could be safely achieved. In some cases, it was not possible to completely capture the participant in the DXA scanner, and half of the participant was scanned then measurements were doubled when this occurred. It was, however, not possible to measure visceral fat volume in these cases.

12.4.4 Conclusion

In this chapter it has been demonstrated that a routine low energy carbohydrate-restricted diet in patients scheduled for bariatric surgery results in significant improvements in liver steatosis, markers of metabolic health and in postprandial response to the test meal. These observations are of value in better defining the effects of this intervention, which is frequently prescribed in bariatric surgical centres. It appears that intraoperative liver biopsies taken low calorie diet completion are likely to underestimate the baseline prevalence and severity of steatosis and NAS in bariatric surgical patients. These results highlight the importance of clearly defining the timing of baseline measurements and of recording the utilisation and composition of any preoperative diet co-intervention in bariatric surgical research studies.

It is uncertain whether this diet would be sustainable for a longer period of time since previous studies of low calorie dietary interventions have reported weight regain[63]. However, our routine 3-week 900kcal carbohydrate-restricted diet resulted in preoperative improvements in NAFLD and in a range of metabolic health markers, with measurements after this intervention comparable to the NOC group for some outcome measures.

13 Effects of Bariatric Surgery on NAFLD

13.1 Introduction

13.1.1 Background

In the previous chapter, it was shown that a 3-week low energy carbohydrate-restricted diet had beneficial effects on NAFLD and metabolic health in individuals with severe obesity. However, substantial weight regain is usual after such diets, and their long term palatability and sustainability is uncertain[258]. Additionally, it is unclear whether the 5-10% total body weight loss recommended in national clinical guidelines[259] is sufficient to stimulate NAFLD regression in individuals with severe obesity such as those undergoing bariatric surgery.

Bariatric surgery has now been established as the most effective treatment for achieving weight loss and its effects have been shown to be durable in longitudinal studies[260]. It not only results in reduced obesity-associated morbidity and mortality, but additionally promotes histological NAFLD improvement[186]. It is unknown, however, whether differences in liver metabolism and NAFLD severity that may exist pre-operatively are normalised relative to non-obese control individuals after surgery.

Approximately half of individuals undergoing bariatric surgery have T2D, which is recognised as a risk factor for advanced NAFLD severity. Bariatric surgery is an effective intervention for T2D, and diabetes medications can often be reduced or eliminated postoperatively. Its 5-year superiority to non-surgical T2D treatment has now been established in randomised clinical studies[44,234]. Whilst reduced energy intake appears likely to be an important reason for the T2D remission often achieved after bariatric surgery, additional contributory mechanisms have been suggested. These include incretin effects induced by changes in gut peptide hormones (such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY)[261,262]), and synthetic GLP-1 analogues have subsequently been approved as treatments for obesity and T2D. However, PYY receptors are not known to be expressed in the liver, and the presence of hepatic GLP-1 receptors has been inconsistently reported[263,264]. Therefore, different humoral factors may be responsible for NAFLD improvement after bariatric surgery than those promoting T2D remission. The mechanisms underpinning the histological improvement in patients with NAFLD have not been examined. An enhanced understanding

of the effects of bariatric surgical procedures on NAFLD, at matched weight loss to control for this confounding factor, would provide important mechanistic insight into the ways in which bariatric surgery can improve liver outcome.

It is also unclear whether SG and RYGB surgery have differential effects on NAFLD since previous studies have had non-randomised retrospective designs and often used surrogate markers with poor diagnostic accuracy such as isolated changes in liver blood tests. Some studies have suggested that SG has a greater effect[179], some found no difference between procedures[180] and others have suggested that RYGB is more beneficial[265]. These studies have concluded that there is a need for randomised studies designed to assess whether differential changes in NAFLD may occur after alternative types of bariatric surgery but no such study has previously been conducted. Answering this question would aid clinical practice by personalising the choice of surgical intervention according to an individual's metabolic disease profile, but it is currently unclear whether such a study is feasible.

13.1.2 Aims

The key aims of this chapter are:

1) To define the effects of bariatric surgery (SG or RYGB) on liver fat in bariatric surgical patients

2) To define the effects of bariatric surgery (SG or RYGB) on fasting and postprandial plasma metabolites

3) To define the changes in body composition (total and regional muscle and fat mass) and grip strength after bariatric surgery (SG or RYGB)

4) To define differences in the above outcome measures for participants with obesity before and after bariatric surgery (SG or RYGB), and 'normal' results, as recorded from non-obese control participants

5) To determine whether differences in outcome measures occur after matched (20%) postoperative weight loss in the SG and RYGB participant groups and whether randomisation to these interventions for a NAFLD-focused study is feasible

13.2 Patients and Methods

13.2.1 Research strategy

This chapter assesses the effects of bariatric surgical intervention (SG or RYGB surgery) on NAFLD. It represents a continuation of the Effects of Dietary Intervention and Surgery on NAFLD (EDISON) study, the dietary component of which was discussed in the previous chapter.

13.2.2 Study design, methods and statistical analysis

The EDISON study design, methodology and statistical analysis plan for this chapter were described in the previous chapter so will not be repeated in detail here.

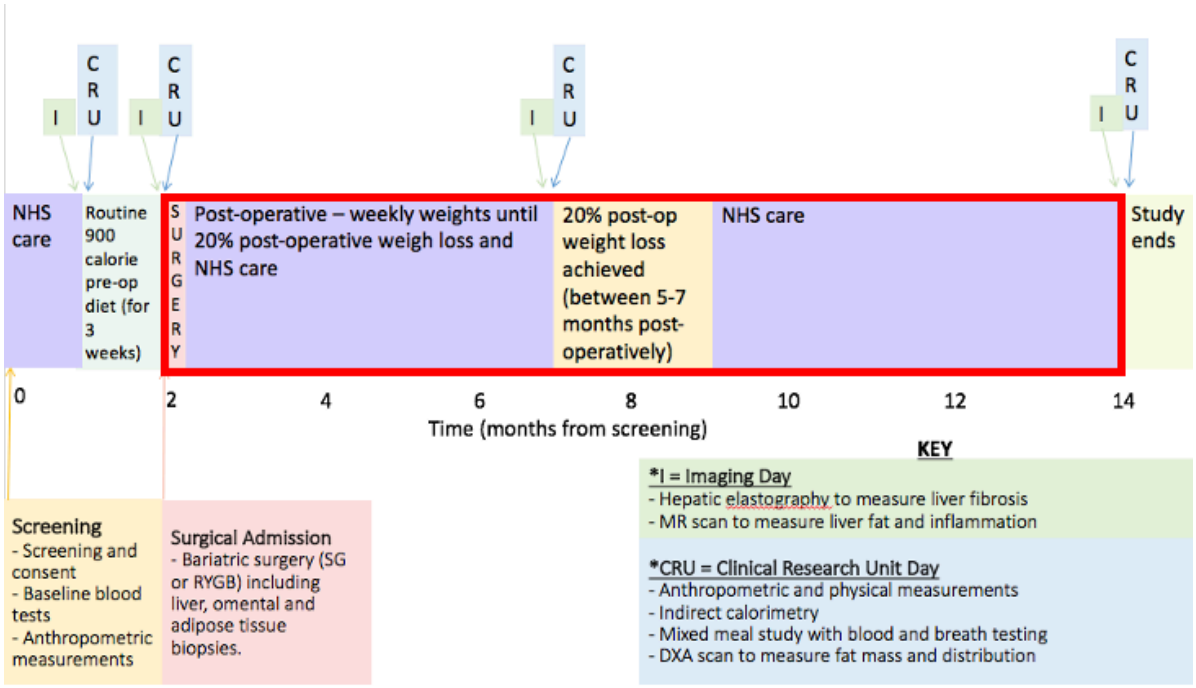


Figure 13-1: Schematic representation of overall EDISON study design. The study phases analysed and discussed in this chapter are contained within red outline.

In the current chapter, the study's outcome measures were compared just before surgery, at the end of the preoperative dietary intervention (Post Diet visit/Research Visit 2) and at

20% total weight loss after bariatric surgery. The study's primary end point was reached at this time point. Preliminary data is also presented from the final follow-up visit (Research Visit 4), which occurs 1 year after surgery. Of the 24 participants recruited to the bariatric surgical group, 1 participant dropped out before Research Visit 1 (declined allocated surgical intervention), 1 after Research Visit 1 (did not proceed to surgery). 22 participants in the BS group were therefore included in our primary endpoint analysis and were compared to 16 NOC participants.

13.3 Results

13.3.1 The effects of 20% weight loss after bariatric surgery

The 20% postoperative weight loss visit took place at a mean of 154.5 days after bariatric surgery. No participants who completed the Post-Diet research visit withdrew from the study before the 20% weight loss visit (n=22). However, only fasting blood tests were taken for one participant who had undergone an abbreviated Post-Diet visit then experienced a complicated postoperative course (staple line leak following sleeve gastrectomy resulting in extended postoperative stay and admission to the Intensive Care Unit). It was felt that conducting the full postprandial component of the protocol was inappropriate for this participant as it assessed secondary outcome measures that may not have been representative of the postoperative clinical course. If measured, postprandial values could not have been included in our pre-specified paired analyses due to a previously abbreviated post-diet visit (due to challenging venous access).

13.3.1.1 Intraoperative liver biopsy histology

Liver histology was assessed for all participants using liver biopsy tissue that was obtained during bariatric surgery. Histological assessment was performed by a consultant histopathologist with a liver interest (Dr Eve Fryer). NAFLD was assessed using the NAFLD Activity Score (NAS) and Kleiner fibrosis staging classification. Histological NAFLD severity across the participant groups is reported in Table 13-1.

Table 13-1 Table detailing intraoperative liver biopsy histology for EDISON study participants.

Histological feature	**Participant Group**			
	NOC (n=16)	**All BS (n=22)**	**SG (n=11)**	**RYGB (n=11)**
Biopsy size (mm)	5.88 (2.06)	9.18 (1.87)	8.82 (1.83)	9.55 (1.92)
Steatosis (%)	0 (5) †	24.43 (23.83)	24.55 (26.69)	24.32 (21.91)
Kleiner F0 (n=)	10/16	3/22	2/11	1/11
Kleiner F1 (n=)	6/16 (all 1c)	6/22 (all 1c)	3/11 (all 1c)	3/11(all 1c)
Kleiner F2 (n=)	0/16	12/22	5/11	7/11
Kleiner F3 (n=)	0/16	1/22	1/11	0/11
Kleiner F4 (n=)	0/16	0/22	0/11	0/11
NAS (/8)	0 (0) †	1.82 (1.47)	2 (1.67)	1.64 (1.29)
Steatosis (/3)	0 (0) †	0.95 (0.90)	0.91 (1.04)	1.00 (0.77)
Ballooning (/2)	0 (0)	0 (1)†	1 (1)†	0 (1)†
Lobular inflammation (/3)	0 (0)	0 (1)†	1 (1)†	0.27 (0.47)
Ishak Fibrosis Stage (/6)	0.44 (0.63)	1.73 (0.88)	1.64 (1.12)	2 (0) †
Ishak Grade (/18)	0 (0)	1.5 (0.80)	1.73 (0.79)	1.27 (0.79)

Data marked † is not normally distributed. For normally distributed variables, mean (SD) values are presented. For non-normally distributed variables, median (IQR) values are presented.

Table 13-1 shows a significant increase in obesity-associated hepatic steatosis in the BS compared to NOC group (median 17.5 vs 0 %, $p<0.0001$ with Mann-Whitney test). The BS group also had significantly higher median NAS (2 vs 0, $p<0.0001$) and Kleiner fibrosis stage (2 vs 0, $p<0.0001$) than the NOC group.

13.3.1.2 Effects of 20% postoperative weight loss: anthropometric and physiological measures

Anthropometric and physiological measures were again recorded after 20% post-surgical weight loss. These were compared to those recorded just before surgery in the post-diet visit in Table 13-2. Outcome measures after 20% postoperative weight loss were also compared to those recorded in the NOC group.

Table 13-2 Table of anthropometric and physiological measures on completion of a routine pre-operative low energy diet before bariatric surgery (V2) and after 20% surgically induced total body weight (TBW) loss (V3).

	Participant Group						
	BS					**NOC**	
Outcome measure	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 vs NOC**
	Mean (SD) / Median (IQR)	*Mean (SD)*				*Mean (SD)*	*p value*
TBW (kg)	123.2 (11.5)	98.2 (9.2)	-25.0	-26.3 to -23.8	<0.0001	67.2 (8.6)	<0.0001
BMI (kg/m^2)	45.5 (4.2)	36.3 (3.3)	-9.3	-9.8 to -8.7	<0.0001	25.1 (3.1)	<0.0001
Waist circumference (cm)	125.2 (11.5)	111.6 (9.3)	-13.6	-17.0 to -10.3	<0.0001	85 (10)	<0.0001
Systolic Blood Pressure (BP) (mmHg)	128 (13)†	123 (12)	-5	N/A	0.1087	131 (19)	0.1140
Diastolic BP (mmHg)	80 (8)	79 (12)	-1	-6 to 4	0.6057	77 (8)	0.4784
Heart rate (bpm)	65 (8)	66 (12)	1	-3to 6	0.5830	69 (10)	0.5485
Max. dominant hand grip strength (kg)	33.5 (7.2)	31.9 (5.7)	-1.6	-3.4 to 0.2	0.0732	31.2 (7.1)	0.7434
Max. non-dominant hand grip strength (kg)	33.0 (7.1)	30.0 (6.4)	-2.9	-4.7 to -1.1	0.0028	28.8 (7.2)	0.5830

BS Group, n=22 paired values for each outcome measure. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to 20% post-surgical weight loss (V3) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of surgical weight loss on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V3 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data.

Whilst there were significant improvements in a range of anthropometric outcome measures at 20% Weight Loss (Table 13-2) and also in more detailed body composition analysis through DXA scanning (Table 13-4), these measurements did not normalise relative to Non-Obese Control (NOC) participants. There was a reduction in total lean mass following surgery, which was associated with a significant decline in hand grip strength (Table 13-2). This has been reported previously[266], but the inclusion of the NOC group in the present study highlights that this functional decline reduced strength only to levels observed in the healthy NOC group.

13.3.1.3 Effects of 20% postoperative weight loss: Imaging Investigations

Comprehensive liver imaging was repeated to non-invasively stage postoperative NAFLD severity. The effects of 20% postoperative weight loss on these outcome measures, and on pancreatic steatosis, are shown in Table 13-3.

Table 13-3 Table of imaging outcome measures on completion of a routine pre-operative low energy diet before bariatric surgery (V2) and after 20% surgically induced total body weight (TBW) loss (V3).

	Participant Group						
	BS					**NOC**	
Outcome measure	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 vs NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
Liver steatosis (%)	6.9 (10.9)†	3.8 (5.6)†	-3.0	N/A	0.0015	3.2 (4.3)†	0.4555
Liver cT1 (ms) *n=20 pairs*	776.0 (110.5)	753.0 (78.3)†	-27.5	N/A	0.0221	758.1 (85.9)	0.5937
Liver elasticity (kPa)	8.0 (6.8)†	5.1 (2.3)†	-2.6	N/A	0.0004	3.8 (0.7)	<0.0001
Pancreatic steatosis (%) *n=20 pairs*	5.8 (2.9)	4.0 (3.7)†	-0.4	N/A	0.3884	*n=15:* 2.5 (2.7)†	0.0303

BS Group, n=22 paired values for each outcome measure unless otherwise specified. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to 20% post-surgical weight loss (V3) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of surgical weight loss on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V3 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

Table 13-3 shows that 20% TBW loss after bariatric surgery resulted in significant improvements in liver steatosis beyond those observed after the preoperative diet. These further changes were sufficient to normalise levels when compared to NOC participants, suggesting preferential clearance of ectopic fat over that stored in adipose tissue depots, which remained heavier than in NOCs (Table 13-4). Similarly, significant reductions in liver fibro-inflammation were achieved, reducing liver cT1 values to those seen in the NOC group. Whilst there were also significant reductions in liver elasticity, which has been validated as a biomarker of fibrosis, values remained elevated compared to controls. This result may partially be explained, particularly in view of relatively mild histological fibrosis in the BS group at the time of surgery, by the recognised challenges associated using the Fibroscan device (Echosens, Paris, France) in individuals with severe obesity. Even using the XL probe, fibrosis is reported to be overestimated, and for this reason an XXL probe has been designed and is undergoing evaluation for patients with BMI≥35kg/m^2 [189].

Table 13-4 details the effects of 20% surgical TBW loss on body composition, which was assessed using DXA scanning. Values for these outcome measures in NOC participants are also displayed and compared to those after 20% surgical weight loss.

Table 13-4 Table of DXA scan-derived outcome measures on completion of a routine pre-operative low energy diet before bariatric surgery (V2) and after 20% surgically induced total body weight (TBW) loss (V3).

	Participant Group						
	BS					**NOC**	
Outcome measure	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 vs NOC**
	Mean (SD) / Median (IQR)	*Mean (SD)*				*Mean (SD) / Median (IQR)*	*p value*
Visceral fat mass (kg) *n=17*	2.25 (0.58)	1.43 (0.45)	-0.81	-1.01 to -0.62	<0.0001	0.64 (0.44)	<0.0001

	Participant Group						
	BS					NOC	
Outcome measure	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 vs NOC**
	Mean (SD) / Median (IQR)	*Mean (SD)*				*Mean (SD) / Median (IQR)*	*p value*
Android fat mass (kg)	6.51 (1.14)†	4.28 (0.90)	-2.27	N/A	<0.0001	1.96 (7.38)	<0.0001
Android fat (%)	63.9 (4.2)	54.5 (5.0)	-9.4	-11.0 to -7.9	<0.0001	40.1 (10.4)	<0.0001
Gynoid fat mass (kg)	9.77 (2.34)†	6.91 (1.15)	-3.00	N/A	<0.0001	4.17 (0.85)	<0.0001
Gynoid fat (%)	56.2 (7.2)†	48.2 (3.8)	-6.3	N/A	<0.0001	40.6 (4.7)	<0.0001
Android/Gynoid fat ratio	0.65 (0.10)†	0.62 (0.09)	-0.04	N/A	0.0115	0.47 (0.16)	0.0007
Total fat mass (kg)	64.63 (8.71)	46.00 (6.07)	-18.62	-20.23 to -16.95	<0.0001	24.80 (5.94)	<0.0001
Total tissue fat (%)	55.4 (2.8)	48.5 (3.4)	-6.9	-7.8 to -5.9	<0.0001	38.3 (6.1)	<0.0001
Total arms lean mass (kg)	4.99 (1.35)	4.72 (0.79)	-0.28	-0.88 to 0.33	0.3538	4.24 (0.76)	0.0688
Total lean mass(kg)	51.86 (5.09)	48.62 (4.72)	-3.24	-5.34 to -1.14	<0.0043	39.54 (4.98)	<0.0001

BS Group, n=22 paired values for each outcome measure unless otherwise specified. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to 20% post-surgical weight loss (V3) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of surgical weight loss on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V3 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed

paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

This table shows significant reductions in fat mass across all depots, although these remained increased compared to non-obese controls. There appeared to be a preferential clearance from android over gynoid depots, resulting in a significantly improved android/gynoid fat ratio, an index of metabolic health. Whilst there was also a reduction in lean mass in the BS group, it was relatively spared, meaning that percentage body fat decreased. Lean mass remained higher than in NOCs after 20% postoperative weight loss.

13.3.1.4 Effects of 20% postoperative weight loss: indirect calorimetry

Fasting and postprandial indirect calorimetry was performed after 20% postoperative weight loss and measurements were compared to those taken just before surgery on completion of the preoperative low energy diet. The results of these investigations are shown in Table 13-5:

Table 13-5 Table of indirect calorimetry outcome measures on completion of a routine pre-operative low energy diet before bariatric surgery (V2) and after 20% surgically induced total body weight (TBW) loss (V3).

	Participant Group						
	BS					**NOC**	
Outcome measure	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit *(n=16)***	**BS V3 vs NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
T0 Energy Expenditure (kcal/24hrs)	1577 (221.0)	1181 (302)†	-388.8	N/A	0.0014	1243 (204.7)	0.8689
T120 Energy Expenditure (kcal/24hrs)	1641 (325.4)	1332 (191.0)	-344.0	-524.6 to -163.4	0.0009	1254 (217.9)	0.2524
T0 Respiratory Quotient	0.73 (0.10)	0.73 (0.09)	0.01	-0.07 to 0.08	0.8528	0.86 (0.17)†	0.0102
T120 Respiratory Quotient	0.73 (0.12)†	0.72 (0.12)	-0.02	N/A	0.3836	0.85 (0.08)	0.0003

BS Group, n=22 paired values for each outcome measure unless otherwise specified. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to 20% post-surgical weight loss (V3) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of surgical weight loss on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V3 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

Table 13-5 shows that fasting and postprandial energy expenditure decreased to values observed in NOCs despite a higher body mass, suggesting relative suppression of metabolic rate in order to conserve energy stores.

13.3.1.5 Effects of 20% postoperative weight loss: clinical chemistry

Biochemical outcome measures were recorded at the 20% Weight Loss research visit to determine the effects of this achievement on fasting clinical chemistry. Table 13-6 details the results of these analyses and compares 20% Weight Loss values to those in the NOC group.

Table 13-6 Table of fasting biochemical outcome measures on completion of a routine pre-operative low energy diet before bariatric surgery (V2) and after 20% surgically induced total body weight (TBW) loss (V3).

	Participant Group						
	BS					**NOC**	**BS vs NOC comparisons**
Fasting clinical chemistry (plasma).	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 *vs* NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
ALT (IU/L)	24 (16) †	21 (12) †	-3	N/A	0.1227	17 (12)†	0.3436
AST (IU/L)	21 (12) †	19 (8) †	-1	N/A	0.2099	23 (9)	0.2191
Enhanced Liver Fibrosis (ELF) Test *n=20 pairs*	7.87 (0.72)	8.41 (0.82)	0.53	0.21 to 0.85	0.0025	7.99 (0.74)	0.2412
Glucose (mmol/L)	5.6 (0.5)	5.3 (0.7)	-0.2	-0.6 to 0.1	0.1249	5.7 (1.2)†	0.1077

	Participant Group						
	BS					**NOC**	**BS vs NOC comparisons**
Fasting clinical chemistry (plasma).	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 *vs* NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
Insulin (mU/L)	13.23 (14.62)†	6.03 (7.68) †	-7.24	N/A	0.0037	5.53 (5.38)†	0.4207
Beta cell Function: HOMA2 %B	139.8 (74.4)	70.5 (72.4)†	-38.5	N/A	0.0263	59.15 (31.5)†	0.0507
Insulin sensitivity: HOMA2 %S	56.3 (25.7)	125.9 (80.6)	69.6	33.9 to 105.2	0.0006	135.4 (123.0)†	0.3385
NEFA (µmol/L)	1017.0 (278.4)	895.2 (235.7)	-122.1	-249.6 to 5.32	0.0594	801.2 (243.1)	0.2386
TAG (µmol/L)	983.1 (866.5)†	1087 (433.9)	49.6	N/A	0.6327	1267 (546.4)	0.2658
Lactate	0.7 (0.2)	0.7 (0.2)†	0.0	N/A	0.7441	0.8 (0.2)	0.3294
3-OHB(µmol/L)	249.0 (306.6) †	392.8 (279.5)	23.91	N/A	0.3209	64.05 (114.75)†	0.0001
Total chol. (mmol/L)	4.2 (1.0)	4.2 (1.0)	-0.03	-0.4 to 0.3	0.8613	5.0 (1.3)	0.0522
HDL chol. (mmol/L)	1.0 (0.2)	1.1 (0.3)	0.1	-0.0 to 0.2	0.0532	1.3 (0.3)	0.0337

	Participant Group						
	BS					**NOC**	**BS vs NOC comparisons**
Fasting clinical chemistry (plasma).	**Post-diet visit (V2)**	**20% weight loss visit (V3)**	**Difference (Diff.)**	**95% CI of Diff.**	**p value**	**Control visit**	**BS V3 *vs* NOC**
	Mean (SD) / Median (IQR)	*Mean (SD) / Median (IQR)*				*Mean (SD) / Median (IQR)*	*p value*
Total chol./HDL ratio	4.2 (1.0)	3.9 (0.9)	-0.3 (0.7)	-0.6 to 0.0	0.0601	4.0 (1.3)	0.8638
ApoB (g/L)	0.8 (0.2)	0.8 (0.3)	-0.1 (0.2)	-0.2 to 0.0	0.1548	0.9 (0.3)	0.1933
VLDL ApoB (g/L) *n=21 pairs*	0.1 (0.1)†	0.2 (0.1)	0.0	N/A	0.4070	0.2 (0.1)	0.9903
Chylomicron TAG (µmol/L) *n=21 pairs*	10.6 (27.7)†	3.7 (10.1)†	-6.8	N/A	0.0195	8.2 (24.4)†	0.6600
VLDL TAG (µmol/L) *n=21 pairs*	501.2 (618.5)†	522.9 (243.8)	-11.73	N/A	0.7351	609.9 (434.2)	0.2452

BS Group, n=22 paired values for each outcome measure unless otherwise specified. Values for NOC participants (n=16 unless otherwise specified) also shown and compared to 20% post-surgical weight loss (V3) values in the BS Group. Paired two-tailed T-tests used to analyse the effects of surgical weight loss on each normally distributed outcome measure. Unpaired two-tailed T-tests used to compare normally distributed BS V3 values to those of NOC participants. Wilcoxon matched-pairs signed rank test for non-normally distributed paired data. Mann-Whitney test used to compare non-normally distributed unpaired data. † indicates non-normally distributed data.

This table shows that the increase in liver enzymes observed after the preoperative diet was transient. However, in contrast to this suggestion of improving liver function there was a deterioration in ELF score. It is noted that all the input variables for this non-invasive

biomarker of liver fibrosis are measurements of extracellular matrix proteins and that these are not specifically expressed in the liver. Therefore, the ELF score increase may reflect increased postoperative whole body remodelling rather than adverse changes in the liver. Liver biopsies were not available for comparison after 20% weight loss.

13.3.1.6 Effects of 20% postoperative weight loss: postprandial biochemical measures

Postprandial blood sampling was performed for six hours following ingestion of the test meal. TAG responses are characterised in Figure 13-2 and compared to those measured on completion of the preoperative low energy diet and in control participants.

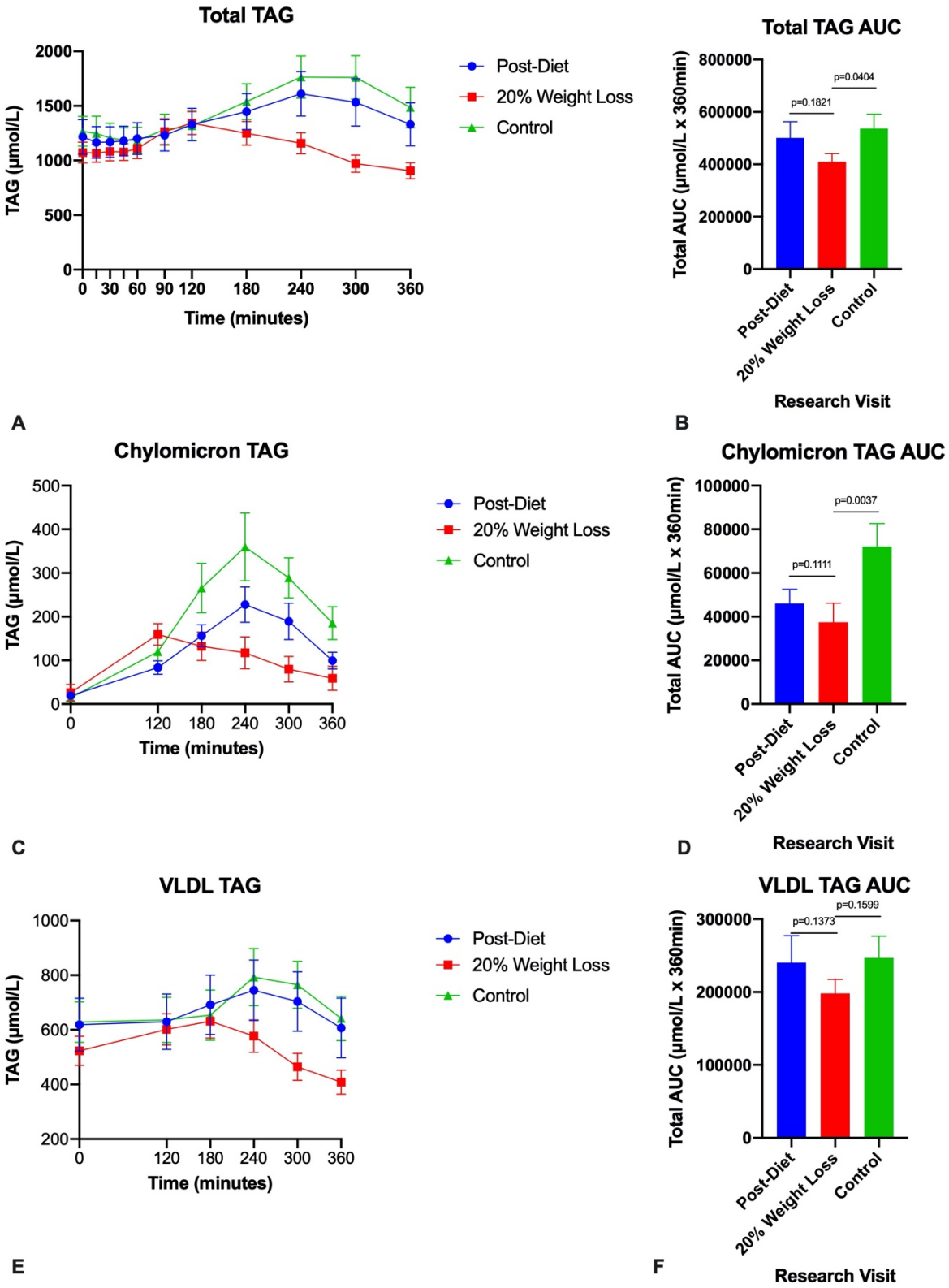


Figure 13-2 Postprandial peripheral venous (A) total triacylglycerol (TAG) level and (B) total TAG Area Under the Curve (AUC) following standardised test meal consumed on completion of routine preoperative diet and after 20% weight loss following bariatric surgery. First samples taken after overnight fast, timing for subsequent samples initiated after complete ingestion of test meal. Postprandial chylomicron (C) and VLDL

(E) TAG changes and chylomicron (D) and VLDL (F) TAG AUC also recorded. Data plotted are mean ± SEM, n=21 for bariatric group (Pre-diet and Post-diet), n=16 for control group. AUCs compared in (B), (D) and (F) with paired analysis for Post-Diet and 20% Weight Loss visits, and unpaired analysis to compare 20% Weight Loss and Control AUCs. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 13-7: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison					
	Post-Diet (V2) vs 20% Weight Loss (V3)			20% Weight Loss (V3) vs Control		
Variable	Total TAG	Chylo TAG	VLDL TAG	Total TAG	Chylo TAG	VLDL TAG
Variation Source	p value			p value		
Participant Group/Research Visit	0.1762	0.2134	0.1312	0.0973	0.0060	0.1114
Postprandial Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

In Figure 13-2, earlier peaks in the measured metabolites were observed compared to those measured at the end of the preoperative diet. These changes likely reflect accelerated postsurgical gastric emptying and led to interaction effects between postprandial time and these research visits for chylomicron-TAG and VLDL-TAG in Figure 13-2 and Table 13-7. Reduced plasma total TAG levels relative to NOCs were also seen.

Additional plasma metabolites were also measured during the postprandial period and measurements were compared to those recorded just before surgery and to NOC participants. The results are shown in Figure 13-3 and in Figure 13-4, which additionally includes $^{13}CO_2$ production data from the 20% Weight Loss research visit.

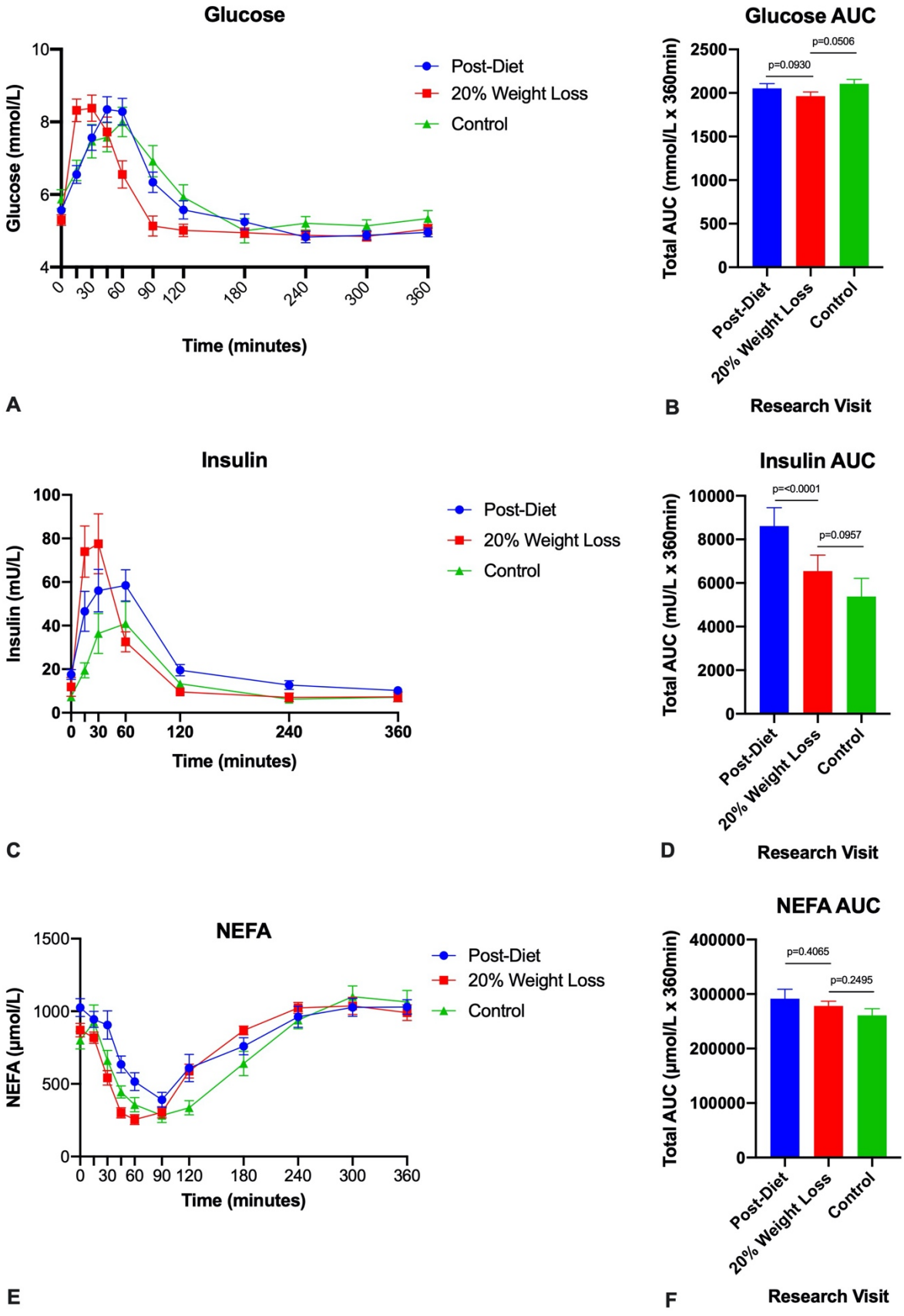


Figure 13-3: Postprandial peripheral venous (A) Glucose, (C) Insulin and (E) NEFA levels in response to standardised test meal. First samples taken after overnight fast, timing for subsequent samples initiated

after complete ingestion of test meal. AUCs for Glucose (B),(D) Insulin and (E) NEFA also calculated and compared. Data plotted are mean ± SEM, n=21 for bariatric group (Pre-diet and Post-diet), n=16 for control group. AUCs compared in (B), (D) and (F) using paired analysis for Post-Diet and 20% Weight Loss visits, and unpaired analysis to compare 20% Weight Loss and Control AUCs. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 13-8: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison					
	Post-Diet (V2) vs 20% Weight Loss (V3)			20% Weight Loss (V3) vs Control		
Variable	Glucose	Insulin	NEFA	Glucose	Insulin	NEFA
Variation Source	p value			p value		
Participant Group/Research Visit	0.2518	0.9477	0.0135	0.2597	0.0366	0.9266
Postprandial Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Figure 13-3 and Table 13-8 show earlier and greater NEFA suppression after surgery, which reflects increased insulin sensitivity. 3-OHB remained elevated after 20% surgical weight loss relative to the NOC group, reflecting ongoing increased hepatic fat oxidation. Earlier peak glucose and insulin responses to the test meal are also apparent in this figure.

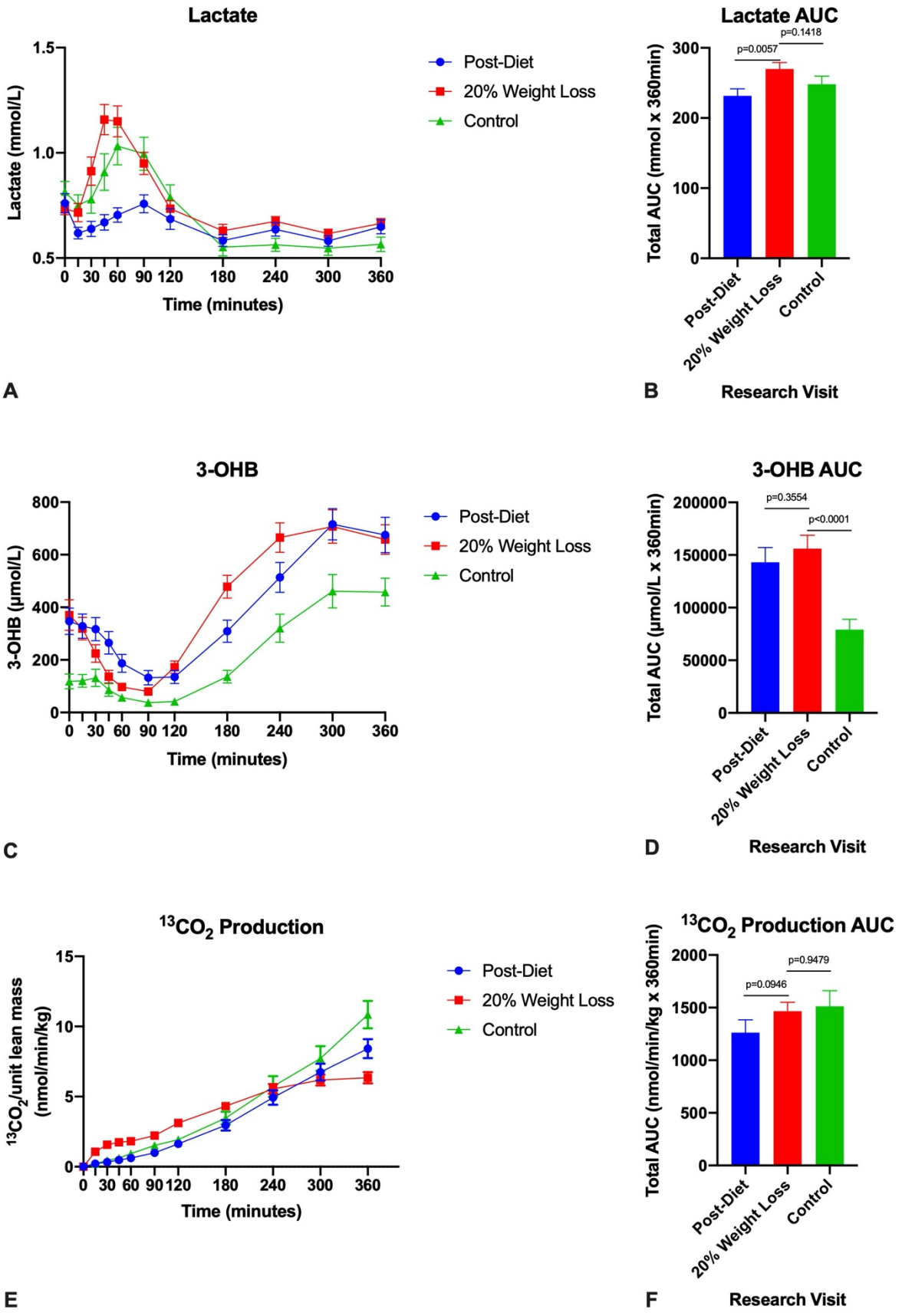


Figure 13-4 Postprandial peripheral venous (A) Lactate, (C) 3-OHB and (E) lean mass adjusted $^{13}CO_2$ production levels in response to standardised test meal. First samples taken after overnight fast, timing for subsequent

samples initiated after complete ingestion of test meal. AUCs for Lactate (B), 3-OHB (D) and $^{13}CO_2$ production (F) also calculated and compared. Data plotted are mean ± SEM, n=21 for bariatric group (Pre-diet and Post-diet), n=16 for control group, except for $^{13}CO_2$ production (n=16 for Post-Diet and 20% Weight Loss visits, n=12 for Control group). AUCs compared in (B), (D) and (E) using paired analysis for Post-Diet and 20% Weight Loss visits, and unpaired analysis to compare 20% Weight Loss and Control AUCs. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 13-9: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison					
	Post-Diet (V2) vs 20% Weight Loss (V3)			20% Weight Loss (V3) vs Control		
Variable	Lactate	3-OHB	$^{13}CO_2$ Production	Lactate	3-OHB	$^{13}CO_2$ Production
Variation Source	p value			p value		
Participant Group/Research Visit	0.0011	0.9761	0.0282	0.2431	0.0002	0.5883
Postprandial Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	<0.0001	<0.0001	<0.0001	0.0021	<0.0001	<0.0001

There was a postsurgical increase in lactate production in the early postprandial period as shown in Figure 13-4 and Table 13-9. These postprandial changes in lactate concentration are similar to those observed in the first research visit, and may be explained by increased postoperative carbohydrate intake and glycogen stores after bariatric surgery than on completion of the low energy carbohydrate-restricted dietary intervention. Earlier whole-body fatty acid oxidation of the test meal is also highlighted in Figure 13-4.

13.3.2 Comparison of outcomes in SG and RYGB groups at 20% weight loss

Exploratory comparisons of the effect of SG or RYGB surgery on the postprandial response to the test meal after matched (20%) total body weight loss were also made. A similar time was taken to achieve this target after SG and RYGB surgery (154.4 (43.6) *vs* 154.6 (42.51) days respectively, p=0.9883). The study protocol designed to achieve matched (20%) total body weight loss after SG and RYGB was effective, with a mean of 20.4 (1.3)% weight loss in the SG group and 20.2 (1.3)% weight loss in the RYGB group (p=0.7060). Histological liver steatosis, inflammation (NAS) and fibrosis (Kleiner stage) were similar in SG and RYGB groups (p>0.05 for all comparisons) at the time of surgery.

Total TAG, chylomicron TAG and VLDL TAG changes in response to the test meal in SG and RYGB groups are shown in Figure 13-5.

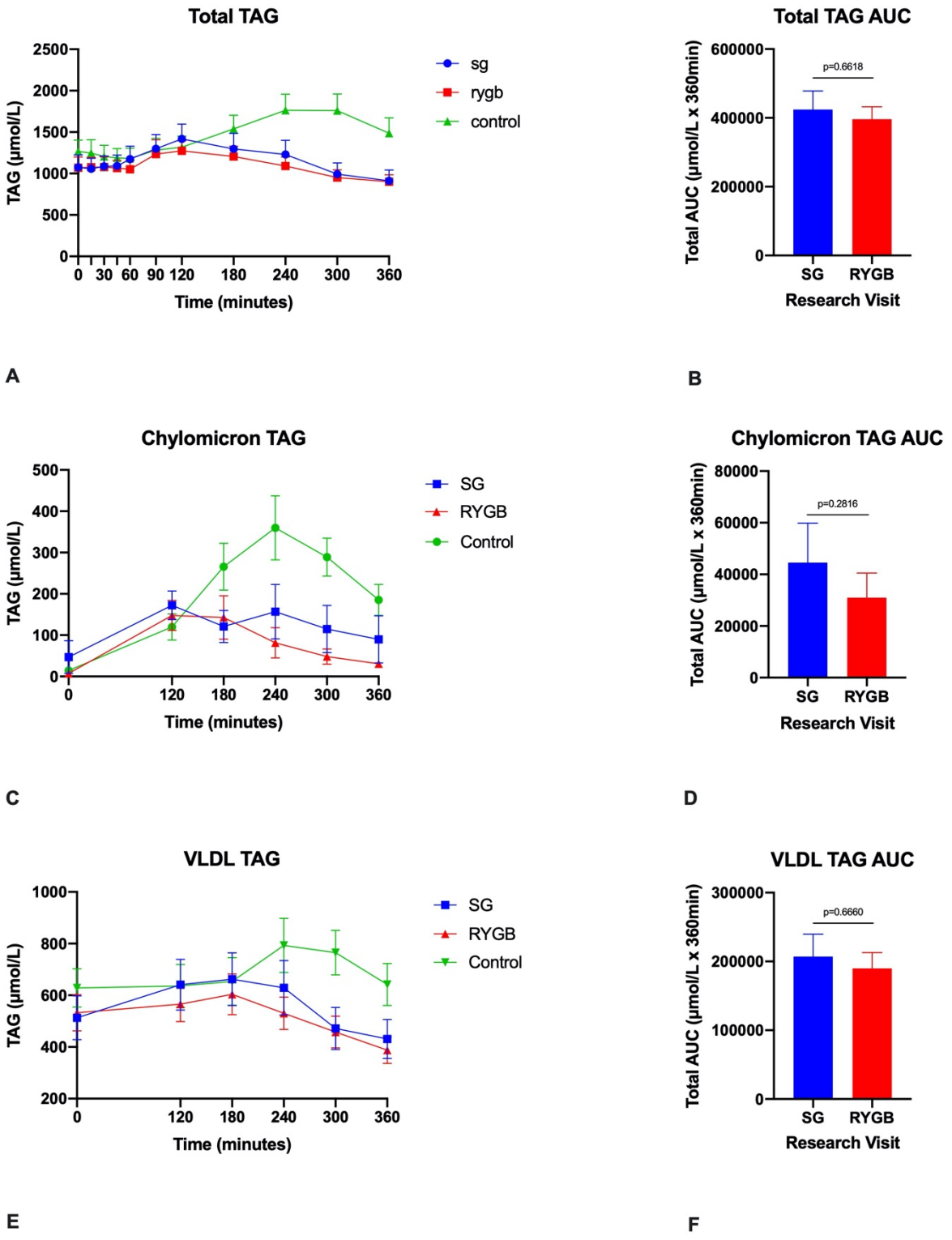


Figure 13-5: Postprandial peripheral venous (A) Total TAG (C) Chylomicron TAG and (E) VLDL TAG levels in response to standardised test meal at matched (20%) weight loss following SG (n=10) or RYGB (n=11) surgery and in non-obese control participants (n=16). First samples taken after overnight fast, timing for subsequent samples initiated after complete ingestion of test meal. AUCs for Total TAG (B), Chylomicron TAG (D) and VLDL TAG (F) also calculated and compared. Data plotted are mean ± SEM. AUCs compared in (B), (D) and (E) using

unpaired analysis to compare SG and RYGB groups. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 13-10: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison		
	SG vs RYGB		
Variable	Total TAG	Chylo TAG	VLDL TAG
Variation Source	p value		
Operation	0.7391	0.4269	0.6767
Postprandial Time	<0.0001	0.0002	<0.0001
Interaction	0.9242	0.3046	0.2743

Figure 13-5 and Table 13-10 illustrate that reduced total, chylomicron and VLDL TAG AUC, and earlier peak are seen after both SG and RYGB relative to non-obese control participants. However, the TAG response is relatively similar after these operations, with no significant differences detected at matched weight loss.

Figure 13-6 shows the glucose, insulin and NEFA response to the test meal in SG and RYGB groups after 20% postoperative weight loss:

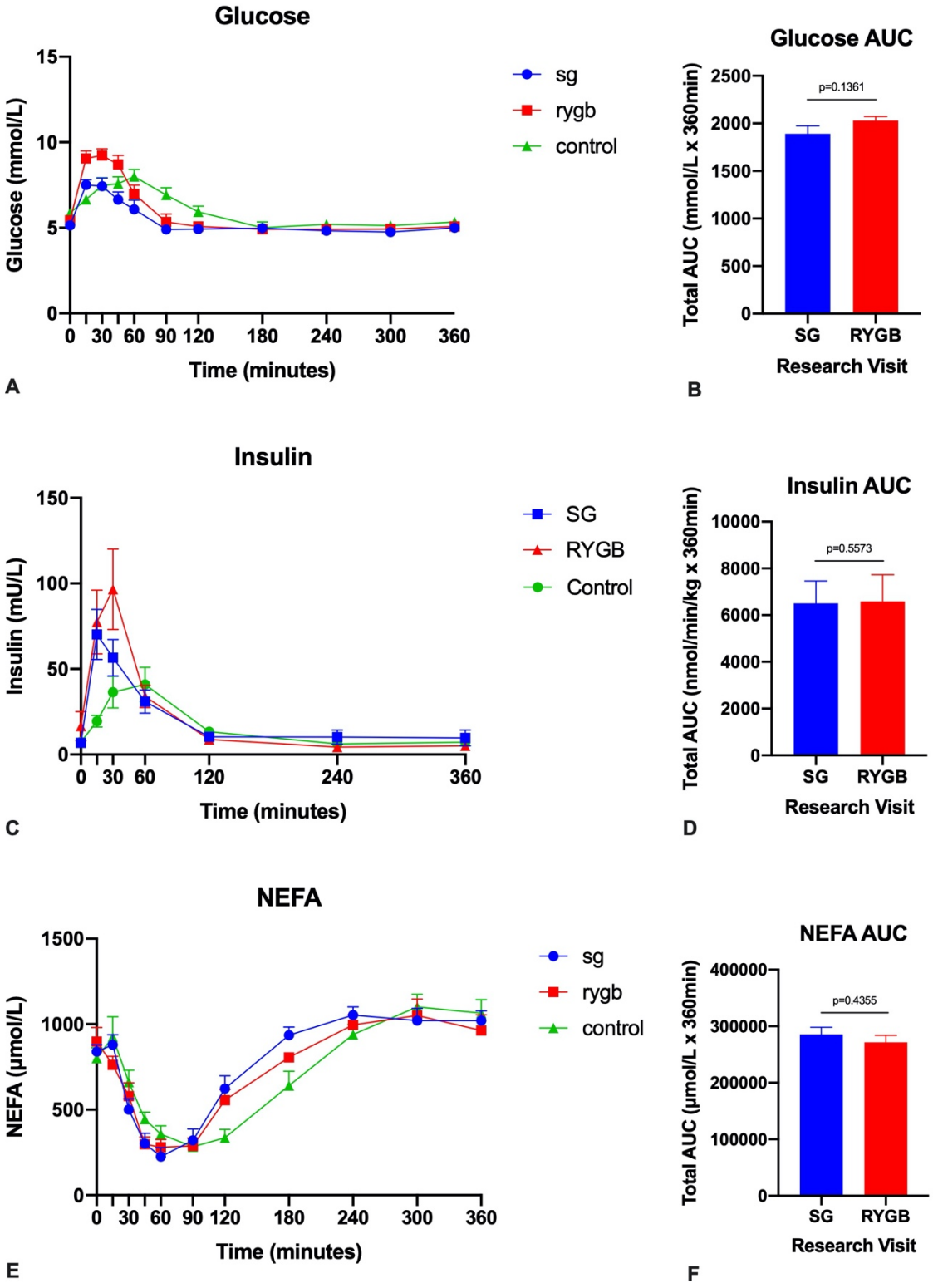


Figure 13-6: Postprandial peripheral venous (A) Glucose, (C) Insulin and (E) NEFA levels in response to standardised test meal at matched (20%) weight loss following SG (n=10) or RYGB (n=11) surgery and in non-obese control participants (n=16). First samples taken after overnight fast, timing for subsequent samples initiated after complete ingestion of test meal. AUCs for Glucose (B), Insulin (D) and NEFA (F) also calculated

and compared. Data plotted are mean ± SEM. AUCs compared in (B), (D) and (E) using unpaired analysis to compare SG and RYGB groups. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 13-11: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison		
	SG vs RYGB		
Variable	Glucose	Insulin	NEFA
Variation Source	p value		
Operation	0.0319	0.4525	0.6716
Postprandial Time	<0.0001	<0.0001	<0.0001
Interaction	<0.0001	0.1644	0.5382

Significant differences in early postprandial glucose levels are observed between SG and RYGB levels after the test meal (Figure 13-6 and Table 13-11). There is a suggestion of a corresponding difference in insulin concentration at 30 minutes, but there was no overall difference between groups or interaction effect for insulin across the test meal.

Products of carbohydrate, hepatic fatty acid metabolism and whole-body test meal fatty acid oxidation were also compared in participants who had undergone SG or RYGB bypass surgery. The results of these comparisons are illustrated in Figure 13-7.

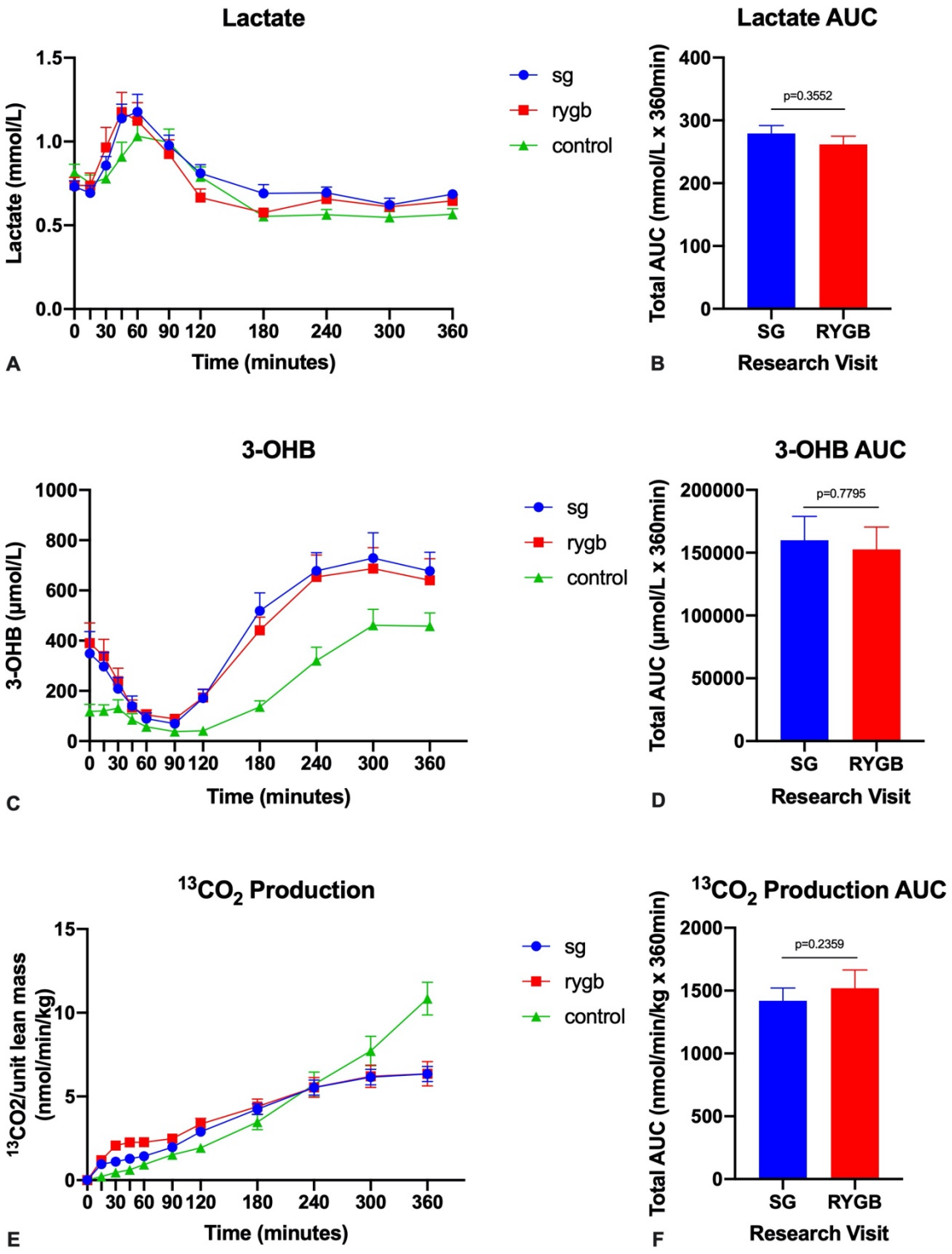


Figure 13-7 Postprandial peripheral venous (A) Lactate, (C) 3-OHB and (E) lean mass adjusted $^{13}CO_2$ production levels in response to standardised test meal at matched (20%) weight loss following SG or RYGB surgery and in non-obese control participants. First samples taken after overnight fast, timing for subsequent samples initiated after complete ingestion of test meal. AUCs for Lactate (B), 3-OHB (D) and $^{13}CO_2$ production (F) also calculated and compared. Data plotted are mean ± SEM. Participants numbers (SG n=10, RYGB n=11, Control n=16) consistent throughout except for 13CO2 production (SG n=8, RYGB n=8, Control n=11). AUCs compared in

(B), (D) and (E) using unpaired analysis to compare SG and RYGB groups. Normality testing then appropriate parametric or non-parametric statistical tests used throughout.

Table 13-12: Postprandial response comparison using two-way repeated measures ANOVA

	Comparison		
	SG vs RYGB		
Variable	Lactate	3-OHB	$^{13}CO_2$ Production
Variation Source	p value		
Operation	0.7208	0.9616	0.2999
Postprandial Time	<0.0001	<0.0001	<0.0001
Interaction	0.5156	0.9427	0.3205

Overall postprandial responses to the standardised test meal were relatively similar after SG or RYGB surgery, with no significant differences in measured postprandial metabolite AUCs were observed for the overall test meal period (Table 13-12). However, there were significantly different early glucose responses to the test meal, with a higher peak glucose concentration observed in the RYGB group (Figure 13-6). These results are consistent with one previous study comparing SG and RYGB responses to a standardised test meal[262]. Additionally, it appears that there was earlier oxidation of the labelled palmitate in the test meal after RYGB (Figure 13-7).

13.3.3 Preliminary 1-year outcomes analysis: liver parameters

This research study was powered to detect a change in liver steatosis at the primary endpoint research visit, which was pre-specified as being the 20% Weight Loss visit. However, since additional weight loss was expected beyond this visit, a fixed timepoint of 1 year after surgery was also included in the EDISON study's protocol. Full Imaging and CRU research visits were performed at this timepoint, some of which are ongoing. Whilst full

results from this visit are outside the scope of this thesis, mixed effects models have been generated that include all available data from 1-year research visits.

The effects of our clinical care pathway that includes preoperative low energy diet followed by bariatric surgery on liver-related outcome measures in the bariatric surgical group are shown in Figure 13-8. The effects of our interventions on a range of metabolic health parameters was also assessed and Figure 13-9 provides a summary of these results.

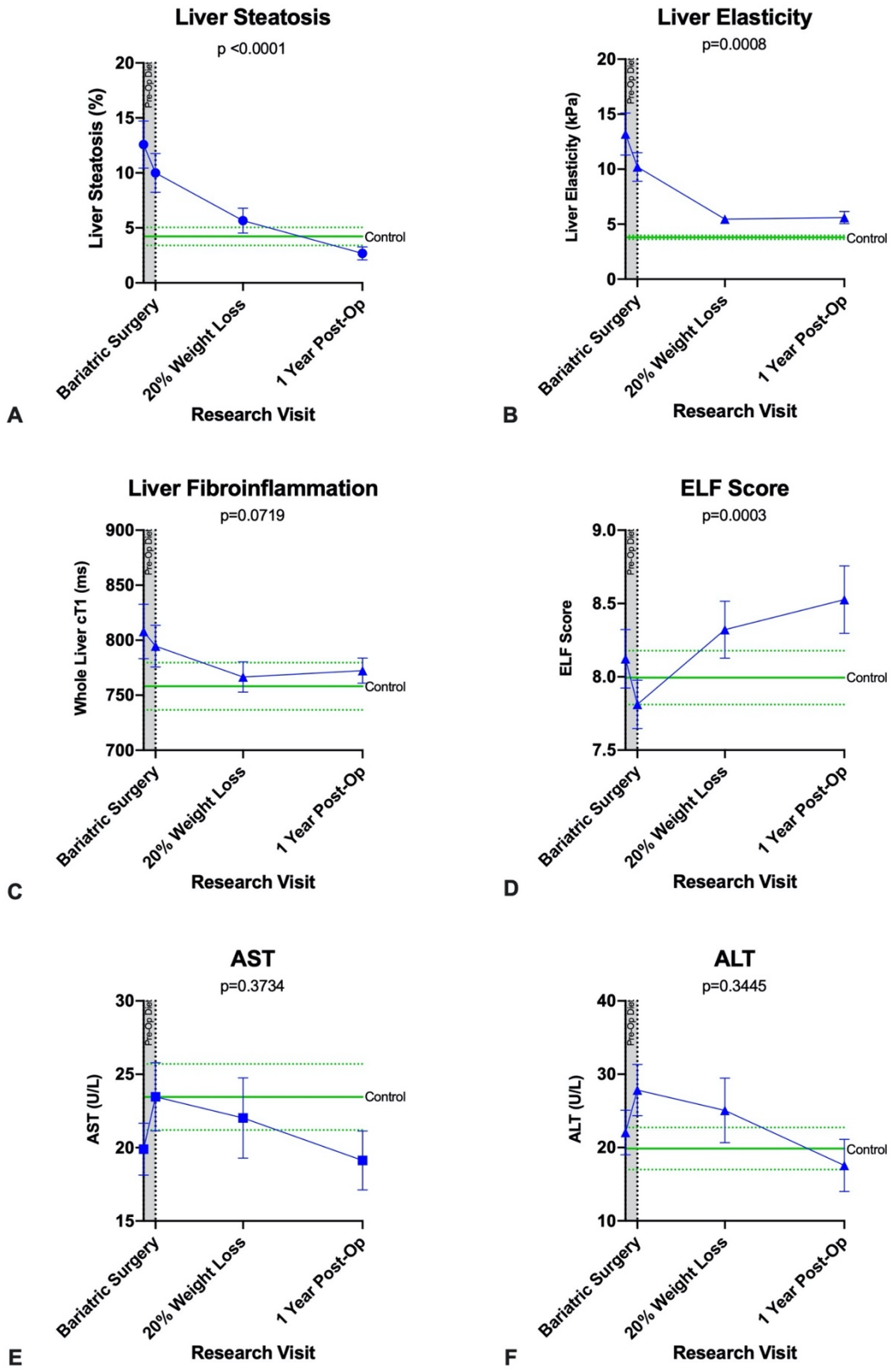


Figure 13-8 Mixed effects model analysis of the effects of dietary and bariatric surgical interventions on liver-related outcome measures across EDISON study research visits. Preliminary 1 year postoperative outcomes data included (available for 16 bariatric surgical participants). 3 Week Pre-operative Diet period highlighted in grey and labelled. Data plotted are mean ± SEM for Bariatric Surgical and Control groups.

As shown in Figure 13-8, our bariatric surgical clinical care pathway appears to promote favourable changes in this panel of liver-related outcome measures. Liver steatosis[200], fibroinflammation[267] and elasticity[268] all reduced to published low NAFLD risk reference ranges by the end of the first postoperative year. NOC values for these outcome measures provide additional confirmation of reduced postoperative risk following dietary and surgical interventions in these mixed effects models for the Bariatric Surgical group. The ELF test changes are discordant and requires further study against postoperative liver biopsy histology in order to determine the accuracy of this non-invasive biomarker in this setting.

13.3.4 Preliminary 1-year outcomes analysis: metabolic health parameters

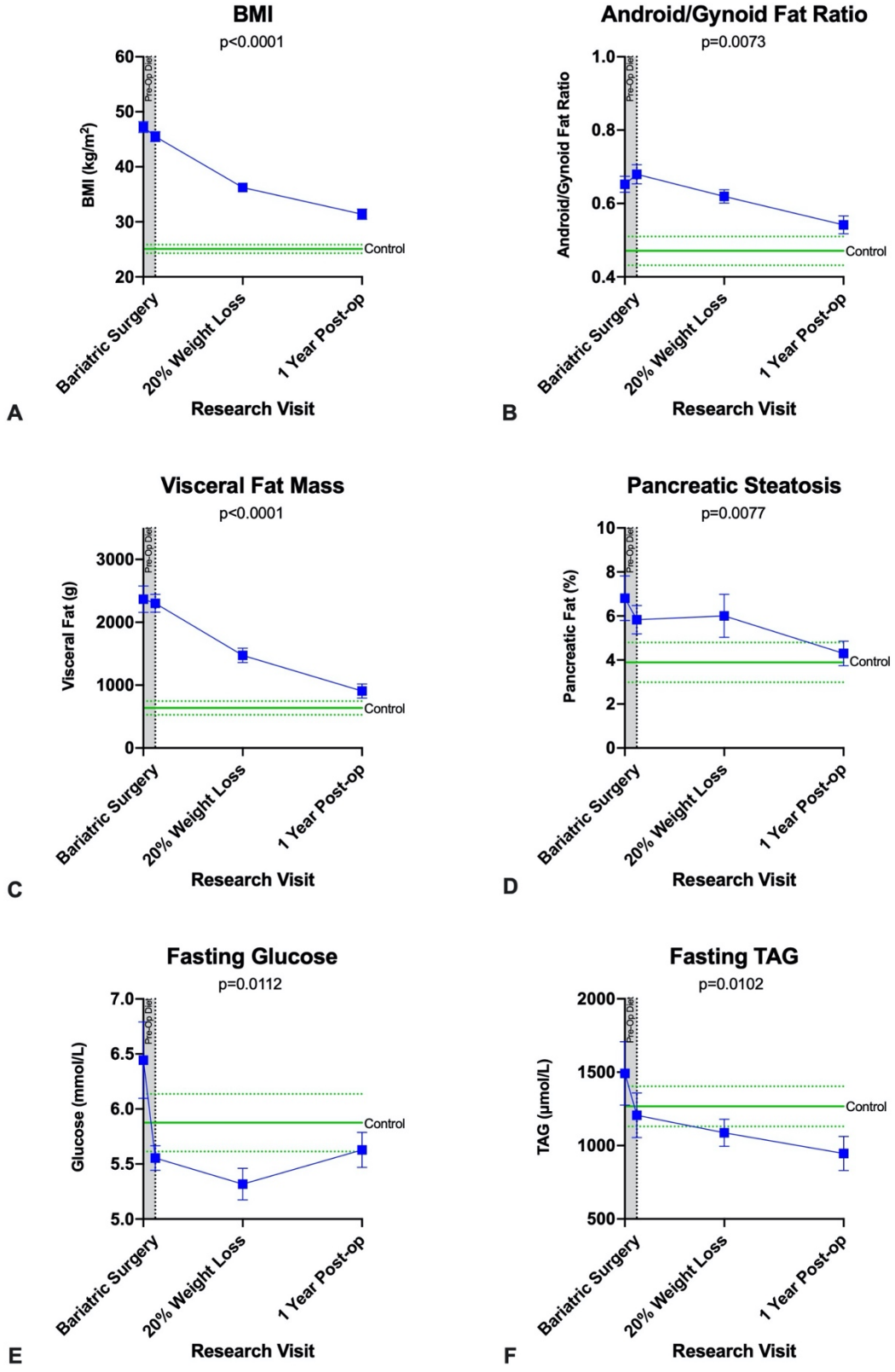


Figure 13-9 Mixed effects model analysis of the effects of dietary and bariatric surgical interventions on a selection of metabolic health parameters across EDISON study research visits. Preliminary 1 year postoperative

outcomes data included (available for 16 bariatric surgical participants). 3 Week Pre-operative Diet period highlighted in grey and labelled. Data plotted are mean ± SEM for Bariatric Surgical and Control groups.

Similar beneficial changes were seen in other markers of metabolic health highlighted in Figure 13-9, and it is notable that these were achieved despite persistent obesity 1 year after surgery. Android/gynoid fat ratio and visceral fat mass did, however, approach NOC levels at the 1-year timepoint in this preliminary analysis. Fat distribution is prognostically relevant, as android fat is associated with adverse cardiometabolic risk, whilst gynoid fat has been reported to be protective against NAFLD progression[269]. Visceral fat is known to be particularly metabolically active and associated with fibro-inflammation in NAFLD, purportedly due to increased expression of proinflammatory adipocytokines and its drainage into the hepatic portal vein[270,271].

It is notable that the magnitude of the changes in glucose and triglyceride concentrations observed in the mixed effects models in Figure 13-9 were more marked during the dietary component of the study than after surgery. Sustained adherence to such diets can, however, be challenging. Whilst the participants were non-diabetic, marked changes in fasting glucose occurred that reduced mean values from levels suggestive of impaired glucose tolerance to within the normal range. Decreased pancreatic steatosis, which has been associated with impaired β cell function[158], was also observed, and continued improvement in this outcome measure and in fasting plasma TAG was seen longitudinally across the study.

13.4 Discussion

13.4.1 Principal findings

In this chapter, I aimed to study the effects of 20% weight loss after bariatric surgery on liver steatosis and on a range of fasting and postprandial plasma metabolites and anthropometric measures. I also aimed to compare postoperative values against those recorded for NOC participants and to perform an exploratory comparison of the effects of SG or RYGB surgery on our outcome measures.

This chapter's results show that at the time of surgery, participants with obesity had increased hepatic steatosis compared to NOC participants, but relatively low NAS and fibrosis stage. Bariatric surgical intervention normalised liver steatosis relative to non-obese controls in the first postoperative year despite persistent obesity, suggesting that BMI normalisation may not be required for NAFLD remission. Bariatric surgery resulted in marked changes in the postprandial response to the test meal and, whilst AUCs were similar to those achieved with the dietary intervention, more rapid alterations in metabolite levels and in the oxidation of the test meal were apparent. Similar postprandial AUCs were observed after SG or RYGB at matched weight loss, although there was a suggestion that RYGB resulted in a more rapid glucose response and earlier oxidation of the test meal.

13.4.2 Interpretation and comparison with previous studies

Patients with NAFLD are generally advised to lose 5% or more of TBW by lifestyle modification[272], but in the previous chapter it was demonstrated that a similar degree of weight loss (4.6%) was insufficient to reverse hepatic steatosis or inflammation in individuals with severe obesity. Histological NAFLD severity was relatively mild following this preoperative dietary intervention, with persistent steatosis but minor fibroinflammatory changes. This is likely because this study recruited non-diabetic female participants (in order to eliminate confounding due to postoperative T2D medication changes) and T2D is known to drive NAFLD progression[273]. In this chapter, we conducted the initial postsurgical research visit after 20% total body weight (TBW) loss since this exceeds reported levels of weight loss after currently available dietary or pharmacological interventions.

Two previous observational diabetes-focused studies have assessed the effects of 20% TBW loss after bariatric surgery[242,274]. These studies had no liver-related outcome measures, did not incorporate randomisation and included operations that are performed infrequently (adjustable gastric banding and biliopancreatic diversion). This study is the first to randomise participants between the two most commonly performed bariatric operations in order to study their effects on NAFLD. At present, the only additional randomised data comparing the effects of these interventions is provided in a secondary analysis of a randomised controlled trial designed to compare weight loss outcomes, which reported greater improvements in liver aminotransferases after SG than after RYGB[275]. Since this

previous study was not designed to assess the effects of bariatric surgery on NAFLD, it did not include liver imaging or any recognised non-invasive biomarkers of NAFLD severity.

There were marked differences when comparing outcome measures for the BS group at 20% Weight Loss to Post-Diet results. Fasting and postprandial energy expenditure declined to meet NOC levels in response to prolonged caloric restriction, a metabolic response that has been linked to reduced oxidative stress and physiological aging[276].

In contrast to all other liver outcome measures, the Enhanced Liver Fibrosis (ELF) score deteriorated both after 20% weight loss and 1 year after surgery (Figure 13-8). Whilst this test is recommended in UK national guidelines for staging and monitoring NAFLD severity[145], its longitudinal use after bariatric surgery has not been validated. The ELF score is derived from a panel of extracellular matrix (ECM) proteins (hyaluronic acid, tissue inhibitor of metalloproteinases 1 and amino-terminal propeptide of type III procollagen)[277], none of which are expressed exclusively in the liver. Therefore, it is unclear whether the changes in ELF score observed after bariatric surgery accurately reflect changes in liver health, or are increased due to extrahepatic weight loss-associated ECM remodelling. These findings highlight the importance of using validated non-overlapping non-invasive NAFLD biomarkers, and they are of particular interest since the ELF score is specified as the biomarker that will be used to evaluate changes in NAFLD after bariatric surgery in the By-Band Sleeve study, which is the largest ongoing bariatric Randomised Controlled Trial (RCT) worldwide[278].

13.4.3 Strengths and limitations

A strength of this study is in the novelty of studying patients randomised to SG or RYGB surgery at matched weight loss rather than at a fixed time point. This design is more intensive for the study team and participants, but the home weight monitoring and participant follow-up protocol was successful in enabling participants to attend promptly when they neared their 20% weight loss target. Whilst it was not the aim of the investigators, SG and RYGB participants also attended this visit after an equal mean number of days after surgery, reducing the impact of an additional potential confounding factor. Another strength of this study design is in the inclusion of the NOC group, which aids the

interpretation of the study's results, particularly for more exploratory secondary outcome measures such as pancreatic steatosis, which was measured using MRI-PDFF.

The inclusion of randomisation in the study design represents another achievement and this study is the first to have randomised participants to different surgical interventions in order to study their effects on NAFLD and postprandial liver physiology. It is accepted that the present study may not have the statistical power to detect differences in some outcome measures between SG and RYGB interventions as this was not its primary aim. However, our experience suggests that conducting a larger NAFLD-focused surgical trial in the future may be feasible. The mechanistic nature of the present study led to the exclusion of participants with diabetes to reduce confounding, resulting in the inclusion of participants with less severe NAFLD.

An additional strength was in our retention of participants at the 20% Weight Loss visit, with no participant dropout or loss to follow-up amongst those who completed the Post-Diet visit. Primary outcome measure data was recorded at every research visit and there was minimal missing data for secondary outcome measures. Complete postprandial blood sampling was achieved for all but one participant. Our study included female non-diabetic participants suitable for SG or RYGB surgery and our findings may not be generalisable to individuals with different characteristics or undergoing alternative bariatric surgical interventions.

A pragmatic decision was made not to provide food to participants between the time of surgery and the achievement of 20% postoperative total body weight loss. Instead, specialist dieticians for the clinical bariatric service provided standard dietary advice and support, enhancing the 'real world' applicability of the study.

One weakness is that, for clinical reasons, one participant underwent a one-anastomosis gastric bypass (OAGB) rather than the Roux-en-Y gastric bypass to which they had been randomised. However, the comparison of surgical operations represents an exploratory secondary analysis. For the purposes of analysis, the participant was included in the RYGB group on an intention-treat basis in adherence to the pre-specified statistical analysis plan.

Another weakness is that some of the secondary outcome measures, such as non-invasive biomarkers of NAFLD severity, have not previously undergone thorough validation after bariatric surgical intervention. A range of imaging-derived and serological biomarkers have been included enable the identification of discordant biomarker results and aid interpretation of the effects of bariatric surgery on the liver.

13.4.4 Conclusion

The achievement of 20% postoperative weight loss following bariatric surgery led to substantial improvement in NAFLD and obesity phenotypes. SG and RYGB surgery had relatively similar effects at matched weight loss. More extensive validation to confirm the diagnostic accuracy of non-invasive biomarkers of NAFLD severity following bariatric surgery would be beneficial. It appears feasible to conduct a randomised trial evaluating the potential of surgical interventions for NAFLD, and future larger and more clinically-focused studies should recruit participants with NASH or greater baseline fibrosis severity.

14 Conclusions and Future Directions

14.1 Ongoing Work

The final results chapter in this thesis focused on the effects of 20% total body weight loss after bariatric surgery on our outcome measures, and it is at this time that primary endpoint data is recorded. However, the EDISON study has also been designed to include an additional research visit 12 months after surgery, in order to determine whether the observed changes in NAFLD-related outcomes are maintained and if additional improvements occur.

The final study visits have recently been completed and final analysis is now being performed to determine the effects of our interventions on the serum metabolome (with a focus on bile acids, proteomics and peptidomics in collaboration with Novo Nordisk Research Centre Oxford), gut microbiome (in collaboration with Oxford's Translational Gastroenterology Unit) and on liver fat metabolism as measured using stable isotope 'tracers'.

If our preliminary 12-month outcomes are confirmed in this final analysis, it appears that our interventions are effective in improving NAFLD despite continued obesity and impaired traditional markers of metabolic health (such as waist circumference). This raises the possibility of weight loss-independent effects on the liver (perhaps driven by changes in serum bile acids or other factors).

We are keen to test this hypothesis in addition to completing the clinical component of the EDISON study. We have stored serum at all EDISON study research visits and propose to use this to treat a cellular model of NAFLD to determine whether post-intervention differences in humoral factors have direct beneficial effects on human hepatocytes.

In brief, in the proposed project, *in vitro* cellular models of NAFLD will be used to gain mechanistic insight into differential changes that occur in the ability of hepatocytes to store and process lipid before and after SG or RYGB surgery.

Changes in the expression of relevant genes and proteins will be measured as well as determining the impact upon lipid accumulation in the hepatocytes. Comparison will be made with supplementation of media from the non-obese control participants *vs.* bariatric participants, as well as comparing the response with serum obtained from the same individuals pre- and post-surgery and across different surgical interventions (SG vs. RYGB).

If distinct phenotypes are seen, especially at matched weight loss, the proposed complimentary metabolomics profiling will potentially be highly informative. It may identify candidate molecules that could be tested in further experiments with a view towards the development of novel pharmaceuticals.

14.2 Final Conclusions and Future Directions

In the opening results chapter, it was demonstrated that hepatologists and bariatric surgeons view bariatric surgery as an intervention that is likely to be beneficial for NAFLD. However, there is widespread variability in the surgical management of patients with NAFLD, which may reflect a lack of clinical guidance and evidence in the existing literature. Whilst it appears that few patients with obesity and NAFLD currently access bariatric surgery, it appears that the majority of patients are open to surgical treatment of these diseases. The cross-sectional design of the surveys highlighted that many of the surveyed patients with NAFLD did not receive information on bariatric surgery from health professionals involved in their care. It is uncertain whether the provision of such information might increase the proportion of patients with obesity and NAFLD who would consider bariatric surgical treatment further.

One area of clinical uncertainty identified through our surveys is in the staging of NAFLD severity. Liver biopsies are considered the 'gold standard' for determining the severity of liver fibrosis, but most bariatric surgeons do not routinely use non-invasive biomarkers to predict fibrosis severity before bariatric surgery or perform intraoperative liver biopsies. This may be because existing non-invasive biomarkers were not developed for use in people with severe obesity, such as those undergoing bariatric surgery, and we have demonstrated

that their performance is suboptimal in such individuals. We have used an innovative machine learning-based approach to develop predictive models that are optimised for people with severe obesity. These offer improved prediction of NAFLD histology and are attractive as they only require the input of routinely recorded clinical data. More extensive and independent validation of these models is now required. Additional work to determine the diagnostic accuracy of our biomarkers after bariatric surgery, especially in patients with advanced fibrotic NAFLD at baseline, will also be required using data from patients with longitudinal liver biopsies.

The omission of bariatric surgery as a recommended intervention for NAFLD in national guidelines was highlighted by hepatologists as a major barrier to its implementation in clinical practice. However, it is also acknowledged that a significant minority of individuals with obesity and NAFLD do not want bariatric surgery. It is apparent that there are a lack of studies comparing outcomes following bariatric surgical and conventional hepatology-led management of patients with NAFLD. Our adjusted analyses suggest that bariatric surgery is associated with greater improvements in a range of NAFLD-related outcome measures (including 5-year mortality) than non-surgical management. This initial data supports the performance of a randomised trial comparing these interventions for their effects on NAFLD in order to provide the high quality evidence required for the inclusion of bariatric surgery in national guidelines for NAFLD management.

In the EDISON study, we have demonstrated that bariatric surgery is an effective intervention for NAFLD in the participants who were studied. Extensive phenotyping was performed at each research visit, and this highlighted the relatively substantial contribution of the preoperative diet to the overall improvements in liver steatosis that were. Whilst the longer term sustainability of such diets is uncertain, the potential of an extended low energy carbohydrate-restricted diet merits further study, and future studies assessing the effects of bariatric surgery on NAFLD should specify details of any preoperative diets used and the timing of the baseline research visit in relation to this. Non-diabetic female participants were chosen for the EDISON study to reduce the potential confounding effects of sex and changes in T2D status (and medication usage) on our outcome measures. The relatively mild

fibrosis stage of participants reflects this decision, and the role of bariatric surgery as an intervention for individuals with T2D and/or advanced NAFLD requires further study.

Since a minority of patients with progress through NAFLD's spectrum of severity, the development of 'hard' clinical outcomes in NAFLD is relatively unusual. Nevertheless, there is a need to determine the safety profile of bariatric surgery compared to medical management in advanced stages of NAFLD such in individuals with cirrhosis. There is the potential for future studies use national resources, such as Hospital Episode Statistics (HES) or The Health Improvement Network (THIN) databases to provide preliminary evidence, and this would ideally provide a foundation for a subsequent multicentre randomised clinical study that definitively addresses this research question. In the EDISON study, it was observed that SG and RYGB had similar effects on NAFLD after 20% postoperative weight loss but it is likely that it was underpowered to compare these operations. An additional multicentre randomised clinical trial would be required to determine whether there are clinically meaningful differences in the effects of these operations on NAFLD.

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16 Appendix: Standard Operating Procedures

16.1 Bariatric Surgery and NAFLD: Survey for Surgeons

Bariatric Surgery and Non-Alcoholic Fatty Liver Disease: Clinical Practice Survey

Prof. Jeremy Tomlinson and Prof. Leanne Hodson at the University of Oxford are investigating the effects of bariatric surgery on Non-Alcoholic Fatty Liver Disease (NAFLD) in collaboration with the Oxford Bariatric Unit. NAFLD is the hepatic manifestation of the metabolic syndrome and encompasses a spectrum of pathology including steatosis, inflammation and fibrosis.

We would appreciate if you could complete this brief survey, which takes approximately 5 minutes to complete. It aims to determine bariatric surgeons' perceptions of NAFLD severity in patients undergoing bariatric surgery and define current approaches to its investigation and management.

1) **Please confirm that you are a consultant bariatric surgeon and have not previously completed this survey:**
   - Yes – I am a consultant bariatric surgeon and have not previously completed this survey ☐
   - No – I do not meet the above criteria ☐

2) **In which country/countries do you work?**.....

3) **Approximately how many of the below operations do you perform annually?**
   - Gastric Banding ……
   - Sleeve Gastrectomy ……
   - Roux-en-Y Gastric Bypass ……
   - One Anastomosis Gastric Bypass/"Mini" Gastric Bypass ……
   - Other ……

4) **Does your bariatric unit have a local guideline or preoperative pathway for NAFLD investigation and treatment?**
   - Yes ☐
   - No ☐
   - What is the name and location (city) of the hospital you work in?
   ……

5) **Please indicate the availability of the following services in your hospital:**

- Hepatobiliary surgery ☐
- Liver transplant surgery ☐
- Liver services provided by gastroenterology ☐
- Liver services provided by specialist hepatologists ☐

6) Please estimate the intraoperative prevalence (%) of the following in your bariatric patients:

- NAFLD ……%
- Advanced fibrotic NAFLD……%
- Cirrhosis ……%
- Non-alcoholic steatohepatitis ……%
- There is insufficient data to provide these estimates ☐

7) How would you manage a patient scheduled for bariatric surgery who is found to have well compensated cirrhosis?

- Cirrhosis shouldn't affect bariatric intervention choice or perioperative management ☐
- Favour gastric band surgery (in the absence of contraindications) ☐
- Favour sleeve gastrectomy surgery (in the absence of contraindications) ☐
- Favour gastric bypass surgery (in the absence of contraindications) ☐
- Favour lifestyle rather than surgical approach to weight loss ☐
- Hepatology referral for perioperative investigation / management advice ☐
- Other *(please specify)*: ☐

8) Which of the following tests and investigations do you routinely arrange pre-operatively in patients scheduled for bariatric surgery?
(please select all that apply)

- ALT ☐
- AST ☐
- Coagulation profile ☐
- Enhanced Liver Fibrosis (ELF) testing ☐
- NAFLD risk score calculation ☐
- HbA1c testing ☐
- Alcohol intake history ☐
- Scan(s) (please specify type):……

9) When would you perform intraoperative liver biopsy during bariatric surgery for NAFLD staging? (please select all that apply)

- Never ☐
- Selectively if NAFLD diagnosed preoperatively ☐
- Selectively if liver looks fatty or cirrhotic during bariatric surgery ☐
- Routinely (normal practice) ☐

10) In your experience, can advanced NAFLD be visually identified intraoperatively?

Yes ☐

Usually ☐

Occasionally ☐

No ☐

11) How does liver function usually change postoperatively in bariatric patients with advanced NAFLD?

No change ☐

Improvement ☐

Improvement then deterioration ☐

Deterioration the improvement ☐

Deterioration ☐

There is insufficient data (local or published) to answer this question ☐

12) Has the liver function of any of your bariatric patients deteriorated post-operatively due to advanced NAFLD?

Yes ☐ - *please proceed to Question 13*

No ☐ *– please proceed to Question 14*

Unsure ☐ *– please proceed to Question 14*

13) If you have experienced cases of liver function deterioration after bariatric surgery due to advanced NAFLD:

In approximately how many cases has this occurred?.....

After which type(s) of bariatric surgery did this occur? .....

In what way(s) did liver function deteriorate (e.g. blood tests, jaundice, ascites, encephalopathy,

death)?.....

How was this managed (e.g. reversal of bariatric surgery, referral to endocrinologist, dietary advice)? .....

Were other contributory factors identified (e.g. alcohol, viral hepatitis)?

……

14) How are bariatric patients who are identified as having NAFLD before or during surgery managed postoperatively in your hospital (Please select all that apply)

Standard care ☐

Family practice physician informed, further management at their discretion ☐

Referral to hepatologist ☐

Arrange scan (*please specify type in box below*) ☐

……

15) Does the investigation and management of NAFLD differ in your publicly funded (e.g. Medicaid or National Health Service) and privately funded practice?

Not applicable ☐

No ☐

Yes (*please specify differences*): ☐ ……..

Would you be interested in collaborating with our researchers on planned research studies investigating the effects of bariatric surgery on NAFLD?

Yes ☐

No ☐

If you may be interested in collaborating in future research or if you would like survey's results emailed to you then please write your name and email address below. <u>Your contact details will not be shared and responses will be anonymised and remain confidential.</u>

Name: ……Email address: …….

If you have additional comments you'd like to share please enter them below.

<u>Thank you for completing this survey!</u>

……

16.2 Bariatric Surgery and NAFLD: Survey for Hepatologists

<u>Non-Alcoholic Fatty Liver Disease and Bariatric Surgery: Clinical Practice Survey</u>

Prof. Jeremy Tomlinson and Dr Jeremy Cobbold at the University of Oxford are investigating the effects of bariatric (weight loss) surgery on Non-Alcoholic Fatty Liver Disease (NAFLD) in collaboration with the Oxford Bariatric Unit.

We would appreciate if you could complete this brief survey, which will take approximately 5 minutes. It aims to determine the views of gastroenterologists/ hepatologists as to the role of bariatric surgery in the treatment of NAFLD.

1) **Please confirm that you are a consultant gastroenterologist / hepatologist and have not previously completed this survey:**

- Yes – I am a consultant/attending gastroenterologist / hepatologist and have not previously completed this survey ☐
- No – I do not meet the above criteria ☐

2) **In which country/countries do you work?**.....

3) **Please indicate the availability of the following services in your hospital or Trust:**

- Liver services as part of combined gastroenterology/hepatology service ☐
- Liver services provided by dedicated hepatology (non-transplant centre) ☐
- Liver services provided by dedicated hepatology (transplant centre) ☐
- Bariatric Surgery ☐

4) **Which of the following patients do you think would benefit from bariatric surgery? (please select all that apply)**

    - Patients with severe obesity ☐
    - Patients with severe obesity and NASH ☐
    - Patients with severe obesity and advanced fibrosis ☐
    - Patients with severe obesity and cirrhosis ☐
    - Patients with severe obesity and decompensated cirrhosis ☐
    - Nobody - Bariatric surgery is not beneficial for NAFLD ☐

5) **What is the pathway through which patients access bariatric surgery in your hospital?**

    - I refer patients directly to the bariatric surgeons ☐

- I ask their GP/family doctor to arrange referral ☐
- I am unsure ☐

6) **Approximately what percentage of patients eligible for bariatric surgery would you estimate undergo this operation locally?**:…….%

7) **Does your hospital have a defined pathway for the staging of NAFLD severity pre-operatively in bariatric surgical patients?**
   - Yes ☐
   - No ☐
   - If yes, approximately how many patients are referred to you through this pathway each year? ……

8) **Which tests do you use to stage NAFLD severity in bariatric surgical patients pre-operatively? (please select all that apply):**
   - Liver Biopsy ☐
   - ALT ☐
   - AST ☐
   - Enhanced Liver Fibrosis (ELF) testing ☐
   - NAFLD risk score/non-invasive biomarker ☐
   - Please specify which score/biomarker(s):……
   - Scan(s) (please specify type):……

9) **Do you recommend liver biopsy at the time of bariatric surgery?**
   - **a.** No, this is not clinically beneficial ☐
   - Yes, in all patients ☐
   - Yes, in selected patients ☐

   Please specify your selection criteria:…….

10) **Which tests do you use to longitudinally monitor NAFLD in bariatric surgical patients post-operatively? (please select all that apply)?:**

- Liver Biopsy ☐
- ALT ☐
- AST ☐
- Enhanced Liver Fibrosis (ELF) testing ☐
- NAFLD risk score/non-invasive biomarker ☐
- Please specify which score/biomarker(s):……
- Scan(s) (please specify type):……

11) Do you recommend bariatric surgery in patients with decompensated liver cirrhosis?

- No ☐
- Yes – prior to liver transplant ☐
- Yes – at time of liver transplant ☐
- Yes – after liver transplant ☐

12) Are you aware of any cases from you hospital and practice, where liver function has deteriorated after bariatric surgery?

Yes ☐ *- please proceed to Question 13*

No ☐ *– please proceed to Question 14*

Unsure ☐ *– please proceed to Question 14*

13) If you have managed cases of NAFLD-related liver dysfunction after bariatric surgery:

- In approximately how many cases has this occurred?.....
- After which type(s) of bariatric surgery did this occur? .....
- In what way(s) did liver function deteriorate (e.g. blood tests, jaundice, ascites, encephalopathy, death)?.....
- How was this managed (e.g. reversal of bariatric surgery, dietary advice, transplantation)? .....
- Were other contributory factors identified (e.g. alcohol, malnutrition)?

……

14) Do you believe that there are local / national barriers to the wider implementation of bariatric surgery as a treatment for NAFLD? (please select all that apply)

- Bariatric surgery not included in national guidelines for NAFLD management ☐
- Insufficient evidence of benefit ☐
- Risks of postoperative liver dysfunction too high ☐
- Too costly ☐
- Insufficient surgical capacity to provide bariatric operations ☐
- I cannot directly refer patients to the bariatric surgery team ☐
- Patients are not interested in surgery as a treatment for obesity and NAFLD ☐
- Other (please specify):…….

If you have additional comments you'd like to share please enter them below:

Thank you for completing this survey!

16.3 Bariatric Surgery and NAFLD: Survey for Patients

Non-Alcoholic Fatty Liver Disease and Bariatric (Weight Loss) Surgery: Patient Survey

Prof. Jeremy Tomlinson and Dr Jeremy Cobbold are keen to learn more about the views of patients with Non-Alcoholic Fatty Liver Disease (NAFLD) who are attending the metabolic hepatology clinic. Bariatric (weight loss) surgery was originally designed to treat obesity but it has also been shown to improve other associated diseases such as diabetes and NAFLD.

We would appreciate if you could complete this brief survey, which will take around 5 minutes. Participation will not affect your NHS care in any way and is voluntary and confidential.

1) Please confirm that you have been previously diagnosed with NAFLD and have not already completed this survey:

- Yes – I have been diagnosed with NAFLD and have not previously completed this survey ☐

- No – I do not meet the above criteria ☐

2) What treatments have been advised? (please select all that apply):

- Weight reduction by diet ☐
- Weight reduction by exercise ☐
- Alcohol reduction ☐
- Medicines to improve the liver ☐
- Medicines to improve diabetes control ☐
- Bariatric (weight loss) surgery ☐

3) If you have previously tried weight reduction through diet/exercise, please specify:

- Which programme(s) have you tried:……..

  ……..

- How much weight did you gain or lose (with each)……

  ……..

  ……..

  ……

4) If you previously stopped a weight management programme, what were the reasons for this? (please select all that apply):

- It was effective and I achieved my goals ☐
- It wasn't effective ☐
- I was too busy ☐
- It was too expensive ☐
- I do not see my body weight and NAFLD as health issues ☐

5) Have you previously heard of bariatric (weight loss) surgery?:

- Yes ☐
- No ☐

6) If you have previously heard of bariatric surgery, how did you hear about it?:

- From a health worker (e.g. doctor, nurse, dietician) ☐

- From friends or family ☐
- From TV, the internet or a newspaper ☐
- From an advert ☐

7) If your doctor recommended bariatric (weight loss) surgery, is this something you would consider?:

- Yes ☐
    - Please specify the reasons for this:……
      ……..
- No ☐
    - Please specify the reasons for this:……
      ……..

If you have additional comments you'd like to share please enter them below:

……

Thank you for completing this survey!

16.4 CRU Day Test Meal Preparation

A 'hot chocolate drink' was prepared as follows for all participants at all research visits:
A bottle warmer was filled with water and heated at 65°C. The following were then weighed out using calibrated scales:

- 40g olive oil (into cup)
- 30g glucose powder (into cup)
- 200mg [$U^{13}C$] palmitic acid
- 400mg Myverol 18-04k emulsifier (into weighing boat)
- 2.4g Cocoa powder (into weighing boat)
- 35g boiled deuterated water

The olive oil was then transferred to a 600mL glass beaker and heated for 20 seconds in a microwave. The temperature was checked and microwave heating was repeated until the oil's temperature was recorded as being 50-60°C.

The emulsifier and tracer were then added to the oil and mixed before heating the solution for a further 10 seconds in the microwave. The drink was checked to ensure it had completely dissolved.

The beaker was then placed in a bowl of hot water and boiling water, cocoa and glucose were added whilst stirring to ensure emulsification.

The drink was stored in the bottle warmer until required.

The reagents were used in the above method were prepared in batches according to the following protocols:

16.5 Laboratory Solution and Reagent Preparation

16.5.1 0.1 Mol l^{-1} EDTA, di-sodium salt

Weigh out 1.86 g of di-sodium EDTA
Add approximately 35 ml distilled water to dissolve
Adjust pH to 7.4 and make up to 50 ml with distilled water.
Store at 4°C.

16.5.2 0.5 Mol l^{-1} EDTA, di-sodium salt

Weigh out 18.62 g di sodium-EDTA
Add approximately 80 ml distilled to dissolve (heat may be required plus a little more volume)
Adjust pH to 7.4 and make up to 100 ml with distilled water.
Store at room temperature.

16.5.3 10 mmol l^{-1} PMSF

The 0.1 Mol l^{-1} solution of PMSF is stored in the Sanyo fridge in lab F40. This stock solution must be replaced every six months.

Prepare a 1 in 10 dilution of stock PMSF in anhydrous propan-2-ol:

Using a 5 ml syringe and a hypodermic needle withdraw 4.5ml of anhydrous prop-2-ol and transfer this to a 5ml glass screw cap tube.

Pipette 500µl of 0.1 mol l^{-1} PMSF (using a positive displacement pipette) into the 4.5ml of anhydrous propan-2-ol. Tighten the cap and mix well.

Store at 4°C in the Sanyo fridge in lab F40 and make up a fresh solution every three months.

16.5.4 Trasylol, 10,000KIE

This is used as supplied from Bayer. Remove a small amount from the 50ml bottle and transfer to a plastic tube. This is stored at 4°C.

16.5.5 Sodium Bromide, density 1.42 kg l^{-1}, containing 10µmol l^{-1} EDTA

Weigh out 574 g NaBr

Dissolve in 846 ml distilled water. (volume should equal 1 litre)

Add 100µl 0.1Mol l^{-1} EDTA

Check density on Paar Density meter and adjust if necessary.

Store at 4°C but allow to room temperature before use.

16.5.6 Sodium chloride, density 1.063 kg l^{-1}, containing 10µmol l^{-1} EDTA

Weigh out 98.35 g NaCl

Dissolve in 967 ml distilled water. (volume should equal 1 litre)

Add 100µl 0.1Mol l^{-1} EDTA

Check density on Paar Density meter and adjust if necessary.

Store at 4°C but allow to room temperature before use.

16.5.7 Sodium chloride, density 1.02 kg l^{-1}, containing 10µmol l^{-1} EDTA

Weigh out 31.17 g NaCl

Dissolve in 989 ml distilled water. (volume should equal 1 litre)

Add 100µl 0.1Mol l^{-1} EDTA

Check density on Paar Density meter and adjust if necessary.

Store at 4°C but allow to room temperature before use.

16.5.8 Sodium chloride, density 1.006 kg l^{-1}, containing 10µmol l^{-1} EDTA

Weigh out 11 g NaCl

Dissolve in 995 ml distilled water. (volume should equal 1 litre)

Add 100µl 0.1Mol l^{-1} EDTA

Check density on Paar Density meter and adjust if necessary.

Store at 4°C but allow to room temperature before use.

Measurement of triglyceride concentration

Reagent: Triglyceride assay reagent (Ref 0018255640, Instrumentation Laboratory, Bedford, USA) was used.

Plasma calibration: ReferrIL G calibrator vial (Ref 0018257000, Instrumentation Laboratory, Bedford, USA) was reconstituted with 3mL of deionised water.

Plasma quality control: Serachem 1 and 2 vials (Refs 0018162412, 0018162512 respectively, Instrumentation Laboratory, Bedford, USA) were reconstituted with 5mL of deionised water.

Lipoprotein fraction (chylomicron, VLDL) calibration: 50µM, 100µM, 200µM and 1000µM calibration agents were made from a 10mM stock.

Lipoprotein fraction (chylomicron, VLDL)quality control: R1 and R2 vials (Refs HN1530 and HE1532 respectively, Randox Quality Control, Crumlin, UK) were reconstituted with 5mL of deionised water then diluted 1 in 10 to produce GO1 and GO2 Quality Control (QC) agents.

Measurement of NEFA concentration

Reagent: This was prepared fresh by adding 10.5mL of R1a NEFA buffer to R1b NEFA enzyme (ref FA115, Randox Quality Control, Crumlin, UK). R2c reagent was prepared by adding R2a enzyme dilutent to R2b (Maleimide) then using this to reconstitute R2c NEFA enzyme reagent (ref FA115, Randox Quality Control, Crumlin, UK).

Calibration: 150µL of neat NAFA calibrator (ref FA115, Randox Quality Control, Crumlin, UK).

Quality control: R1 and R2 vials (Refs HN1530 and HE1532 respectively, Randox Quality Control, Crumlin, UK) were reconstituted with 5mL of deionised water. R3, a 1/3 dilution of R1, was also used in quality control.

Measurement of glycerol concentration

Reagent: 4.6ml R1a buffer was added to the R1b vial (Ref GY105, Randox Quality Control, Crumlin, UK).

Calibration: Dilutions of glycerol calibrator agent were made (150µL neat, 75µL calibrating agent mixed with 75µL deionised water, 150µL deionised water. Ref GY105, Randox Quality Control, Crumlin, UK).

Quality control: R1 and R2 vials (Refs HN1530 and HE1532 respectively, Randox Quality Control, Crumlin, UK) were reconstituted with 5mL of deionised water then diluted 1 in 10 to produce GO1 and GO2 QC agents.

Measurement of 3-OHB concentration

Reagent: 10mL R1a reagent was added to reconstitute R1b RanBut vials (Ref RB1007, Randox Quality Control, Crumlin, UK).

Calibration: Neat 3-OHB standard (Ref RB1007, Randox Quality Control, Crumlin, UK) was diluted with deionised water to make 62.5µM/L, 125µM/L and 500µM/L 3-OHB dilutions for calibration.

Quality control: R1 and R2 vials (Refs HN1530 and HE1532 respectively, Randox Quality Control, Crumlin, UK) were reconstituted with 5mL of deionised water then diluted 1 in 10 to produce GO1 and GO2 QC agents.

Measurement of lactate concentration

Reagent: R1b enzyme reagent was reconstituted using R1a buffer (Ref LC3980, Randox Quality Control, Crumlin, UK).

Calibration: 150µL of lactate calibrator (neat). (Ref LC3980, Randox Quality Control, Crumlin, UK).

Quality control: R1 and R2 vials (Refs HN1530 and HE1532 respectively, Randox Quality Control, Crumlin, UK) were reconstituted with 5mL of deionised water.

Measurement of glucose concentration

Reagent: Glucose (Oxidase) reagent was used (Ref 0018250840, Instrumentation Laboratory, Bedford, USA).

Calibration: ReferrIL G calibrator vial (Ref 0018257000, Instrumentation Laboratory, Bedford, USA).

Quality control: Serachem 1 and 2 vials (Refs 0018162412, 0018162512 respectively, Instrumentation Laboratory, Bedford, USA) were reconstituted with 5mL of deionised water.

Measurement of apolipoprotein B concentration

Reagent: R1 and R2 reagents used (Ref LP 3839, Randox Quality Control, Crumlin, UK).

Plasma calibration: ApoB calibrator (Ref LP303, Randox Quality Control, Crumlin, UK) aliquot was thawed. Serial dilutions were prepared for calibration as follows:

Calibrator Standard Cup	Water Volume	ApoB Volume
5	0µL	Whole aliquot
4	50µL	50µL from Cup 5
3	50µL	50µL from Cup 4
2	50µL	50µL from Cup 3
1	100µL	0

Plasma quality control: L1, L2 and L3 aliquots were thawed and used for quality control (Refs LE2668, LE2669 and LE2670 respectively, Randox Quality Control, Crumlin, UK).

Lipoprotein (chylomicron, VLDL) fraction calibration: ApoB calibrator (Ref LP303, Randox Quality Control, Crumlin, UK) aliquot was thawed and diluted for calibration curve as follows:

Calibrator Standard Cup	0.9% Saline Volume	ApoB Volume
S1	200µL	0
S6	100µL	100µL from S2

S2	150µL	150µL from S3
S3	300µL	100µL ApoB calibrator

Lipoprotein (chylomicron, VLDL) fraction quality control: L1, L2 and L3 aliquots were thawed and diluted prior to use for quality control (Refs LE2668, LE2669 and LE2670 respectively, Randox Quality Control, Crumlin, UK). Dilutions were as follows:

Calibrator Standard Cup	0.9% Saline Volume	ApoB Calibrator Volume
Lipoprotein L1	50µL	50µL L1
Lipoprotein L2	150µL	50µL L2
Lipoprotein L3	180µL	20µL L3

Measurement of total cholesterol concentration

Reagent: Cholesterol reagent (Ref 0018250540, Instrumentation Laboratory, Bedford, USA)
Calibration: ReferrIL G calibrator vial (Ref 0018257000, Instrumentation Laboratory, Bedford, USA)
Quality control: Serachem 1 and 2 vials (Refs 0018162412, 0018162512 respectively, Instrumentation Laboratory, Bedford, USA) were reconstituted with 5mL of deionised water.

Measurement of HDL cholesterol concentration

Reagent: HDL R1 and R2 reagents used (Refs CH 2652, Randox Quality Control, Crumlin, UK)
Calibration: HDL calibrator (Ref CH 2652, Randox Quality Control, Crumlin, UK) aliquot was thawed then used neat.
Quality control: Serachem 1 and 2 vials (Refs 0018162412, 0018162512 respectively, Instrumentation Laboratory, Bedford, USA) were reconstituted with 5mL of deionised water.