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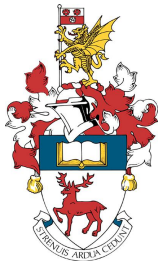

Faculty of Medicine

Human Development and Health, Nutrition and Metabolism

Endoscopic bariatric therapies and the effect of duodenal exclusion using a duodenal-jejunal bypass liner (EndoBarrier®) on insulin sensitivity and blood lipid profile in patients with obesity and type 2 diabetes.

by

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Thesis for the degree of DM

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ABSTRACT

FACULTY OF MEDICINE
Human Development and Health, Nutrition and Metabolism
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The effect of duodenal exclusion using a duodenal-jejunal bypass liner (EndoBarrier®) on insulin sensitivity and blood lipid profile in patients with obesity and type 2 diabetes.

Mr Michael Alan Glaysher BM Hons MRCS Eng

Background: The Endobarrier® duodenal-jejunal bypass liner (DJBL) shows promise as a safe and effective therapeutic option for patients with obesity and type 2 diabetes (T2DM) by reducing weight and improving glycaemic control.

Objective: Effectiveness of the Endobarrier® DJBL compared with intensive medical therapy, diet and exercise in the treatment of patients with obesity and T2DM.

Design, setting and participants: Multicentre randomised controlled, open-label, trial at two University hospitals in England. 170 patients, aged 18 – 65 years, BMI 30–50 kg/m^2, with inadequately controlled T2DM (HbA1c 58 – 97 mmol/mol) on oral anti-hyperglycaemic medications, were randomised at a ratio of 1:1 by a centralised computer system. Trial Registration: ClinicalTrials.gov Identifier NCT02459561.

Intervention: 12 months of intensive medical therapy with (n = 85) vs without (n = 85) the DJBL. Follow-up for 12 months.

Outcome Measures: Primary outcome was the percentage of participants (using Intention to Treat) achieving a glycated haemoglobin (HbA1c) reduction of ≥20% at 12 months (reported elsewhere). Secondary outcomes included weight loss and cardiometabolic risk factors at 12 and 24 months. Nested mechanistic studies examined alterations in insulin sensitivity, ß-cell function, lipid profile and blood concentrations of polyunsaturated fatty acids (PUFAs).

Results: DJBL treatment resulted in significantly greater weight loss (DJBL -10.6 ± 6.2% vs. control -5.4 ± 5.8%, p<0.0001) and significantly more DJBL-treated patients achieved a ≥ 15% weight loss at 12 months (DJBL 24.2% vs. control 3.7% (OR 8.3, 95% CI: 1.8, 39.0; p=0.0071)). At 24 months there were no significant differences in weight loss between the two groups. Hepatic glucose production and fasting plasma glucose (FPG) concentrations significantly reduced within both groups at 10 days but were not significantly different between groups at any time-point. Peripheral insulin sensitivity increased significantly within both groups at 10 days and was significantly higher in the DJBL group at 6 months (rate of peripheral glucose disposal: DJBL 39.5 $\pm$ 15.8 μmol/kg/min vs. control 26.4 $\pm$ 11.1 μmol/kg/min (mean difference [95% CI] = 12.9 [5.0, 20.7], p = 0.002) and 11.5 months (HOMA2-%S, median (range): 90.4 (70.6 – 114.0) vs. 66.6 (50.4 -106.6), p = 0.02). Early improvements in beta cell function (HOMA2-%B) were observed but there were no differences between groups. Absolute concentrations of long-chain PUFAs, total serum cholesterol and LDL-cholesterol were significantly lower in DJBL-treated patients after 1 year compared to controls (p<0.05). After the Endobarrier was removed, patients regained weight, insulin sensitivity decreased and insulin resistance, FPG and insulin concentrations increased. At 24 months, all values remained significantly improved from baseline but were not statistically different from control arm participants.

Conclusions: One year of therapy with the Endobarrier DJBL in patients with obesity and T2DM resulted in superior weight loss, greater late improvements in peripheral insulin sensitivity and significant reductions in total serum cholesterol and LDL-C concentrations when compared to intensive medical therapy, diet and lifestyle advice alone. Early reductions in FPG may be as a consequence of improvements in hepatic insulin sensitivity but the DJBL provided no additional improvements to early changes in glucose homeostasis above that achieved through calorie restriction alone. Absolute blood concentrations of essential fatty acids and their long chain derivatives are depleted during 1 year of Endobarrier therapy and should be offset by maintaining an adequate dietary intake of PUFAs or by supplementation.

Funding: Efficacy and Mechanism Evaluation Programme, a Medical Research Council and National Institute for Health Research (NIHR) partnership reference 12/10/04.

Endoscopic sleeve gastroplasty: a modified technique with greater curvature compression sutures

Mr Michael Alan Glaysher BM Hons MRCS Eng

Background: Endoscopic sleeve gastroplasty (ESG) is rapidly becoming established as a safe and effective means of achieving substantial weight loss via the transoral route. New ESG suture patterns are emerging.

Objective: Effectiveness and safety of performing ESG with a modified suture pattern.

Design, setting and participants: Retrospective comparative study of all patients who underwent ESG by a single operator in a single UK centre.

Intervention: ESG performed using a unique combination of longitudinal compression sutures and "U"-shaped sutures versus ESG performed with a conventional triangular pattern of plications.

Outcome Measures: Procedural times, morbidity and weight loss at 1, 3 and 6 months.

Results: Between January 2016 and December 2017, 32 patients (23 women) underwent ESG; n = 9 cases were completed utilizing a commonly used triangular suture pattern ("no longitudinal compression") and n = 23 cases were completed using our unique "longitudinal compression" suture pattern. In the no compression and compression groups, the mean ages were 45 ± 12 years and 43 ± 10 years, the median baseline weights were 113.6 kg (range 82.0–156.4) and 107 kg (range 74.0–136.0), and the median baseline body mass indexes (BMIs) were 35.9 kg/m^2 (range 30.9–43.8) and 36.5 kg/m^2 (range 29.8–42.9), respectively. After 6 months, body weight decreased by 21.1 kg (range, 12.2–34.0) in the compression group (n = 7) versus 10.8 kg (range, 7.0–25.8) in the no compression group (n = 5). Correspondingly, BMI decreased by 7.8 kg/m^2 (range, 4.9–11.2) and 4.1 kg/m^2 (range, 2.6–7.2) in each group, respectively. Total body weight loss was greater in the compression group at 19.5% (range, 12.9–30.4%) compared to 13.2% (range, 6.2–17.1%) in the non-compression group. There was no significant difference in procedural time between the two cohorts (no compression: 96 minutes (range, 85 – 135 minutes) versus longitudinal compression: 105 minutes (range, 65 – 139 minutes)). No significant adverse events were reported in this series.

Conclusions: The technique of ESG is evolving and outcomes from endoscopic bariatric therapies continue to improve. We provide preliminary evidence of superior weight loss achieved through a modified gastroplasty suture pattern.

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Declaration of Authorship

I, Michael Alan Glaysher

declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

Endoscopic bariatric therapies and the effect of duodenal exclusion using a duodenal-jejunal bypass liner (EndoBarrier®) on insulin sensitivity and blood lipid profile in patients with obesity and type 2 diabetes.

I confirm that:

1) This work was done wholly or mainly while in candidature for a research degree at this University;
2) Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3) Where I have consulted the published work of others, this is always clearly attributed;
4) Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5) I have acknowledged all main sources of help;
6) Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7) Parts of this work have been published as journal articles, presented as oral presentations and published in abstract form as listed overleaf.

Signed:

Date:

Parts of this work have been published in the following journal articles:

- Glaysher MA, Mohanaruban A, Prechtl CG, Goldstone AP, Miras AD, Lord J, Chhina N, Falaschetti E, Johnson NA, Al-Najim W, Smith C, Li JV, Patel M, Ahmed AR, Moore M, Poulter N, Bloom S, Darzi A, Le Roux C, Byrne JP, Teare JP. A randomised controlled trial of a duodenal-jejunal bypass sleeve device (EndoBarrier) compared with standard medical therapy for the management of obese subjects with type 2 diabetes mellitus. *BMJ open*. 2017;7(11): e018598.
- Ruban A, Prechtl CG, Glaysher MA, Chhina N, Al-Najim W, Miras AD, Smith C, A PG, Patel M, Moore M, Ashrafian H, Byrne JP, Teare JP. Effectiveness of different recruitment strategies in an RCT of a surgical device: experience from the Endobarrier trial. *BMJ open*. 2019;9(11): e032439.
- Glaysher MA, Moekotte AL, Kelly J. Endoscopic sleeve gastroplasty: a modified technique with greater curvature compression sutures. *Endosc Int Open*. 2019;7(10): E1303-e9.

Parts of this work formed the basis of an oral presentation delivered at the 10th British Obesity and Metabolic Surgery Society (BOMSS) Annual Scientific Meeting, Belfast, 2019 and the abstract has been published:

- Glaysher MA, Moekotte AL, Kelly J. Endoscopic sleeve gastroplasty: a modified technique with greater curvature compression sutures. *Obes Surg.* 2019; 29(2): Suppl 1, S11.

Parts of this work formed the basis of an oral and poster presentation delivered at the 11th British Obesity and Metabolic Surgery Society (BOMSS) Annual Scientific Meeting, Aberdeen, 2020 and the abstracts have been published:

- Glaysher MA, Ward J, Aldhwayan M et al. The effect of a duodenal-jejunal bypass liner device (Endobarrier®) on lipid profile and blood concentrations of long chain polyunsaturated fatty acids. *Obes Surg* 2020; 30: S16-S17.
- Glaysher MA, Miras AD, Ruban A et al. The effect of a duodenal-jejunal bypass liner device (Endobarrier®) on insulin sensitivity. *Obes Surg* 2020; 30: S25.

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I acted as co-investigator for the herein reported multicentre randomized controlled trial of a duodenal jejunal bypass liner (EndoBarrier®) compared with standard medical therapy for the management of patients with obesity and type 2 diabetes. I was an active member of the trial management group and also an independent observer within the trial steering committee. I performed the majority of the work involved in the design, execution and analysis of this trial and the nested mechanistic studies at the University Hospital Southampton site. I recruited, consented, and randomised all patients at this site; prepared and conducted all study visits; obtained and reported study samples and data, and assisted in the implantation and removal of the Endobarrier devices. I designed the insulin clamp sub-study; prepared and conducted all visits; derivatised the clamp samples and prepared them for gas chromatography-mass spectrometry; analysed and performed statistical analysis on the study results; interpreted and drew conclusions from the clamp data. I also conducted the mixed meal tolerance tests, collected the samples for fatty acid analysis and completed the analysis and interpretation of the results. In parallel to this work, I also conducted, analysed, interpreted and presented a retrospective evaluation of the Endoscopic Sleeve Gastroplasty. I am very grateful for the contribution of my colleagues who assisted me and I would like to acknowledge the help and support of the following people:

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Definitions and Abbreviations

AA	arachidonic acid
Akt/PKB	protein kinase B
ALA	α-Linolenic acid
ALT	alanine aminotransferase
ASBT	apical sodium bile salt transporter
AST	aspartate aminotransferase
AUC	area under the curve
BA	bile acid
BPD $\pm$ DS	biliopancreatic diversion $\pm$ duodenal switch
BMI	body mass index
CCK	cholecystokinin
CI	confidence interval
CRF	case report form
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
DI	disposition index
DIO	diet-induced obesity
DJBL	duodenal jejunal bypass liner
EBT	endoscopic bariatric therapy
EFA	essential fatty acid
EGP	endogenous glucose production
EPA	eicosapentaenoic acid
EPIC FFQ	European Prospective Investigation of Cancer Food Frequency Ques
ESG	endoscopic sleeve gastroplasty
EWL	excess weight loss
FA	fatty acid
FAME	fatty acid methyl ester
FGF	fibroblast growth factor
FID	flame ionisation detection
FIRKO	fat insulin receptor knockout
FPG	fasting plasma glucose
FXR	farnesoid X receptor
GC	gas chromatography
GGT	γ-glutamyltransferase

GI	gastrointestinal
GIP	gastric inhibitory peptide
GK	Goto-Kakizaki
GLP-1	glucagon-like peptide 1
GLUT	glucose transport protein
HA	hepatic abscess
HbA_{1C}	glycated haemoglobin
HDL-C	high density lipoprotein cholesterol
HEC	hyperinsulinaemic euglycaemic clamp
HGP	hepatic glucose production
HOMA-β	homeostasis model assessment-β
HOMA-IR	homeostasis model assessment – insulin resistance
HOMA-S	homeostasis model assessment – sensitivity
IGF	Insulin-like growth factor
IGFR	Insulin-like growth factor receptor
IGT	impaired glucose tolerance
II	insulinogenic index
IL	interleukin
IMT	intensive medical therapy
IR	insulin receptor
IRS	insulin-responsive substrate
ISI	insulin sensitivity index
LA	linoleic acid
LAGB	laparoscopic adjustable gastric banding
LDL-C	low density lipoprotein cholesterol
LIRKO	liver insulin receptor knockout
LSG	laparoscopic sleeve gastrectomy
MIRKO	muscle insulin receptor knockout
MMTT	mixed meal tolerance test
MOX-TMS	methyloxime penta-O-trimethylsilyl
MS	mass spectrometry
MUFA	monounsaturated fatty acid
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NEFA	non-esterified fatty acid
NF-kB	nuclear factor-kappa B

NGT	normal glucose tolerant
OFN	oxygen-free nitrogen
OGTT	oral glucose tolerance test
OA	oleic acid
PA	palmitic acid
PACAP	pituitary adenylate cyclase-activating polypeptide
PIS	patient information sheet
PPI	proton pump inhibitor
PUFA	polyunsaturated fatty acid
PYY	peptide YY
RCT	randomised controlled trial
RPB-4	retinol binding protein-4
RYGB	Roux-en-Y gastric bypass
SAE	serious adverse event
SBP	systolic blood pressure
SD	standard deviation
SGLT	sodium-glucose linked transporter
SST	somatostatin
T2DM	type 2 diabetes mellitus
TAG	triacylglycerol
TBW	total body weight
TBWL	total body weight loss
TIC	total ion chromatogram
TNF-α	tumour necrosis factor alpha
TSC	total serum cholesterol
VBG	vertical banded gastroplasty
VIP	vasoactive intestinal peptide
VLCD	very low calorie diet
WHO	world health organisation

Chapter 1:

Introduction

1.1 Clinical Context of the Study

The global prevalence of obesity is increasing and it is estimated that the number of individuals who are either overweight (clinically defined as a body mass index (BMI) of 25 – 30 kg/m^2) or obese (BMI ≥ 30 kg/m^2) exceeds 2.1 billion.[1,2] The metabolic complications of obesity, including type 2 diabetes mellitus (T2DM), hyperlipidaemia, and hypertension, are well recognized. In correlation with obesity trends, the prevalence of T2DM continues to increase and it is estimated that 8.6% of the UK population are currently affected. This is projected to increase to 9.7% by 2035.[3] Compared to the general population, patients with T2DM are 87.6% more likely to be admitted to hospital for a myocardial infarction, 121.1% more likely to be admitted for heart failure, 59.1% for a stroke, and are 32% more likely to die prematurely.[4] In a meta-analysis of 57 prospective studies and 900,000 adults, each increase in BMI > 25 kg/m^2 by 5 kg/m^2 was associated with a 30% increase in overall mortality.[5] This represents an overwhelmingly significant socioeconomic burden for largely preventable conditions and combined healthcare costs are estimated to increase by up to 2 billion pounds each year in the UK.[6]

Extensive evidence exists for the efficacy of combined medical therapy, intensive lifestyle interventions and weight loss in patients with T2DM on improving glycaemic control, reducing morbidity and mortality, and improving quality of life.[7] Unfortunately, despite this, currently available pharmacological, behavioural and dietary strategies provide only mild to modest levels of weight reduction that are often not sustained.[8,9] Metabolic surgery remains the most efficacious and cost-effective means of treating individuals with obesity and T2DM by producing often profound and sustained weight loss, as well as improvements in metabolic health. However, surgery carries with it a significant risk of complications and there continues to be numerous, multifactorial barriers to accessing these procedures. As such, only approximately 1% of eligible, obese adults currently undergo metabolic operations each year.[10]

The search for an intermediate treatment modality to bridge the 'treatment gap' between lifestyle interventions, medical therapy and surgery has led to the development of several therapeutic endoscopic procedures for obesity, which principally involve duodenal exclusion, gastric restriction and/or altering the delivery of gastric content to the duodenum. These endoscopic bariatric therapies (EBTs) remain in their relative infancy and ongoing research of their safety, efficacy and effectiveness is required. Furthermore, metabolic surgery is an evolving concept and we are only just beginning to increase our understanding of the effects of these interventions on gastrointestinal physiology and human metabolism. Many of the underlying mechanisms and pathways that are modified by these interventions remain uncertain. Only by understanding these pathways can we optimise current therapies and design new, targeted

treatments for patients with obesity and obesity-related diseases. The herein reported research therefore sought to further examine two evolving EBTs: a duodenal jejunal bypass liner (DJBL) and the endoscopic sleeve gastroplasty (ESG).

1.2 Literature Review

1.2.1 Physiology of Insulin

1.2.1.1 Insulin: biosynthesis, secretion and mechanisms of action

Insulin is a 51-residue peptide hormone that is manufactured within and secreted from the β-cells of the Islets of Langerhans of the pancreas. It has essential roles in carbohydrate, lipid and protein metabolism. It is coded for by the human insulin gene on the short arm of chromosome 11,[11] which is a 1355 base sequence from which the mRNA transcript encodes the inactive precursor preproinsulin within the rough endoplasmic reticulum of the β-cell.[12] This signal peptide is subsequently cleaved by a signal peptidase to form proinsulin, comprising of the 'active' two-chain heterodimer of insulin with a 21-residue A-chain and a 30-residue B-chain connected via an inactive C-domain (C-peptide). This proinsulin is packaged into secretory granules within the Golgi apparatus of the β-cell before then being cleaved by a trypsin-like enzyme to release the mature hormone and a free C-peptide chain.[13,14]

The major determinant of insulin secretion in humans is the concentration of circulating glucose and other nutrients originating from the diet, such as amino and fatty acids. Elevated glucose concentrations generated from the intravenous administration of glucose result in the secretion of insulin into the hepatic portal circulation in a biphasic fashion. In the acute first phase, glucose entering the β-cells via glucose transporter 2 (GLUT-2) activity initiates an intracellular cascade that culminates in: (1) the increased translation and transcription of insulin mRNA, (2) calcium-dependent exocytosis of secretory granules and pulsatile secretion of insulin and equimolar concentrations of C-peptide that have already been synthesised and stored.[14,15] Following this initial rapid phase, which lasts several minutes, there is a second attenuated phase with sustained release of both stored and newly-synthesised hormone that lasts for the duration of the hyperglycaemia. This secretory response however is augmented by other mediators and pathways as summarised in **Table 1.1,** which allows for the release of sufficient quantities of insulin that, in healthy individuals, match the prevailing metabolic status.[12,14,16,17] The insulin response to an oral glucose load or a mixed meal is much more variable and is dependent on: (a) the nutritional composition and physical form of the meal, (b) rate of gastric emptying, (c) gastrointestinal (GI) motility, (d) GI hormones, and (d) neural inputs. Oral intake of glucose also

induces an incretin effect whereby certain GI hormones, such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP), are released in response to nutrients in the GI-tract that will amplify glucose-induced insulin release and produce a greater insulin response when compared to intravenous administration (see **section 1.2.1.4**).[18]

Type of signal	Stimulatory	Inhibitory
Nutritional/Metabolic	Glucose Amino acids e.g. Arginine and Leucine	
Hormonal/Other	Growth hormone Glucagon GLP-1 GIP Secretin CCK Gastrin/GRP VIP Gastrin releasing peptide Adiponectin PACAP	Adrenocorticoids Somatostatin Adrenaline Noradrenaline Galanin Neuropeptide Y Calcitonin gene related peptide Prostaglandin E Ghrelin Leptin Resistin SST-14 and 28
Neural	B-adrenergic Vagal Parasympathetic	α-adrenergic

Table 1.1 Mediators regulating insulin secretion
GLP-1, glucagon-like peptide 1; GIP, gastric inhibitory peptide; CCK, cholecystokinin; VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; SST, somatostatin. Modified from Wilcox.[14]

After insulin is released into the hepatic portal circulation approximately 60% is removed by the liver before then entering the systemic circulation.[14] Here its actions are mediated in peripheral cells through a specific tyrosine kinase insulin receptor (IR), which exists in two isoforms: IR-A and IR-B.[19] These receptors are also activated by Insulin-like growth factor (IGF), secreted largely from hepatocytes following stimulation by growth hormone.[13,19,20] Insulin and IGF have distinct physiological roles but have a shared signalling pathway through IRs and IGF receptors (IGFRs) (see **Figure 1.1**). The IR-B receptor is the predominant receptor type in humans after birth in muscle, liver and fat cells and is largely activated by insulin.[19-21]

Binding of insulin (and IGF) to the extracellular domain of the IR triggers an intracellular cascade leading to the phosphorylation and activation of 4 specific insulin-responsive substrates (IRS 1-4). These, in turn, bind multiple signal molecules that potentiate the effects of the insulin in

regulating cellular energy supply and macronutrient balance via various signalling pathways.[21,22] IRS-1 is the major substrate in skeletal muscle and adipose tissue and is phosphorylated by coupling with IR-A, IR-B and IGF1R to mediate the mitogenic effects of insulin. IRS-2 is predominantly found in hepatic tissue to mediate the peripheral action of insulin and also augments pancreatic β-cell growth and survival.[14,22] IRS-3 and -4 are less well characterised and are found to be distributed throughout adipose tissue, β-cells, thymus, brain and kidney.[23]

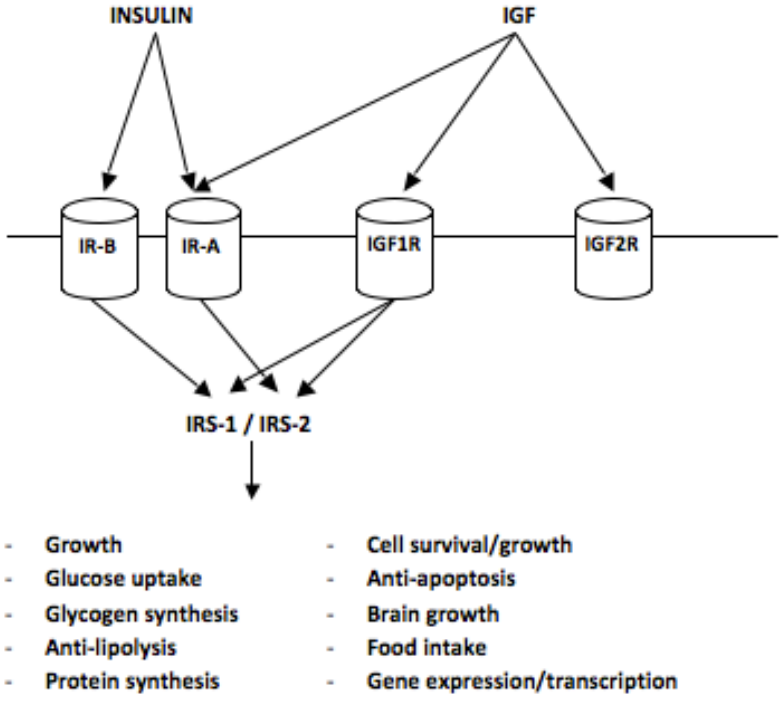


Figure 1.1 Overview of the insulin-IGF signalling pathway and its targets.
IGF, Insulin-like growth factor; IR, insulin receptor; IRS, insulin-responsive substrates.

1.2.1.2 Functions of insulin

Insulin acts to direct anabolism during the fed state, predominantly through its action on skeletal muscle, hepatic and adipose tissue. After binding to IRs, insulin stimulates mitogenic processes (cell growth and differentiation); promotes the storage of substrates by stimulating lipogenesis, glycogenesis and protein synthesis; and simultaneously inhibits lipolysis, glycogenolysis and proteolysis within insulin-sensitive tissues. The main mitogenic and metabolic functions of insulin are summarised in **Table 1.2**. Insulin is considered to be integral to all intermediary metabolic pathways but its principle function is in carbohydrate metabolism and the regulation of glucose homeostasis.

	Stimulates	Inhibits
Carbohydrate metabolism	▪ Glucose uptake (adipose tissue and skeletal muscle via GLUT 4 receptors) ▪ Glycogenesis ▪ Glycolysis	▪ Glycogenolysis ▪ Gluconeogenesis
Lipid metabolism	▪ Fatty acid synthesis ▪ Formation and storage of triglycerides in adipose tissue ▪ Cholesterol synthesis	▪ Lipolysis ▪ Ketogenesis
Protein metabolism	▪ Amino acid uptake ▪ Protein synthesis (e.g. enzymes and albumin)	▪ Proteolysis
Other	▪ HCl secretion by parietal cells ▪ Cell growth and differentiation ▪ Beta cell survival and growth ▪ Appetite regulation, satiety, olfaction and memory ▪ Enhances steroidogenesis in gonadal tissue ▪ Bone formation/osteoblast activity	▪ Renal sodium excretion ▪ Osteoclast activity

Table 1.2 Summary of the actions of insulin
HCl, hydrochloric acid

1.2.1.3 Glucose homeostasis

Insulin, together with its principal counter-regulatory hormone glucagon, acts to regulate blood glucose concentrations. Glucose enters different cells via a family of 14 glucose transport (GLUT) proteins, which are encoded by the SLC2 gene and utilise ATP to actively transport glucose across the plasma membrane (**Figure 1.2**).[24] Each of the GLUT transporters differ in their Km value, which is the relative affinity of the transporter protein for glucose molecules, and also their dependency on insulin. This allows for glucose uptake into cells to be regulated according to the metabolic functions of each specific cell/tissue type in view of the prevailing circulating glucose concentration. For example, GLUT-1 and GLUT-3 transporters have a low Km, reflecting a high affinity for glucose, and uptake into cells occurs constantly at low levels of glucose without the need for insulin stimulation. GLUT-1 transporters are the most abundant transporter type in the brain, therefore allowing neural tissue to continuously utilise glucose for metabolic activities during the fasted state. Conversely, skeletal muscle, adipose and cardiac muscle cells are predominated by GLUT-4 proteins, which have a higher Km value and require insulin for their

action. Therefore, when glucose levels are elevated (i.e. during the fed state), insulin acts on these insulin-sensitive tissues via IR-binding and IRS activation to move glucose intracellularly via GLUT-4 transporters, reduce circulating plasma glucose concentrations and direct metabolic/mitogenic cellular processes. Translocation of GLUT-4 glucose transporters to the plasma membrane in skeletal muscle, adipose and cardiac muscle cells occurs within 30 seconds of insulin stimulation.[13,14]

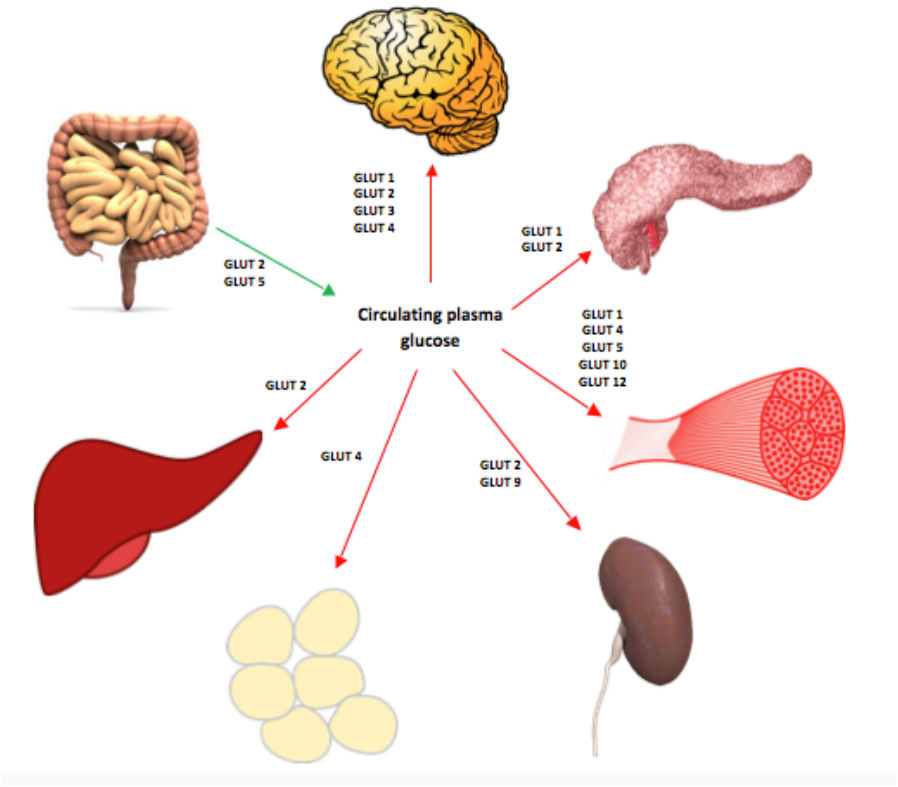


Figure 1.2 Distribution of GLUT transporter proteins and their role in plasma glucose regulation. Adapted from Mueckler et al.[24] GLUT, glucose transport protein.

During the fasted state, metabolic processes are driven by the counter-regulatory hormone glucagon (promoting glycogenolysis, gluconeogenesis and ketogenesis), as well other mediators such as catecholamines (lipolysis and glycogenolysis) and glucocorticoids (proteolysis, gluconeogenesis and lipolysis).[14] β-cells however continue to produce insulin at a rate of 0.25 – 1.5 units per hour,[14] which allows for glucose to enter cells via insulin-dependent transporters, limits uncontrolled lipolysis and limits gluconeogenesis, thereby maintaining normal fasting glucose levels (3.9 – 5.5 mmol/L). Islet β-cells respond acutely to small changes in extracellular glucose and the rate of insulin secretion is coupled closely to nutrient metabolism in a sigmoidal

fashion in order to keep plasma glucose concentrations within a narrow range; concentrations of glucose < 5 mmol/L do not affect the rate of insulin release but this increases progressively for extracellular glucose concentrations between 5 and 15 mmol/L before then plateauing.[12] This insulin secretion follows a biphasic pattern as discussed in **section 1.2.1.1**.

1.2.1.4 The role of the gastrointestinal tract in glucose homeostasis.

Nutrients within the GI tract stimulate a cascade of neuro-hormonal pathways that regulate digestion, energy and glucose homeostasis, and metabolism. Over 100 metabolically active peptides are actively secreted within the GI tract from mucosal neuroendocrine cells that act on peripheral or central targets via the circulation or afferent nerves.[25] Those hormones which act to increase insulin secretion and thus regulate glucose homeostasis are termed *incretins* and these are responsible for the observed phenomenon by which oral glucose promotes greater insulin release than what occurs through intravenous administration; this so called *incretin effect* accounts for 50-70% of insulin secreted following oral glucose ingestion.[26] This effect is predominantly mediated by GIP, secreted from the I and K cells of the proximal intestine, and GLP-1, originating from the L-cells of the distal GI tract. Together, these hormones act to enhance glucose-stimulated insulin biosynthesis and secretion, increase insulin sensitivity and β-cell function, decrease glucagon secretion from pancreatic alpha-cells, decrease GI motility, decrease appetite and food intake, alongside numerous other roles as discussed by Baggio et al.[26,27]

In the post-prandial state, there are also elevated levels of numerous other anorexigenic hormones in addition to GLP-1, such as peptide YY (PYY), cholecystokinin (CCK) and oxyntomodulin. These act to delay gastric emptying, decrease GI motility, reduce the rate of small intestinal transit and, hence, promote satiation and limit food intake.[28] Conversely, concentrations of the orexigenic hormone ghrelin decline post-prandially. Ghrelin originates from neuroendocrine cells of the stomach, duodenum and pancreas and has many complex functions relating to energy homeostasis, nutrient sensing, and appetite. Its primary functions include: (1) stimulating hunger, reward-behaviours and meal initiation (concentrations increase pre-prandially),[29] (2) increasing GI motility, (3) promoting adipogenesis and lipogenesis, (4) inhibiting glucose-stimulated insulin secretion, reducing hepatic insulin signalling and increasing glycogen storage, and (5) decreasing energy expenditure – with the net result being weight gain.[30]

1.2.2 Insulin sensitivity and resistance

Insulin sensitivity exists as a spectrum and refers to the ability of cells to respond to a given concentration of insulin and consequently regulate metabolic activity via normal signalling

pathways. The sensitivity of tissues will fluctuate throughout an individual's lifetime and Increasing age,[31] puberty and pregnancy are associated with increased insulin resistance.[14,32-34] As a reciprocal to sensitivity, insulin resistance can therefore be defined as reduced responsiveness of insulin-sensitive tissues to the metabolic and mitogenic actions of insulin.[35] Insulin resistance in the liver, skeletal muscle, and adipose tissue is considered central to pathogenesis of insulin resistance syndromes and impaired insulin activity within these tissues results in: impaired peripheral uptake and utilisation of glucose in skeletal muscle and adipose tissue; increased hepatic glucose output as a consequence of reduced suppression of hepatic glycogenolysis and gluconeogenesis; and increased lipolysis within adipose tissue as a consequence of reduced antilipolytic insulin activity.[36] The net effect of which is hyperglycaemia and hyperlipidaemia. However, insulin also has broad metabolic and mitogenic actions on numerous other cell types and resistance to insulin manifests in different tissues at variable times and to differing extents. Furthermore, insulin resistance in one or more tissues results in compensatory hyperinsulinaemia that leads to increased insulin action in other insulin-regulated pathways that are not as resistant to those that regulate glucose homeostasis. For example, hyperinsulinaemia acting on adipose tissue, the liver, kidneys, endothelium and ovaries can result in obesity, hypertriglyceridaemia, hyperandrogenism, increased sodium retention and hypertension.[37] An individual can therefore not be considered as a dichotomy of either being insulin sensitive or insulin resistant, but instead insulin sensitivity/resistance manifests as a broad range of phenotypes with considerable overlap between physiological and pathological states.

1.2.2.1 The insulin resistance syndrome

The insulin resistance or metabolic syndrome describes a constellation of metabolic abnormalities which co-exist and increase an individual's risk of cardiovascular disease. There remains no unified definition of this syndrome but it is a cluster of central obesity, hyperglycaemia, elevated blood pressure and dyslipidaemia.[38] As an umbrella term for many interrelated conditions, the metabolic syndrome has a broad and complex pathophysiology predominated by a positive energy balance. This results in excess adiposity and insulin resistance, which contribute to the pathogenesis of T2DM, hypertension, hyperlipidaemia and a myriad of other related conditions, such as non-alcoholic fatty liver disease (NAFLD), hyperuricaemia, microalbuminuria, hypogonadism, platelet hyperaggregation, anti-fibrinolysis and obstructive sleep apnoea. The prevalence of metabolic syndrome is increasing in parallel to trends for obesity and T2DM, but estimates of this differ widely depending on which definition of metabolic syndrome is used.[39]

Since the concept of metabolic syndrome was first proposed,[40] there have been different criteria proposed for identifying affected individuals, notably from the WHO, The European Group for the

Study of Insulin Resistance (EGIR), The American Association of Clinical Endocrinologists (AACE), The National Cholesterol Education Program's Adult Treatment Panel III (NCEP:ATPIII) and the International Diabetes Federation (IDF).[41-45] Irrespective of definition, strong evidence supports the fact that individuals with metabolic syndrome are at increased risk of cardiovascular disease and that this is proportional to the number of components of the metabolic syndrome present.[46-49] However, with so many differing definitions, no consensus exists with regards to nomenclature, diagnostic criteria, nor whether clustering conditions as a syndrome can superiorly predict increased risk of cardiovascular disease and T2DM above that of the risk imposed by the sum of risk factors for each respective condition.[38,50]

1.2.2.2 Tissue-specific insulin sensitivity and resistance

In the majority of individuals, insulin resistance results from a combination of factors acting to create partial resistance in several different pathways of insulin action. Thus, some pathways retain sensitivity to insulin and the degree to which a particular pathway becomes resistant varies between tissue types. Ultimately, the phenotype of insulin resistance will depend on the components affected in any particular pathway and the exact tissues in which they are affected. Examinations of how altered insulin signalling in different tissues results in the wide range of phenotypes associated with the metabolic syndrome have been made possible by using knockout models of various components of the insulin signalling cascade and tissue-specific insulin receptor knockout mice.[20,37] Some of the key phenotypes from these knock-out models are summarised in **Table 1.3**.

Insulin acts on hepatocytes to stimulate glycogenesis, inhibit gluconeogenesis and modulate protein synthesis and lipoprotein metabolism. Although glucose uptake into the liver is not wholly insulin-dependent, hepatic uptake accounts for approximately 30% of whole body insulin-mediated glucose disposal.[14] It has been shown that liver insulin receptor knockout (LIRKO) mice develop mild fasting hyperglycaemia and impaired glucose tolerance due to reduced suppression of hepatic glucose output and an increase in enzyme concentrations associated with gluconeogenesis.[51] Furthermore, LIRKO mice develop compensatory hyperinsulinaemia, as a consequence of β-cell hypertrophy and reduced hepatic clearance of insulin, resulting in secondary insulin resistance in non-hepatic tissues, which may contribute to post-prandial hyperglycaemia. However, these mice also exhibit improvements in their lipid profile due to a decline in hepatic triglyceride secretion and increased suppression of extrahepatic lipolysis. Glucose intolerance in LIRKO mice resolves with age as result of hepatic failure and a reduction in synthetic function, confirming that insulin is required for normal liver function.

	Knockout model	Phenotype
Liver insulin receptor knockout (LIRKO)	LIRKO mice	Fasting hyperglycaemia Impaired glucose tolerance Hyperinsulinaemia Insulin resistance in non-hepatic tissues Decreased triglycerides Decreased free fatty acids
Muscle insulin receptor knockout (MIRKO)	MIRKO mice	Normoglycaemia Normal glucose tolerance Normal insulin concentrations Obesity, Muscle wasting Increased triglycerides Increased free fatty acids
Fat insulin receptor knockout (FIRKO)	FIRKO mice	Normal glucose tolerance Improved insulin sensitivity Normal insulin concentrations Decreased fat mass Decreased triglycerides Increased longevity
β-cell insulin receptor knockout (βIRKO)	βIRKO mice	Reduced first-phase insulin response Reduced glucose tolerance β-cell atrophy
Vascular endothelium insulin receptor knockout (VENIRKO)	VENIRKO mice	Normal glucose homeostasis Decreased triglycerides Protection against neovascularisation
Neuronal insulin receptor knockout (NIRKO)	NIRKO mice	Hyperphagia Increased body weight/fat mass Normoglycaemia Normal glucose tolerance Whole-body insulin resistance Hyperinsulinaemia Increased triglycerides Hypogonadism/reduced fertility
Hypothalamic insulin receptor knockdown (HIRKD)	HIRKD mice	Hyperphagia Obesity Normoglycaemia Normal insulin concentrations Increased hepatic glucose production

Table 1.3 Phenotypes associated with tissue-specific insulin resistance

Skeletal muscle normally accounts for the majority of peripheral glucose disposal (70-90%) via insulin-dependent GLUT-4 transporters. In the fed state, insulin dephosphorylates glycogen synthase and glycogen phosphorylase resulting in increased glycogenesis and reduced glycogenolysis. Glycolysis also increases. At times of higher circulating insulin concentrations, protein synthesis occurs with inhibition of proteolysis occurring at much lower insulin concentrations. The earliest development of insulin resistance generally occurs in skeletal muscle and has been investigated using muscle insulin receptor knockout (MIRKO) mice.[52,53] As expected, MIRKO mice demonstrate a 70-80% decrease in insulin-stimulated glucose transport, glycolysis and glycogen synthesis. Surprisingly however, despite this, MIRKO mice display normoglycaemia, normoinsulinaemia and normal glucose tolerance. This is the consequence of an increase in insulin-stimulated glucose transport and uptake into adipose tissue, which results in obesity and dyslipidaemia (elevated circulating free fatty acids and triglycerides) but normal glucose homeostasis.

Insulin has several important effects of adipose tissue; namely lipogenesis, adipogenesis, and glucose uptake via insulin-dependent GLUT-4 transporters. Furthermore, white adipose tissue sequesters 5-15% of an oral glucose load as triglycerides and secretes numerous factors that regulate appetite and energy homeostasis. In contrast to LIRKO and MIRKO models, fat insulin receptor knockout (FIRKO) mice demonstrate reductions in insulin concentrations, improvements in insulin sensitivity and display normal glucose tolerance. They also have reduced circulating concentrations of triglycerides and a decrease in fat mass. As a consequence of their leanness and overall improved insulin sensitivity, FIRKO mice also have a longer lifespan.[54,55]

Knockout and knockdown models have also been used to evaluate insulin resistance in neural tissue, the hypothalamus, β-cells, and vascular endothelium, which each create unique murine phenotypes (**Table 1.3**). However, pure insulin resistance in humans as a consequence of defects in the IR only occurs in rare genetic conditions and, instead, insulin resistance is predominantly manifested at the molecular level via multiple post-receptor defects in the insulin-signalling pathway. Proposed mechanisms include abnormalities of tyrosine phosphorylation of the insulin receptor, IRS proteins, the mitogen-activated protein (MAP) kinase pathway, phosphatidylinositol 3-kinase (PI 3-kinase) pathway, the sterol regulatory element-binding protein (SREBP) transcription factor or defects in the translocation and function of GLUT 4 transporter proteins. Again, each of these have been elegantly investigated using specific knockout mouse models and tissue-specific defects in cell signalling that contribute to the stepwise progression of tissue-specific insulin resistance has been the focus much research.[37,56] The assessment of insulin

signalling and resistance in humans is much more complex and the defective pathways do differ from rodents in the magnitude of insulin resistance produced. However, these murine models have allowed us to robustly investigate the positive and negative effects of resistance in some insulin-sensitive pathways and the consequence of hyperinsulinaemia in others in creating a heterogeneous range of phenotypes associated with the insulin resistance syndrome.

1.2.2.3 Aetiology of insulin resistance

Predisposition to insulin resistance and T2DM is multi-factorial (**Table 1.4**), with strong genetic and environmental factors contributing to the pathogenesis of these conditions through alterations in insulin signalling and beta cell physiology.[57-61] It is well recognised that the risk of T2DM is higher in first-degree relatives of individuals with T2DM, gestational diabetes or polycystic ovarian syndrome, and there is also high concordance between monozygotic twins.[62] Furthermore, insulin resistance predates the development of T2DM and is typically found in unaffected first-degree relatives. Multiple candidate genes have been identified as being partially responsible for this predisposition, but the currently identified gene loci explain only approximately 10% of its heritability.[56,62,63]

	Risk factors for insulin resistance and T2DM
Environmental and lifestyle factors	Dietary factors (saturated and trans-fat intake, red and processed meat, high-sugar foods and beverages, excess alcohol) Central obesity Sedentary lifestyle Sleep deprivation Environmental toxins
Metabolic syndrome and co-morbidities	History of gestational diabetes Low birthweight and foetal malnutrition Polycystic ovarian syndrome Hypertension Dyslipidaemia Depression Drugs (Thiazide diuretics, β-blockers, corticosteroids, oral contraceptives, protease inhibitors, nucleoside reverse transcriptase inhibitors) Severe illness
Heritable factors	Increasing age Family history / candidate genes Ethnicity Maternal obesity

Table 1.4 Aetiology of insulin resistance and type 2 diabetes mellitus

The amalgamation of all heritable components therefore contribute minimally to the prediction of developing T2DM. Conversely, interventions that focus on reducing insulin resistance significantly reduce the incidence of T2DM, which reflects the fact that the largest determinants of insulin sensitivity are in fact exogenous lifestyle factors; high carbohydrate diets,[64] dietary fibre/starches,[65] and exercise[66] confer increased sensitivity whereas high fat diets (especially diets that are rich in saturated fats and trans-fatty acids), central obesity, high alcohol consumption, and physiological stress are associated with developing insulin resistance.[14,32,67-72] The rising prevalence of T2DM is therefore largely ascribed to energy-dense, westernised diets and sedentary lifestyles, thus making excess adiposity the main modifiable risk factor for developing insulin resistance.

1.2.2.4 Obesity, adipose tissue and insulin resistance

The World Health Organisation (WHO) defines obesity and overweight as abnormal or excessive fat accumulation that presents a risk to health and arises due to an imbalance between calories consumed and calories expended. The cause of obesity at a population level is complex; it arises from an interplay of genetic, behavioural and neuroendocrine determinants. Twin studies have suggested that heritability of obesity to be in the order of 40-70%[73,74] and over 75 susceptibility genes have been identified (with the fat mass and obesity-associated (FTO) gene being the most common). These however are thought to account for less than 2% of BMI variability within a population.[75] Gene mutations, such as that of the melanocortin 4 receptor (MC4R), leptin (LEP), and leptin receptor (LEPR), do exist to cause monogenic obesity but again only account for a small proportion of obese adults. For example, MC4R mutations are the most common cause of monogenic obesity and account for only 0.5-1.0% of obese adults.[75]

Epidemiological data therefore confirm that environmental factors that promote a positive energy balance, such as sedentary lifestyle, increased energy consumption and eating patterns, are predominantly responsible for the current obesity epidemic. Regulation of body weight, energy intake and expenditure however is further moderated by the complex interaction of physiological neuroendocrine feedback mechanisms, which fundamentally seek to promote energy intake and protect against weight loss. Hormone signals from the GI tract (e.g. ghrelin, CCK, peptide YY, pancreatic polypeptide and GLP-1), pancreas (insulin), and adipose tissue (leptin and adiponectin) are integrated in the hypothalamus with hedonic peripheral (dopaminergic and opioidergic) and central (cortex and reward circuits in the limbic system) reward pathways, which act to decrease energy expenditure, influence appetite regulation and promote fat storage. Weight maintenance following weight-loss therefore becomes inherently difficult due to these counter-regulatory mechanisms.[76] The orexigenic hormone ghrelin, for example, has been

demonstrated to increase in proportion to weight-loss and dieting, which may correlate with adaptive increases in hunger and appetite that act to resist non-surgical weight-loss.[28,29] There is also expanding research to suggest that obesity is influenced by alterations in the composition of the gut microbiota, with increased populations of Bacteroidetes and Firmicutes inferring an 'obese microbiome'.[77]

Being overweight or obese accounts for approximately 80% of the overall risk for developing insulin resistance and T2DM.[57] Having a BMI of > 25 kg/m^2 increases the risk of developing T2DM by 5 times and 90% of adult patients with T2DM are obese or overweight.[78] Abdominal or centrally placed adipose tissue is associated with a higher risk of developing obesity-related conditions, independently of an elevated BMI. Specifically, men are at higher risk of T2DM if they have a waist circumference of 94-102 cm and are at very high risk if it is more than 102 cm. Women are at higher risk if they have a waist circumference of 80-88 cm and at very high risk if it is more than 88 cm.[79]

Adipose tissue is a fibrous connective tissue matrix containing adipocytes that exists in white or brown forms. White adipose tissue is the most common type and is found in abundance throughout the body, particularly in the deep layers of the skin, whereas brown adipose tissue is found in smaller amounts and acts to regulate body temperature, particularly in newborn mammals. Primarily adipocytes act as a reservoir for lipid storage, which is derived from three main sources: dietary fat circulating in the bloodstream as chylomicrons; triglycerides synthesised in the liver and transported in the blood as very-low-density lipoprotein; and triglycerides synthesised from circulating glucose in response to insulin within adipocytes. Deposition and utilisation of triglycerides from adipose tissue is therefore largely determined by dietary intake and energy expenditure, but is further influenced by numerous neural and hormonal mediators.

Adipose tissue exists not only as a passive reservoir for energy storage but also functions as a highly active endocrine organ that influences inflammation and acts to modulate metabolism by releasing adipokines (adiponectin, resistin, acylation-stimulating protein, visfatin and retinol binding protein-4),[80] pro-inflammatory cytokines (tumour necrosis factor alpha (TNF-α), interleulin-6 (IL-6), monocyte chemoattractant protein-1),[81] hormones, glycerol and non-esterified fatty acids (NEFAs).[32,68,70,71,82] As the adipose tissue compartment expands, the concentration of these mediators is altered and they subsequently act to influence systemic metabolic processes. Increased concentrations of NEFAs are particularly associated with increased insulin resistance, which has been observed to occur within hours of an acute increase in plasma NEFA levels.[71] This is believed to occur through phosphorylation of IRS-1 and -2 by fatty acid metabolites, which renders them incapable of activating PI(3)K pathways and thus diminishes

the downstream insulin-receptor signalling pathway.[83] Peripheral glucose uptake (primarily within skeletal muscle) and insulin-induced suppression of hepatic glycogenolysis and adipose tissue lipolysis are therefore reduced.[84] NEFAs have also been implicated in potentiating obesity as an 'inflammatory state' by activating the canonical pro-inflammatory nuclear factor-kappa B (NF-kB) pathway and increasing the expression of several inflammatory cytokines (TNF-α, IL-6, MCP-1),[85] which may interfere with insulin receptor binding with IRS-1/2.[86]

Those with T2DM have a high intramyocellular lipid content and this is an important feature of the insulin resistant state as exposure of skeletal muscle to excessive NEFAs (and lipid derived metabolites i.e. ceramide, diacylglycerol, fatty acyl coenzyme A) directly impairs insulin action.[87] Centrally placed adipose tissue however confers a higher degree of insulin resistance as a consequence of differing gene expression and secretory functions between the adipocytes found in these truncal stores and those found within the subcutaneous tissue.[32]

1.2.2.5 Insulin resistance and β-cell function

The secretory function of β-cells varies in response to the prevailing plasma glucose concentration and overall insulin sensitivity i.e. β-cells will increase the supply of insulin in response to the demand by insulin sensitive tissues. When insulin sensitivity declines, as occurs 'normally' during puberty and pregnancy, insulin secretion is reciprocally increased by up to 5 times and β-cell mass is increased by 50% in order to achieve euglycaemia and maintain glucose tolerance.[32-34,88] Potential mechanisms for this relationship between insulin sensitivity and insulin secretion include: (1) glucose-stimulated insulin release via increased intracellular glucose metabolism, (2) NEFA signalling, and (3) response to incretin hormones (e.g. GLP-1). Activation of the insulin-IGF-1 signalling pathway itself also moderates islet mass through activation of IRS-2 and subsequent downstream signalling through mitogenic pathways, which culminates in β-cell proliferation, neogenesis and survival.[89] Increased vagal input associated with diet-induced obesity may also contribute to increased β-cell mass.[90,91]

In conditions hallmarked by hyperglycaemia, individuals have a genetic predisposition that results in β-cell dysfunction whereby they fail to respond adequately to secretagogue stimulation. Numerous genes have been identified as being associated with β-cell dysfunction and these include hepatocyte nuclear factor-4α and 1α, the E23K polymorphism in the islet ATP-sensitive potassium channel Kir6.2, polymorphisms in transcription factor 7-like 2 (TCF7L2) and mutations in the mitochondrial genome. Many other candidate genes, including calpain-10, adiponectin, PPAR-γ coactivator 1 (PGC1) and the glucose transporter GLUT2 have also been implicated.[92] In genetically and epigenetically susceptible individuals, insulin production from β-cells is reduced and is incapable of overcoming the degree of insulin resistance imposed by other lifestyle and

genetic factors.[93] In the Belfast Diet Study they identified two phases of β-cell dysfunction: (1) a slow but constant phase with a decline in function of approximately 2% per year, which precedes the onset of overt diabetes, and then (2) an accelerated decline in β-cell function that occurs at a rate of approximately 18% per year and often coincides with the onset of diabetes.[94] Loss of first-phase insulin secretion is a very early feature of β-cell dysfunction whereby hepatic glucose production is not adequately suppressed and peripheral uptake of glucose by insulin-sensitive tissues via GLUT-4 is insufficient, which together contribute to the early excessive rise in glucose in response to an oral glucose load. Furthermore, the reduced action of insulin on adipocyte metabolism leads to increased lipolysis and elevated circulating NEFA levels, which, together with glucose, synergistically induce oxidative stress within the β-cell (termed glucolipotoxicity) resulting in a further decrease in β-cell function and reduced β-cell mass through apoptosis and autophagy. In addition, NEFA-induced activation of the NF-kB pathway increases TNF-α and IL-6 expression which not only potentiates insulin resistance, but also directly inhibits β-cell function and survival.[95] Islet function therefore progressively declines towards β-cell failure with severe defects in both the early- and late-phase insulin responses, which manifests clinically as elevated post-prandial and, eventually, fasting blood glucose levels.[96] Longitudinal studies of Pima Indians by Weyer et al. have demonstrated that it is the decline of the acute insulin response, as opposed to worsening insulin resistance, which marks the progression from normal glucose tolerance to impaired glucose tolerance and then to T2DM.[97]

1.2.3 Type 2 diabetes mellitus

T2DM is a chronic, progressive disorder of carbohydrate, lipid and protein metabolism, which manifests as persistent hyperglycaemia and the subsequent development of neuropathic, macro- and microvascular disease secondary to the chronic exposure of tissues to elevated ambient glucose concentrations.

A patient is diagnosed with diabetes if they meet one of the following criteria:[98,99]

- Fasting plasma glucose ≥ 7.0 mmol/L*;
- Plasma glucose ≥ 11.1 mmol/L during 2 hour 75 g oral glucose tolerance test*;
- Glycated haemoglobin (HbA_{1C}) ≥ 48 mmol/mol*;
- Classic symptoms of hyperglycaemia or hyperglycaemia crisis with a random plasma glucose ≥ 11.1 mmol/L.

**In an asymptomatic individual, a diagnosis of T2DM should not be based on a single abnormal glucose or HbA_{1c} measurement. At least one additional abnormal test result with a value in the diabetic range is required.*

T2DM becomes clinically apparent when a susceptible individual with insulin-resistance develops progressive beta cell dysfunction and is unable to produce the necessary amount of insulin that is required to maintain normoglycaemia.[14,32,67] Initially, patients will develop impaired glucose tolerance or impaired fasting glycaemia but as β-cell function deteriorates further towards failure and as insulin resistance persists, then the individual progresses to T2DM as suppression of hepatic glucose production and peripheral glucose uptake declines (**Figure 1.3**). Fasting and post-prandial glucagon concentrations are characteristically elevated in T2DM, contributing to increased gluconeogenesis, increased hepatic glucose output and hyperglycaemia.

There is a long asymptomatic phase in the development of T2DM and the majority of newly-diagnosed patients will not have catabolic symptoms, such as polydipsia, polyuria or weight loss. Increasingly, people are being diagnosed incidentally during routine investigations or screening. By the time an individual is diagnosed with T2DM, β-cell numbers are reduced by 50% and secretory function is reduced by approximately 75%,[88,100] and then continues to decline after diabetes has developed. This is largely as a consequence of chronic glucolipotoxicity, systemic inflammation and oxidative stress but is further potentiated by elevated leptin levels (decreasing insulin secretion and causing dysregulation of β-cell proliferation, apoptosis and size),[101] chronic β-cell stimulation (causing amyloid deposition and endoplasmic reticulum stress),[102,103] and an impaired incretin effect (plasma GLP-1 levels are reduced compared to 'normal' individuals).[104,105]

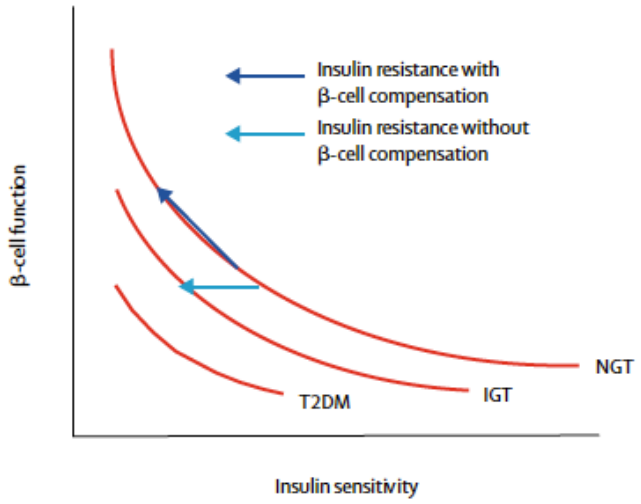


Figure 1.3 Relationship between β-cell function and insulin sensitivity.
IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus.
Source: Stumvoll et al. (2005).[106] Copyright Elsevier.

1.2.4 Measuring and assessing insulin sensitivity

Quantitative measurements of insulin sensitivity and β-cell function in humans are fundamental to the research of disorders associated with insulin resistance, such as T2DM, in order to understand the pathophysiological basis of the disease, monitor its clinical course and evaluate the efficacy of therapeutic interventions. However, due to the ubiquitous nature of insulin in intermediary metabolism and its concentration-dependent action on many different tissues, measurement of insulin sensitivity is complex and not measurable by one single test. The majority of tests quantify insulin resistance based on glucose metabolism and several techniques have been developed that vary widely in their sensitivity, limitations and complexity. These can be broadly categorised as *direct* and *indirect* measures of insulin sensitivity and some of the indications, advantages and limitations of the most common tests are summarised in **Tables 1.5 and 1.6**. Each of these different investigations evaluate different aspects of glucose homeostasis and, as such, a good understanding of these assessments and their limitations is required in order to select the most appropriate test of insulin sensitivity for the study being conducted. Furthermore, there are multiple variables that will impact on these study measurements, such as the time of day, dietary intake, smoking, stress and recent exercise, and so the tests must be performed in accordance with a robust protocol that will control for possible confounding factors. Sample numbers, time, budget and resources available will also heavily influence the choice of test for each study.[107] The methodologies for these techniques are fully reported in the literature.[35,36,107,108]

Method	Indications	Advantages	Limitations/disadvantages	Correlation with HEC
Direct measurements				
Hyperinsulinaemic Euglycaemic clamp[109]	✓ Primary outcome: evaluating insulin sensitivity/resistance. ✓ study population < 100.	❖ Direct measure of insulin sensitivity under steady state conditions. ❖ Addition of radiolabelled tracers of glucose, glycerol or amino acids allows quantification of HGP and whole body glucose disposal, lipolysis and protein metabolism.	▪ Labour-intensive, time-consuming, and expensive (precludes use in large cohorts). ▪ Requires multiple intravenous infusions and regular blood sampling. ▪ No standardised protocol precludes direct comparison across studies. ▪ Does not accurately reflect insulin activity and glucose homeostasis under physiological conditions. ▪ No incretin effect.	Gold standard for the assessment of insulin sensitivity in clinical research
Insulin Suppression Test[110, 111]	✓ Primary outcome: evaluating insulin sensitivity/resistance. ✓ Larger study populations. ✓ Primarily determines sensitivity of skeletal muscle.	❖ Direct measure of ability of exogenous insulin to mediate disposal of intravenous glucose under steady-state conditions. ❖ Highly reproducible. ❖ Less labour-intensive than HEC.	▪ Not designed to reflect hepatic insulin sensitivity. ▪ Labour-intensive, time-consuming, and expensive. ▪ Risk of hypoglycaemia. ▪ Hyperglycaemia in participants with T2DM may lead to glycosuria and underestimation of insulin resistance. ▪ Alterations in splanchnic blood flow and peripheral glucose uptake by somatostatin may influence measure of insulin resistance. ▪ No incretin effect	Participants with T2DM: r = 0.91 Participants without T2DM: r = 0.93
Indirect measurements				
Frequently Sampled Intravenous Glucose Tolerance Test[112]	✓ Evaluating insulin sensitivity, glucose effectiveness and β-cell function. ✓ Larger study populations (> 1000)	❖ Less labour-intensive than HEC. ❖ Steady-state conditions not required.	▪ Requires intravenous infusions with multiple blood samples over several hours. ▪ Measurements of glucose effectiveness and insulin sensitivity are affected by using a minimal model approach and in individuals with impaired insulin secretion and/or resistance (T2DM) – this approach is less accurate than other simple surrogate markers of insulin sensitivity. ▪ No incretin effect. ▪ Requires computer modelling of outcome measures.	r = 0.89

HEC, hyperinsulinaemic euglycaemic clamp; HGP, hepatic glucose production; r, rho (correlation coefficient); T2DM, type 2 diabetes mellitus.

Table 1.5 Direct and indirect assessments of insulin sensitivity and resistance in vivo.

1.2.5 Management of Obesity and Type 2 Diabetes Mellitus

Management of the metabolic syndrome focuses on identifying each of the respective components and using lifestyle changes, targeted pharmacotherapy and/or surgery in order to address modifiable risk factors, reduce adiposity and improve insulin resistance, with the ultimate aim being to attenuate the risk of cardiovascular and metabolic disease. In the following sections I will further discuss some of these management strategies with respect to weight loss and management of T2DM.

1.2.5.1 Lifestyle interventions and medical management

T2DM and obesity are complex, chronic conditions that require a multidisciplinary approach for the delivery of lifelong, multimodal therapies that focus on patient education, dietary guidance, lifestyle modification, weight loss, managing cardiovascular risk factors, managing hyperglycaemia, and preventing, identifying and managing complications. Such management strategies and guidelines are available from the American Diabetes Association (ADA) and National Institute for Health and Care Excellence (NICE).[113,114]

Weight management is a high priority in this treatment algorithm with the ultimate aim being to improve an individual's metabolic health. Just 10kg of weight loss confers a 20-25% reduction in overall mortality and 40-50% of obesity-related cancer deaths are prevented. Furthermore, this degree of weight loss has been shown to significantly reduce cardiovascular risk factors (10% reduction in systolic and diastolic blood pressure, 10% reduction in total cholesterol, 15% reduction in low-density lipoprotein cholesterol (LDL-C), 30% reduction in triglycerides, 8% increase in high-density lipoprotein cholesterol (HDL-C)), improve lung function, reduce obstructive sleep apnoea, reduce joint pain, increase fertility, and improve quality of life.[115,116] In patients with T2DM, sustained weight losses of just 10 kg, or 5-10% of total body weight (TBW), can be expected to improve glycaemic control (30-50% reduction in fasting glucose and 15% reduction in HbA1C), reduce the requirement or dosage for oral and injectable glucose-lowering medications, decrease overall morbidity and reduce deaths specifically attributed to T2DM by 30-40%.[8,116-119] Normalisation of glycaemic control and resolution of T2DM after weight loss is also achievable in some individuals.[119-122]

Extensive evidence exists for the efficacy of combined medical therapy, intensive lifestyle interventions and weight loss in patients with T2DM on improving glycaemic control, reducing the number of medications, improving quality of life, mobility, physical and sexual function.[7] Two such studies are the UK Prospective Diabetes Study (UKPDS), which concluded that intensive glycaemic control was associated with decreased rates of neuropathic and microvascular complications, and

Method	Indications	Advantages	Limitations/disadvantages	Correlation with HEC
Indirect measurements cont…				
Indices derived from Oral Glucose Tolerance Test/Mixed Meal Tolerance Test[108, 123-127]	✓ Evaluating glucose tolerance ✓ Incorporation of surrogate indices allow simultaneous measurement of insulin sensitivity/resistance and insulin secretion: Matsuda ISI, Stumvoll ISI, Avignon ISI, Belfiore ISI, Cederholm ISI, GUTT ISI.	❖ Physiological assessment of insulin sensitivity. ❖ Technically straight-forward and inexpensive.	▪ Poor reproducibility due to multiple physiological confounding factors - incretin effect, neurohormonal actions, rate of glucose absorption and insulin secretion. ▪ No distinction between peripheral and hepatic insulin sensitivity. ▪ Involves taking regular blood samples over several hours.	Matsuda, r = 0.54 - 0.73 Stumvoll, r = 0.62 - 0.79 Gutt, r = 0.63 - 0.68 Belfiore, r = 0.65 – 0.93 Avignon, r = 0.76 – 0.93
Indices derived from fasting values[128-131]	✓ Evaluating basal insulin sensitivity. ✓ Primarily determines hepatic insulin sensitivity but is proportional to sensitivity of skeletal muscle under most conditions. ✓ Applicable in most clinical and research settings when assessing insulin sensitivity is of secondary interest or when direct tests are not possible. ✓ Applicable to large epidemiological or clinical research studies.	❖ Simple, inexpensive tests. ❖ Minimally-invasive. ❖ More acceptable for subjects.	▪ Co-efficient of variation can vary considerably depending on the insulin assay used and the number of fasting samples obtained. ▪ No distinction between peripheral and hepatic insulin sensitivity.	
- Homeostasis Model Assessment (HOMA)	✓ Simultaneous assessment of insulin sensitivity and insulin secretion.	❖ Correlates well with HEC		r = 0.56 - 0.89
- Quantitative Insulin Sensitivity Check Index (QUICKI)	✓ Proportional to 1 / log [HOMA-IR]	❖ Excellent linear correlation between QUICKI and HEC in patients with obesity and insulin resistance/T2DM.		r = 0.78 – 0.90

HEC, hyperinsulinaemic euglycaemic clamp; HGP, hepatic glucose production; ISI, insulin sensitivity index; r, rho (correlation coefficient); T2DM, type 2 diabetes mellitus.

Table 1.6 Indirect assessments of insulin sensitivity and insulin resistance in vivo.

the Steno-2 study where it was demonstrated that intensive medical management with behavioural modification and multiple drug combinations in at-risk patients with T2DM resulted in sustained reductions in vascular complications (29% absolute risk reduction) and mortality (20% absolute risk reduction).[132,133] Furthermore, individuals with impaired fasting glycaemia (IFG) and/or impaired glucose tolerance (IGT) who are at risk of progressing to T2DM can decrease the rate of onset of T2DM through intensive lifestyle interventions and weight loss.[134-139] This was demonstrated during long term follow-up in the Da Qing study (43% reduction in the rate of conversion to T2DM at 20 years),[140] the Finnish Diabetes Prevention Study (DPS) (43% at 7 years)[141] and the Diabetes Prevention Program Outcomes Study (DPPOS) (34% at 10 years).[142]

The Look AHEAD (Action for Health in Diabetes) Study examined the effect of weight loss with intensive lifestyle interventions (ILI) with comprehensive behavioural weight-loss counselling on cardiovascular morbidity and mortality in 5145 overweight/obese patients with T2DM. This group demonstrated that ILI produced clinically meaningful weight losses of ≥5% in 50% of patients and ≥10% in 26.9% of patients after 8 years when compared to usual care. As a consequence, patients in the ILI group had improved biomarkers of glucose and lipid control, reduced obstructive sleep apnoea, less steatosis, improved insulin sensitivity, less urinary incontinence, less kidney disease, reduced requirement for glucose-lowering medications, maintenance of physical mobility, improved quality of life and lower healthcare-associated costs.[121] Of clinical importance however is the fact that this trial was halted for futility after a median follow-up duration of 9.6 years after no reduction in cardiovascular events was demonstrated between the two treatment groups.[9]

For those patients who do not achieve or sustain significant weight loss through diet, activity and/or behavioural interventions then pharmacological treatment may be considered. Orlistat, a lipase inhibitor, is a prescription medicine funded by the UK National Health Service that can be prescribed in conjunction with lifestyle interventions in patients with a BMI ≥ 30 kg/m^2 (or ≥ 28 kg/m^2 with associated risk factors). After 1 year of treatment with Orlistat, alongside lifestyle measures, 35-73% of patients achieve ≥ 5% total body weight loss (TBWL) and 14-41% achieve ≥ 10% TBWL.[143] After 2 years, on average, patients will lose an additional 3.3 kg above placebo, demonstrating a modest benefit above lifestyle interventions alone. However, with long-term usage, weight tends to plateau, adherence is often poor due to undesirable side-effects, and the majority of patients will regain weight after cessation of treatment. Liraglutide (Saxenda) is a GLP-1 agonist and was launched in 2017 in the UK as an adjunct to diet, lifestyle modification and exercise for weight loss in adults with a BMI ≥ 30 kg/m^2 (or ≥ 28 kg/m^2 with associated metabolic co-morbidities).[144] 3.0 mg day^{-1} of Saxenda has been shown in four RCTs to achieve significantly greater weight loss when compared to dietary and lifestyle interventions (54.3-63.2% versus 21.4-27.1% achieved ≥ 5% TBWL after 56 weeks; 25.2-33.1% versus 6.7-10.6% achieved ≥ 10% TBWL

after 56 weeks).[145-148] Saxenda has also been shown to reduce the rate of progression to T2DM in patients with pre-diabetes and to improve obstructive sleep apnoea.[145,147] However, as with orlistat, the treatment effects of Liraglutide are not sustained following withdrawal of treatment and many patients in these studies regained weight. For both Orlistat and Saxenda, NICE recommend that if a patient does not achieve at least 5% of weight loss after 3 months then treatment should be discontinued. Naltrexone-bupropion (Mysimba), a combination of an opioid antagonist and an antidepressant used to promote satiety and reduce food intake, is licensed to augment weight loss in continental Europe but is not currently recommended in the UK as a primary weight-loss treatment.[149]

Of great interest is the potential of achieving sustained weight loss and metabolic improvements through acute caloric restriction. It has been demonstrated that, in those with T2DM of short duration, fasting plasma glucose (FPG) concentrations are normalised within a few days of a very low calorie diet (VLCD) due to a rapid decrease in hepatic fat content and subsequent improvements in hepatic insulin sensitivity.[122,150] Furthermore, after approximately 8 weeks, β-cell function improves.[150] In the recent Diabetes Remission Clinical Trial (DiRECT),[120] Lean et al. examined the delivery of intensive weight management with a VLCD versus standard medical care in overweight or obese subjects with newly diagnosed T2DM (< 6 years) through GP practices as part of an open-label, cluster-randomised trial. Intervention patients (n = 149) received total diet replacement (825-853 kcal/day formula diet) for 3-5 months and immediate discontinuation of glucose-lowering medications, stepped food reintroduction for 2–8 weeks, followed by structured support for long-term weight loss maintenance. The control patients (n = 149) received best-practice care in accordance with local and national guidelines. Mean bodyweight was reduced by 10.0 kg in the intervention group versus 1.0 kg in the control group at 12 months and 24% of the intervention group achieved a weight-loss of $\geq$ 15 kg versus 0% in the control group ($p < 0.0001$). Correspondingly, 46% of patients in the intervention group achieved remission of their T2DM at 1 year versus only 4% in the control group (odds ratio 19.7, 95% CI 7.8-49.8; $p < 0.0001$). At 24 months, 11% of intervention participants and 2% of control participants had a sustained weight loss of at least 15 kg (adjusted odds ratio [aOR] 7.49, 95% CI 2.05 to 27.32; p=0.0023) and remission of T2DM was sustained for more than a third of patients in the intervention group (Intervention 53 (36%) versus 5 (3%) control participants (aOR 25.82, 8.25 to 80.84; p<0.0001).[151] This study therefore highlights the feasibility of achieving sustained weight loss and remission of T2DM in the primary care setting using conservative management strategies by means of acute caloric restriction and structured weight-management.

Unfortunately, despite the clear benefits of weight loss, medical therapy and lifestyle modification, currently available pharmacological, behavioural and dietary strategies provide only

mild to modest levels of weight reduction that are often not sustained.[8,9] Health benefits attained from weight loss are dependent on this loss being maintained long-term and it has been demonstrated that, after 2 years, most ILIs produce an average weight loss of just 2.22 kg in comparison to control participants.[152] Current evidence suggests that fewer than half of adults with T2DM attain adequate glycaemic control or meet therapeutic targets intended to reduce long-term risks of complications. Furthermore, less than 20% of patients will lose and maintain a 10% weight reduction with medical treatment during the first year.[153,154]

1.2.5.2 Metabolic surgery

In a 2016 joint statement by international diabetes organisations it was proposed that metabolic surgery should be recommended to treat T2DM in patients with class III obesity (BMI ≥ 40 kg/m^2) or those with class II obesity (BMI 35.0 – 39.9 kg/m^2) where glycaemic control in not adequately controlled through optimal medical therapy or lifestyle modification. Metabolic surgery should also be considered in those with class I obesity (BMI 30.0 – 34.9 kg/m^2) if glycaemia is not adequately controlled through optimal oral or injectable medications (**Figure 1.4**).[155]

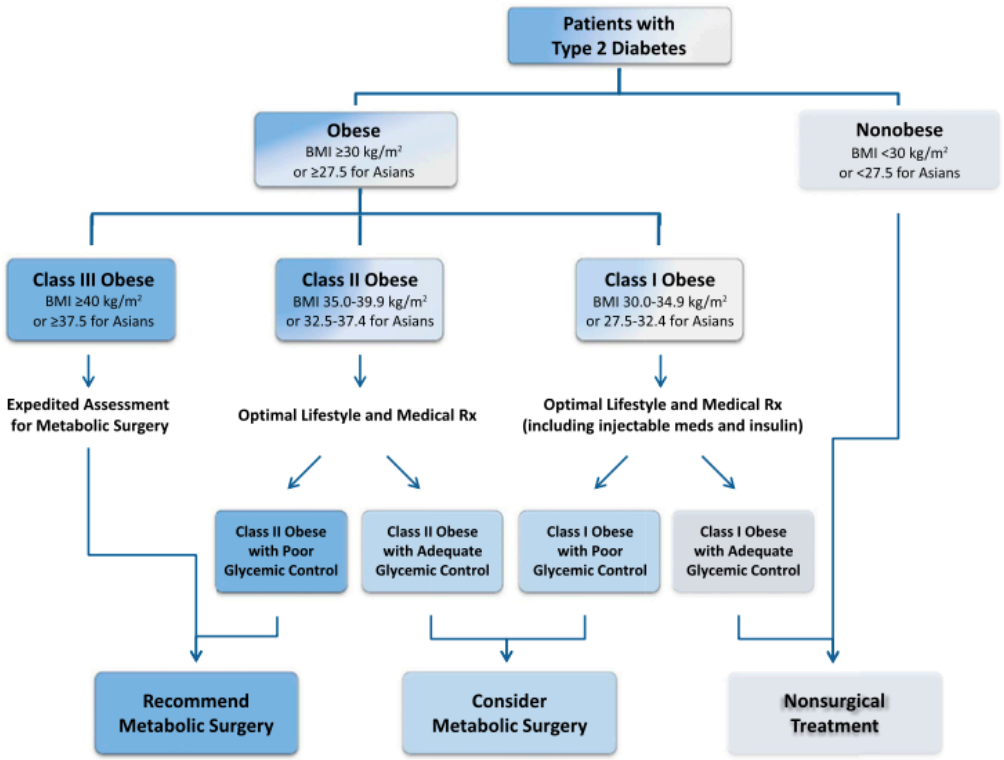


Figure 1.4 Treatment algorithm for T2DM
In accordance with the consensus recommendations of the Diabetes Surgical Summit (DSS).
Source: Rubino et al. 2016,[155] Copyright American Diabetes Association.
T2DM, type 2 diabetes mellitus.

1.2.5.2.1 Types of surgery and efficacy

Several GI operations now exist, including the Roux-en-Y Gastric Bypass (RYGB), Laparoscopic Sleeve Gastrectomy (LSG), Laparoscopic Adjustable Gastric Banding (LAGB) and Biliopancreatic Diversion (BPD) ± Duodenal Switch (DS), which were initially intended to promote weight loss (i.e. bariatric surgery) but can dramatically improve glucose homeostasis through mechanisms other than just weight loss. There is an increasingly-robust body of high-level evidence which has consistently demonstrated that these *metabolic* operations achieve superior weight loss, glycaemic control and reduction of cardiovascular risk factors in patients with obesity and T2DM when compared to medical and lifestyle interventions.[156-170] Furthermore, available economic analyses support the cost-effectiveness of metabolic surgery, especially in patients with T2DM.[155]

A systematic review and meta-analysis of 621 studies and 135,246 bariatric procedures by Buchwald et al. demonstrated an overall mean % excess weight loss (%EWL, [initial weight in kg - final weight in kg]/[initial weight - ideal body weight in kg] x 100%) of 55.9% (CI 54.1 – 57.8). %EWL was more profound with the BPD +/- DS (63.6%, CI 57.5-69.7, n = 3127), followed by RYGB (59.5%, CI 56.5-62.6, n = 15560) and then gastric banding (46.2%, CI 43.1-49.2, n = 8599).[171] Notably, however, this review was limited by the high attrition rate of patients, which is particularly common in studies evaluating weight loss. Mean %EWL for LSG has been reported at 55.4% (n = 1662).[172] The majority of studies to date present excellent short- and medium-term weight loss outcomes (< 5 years) and long-term outcome data is evolving.[169,173] The Swedish Obesity Study (SOS) is the first long-term, prospective trial of bariatric surgery (n = 2010; RYGB 13%, gastric banding 19% and vertical banded gastroplasty (VBG) 68%) vs. obese control participants receiving usual care (n = 2037) over 20 years. Mean TBWL was maximal after 1 – 2 years (RYGB 32 ± 8%, VBG 25 ± 9%, banding 20 ± 10%) after which time weight regain was observed across all surgical subgroups before then plateauing after 8 – 10 years. Mean weight losses below the baseline weight after 10 years were 25 ± 11% for RYGB, 16 ± 11% for VBG and 14 ± 14% for banding and then 27 ± 12%, 18 ± 11% and 13 ± 14% after 15 years. At 20 years, the mean percent weight loss was 26% for RYGB, 18% for VBG, and 13% for gastric banding compared with 1% for control participants. Within the control group the weight change remained within ± 3% during the trial period.[173,174] The STAMPEDE trial (intensive medical therapy (IMT) alone vs. IMT plus RYGB or LSG) reported similar outcomes at 5 years (weight loss from baseline = -23.2 ± 9.6 kg for RYGB, -18.6 ± 7.5 kg for LSG and -5.3 ± 10.8 kg for medical therapy).[169] A rebound weight gain phenomenon does occur between 2 and 10 years and 2-18% of patients will be expected to regain weight back to baseline (18% for LAGB, 5% for LSG and 2% for RYGB).[174] These studies currently represent the best available evidence for the medium- and long-term effectiveness of surgery on weight loss, but are not without their limitations. The SOS trial is

limited by the potential biases imposed by its matched,[175] as opposed to randomised, trial design and the open-label, single-centre design and relatively small sample size of the STAMPEDE study does limit the robustness and generalisability of the data obtained. However, given the impressive weight loss outcomes and reproducibility between independent centres, these operations are now established as the most effective treatments for obesity and obesity-related morbidity.

Metabolic surgery confers a significant improvement in a patient's metabolic state with improvements in their glycaemic control, a reduction in medications or even resolution of T2DM. Physiologically, in the immediate week(s) following surgery, post-prandial first-phase insulin response increases, in association with improvements in β-cell function, and hepatic insulin resistance (and hence hepatic glucose production) decreases.[176,177] From about 4 weeks, peripheral insulin sensitivity increases significantly in proportion to ensuing weight loss and peaks after approximately 2 years.[178,179] In terms of clinical outcomes, reductions in HbA_{1c} by 2–3.5% are expected to be seen following surgery compared to only 1–1.5% following non-surgical weight-loss.[174] Resolution of T2DM is often defined as normalisation of HbA_{1C} without the requirement for diabetes medications and was reported in the meta-analysis by Buchwald et al to occur at an overall rate of 78.1% (95.1% for BPD $\pm$ DS, 80.3% for RYGB, 79.7% for VBG and 56.7% for LAGB).[171] A further meta-analysis of 11 randomised controlled trials (RCTs) (n = 796) by Gloy et al. reported the relative risk to achieve diabetes remission was 22 times higher compared with non-surgical treatment (relative risk 22.1, 95% CI 3.2 to 154.3, P=0.002).[161] This effect held robustly with multiple testing for a conservative case scenario but the effect of using different definitions of diabetes remission between the studies on this outcome was not examined. Furthermore, methodological shortcomings were identified in almost half of the RCTs as potential sources of bias and, as noted with the previous systematic review, risk of attrition bias over an average follow-up period of only two years was high. In the STAMPEDE trial, the primary endpoint of achieving a $HbA_{1C} \leq 6.0\%$ (with or without medications) was met by only 5% of the IMT group compared to 29% (p=0.01) and 23% (p=0.03) in the RYBG + IMT and LSG + IMT groups respectively.[169] In the SOS trial the remission rate for T2DM was 72% at 2 years and 36% at 10 years compared with 21% and 13% respectively for the nonsurgical control patients and surgery reduced the risk of developing T2DM in non-diabetic patients by 78% at 15 years.[173] The high rate of T2DM relapse between 2 and 10 years witnessed in the SOS study demonstrates that the remission of T2DM following surgery is not durable in all patients and it is expected that $\geq$ 35-50% of patients who initially achieve remission of T2DM eventually experience recurrence. This observation correlates significantly with weight regain after 2 years but other predictors of relapse of T2DM that have been identified include: poor preoperative glycaemic control, use of insulin, and longer duration of T2DM (> 8 years).[174,180,181] This finding most likely relates to the

individuals residual β-cell function at the time of surgery. The median disease-free period in RYGB patients however is 8.3 years and, with or without relapse, the majority of patients will maintain a substantial improvement in glycaemic control for 5 – 15 years.[155]

The impact of bariatric surgery on cardiovascular risk factors is well documented with numerous randomised, observational and cohort studies collectively demonstrating the superiority of metabolic surgery over medical treatment in the short- and medium-term in achieving a reduction of diabetes and cardiovascular medications, improving triglyceride, LDL and HDL cholesterol concentrations, ameliorating or resolving hypertension, and improving metabolic syndrome.[182] Several studies have reported the effect of metabolic surgery on the progression of microvascular disease, such as retinopathy, nephropathy, and neuropathy in T2DM, but long-term outcome data from randomised trials for the effect of bariatric surgery on micro- and macrovascular morbidity and mortality compared to medical therapy are lacking.[174] Several existing studies report actual cardiovascular events and mortality outcomes following bariatric surgery and broadly support a cardiovascular event and all-cause mortality benefit conferred by bariatric surgery.[182] The SOS study has the longest follow-up of cardiovascular outcomes to date (median of 14.7 years) in which cardiovascular mortality in the surgical group was significantly lower than in control patients (adjusted hazard ratio 0.47, 95% CI 0.29–0.76, p = 0.002). The surgical group also had a 50% reduction in microvascular complications (cumulative incidence was 41.8 per 1,000 person-years (95% CI 35.3–49.5) for control participants and 20.6 per 1,000 person-years (95% CI 17.0–24.9) in the surgery group (hazard ratio 0.44, 95% CI 0.34–0.56; p = 0.001)). Of interest, it was the baseline degree of insulin resistance, rather than initial BMI or the amount of weight lost, which was most predictive of cardiovascular benefit in the SOS cohort.[183]

The safety profile of metabolic surgery, which requires significant anatomical rearrangement, has markedly improved over recent years, particularly with the routine use of laparoscopy; 95.4% of primary cases currently performed in the UK are laparoscopic.[184] Elective laparoscopic metabolic surgery has a quoted worldwide mortality rate of 0.1-0.5% (0.11% in the UK), similar to that of other common elective procedures such as laparoscopic cholecystectomy (major perioperative morbidity 3.7% and mortality 0.7%) and appendicectomy (4.5% and 0.5%).[155,185] Bypass procedures (BPD +/- DS and RYGB) require more complex anatomical adjustment, longer operating times and therefore have higher rates of associated perioperative mortality and morbidity; early reoperation and readmission rates after RYGB are 2.5% and 5.1%, compared to 0.6% and 2.0% for LAGB, versus 0.6% and 5.5% for LSG.[186] Longer term, however, LAGB is associated with removal or revision rates of 20%.[155] Post-operative micronutrient and nutritional deficiencies are typically seen with the malabsorptive procedures (RYGB and BPD +/- DS), including iron (17-50%), trace minerals (selenium, zinc and copper), calcium, and vitamins (B12,

B1, A, D, E, and K)[174], resulting in varying incidences of anaemia, hypoproteinaemia and bone demineralisation depending on the degree of deficiency and the adequacy of dietary supplementation following surgery.

1.2.5.2.2 Proposed mechanisms of action of metabolic surgery

Profound and sustained weight loss is, in part, achieved through caloric restriction as the GI anatomy is manipulated to (a) reduce the capacity and reservoir function of the stomach (i.e. gastric restriction, such is seen with LAGB and RYGB) and, (b) limit the surface area of the intestine in contact with ingested nutrients (i.e. causing malabsorption, as is seen with RYGB and BPD +/- DS). Reduced caloric intake decreases the mass of adipose tissue which undoubtedly potentiates the positive metabolic effects of surgery as adipokine concentrations are modulated, insulin-receptor concentration and signalling is enhanced, glucose and fatty acid metabolism increases, glucolipotoxicity declines and concentrations of intramuscular and intrahepatic lipid are reduced. Central and peripheral insulin resistance therefore declines, β-cell function and mass increases, and glycaemic control improves.[27,177,178]

However, there are several observations which suggest that weight-loss is not the only determinant of improved glucose homeostasis following intestinal bypass operations (e.g. RYGB and BPD +/- DS): (1) improvements in glycaemic control and T2DM remission occur in the immediate days and weeks following surgery before substantial weight-loss has occurred,[187] (2) greater improvements in glucose homeostasis are observed following bypass procedures when compared to purely restrictive operations or non-surgical interventions producing the same degree of weight-loss,[188] (3) T2DM resolution occurs in leaner individuals to the same degree in obese patients despite greater weight-loss, and (4) glycaemic control is improved following duodenal-jejunal bypass and ileal interposition, independently of changes in food intake, body-weight or malabsorption.[189-191] The precise mechanisms by which these *weight-independent* metabolic improvements occur after surgery are yet to be fully determined but complex entero-neuro-hormonal pathways involving exclusion of food from the duodenum and proximal jejunum, early delivery of food to the terminal ileum, GI tract nutrient sensing, incretins/anti-incretins, altered bile acid metabolism and changes in the intestinal microbiota have all been implicated.[27]

All patients undergoing metabolic surgery are calorically restricted in the immediate post-operative period and goes some way to explaining the rapid metabolic improvements observed post-operatively. It is well evidenced that acute caloric restriction will transiently improve glucose homeostasis and in a study by Jackness et al. a VLCD (500kcal/day) compared to RYGB produced comparable early improvements in both insulin sensitivity and β-cell function.[192] As caloric intake then increases and the patient resumes *ad libitum* eating, weight loss-dependent metabolic

improvements ensue. These findings, however, do not explain the experience of patients undergoing LAGB where, despite the same caloric restriction, rapid diabetes remission seldom occurs and tends to be proportional to the degree of weight loss. This was demonstrated by Dixon et al. (adjustable gastric banding vs. conventional medical therapy for T2DM) where no patients achieved remission of T2DM within the first 6 months but then 73% of the surgical group achieved remission by two years in proportion to the amount of weight lost, versus only 13% in the conventional medical therapy group.[160] This is in contrast to BPD +/- DS and RYGB in which the majority of patients will achieve remission of T2DM within the immediate days and weeks post-operatively. The orexigenic hormone ghrelin has been implicated as potentially causing the observed differences in clinical outcomes between different metabolic operations. Cummings et al. demonstrated that, as 90% of ghrelin is secreted from the stomach or duodenum, operations characterised by exclusion or manipulation of these regions (e.g. RYGB) produced a paradoxical decrease in post-prandial ghrelin concentrations.[29] This has since been corroborated in numerous other studies and occurs not only with RYGB but also with LSG, but not LAGB.[182,193-195] This may contribute to the reduction in appetite, food intake and glucose tolerance post-operatively, which favours ongoing weight-loss and weight maintenance.[179] In contrast, following weight loss with a VLCD, ghrelin levels increase significantly and it is this that is likely to contribute to the high degree of weight regain observed with dieting and lifestyle interventions.[76]

Another potential mechanism for improved glucose tolerance following surgery is the expedited delivery of nutrients to the distal intestines following bypass operations – the *hindgut hypothesis.* The early post-prandial presence of nutrients in the hindgut directly stimulates secretion of GLP-1 from L-cells of the terminal ileum and colon, as well as secretion of PYY and oxyntomodulin, which act to decrease food intake, delay gastric emptying, increase glucose-stimulated insulin secretion, suppress glucagon secretion, increase β-cell function and mass, and enhance insulin sensitivity.[196] Following RYGB and BPD, in which nutrients pass directly from the stomach to the ileum, L-cell density and post-prandial GLP-1 concentrations are significantly elevated and this may explain the rapid improvement of glucose homeostasis and remission of T2DM in these surgical patients.[179,197] This incretin effect is also observed following LSG but does not occur following LAGB.[188,195,198-200] Experiments involving ileal interposition, whereby a segment of ileum is transplanted between the duodenum and jejunum, further supports this theory. Following this procedure, the L-cells of the transplanted ileum are exposed to enteric nutrients earlier in the foregut and similar post-prandial excursions in GLP-1 concentrations and improvements in glycaemic control are observed in the post-operative period. Notably, this occurs in the absence of gastric resection, malabsorption and weight loss.[191,201] This hindgut hypothesis however has recently been contested by studies that have demonstrated that attenuation of GLP-1 action only partially

reduces the metabolic improvements observed following surgery, thus suggesting that GLP-1 and the hindgut are not the sole contributors to improvements in glucose homeostasis.[202,203]

In contrast to the *hindgut hypothesis,* duodenal-jejunal exclusion has also been implicated as the key mediator of metabolic changes that occur following surgery – the *foregut hypothesis*. Rubino et al. provided the first strong evidence in support of this model in which gastro-jejunal bypass was performed on Goto-Kakizaki (GK) rats, resulting in profound and sustained improvements in fasting glucose, glucose tolerance and insulin action compared to sham-operated controls that occurred independently of calorie intake and weight loss.[204] They also subsequently demonstrated that GK rats subjected to duodenal-jejunal bypass with duodenal exclusion followed by duodenal-jejunal bypass without duodenal exclusion, or vice versa, experienced reversible remission and relapse of T2DM dependent on the respective absence or presence of nutrients within the duodenum.[189] This finding has since been supported in several other studies.[205] In particular, Salinari et al. demonstrated that stomach-sparing duodenal-jejunal bypass and, to a lesser extent, jejunectomy in GK rats improved whole-body insulin sensitivity, reduced hepatic insulin resistance and increased overall glucose tolerance in the immediate post-operative period compared to GK rats undergoing ileectomy or sham operations. It was also demonstrated that following all of these operations there were no significant differences in weight or concentrations of GLP-1 or GIP, suggesting that exclusion/resection of the foregut has specific positive effects on glucose homeostasis that occur independently from weight loss and changes in incretin concentration.[206] One hypothesis to explain this observation comes from Rubino et al. who suggest that bypass or resection of proximal segments of the small intestine independently attenuates hormonal, metabolic or neural factors that could induce insulin resistance, so called *anti-incretins.*[27,207-210]

The anti-incretin theory postulates the presence of nutrient-stimulated mechanisms that function as inhibitory signals to oppose incretin-induced insulin secretion and other post-prandial glucose-lowering mechanisms (i.e. suppression of ghrelin and glucagon). Without such a counter-regulatory system in place the result would be post-prandial hyperinsulinaemic hypoglycaemia and uncontrolled β-cell proliferation (i.e. causing nesidioblastosis and insulinomas) but, as these phenomena are quite rare, it would seem apparent that incretin action is balanced by some yet unknown anti-incretin factor. Conversely, it is theorised that dysregulation of GI physiology could result in disproportionate increases in these putative factors, which act to increase insulin resistance, alter insulin secretion and signalling, reduce β-cell mass, and thus predispose an individual to developing T2DM. It is logical therefore that those metabolic operations that bypass the foregut or other processes which reduce nutrient stimuli in the proximal intestine by other means, via a reduction in food intake (e.g. VLCD) or decreased gastric outflow (e.g. following LSG),

could restore the physiological balance between these anti-incretin and incretin signals, thus improving glucose homeostasis in glucose-intolerant individuals. This notion is supported by the observation made by Salinari et al. whereby the metabolic improvements that occurred following surgery only occurred in the GK rats, a non-obese type 2 diabetic model, and not in the normal glucose tolerant Wistar rats.[206]

Several animal models have supported the current incretin/anti-incretin theory. Firstly, Salinari et al. demonstrated that proteins taken from the duodenum/jejunum of db/db mice or insulin-resistant humans impaired insulin signalling and induced insulin resistance in vivo and in in vitro models, suggesting that the foregut may produce diabetogenic/anti-incretin factors.[211] Specific anti-incretins are yet to be identified in humans but limostatin has been discovered in *Drosophila* that possesses anti-incretin properties and deficiency of which leads to hyperinsulinaemia, hypoglycaemia and excess adiposity.[212] Lindqvist et al. have also demonstrated that bypass of the foregut following RYGB in pigs with normal glucose homeostasis resulted in increased β-cell mass, suggesting that improvements seen following surgery are not a consequence of incretins but rather as a result of defects in the regulation of β-cell proliferation.[196]

Cummings et al. (unpublished) have presented mechanistic human studies performed in patients with obesity and T2DM following RYGB, which is in support of the key role of the foregut in regulating metabolic health. They demonstrated that, as expected following RYGB, insulin sensitivity, as assessed by HEC, and glucose tolerance, as assessed by mixed meal tolerance test, improved in the 2 weeks immediately following surgery. After this time, gastrostomy feeding was commenced (i.e. reinstituting duodenal-jejunal passage of nutrients) and glucose tolerance/insulin sensitivity subsequently declined. By stopping gastrostomy feeding after a further 2 weeks, and hence bypassing the proximal small intestine again, improvements in glucose homeostasis were restored.

It is likely that not one single pathway acting in isolation explains the metabolic effects witnessed following surgery but it is instead a complex milieu contributed to by each of these individual factors, which vary in their relative contribution according to the surgical procedure that has taken place. The common pathway however is decreased hepatic glucose output, increased peripheral uptake and utilisation, improved insulin sensitivity and enhanced β-cell function.

1.2.5.3 Endoscopic Bariatric Therapies

Endoscopic Bariatric Therapies (EBTs) provide a minimally-invasive therapeutic option to achieve weight-loss and treat obesity-related diseases by going beyond what can be achieved through medical and lifestyle interventions alone whilst limiting the potential morbidity and mortality

associated with surgery (**Figure 1.5**). EBTs, which include intragastric balloons, duodenal-jejunal bypass liners (DJBLs), transoral gastroplasty procedures (e.g. POSE, ESG), and duodenal mucosal resurfacing, therefore represent an 'intermediate' treatment option for selected patient groups in terms of effectiveness, safety and cost. EBTs can be utilised as a *primary intervention,* whereby they are intended to be stand-alone treatments that achieve weight-loss and improve obesity-related medical co-morbidities. Alternatively, they may be utilised as: (1) *early interventions*, instituted in patients with class I/II obesity in order to achieve modest weight loss and improve their metabolic risk profile; (2) *bridging therapy,* intended to promote weight loss and reduce a patient's risk from a subsequent planned intervention, such as metabolic surgery or another operation what would benefit from weight- and risk-reduction (e.g. cardiac surgery, orthopaedic surgery or organ transplantation); (3) *metabolic therapies* whereby the primary intention is to treat metabolic co-morbidities, which could be achieved through modest weight loss alone.[213-215]

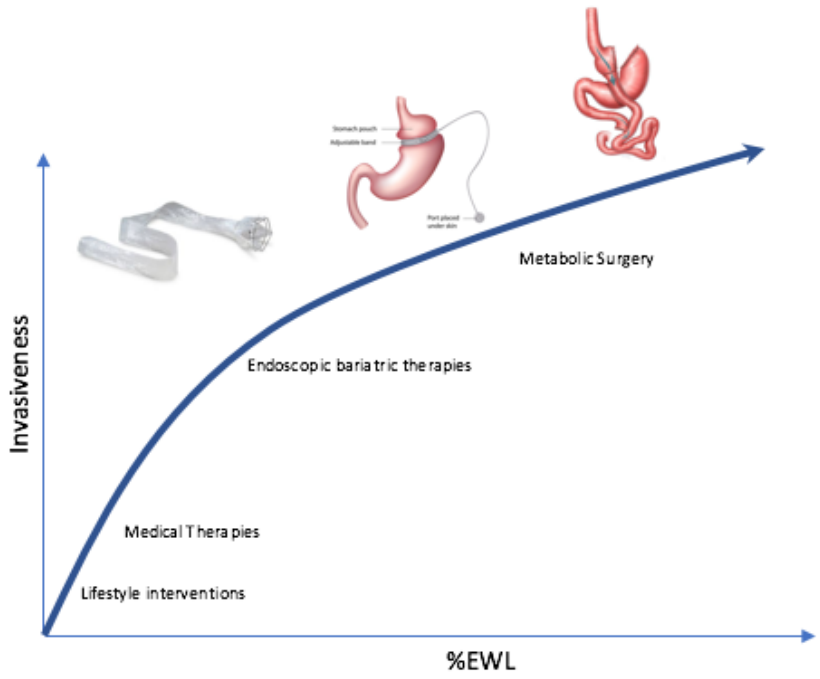


Figure 1.5 Therapeutic approaches to the treatment of obesity and T2DM

Treatments exist as a spectrum in terms of efficacy and potential morbidity and mortality; generally, the more invasive procedures (e.g. BPD +/- DS and RYGB) are able to achieve higher and usually more sustained degrees of weight loss. %EWL, percentage excess weight loss.

Any new EBT should be evaluated in terms of effectiveness, safety, durability and repeatability in accordance with if it is being used as a primary or non-primary intervention. For example, primary

interventions would be expected to achieve higher degrees of %EWL but this may be offset by a relatively higher risk-to-benefit ratio to achieve this goal. Conversely, non-primary EBTs would be expected to be safer and repeatable as a trade-off for lower effectiveness in terms of %EWL achieved. In 2011, a joint task force from the American Society for Gastrointestinal Endoscopy (ASGE) and the American Society for Metabolic and Bariatric Surgery (ASMBS) published a white paper defining acceptable thresholds of safety and efficacy for EBTs:[214]

- **Primary weight-loss threshold:** EBTs intended as a primary intervention in patients with class II/III obesity should achieve a minimum %EWL of 25% at 12 months. In addition, the mean difference in %EWL between a primary EBT and control group should be $\geq$ 15% and statistically significant.
- **Non-primary threshold**: EBTs intended as early intervention, bridging or metabolic therapy should achieve a TBWL of 5%.
- **Safety threshold**: EBTs should have a serious adverse event (SAE) rate of $\leq$ 5%.

1.2.6 Duodenal-jejunal bypass liner

1.2.6.1 Concept and design

The observations made by Rubino and colleagues regarding the role of the foregut in the metabolic changes that occur following surgery have led to the development of endoscopically-delivered duodenal-jejunal bypass liners (DJBLs). These devices line the proximal small intestine and create a non-surgical bypass of the duodenum by delivering undigested chyme directly from the stomach into the mid-jejunum, which is analogous with the bypass component of the RYGB. The EndoBarrier DJBL (GI Dynamics Inc., Lexington, MA) is delivered endoscopically under general anaesthetic or sedation with a custom catheter. It comprises of a nickel and titanium alloy (nitinol) anchor and an impermeable, highly-flexible fluoropolymer sleeve that extends 60 cm through the duodenum and into the jejunum (**Figure 1.6**). Barbs on the circumference of the anchor engage the muscularis of the small intestine to reversibly affix the liner to the wall of the duodenal bulb, proximal to the ampulla of vater, providing a seal to ensure that gastric effluent passes inside the liner (**Figure 1.7**). The implant is open at both ends to allow for passage of chyme from the stomach into the jejunum and prohibits nutrient absorption along its length by creating a barrier between the partially digested food and the absorptive surface of the small intestine. Due to the highly flexible sleeve material, peristaltic movements in the small intestine are capable of propelling nutrients inside the sleeve along the GI tract. Whilst the chyme passes through the inside of the EndoBarrier device, biliopancreatic secretions pass on the outside the liner and only mix with the food when they come into contact at the end of the sleeve.

The Endobarrier is currently licensed for an implant duration of 12 months but is completely reversible and so can be removed at any time. The device is removed under sedation or general anaesthetic using a standard gastroscope fitted with a foreign body retrieval hood. A custom grasper is passed through the working channel of the gastroscope to grab a polypropylene drawstring located on the proximal portion of the anchor. When pulled on, the drawstring collapses the proximal end of the anchor, which can then be pulled into the foreign body hood in order to incorporate the anchor and its barbs and avoid damage to the upper GI tract during withdrawal.

Figure 1.6 EndoBarrier Duodenal Jejunal Bypass Liner

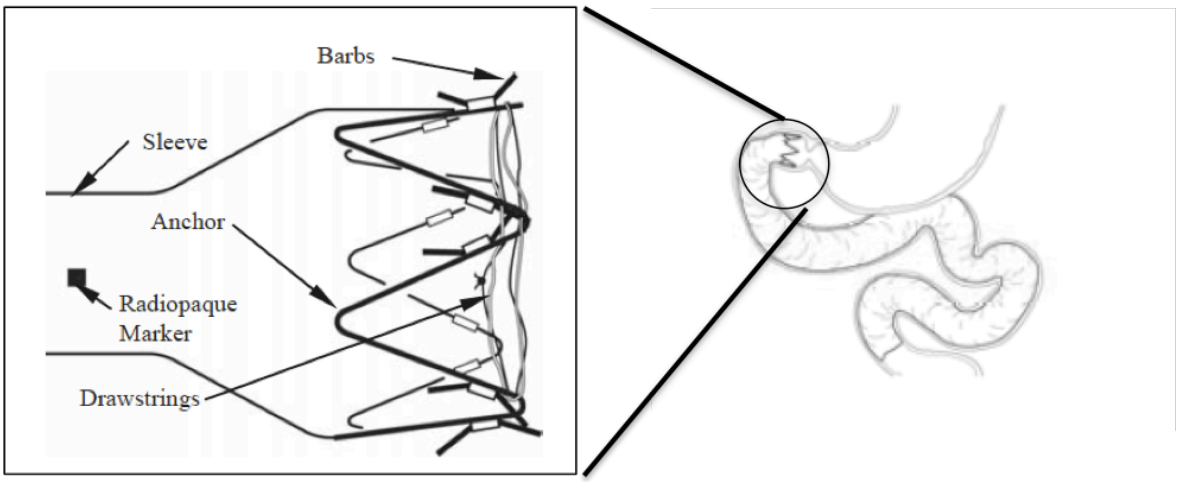


Figure 1.7 Endobarrier anchor system within the proximal duodenum

1.2.6.2 Non-systematic review of the literature

DJBL implantation was first achieved in Yorkshire pigs by Milone et al. in 2006 who observed that DJBL-treated pigs gained weight to a significantly lesser degree than sham-operated control pigs (22.5% vs. 6% respectively, p 0.005).[216] This finding was replicated by Gersin and colleagues who were able to reliably deliver and retrieve a DJBL endoscopically in porcine models before then achieving the first human implantation in 2007, where a 119.5 kg woman (baseline BMI 45.2 kg/m^2) lost 9.1 kg over a 3 month treatment period.[217-219] In a subsequent prospective, observational study by Rodriguez-Grunert et al., DJBL implantation in 10 patients for 3 months resulted in a total weight loss of 10.2 kg, corresponding to an EWL of 23.6% (range 12.5 - 41.5%). Furthermore, it was reported that three of the four patients with T2DM in this study achieved normalisation of their blood glucose without the need for medications (i.e. disease resolution) within 24 hours of DJBL implantation. However, no conclusions could be drawn as to whether or not this was a consequence of weight-loss or due to weight-loss-independent mechanisms induced following bypass of the foregut.[220] Since these preliminary studies, the mechanisms of action and efficacy of the Endobarrier DJBL on weight reduction, glucose homeostasis and metabolic improvements have been reported in five randomised controlled trials[221-225] and numerous other non-randomised or observational studies.[226-256]

1.2.6.2.1 DJBLs and weight loss

It is well evidenced that patients implanted with the Endobarrier DJBL achieve superior weight loss when compared with dietary or sham controls.[257] Patients implanted with the Endobarrier for 12 months can expect to lose 5.9 – 22.4 kg in weight and %EWL > 45% has been reported in several observational studies.[229,244,245,251,252] The national German DJBL registry receives data from

over 30 German centres and currently represents the largest cohort of prospectively collected data on patients treated with the Endobarrier device. A presentation of this registry in 2016 (n = 114) reported a significant mean weight loss of 15.3 kg (BMI reduction -5.4 kg/m^2) and significant improvements in several other metabolic parameters.[258] A further case-control study from this registry in 2018 (n = 235) reported a mean weight reduction of 14.7 $\pm$ 9.0 kg, a reduction in BMI by 5.0 $\pm$ 3.1 kg/m^2 and an EWL of 28.53 $\pm$ 9.0% after a mean implant duration of 47.5 $\pm$ 12.2 weeks. This was superior to matched controls receiving standard treatment.[256] Betzel et al. have published the largest prospective observational study of the Endobarrier to date, reporting on 185 patients implanted with DJBL for a mean duration of 46 $\pm$ 15 weeks, and demonstrated that patients lost on average 12.8 $\pm$ 8.0 kg. This correlated to a %EWL of 46.3 $\pm$ 31.1% at the time of explantation ($p < 0.001$), a TBWL of 11.9 $\pm$ 6.9% ($p < 0.001$) and a reduction in BMI from 35.1 $\pm$ 4.3 kg/m^2 to 30.9 $\pm$ 4.3 kg/m^2 ($p < 0.001$).[244] Corroborating this, Forner et al. subsequently reported on 114 consecutive patients implanted with a DJBL for a mean duration of 51.1 weeks and demonstrated a mean weight loss of 12.0 $\pm$ 8.6 kg ($p < 0.001$), a reduction in BMI by 4.2 $\pm$ 3.2 kg/m^2 and a mean %TBWL of 10.5 $\pm$ 7.3% (range 8.4 – 40.2%).[246] In the largest RCT to date by Koehestanie et al., DJBL (n=34) was found to induce superior weight loss over dietary control patients (n=39) after an implant duration of only 6 months; body weight decreased by 10.6 kg in the DJBL group vs. 5.3 kg in the control group ($p <0.05$), corresponding to a reduction in BMI by 3.3 kg/m^2 vs 1.8 kg/m^2 ($p < 0.05$), an EWL of 32.0% vs 16.4% ($p < 0.05$) and a TBWL of 10.0% vs. 4.7% ($p < 0.05$).[225] In a systematic review by Rohde et al., random effects meta-analysis of 151 patients implanted with DJBL within four RCTs demonstrated that these patients experienced a greater mean weight loss of 5.1 kg and an additional 12.6% EWL compared to control patients.[257] A systematic review by the ASGE Bariatric Endoscopy Task Force sought to review the Endobarrier DJBL against ASGE thresholds for adopting EBTs and concluded that, on available evidence, it did meet the threshold as a primary weight loss therapy with a %EWL of 35% (95% CI, 24-46%) and, in terms of non-primary therapies, a TBWL in excess of 5% was achieved. However, based on the RCT data available, the Endobarrier only achieved a 9.4% EWL (95% CI, 8.26-10.65%) over control patients, thus failing to meet the 15% threshold.[213]

These reported outcomes must be interpreted in light of the fact that the median implant duration for the five RCTs published to date is only 12 weeks and none have examined the effectiveness of DJBL implantation over 12 months. Furthermore, all of these studies have distinct clinical differences, significant methodological shortcomings, and were vulnerable to bias in several domains, thus resulting in considerable inter-trial heterogeneity and funnel-plot asymmetry in the primary analysis of weight loss.[257] Across all of the studies published to date implant durations have varied between three and twelve months with a notable lack of long-term

follow-up data and little focus on patient-reported outcomes. The fact that the majority of the studies have been conducted in South America and Europe, not including the UK, may also limit the generalisability of the results obtained. Robust study data from a well-designed, multicentre, randomised controlled trial of the DJBL implanted for 12 months is therefore required in order to comprehensively evaluate the effectiveness of the Endobarrier DJBL against control interventions and assess whether it meets the ASGE efficacy thresholds.

1.2.6.2.2 DJBLs and glycaemic control

Patients implanted with the Endobarrier DJBL often experience rapid improvements in glucose homeostasis and T2DM, although there continues to be conflicting evidence as to its efficacy in this regard. Following an implantation period of 3 – 12 months, reductions in HbA_{1c} have been reported in the range of 3 – 27 mmol/mol. Most recently, a case control study from the National German DJBL registry (DJBL, n = 111 Vs matched controls receiving standard treatment, n = 222) demonstrated superior reductions in HbA_{1C} in the DJBL group (-1.37 $\pm$ 1.54% vs -0.51 $\pm$ 1.83%, $p < 0.0001$) associated with significantly greater reductions in the use of glucose-lowering medications.[256] In the largest RCT to date conducted by Koehestanie et al., DJBL implantation (n=34) for 6 months resulted in a decrease in HbA_{1C} by 1.3% compared to 0.4% in the dietary control group ($p < 0.05$) and fasting glucose levels were reduced from 11.0 mmol/L to 8.5 mmol/L vs 11.0 mmol/L to 10.0 mmol/L in each group respectively ($p = 0.10$). Additionally, 85.3% of DJBL patients had a decrease in post-prandial glucose excursions versus 48.7% in the control group ($p < 0.05$) and daily insulin or sulphonylurea dosages were more often decreased or discontinued in the DJBL group than in the control group ($p < 0.05$).[225] de Moura et al., demonstrated some of the most significant improvements in glycaemic control where DJBL patients (n = 22), after a mean implantation period of 41.9 $\pm$ 3.2 weeks, reduced their HbA_{1c} by 2.1 $\pm$ 0.3% and their FPG by -30.3 $\pm$ 10.2 mg/dL. Furthermore, 73% of participants reached a final HbA_{1C} measurement of <7%, indicative of adequate glycaemic control.[228] In the largest observational study of patients with T2DM by Betzel et al. (n = 185), both HbA_{1c} and FPG concentrations decreased significantly by 0.6% and 1.2 mmol/L respectively ($p = 0.001$) following an implantation period of 1 year.[244] Similar improvements have been reported with the Endobarrier in numerous other studies, which have demonstrated significant reductions in HbA_{1C}, FPG, and post-prandial glucose excursions, as well as dose-reductions or discontinuation of anti-diabetic medications.[221,227-231,233,237,238,240,242,245,248,251]

However, several studies have failed to observe any statistically significant improvements in either HbA_{1C} or FPG following DJBL implantation.[224,236,239,246,249] Of note, however, these studies are considerably limited by their short implant durations, low participant numbers and high attrition rates, which may have under-estimated the treatment effect. Specifically, Forner et al.

included in their study only a small proportion of patients with T2DM (34%) who already had adequate glycaemic control (HbA_{1c} 6.7 $\pm$ 2%). There were also significant reductions in insulin and oral antidiabetic medications during the course of the trial, which together are likely to explain the lack of any significant HbA_{1c} reduction.[246] In the 2015 systematic review and meta-analysis, the relative risk of reducing or discontinuing antidiabetic medications was 3.28 and 1.13 in DJBL and dietary control groups respectively but it was also concluded that DJBL implantation was associated with a non-significant reduction in HbA_{1C} by -0.9% and in FPG by -3.7 mmol/L when compared with diet alone.[257] Notably, however, these outcomes were derived from only two RCTs and 24 patients with diabetes and, as stated earlier, this meta-analysis is considerably limited by the low number, duration and size of included studies, which suffer from a considerable degree of inter-trial heterogeneity and funnel plot asymmetry in the analysis of glycaemic outcomes. A further systematic review and meta-analysis was therefore conducted in 2018 by Jirapinyo et al to specifically evaluate the effect of a DJBL on glycaemic control in patients with obesity and concomitant T2DM.[259] Seventeen studies and 388 patients were included in the primary analysis and identified a significant reduction in HbA_{1c} by 1.3% or 13.3 mmol/mol after an average implantation period of 8.4 $\pm$ 4.0 months. In a sub-group analysis of RCTs, inclusive of 63 DJBL patients and 53 control patients across 4 studies for an average implantation period of 4.5 $\pm$ 1.7 months, there was a superior reduction in HbA_{1c} by 0.9% or 9.7 mmol/mol in the DJBL patients. Again, heterogeneity across studies was reported to be high and called for larger, high-quality RCTs with longer follow-up.

1.2.6.2.3 DJBLs and cardiovascular risk factors

Studies to date looking at the efficacy and mechanisms of action of the Endobarrier have been limited by their low participant numbers and their low follow-up duration; the majority of studies have a follow-up duration of just 6-12 months with only one study following-up participants for 2 years.[237] As such, no conclusions can be/have been drawn as to the overall effect of the Endobarrier device on diabetic and cardiovascular morbidity and mortality. This being said, many studies provide evidence to support the DJBL as an effective treatment for multiple elements of the metabolic syndrome. One study by Escalona et al., demonstrated a reduction in the prevalence of metabolic syndrome, as defined by the Adult Treatment Panel III criteria,[260] from 83.3% to 41.6% after 1 year of DJBL treatment.[229] Similarly, a study of 40 obese patients who completed 12 months of DJBL implantation demonstrated a significant reduction in the prevalence of metabolic syndrome and 10 year CV risk utilising the Framingham BMI, Framingham Lipid and UKPDS CV risk engine models.[261]

Numerous prospective observational studies have reported improvements in systolic and/or diastolic blood pressure following DJBL implantation and/or a reduction in antihypertensive medications.[228,229,237,242,244,246,248,249,251] Most recently, evidence of this has arisen from the National German DJBL registry.[256] Only one RCT has reported on changes in blood pressure and, after 6 months of treatment, blood pressure was significantly reduced from 147/92 mmHg to 132/81 mmHg in the DJBL group. This, however, was not significantly different from the control group (blood pressure reduced from 152/90 to 137/82, $p = 0.25$) and remained non-significant at 6 month follow-up (mean blood pressure 130/82 vs 140/85, $p = 0.31$).[225] More RCT data for DJBL implant durations of 12 months are therefore required in order to draw conclusions on the effect of duodenal-jejunal bypass on blood pressure.

With regard to lipid modification, in the RCT reported by Koehestanie et al., there were significant reductions in total serum cholesterol (TSC) and LDL-C concentrations when compared to the control group after 6 months of treatment (TSC: 4.4 to 3.7 mmol/L vs 4.4 to 4.5 mmol/L respectively, $p = 0.02$. LDL-C: 2.3 to 2.0 mmol/L vs. 2.4 to 2.3 mmol/L respectively, $p < 0.05$). These changes, however, were not sustained and values in both groups were comparable to baseline at 6 months follow-up (TSC: 4.4 mmol/L in the DJBL group vs 4.4 mmol/L in the control group, $p = 0.79$. LDL-C: 2.4 mmol/L vs. 2.3 mmol/L). In the same study, there was a non-significant reduction in serum triglycerides in both groups and there were no observable changes in HDL-C concentrations between the study groups. Multiple prospective observational studies support the observations made by Koehestanie et al. and have also reported significant improvements in TSC and/or LDL-C following Endobarrier implantation.[227-229,231,237,242,244,246,248,251] Many studies have also demonstrated significant reductions in triglyceride[228,229,231,237,244,250] and/or improvements in HDL-C concentrations[229,237,244,250] and two studies have reported significant reductions in the use of lipid-lowering medications following Endobarrier implantation.[246,251]

In 2013, De Jonge and colleagues examined the effect of a DJBL on plasma parameters of NAFLD and non-alcoholic steatohepatitis (NASH).[232] In this study, 17 patients with obesity and T2DM (mean BMI 37.0 ± 1.3 kg/m^2) were implanted with the Endobarrier for 24 weeks. At the time of device explantation, patients had lost an average of 12.7 ± 1.3 kg, corresponding to an EWL of $29.8 \pm 3.5\%$ and a BMI reduction of 4.1 ± 0.4 kg/m^2 ($p < 0.01$). At baseline, plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) were elevated, giving biochemical evidence of NAFLD, but after 6 months of treatment with the Endobarrier all 3 of these plasma parameters had significantly reduced into the normal range. Plasma levels of liver-fatty acid binding protein (L-FABP) and caspase-cleaved cytokeratin-18 (CK-18) were also reduced over the same time course, reflecting significant reductions in NAFLD-induced liver damage and hepatocyte apoptosis secondary to NASH. Six months following device

explantation, AST and L-FABP levels had returned to baseline levels, which was associated with an increase in weight by 3.4 kg. ALT, GGT and CK-18, however, remained significantly reduced at 6 months follow-up. Taken together, this study provides good biochemical evidence that DJBL implantation and weight loss results in sustained improvements in NAFLD and potentially NASH regression, but this was not confirmed with histological analysis.[232] Gollisch et al. reported similar findings one year after Endobarrier implantation and were able to quantify improvements in liver fibrosis and steatosis utilising liver elastography.[262] Forner et al., similar to the study by De Jonge et al., have also demonstrated significant improvements in liver biochemistry attributable to DJBL therapy. In their study, 82% of patients (23/28) with abnormal LFTs showed improvement or normalisation after a mean implantation duration of 51.1 weeks.[246] However, several other studies, including one RCT and two prospective observational studies, have failed to yield similar results.[220,224,249]

1.2.6.2.4 Proposed mechanisms of action of DJBLs

To date it remains unclear as to whether the metabolic improvements observed following DJBL implantation occur secondary to calorie restriction and subsequent weight loss or occur as a result of the complex neurohormonal mechanisms arising from bypassing the foregut and/or the expedited delivery of partially digested nutrients to the distal small intestine.[220] Similar to the observations made following RYGB, significant improvements in several parameters of glucose homeostasis occur in the immediate weeks following DJBL implantation, before significant weight loss has taken place,[220-222,224,230,231,233,235,240,242,263] thus alluding to the involvement of weight loss-independent mechanisms. However, in order to avoid disruption of the device in the immediate post-implantation period, patients are asked to adhere to 1 – 2 weeks of liquid diet followed by progression to soft diet and then *ad libitum* eating, usually in conjunction with a dietitian-led low-calorie diet. Different authors describe different regimes with varying degrees of calorie restriction during this period (600 – 1500 kcal per day). In addition, up to 100% of patients following DJBL insertion will experience abdominal discomfort, nausea and vomiting, which further reduces appetite, hunger and subsequent caloric intake for up to 1-2 weeks. As previously discussed, acute caloric restriction will transiently increase insulin sensitivity, enhance β-cell function and improve glucose homeostasis, therefore offering an alternative explanation for the observed rapid improvements in metabolic health following this procedure.

Aguirre et al. were the first to evaluate the metabolic effects of the DJBL in a Diet-Induced Obesity (DIO) rat model.[264] This group demonstrated that rats implanted with the DJBL lost 20% more weight than sham-operated controls, associated with a decrease in caloric intake, and that DJBL treatment significantly reduced FPG levels, reduced fasting insulin levels and improved glucose

tolerance, as evidenced by a 40% decrease in the Area Under the Curve (AUC) analysis of glucose excursion. This was mirrored by a 55% improvement in insulin resistance in the DJBL group, measured by Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), and a 36% improvement in β-cell function, as estimated by HOMA-β. As hepatic glucose output is the major determinant of FPG, it was concluded that DJBL specifically reduced hepatic insulin resistance. Furthermore, improved glucose tolerance and a decrease in glucose-stimulated insulin levels reinforced the notion of an overall improvement in peripheral sensitivity. During this study, the authors also performed glucose tolerance testing after intraperitoneal injection of glucose (i.e. bypassing GI absorption) and demonstrated similar degrees of improved glucose tolerance. This suggested that the effects of DJBL on glucose homeostasis were independent of the passage of nutrients within the foregut and, therefore, independent of incretins or other neurohormonal mediators that may be generated by endoluminal nutrient passage. This paper however failed to correlate this with post-prandial incretin hormone measurements. Subsequent work by the same team using the same DIO rat model therefore sought to determine the mechanistic basis of weight loss and improved glycaemic control following DJBL.[263] Through pair-feeding experiments and calorimetry, the authors were able to demonstrate that DJBL implantation resulted in greater degrees of weight loss (by 35%) that was independent of caloric restriction and was associated with increased total and resting energy expenditure (EE). Furthermore, by matching the weight of DIO sham-operated rats, significantly greater improvements in fasting glycaemia, glucose tolerance and HOMA-IR were witnessed in the DJBL-treated rats, which must have occurred through weight-loss-independent mechanisms. Thus, similar to RYGB, DJBL causing isolated duodenal exclusion may exert its effects via weight-dependent and weight-independent mechanisms. These effects were noticeably greater with longer barrier lengths (i.e. with greater degrees of foregut exclusion) and were associated with significantly elevated levels of post-prandial GLP-1 levels. Similar observations have since been made by Schuang et al. who demonstrated significant improvements in plasma glucose levels and resolution of T2DM in steptozotocin-induced diabetic rats following DJBL implantation for 12 weeks compared to sham-operated diabetic rats and diabetic control rats.[265] Similar to the reports by Munoz et al.,[263] these observations were correlated with elevated concentrations of GLP-1. Prior to DJBL placement, post-prandial plasma levels of GLP-1 declined markedly in the diabetic rats compared to normal dietary control rats, as is observed in humans with T2DM.[104,105] 12 weeks following DJBL placement, however, plasma and distal ileal GLP-1 levels were considerably higher than in the sham-operated and diabetic control rats and were associated with significantly increased numbers of GLP-1 positive cells in the distal ileum. There were no observable differences between groups in the number of GLP-1 positive cells in tissues from the proximal jejunum, colon or rectum. It was therefore concluded that isolated duodenal exclusion with a DJBL could potentially

restore the post-prandial GLP-1 response in patients with diabetes, similar to observations made following RYGB.[197]

De Jonge et al. were the first to investigate changes in incretin concentrations in humans following implantation with a DJBL.[233] In this study, 17 patients with obesity (mean BMI 37.0 $\pm$ 1.3 kg/m^2) with inadequately controlled T2DM (mean HbA_{1c} 8.4 $\pm$ 0.2%, FPG 11.6 $\pm$ 0.5 mmol/L) were implanted with the Endobarrier DJBL for 24 weeks. Participants experienced a mean weight loss of 12.7 $\pm$ 1.3 kg (EWL 29.8 $\pm$ 3.5%, BMI reduction -4.1 $\pm$ 0 .4 kg/m^2, $p < 0.01$), HbA_{1C} reduced to 7.0 $\pm$ 0.2% ($p < 0.01$), FPG reduced to 9.0 $\pm$ 0.5 mmol/L within 1 week of implant, 16/17 participants reduced their glucose-lowering medications and post-prandial glucose excursions were reduced with a decreased AUC glucose response. Within 1 week, HOMA-IR had improved in 65% of participants, progressing to 82% by week 24 (HOMA-IR was 14.6 ± 5.8 at baseline vs 9.2 ± 3.5 at week 1 vs 6.3 ± 1.8 at week 24, both $p = 0.06$). Fasting and post-prandial insulin concentrations were unaffected by DJBL placement. At baseline, as expected, these patients demonstrated an attenuated post-prandial GLP-1 response. Within 1 week of DJBL implantation, however, post-prandial GLP-1 concentrations significantly increased and persisted for the duration of the trial, correlating directly with the observed metabolic improvements and corroborating the findings of DJBL in rat models and in humans following RYGB.[263,265] This is in agreement with the hindgut hypothesis whereby increased post-prandial GLP-1 concentrations occur in response to the exposure of L-cells in the distal small intestine to the expedited delivery of partially digested nutrients. In contrast to GLP-1 levels at baseline, a post-prandial rise in GIP was observed, which decreased 1 week following DJBL implantation ($p = 0.06$) reaching statistical significance by week 24 ($p = 0.02$). This finding is consistent with numerous studies where GIP concentrations are decreased following RYGB, which is presumed to occur as a consequence of bypassing the GIP-producing k-cells of the proximal small intestine in accordance with the foregut hypothesis.[178] As both GLP-1 and GIP regulate glucagon secretion (GLP-1 inhibits glucagon secretion, GIP promotes post-prandial glucagon secretion),[266] this was also assessed. At baseline, as expected for patients with T2DM, glucagon levels peaked immediately following a meal. One week following DJBL placement, however, the AUC post-prandial glucagon response was significantly decreased and was maintained for the duration of the study. The authors therefore provide convincing preliminary evidence that the rapid improvements in glucose homeostasis witnessed following implantation of a DJBL in humans are a consequence of improvements in insulin sensitivity and/or decreased hepatic glucose production associated with DJBL-associated changes in incretin hormones and glucagon. Significant reductions in plasma glucagon concentrations following placement of the Endobarrier have also been observed in several other studies.[242,249,251] Kaválková et al. reported similar trends in hormone profiles, although increases in GLP-1 and decreases in

GIP in this study failed to reach statistical significance and they offered sustained increases in circulating bile acids and fibroblast growth factor-19 (FGF19) as a possible explanation for the positive improvements in weight and glycaemic control observed in their study (see below).[242]

Rohde et al. have also examined the post-prandial physiology of individuals implanted with the DJBL, but their findings were less conclusive.[249] 10 normal glucose-tolerant (NGT) and 9 BMI-matched metformin-treated patients with T2DM were compared over a treatment period of 6 months. Both groups achieved only a modest weight loss of 6-7 kg and only minor, non-significant reductions in fasting and post-prandial glucose concentrations were observed in the T2DM population. In line with this, HbA_{1C} values for both groups were not significantly different at 26 weeks and negligible adjustments to glucose lowering medications were made. Fasting plasma glucagon concentrations were marginally but significantly reduced in the NGT group 1 week following implantation but normalised by 26 weeks and were unchanged in the T2DM group throughout the study period. Post-prandial insulin and c-peptide concentrations were unaffected by DJBL placement. Before DJBL placement, patients with T2DM had an attenuated post-prandial GLP-1 response compared to NGT patients. Similar to de Jonge et al., after 1 week of treatment, post-prandial GLP-1 concentrations increased significantly in patients with T2DM (matching the NGT cohort) and this response persisted until week 26. PYY concentrations were also noted to be elevated in the T2DM group at week 1 but normalised by week 26. Given the apparent lack of effect of this elevated GLP-1 (and PYY) response on insulin and glucagon concentrations, this may go some way to explaining why no significant reductions in post-prandial glucose excursions were observed. Similar to de Jonge et al., post-prandial GIP concentrations were also observed to be attenuated in NGT patients throughout the study period, but in the context of unchanged glucose and glucagon concentrations, the significance of this remains uncertain. Of note, unlike the significant increases in EE seen in murine models,[263] REE was only mildly increased in NGT patients in this study and decreased in the T2DM patients. This study therefore gives less convincing evidence for the ability of the DJBL to induce weight loss, modify incretin hormones and improve glucose homeostasis. However, unlike in the Dutch study by De Jonge et al.[233] this cohort of patients with T2DM were already optimally controlled at the time of DJBL implantation (mean HbA_{1c} 50 mmol/mol vs. 68 mmol/mol) and the patients were not subjected to adjunctive caloric restriction. As such, it is difficult to draw direct comparisons between these studies and a treatment effect may have been underestimated.

Further conflicting evidence is provided by a recent prospective observational study by Vilarrasa et al.,[251] where they failed to observe any significant changes in post-prandial GLP-1 levels whatsoever (GIP concentrations were not reported in this study). In this cohort, 21 obese patients with longstanding, poorly-controlled T2DM (12 men, aged 54.1 $\pm$ 9.5 years, BMI 33.4 $\pm$ 1.9 kg/m^2,

HbA_{1C} 9.1 ± 1.3%, mean duration of T2DM 14.8 ± 8.5 years) were implanted with the DJBL and 19 completed 12 months of treatment. An average EWL of 45.5% (TWL 14.9 ± 5.7%) was achieved, maximally by 24 weeks, and a peak reduction in HbA_{1c} of -1.3% was achieved at 4 weeks, increasing thereafter to produce an overall reduction in HbA_{1c} by -0.6% at month 12. A lower baseline HbA_{1c} value and increased weight loss were associated with better outcomes and, in multiple linear regression analysis, weight at 1 month was predictive of metabolic control 12 months after the procedure, suggesting that improvements in glycaemic control were achieved through weight loss-dependent mechanisms.

One suggestion offered for the lack of augmented GLP-1 response in this study was that the anchor of the Endobarrier (and the presence of chyme within it) may reduce the rate of gastric emptying by distending and irritating the duodenal bulb, thus activating mechanoreceptors in this region and setting up local neuro-hormonal enterogastric feedback loops, as described by several other studies.[238,263,264] This is in contrast to the RYGB in which this region of the foregut is bypassed of all stimuli. Decreased gastric emptying would logically reduce the rate of flow of nutrients along the GI tract, thereby decreasing the rate of stimulation of GLP-1-producing L cells in the distal small intestine. Furthermore, delayed gastric emptying would promote early satiety and caloric restriction and would go some way to explaining the early moderate weight-loss in Endobarrier patients and their associated metabolic improvements. Other studies, however, have contradicted the notion of decreased gastric emptying secondary to DJBL and this theory also fails to explain why fasting PYY concentrations were increased in this study, as this hormone is also secreted from the L cells of the ileum.[249] One other explanation for the blunted GLP-1 response in this study could therefore be as a result of dysregulated incretin secretion because of longstanding T2DM in the included patients (mean duration of T2DM 14.8 ± 8.5 years).

Koehestanie et al. have also investigated the potential role of gut hormones in augmenting metabolic physiology following DJBL implantation and, conversely, identified an initial decrease in fasting GLP-1 levels within 1 week of implantation with a subsequent increase back to baseline levels by week 4.[235] It was postulated that the prescription of a liquid diet in the immediate post-procedural period could be responsible for this observation as liquid would be more easily absorbed in the proximal gut and cause less stimulation of distal L cells. In a Pearson's correlation analysis using AUC data, there was a direct correlation between these changes in fasting GLP-1 levels and improvements in fasting insulin levels and HOMA-IR, but no correlation with reduction in weight, BMI and/or fat mass. The authors suggested, therefore, that GLP-1 may be responsible for early improvements in glycaemic control following Endobarrier implantation and that these occur independently of weight loss. Four weeks following implantation there was also a tendency

for fasting GIP levels to decrease, similar to the aforementioned reports,[233,242,249] but this did not reach statistical significance (p = 0.09).

Other gut hormones that have been implicated in regulating metabolic changes following DJBL implantation include PYY, CCK, and ghrelin. The anorexigenic hormone PYY, released from the distal intestinal L-cells and partly responsible for lower-intestinal satiation,[28] has been found to be significantly elevated following DJBL implantation.[241,249,251] De Jonge et al. provided convincing evidence of this and participants reported increased satiety (88%) and reduced caloric intake (100%), similar to observations made following RYGB.[27,198,241] Rohde et al. were also able to demonstrate greater satiety, fullness and decreased food consumption 1 week following DJBL insertion in NGT patients but these findings were not sustained at 26 weeks and were not correlated with changes in PYY (but may be related to elevated post-prandial GLP-1 concentrations up until week 26).[249] The precise role of PYY following Endobarrier insertion therefore remains unclear and warrants further investigation. CCK is released from the I cells of the duodenum and jejunum and is involved with upper-intestinal satiation. Evidence to date suggests that this hormone remains unchanged following bariatric surgery.[199] De Jonge et al. however have provided evidence that there is a decrease in CCK concentrations following DJBL implantation, presumed to occur as a result of foregut exclusion and decreased I cell stimulation,[241] but these findings are yet to be reproduced.[257] Rohde et al. also failed to observe any changes in gallbladder volume or postprandial gallbladder emptying associated with DJBL therapy.[249] The orexigenic hormone ghrelin has been demonstrated to be significantly elevated following Endobarrier insertion,[235,236,241,251] which is in contrast to RYGB where the majority of reports describe a post-operative decrease in ghrelin. During RYGB there is surgical manipulation of the anatomical regions of ghrelin secretion and possible vagal dysregulation to explain this phenomenon. As these surgical adaptations do not occur with DJBLs then the rise in ghrelin is likely to occur as a physiological response to weight loss and caloric restriction, as is seen in starvation and following a VLCD. However, despite the rise in this 'hunger hormone', following insertion of a DJBL, patients still seem to report increased satiety and less hunger, plus significant improvements in weight and glycaemic control are observed. The precise role of ghrelin in this context, therefore, remains unclear.

DJBL implantation has also been associated with increased bile acid (BA) concentrations in obese patients with T2DM and these are believed to be important regulators of energy balance and metabolism, primarily through their action via the nuclear farnesoid X receptor (FXR) and the G-protein-coupled receptor 5 (TGR5).[242,267] As part of normal post-prandial physiology, the presence of lipids and proteins in the duodenum stimulates gall bladder contraction (partly in response to CCK secretion) and BAs are secreted into the duodenum to mix with and aid digestion of ingested

lipids through micelle formation. 95% of bile acids are reabsorbed back into the hepatic portal circulation, either actively through terminal ileal enterocytes via the apical sodium bile salt transporter (ASBT) or passively along the length of the small intestine and colon. Trans-intestinal flux of BAs in the ileum via the ASBT activates FXR, which induces secretion of the enterokine, FGF-19 into the circulation. Primarily, FGF-19 acts to regulate BA synthesis through negative feedback mechanisms, by down-regulating hepatocyte 7α-hydroxylase activity, and also promotes gall bladder filling. With regards to the regulation of energy homeostasis, it has been demonstrated that TGR5 activation by BAs in the distal small intestine causes energy expenditure to increase in brown adipose tissue through deiodination of inactive thyroxine (T_4) to active triiodothyronine (T_3).[268] Additionally, BA-induced activation of the FXR-FGF19 pathway and TGR5 receptor on L cells of the terminal ileum regulate GLP-1 secretion and, hence, potentially play an important role in glucose homeostasis and metabolism.[269,270] Certainly FXR knockout mice are proven to be insulin resistant and, in addition to potentiating GLP-1 activity, FGF-19 has been shown to activate glycogen synthase and inhibits gluconeogenesis. Furthermore, an FXR agonist has been shown to restore glucose homeostasis in a DIO mouse model and improve glucose tolerance by reducing hepatic glucose production.[271-274]

Patients with T2DM have reduced circulating BA and FGF-19 concentrations compared with normal glucose tolerant individuals, irrespective of BMI, and the levels of both of these increase following RYGB and LSG (but not following LAGB or VLCD).[27] This change correlates directly with improvements in glucose tolerance and those who go into remission of their T2DM exhibit higher circulating levels of BAs and FGF-19 compared to non-remitters.[275] The mechanisms behind these observed changes in BA/FGF-19 concentrations are yet to be fully understood but may occur as a consequence of: (1) increased BA cycling, secondary to exposure of the terminal ileum to 'undiluted' bile acids,[276] (2) alterations in ASBT number and function following surgery,[277] or (3) changes in the gut microbiome, which alters BA composition.[278] How these then alter glucose metabolism in humans following metabolic surgery is also yet to be fully elucidated, but alterations in sodium-glucose intestinal co-transport, increased circulating levels of GLP-1, and changes in the microbiome and BA composition have all been implicated.[278,279] Following DJBL insertion there is preliminary evidence of increased fasting and post-prandial concentrations of BAs and FGF-19, which appear to return to baseline levels after removal of the device.[242,280] Therefore, similar to RYGB, BA-FGF19 signalling may, at least in part, be responsible for the metabolic improvements observed following Endobarrier implantation and further research into this is required.

1.2.6.2.5 Changes in insulin resistance following DJBL implantation

Although it remains unclear as to the precise mechanism(s) of action of the DJBL, current evidence supports an overall improvement in peripheral insulin sensitivity and glucose homeostasis through weight loss-dependent and –independent mechanisms. Similar to what is observed following surgical duodenal-jejunal bypass,[205] murine models have confirmed early (within 1 week) improvements in insulin resistance following implantation with an endoluminal sleeve, as demonstrated by a 55% decrease in HOMA-IR.[264] This was associated with a decrease in fasting insulin and plasma glucose concentrations and, as hepatic glucose output is the major determinant of FPG, it would be apparent that the improvements in glycaemic control are a consequence of improvements in hepatic insulin resistance. Improved oral glucose tolerance with a concurrent decrease in glucose-stimulated insulin levels also imply an overall improvement in peripheral insulin sensitivity with increased peripheral glucose utilisation and disposal.[263,264]

Model assessments have been utilised in several human studies to demonstrate rapid improvements in insulin sensitivity and rapid reductions in hepatic glucose output following Endobarrier implantation.[229,230,233,235,242,243,248] In addition to the studies previously discussed, Cohen et al. showed evidence of this in a prospective observational study of 16 patients implanted with the Endobarrier for 1 year (mean BMI 30 kg/m^2, HbA_{1C} 8.6%). In this cohort, HOMA-IR significantly decreased and the Matsuda index significantly increased within 1 week of implantation and remained improved for the duration of the implantation period. Insulin secretion demonstrated a trend towards decreasing, but this was not significant for both fasting values ($p = 0.051$) and AUC analysis ($p = 0.28$) and there were non-significant changes in the insulinogenic index (a measure of first-phase insulin response). Patients were followed up for 6 months following removal of the Endobarrier and there was a trend towards an increase in insulin resistance when compared to the implant period, but this increase was not statistically significant.[230]

Only one study to date has evaluated glucose homeostasis following DJBL implantation using the gold-standard method of Hyperinsulinaemic Euglycaemic Clamps (HEC). In this study by Miras et al,[247] 7 obese patients with a mean baseline BMI of 48.5 kg/m^2 underwent serial clamps in order to evaluate the early effects of DJBL implantation on insulin sensitivity and HGP, whilst controlling for the effects of caloric restriction in the peri-implantation period. Clamps were performed at three time points: (1) at baseline, (2) 1 week after commencing a low-calorie liquid diet (Fortisip® Compact, 1500 kcal d^{-1}), and (3) 1 week following DJBL implantation, whilst the patient continued on the liquid diet. 6 of the 7 patients had T2DM with a mean baseline HbA_{1C} of 7.2% and were treated with glucose-lowering tablets (n = 6), GLP-1 agonists (n = 2) or insulin (n = 3), which were

unchanged during the study period in order to control for this as a possible confounder. Following the initial 1 week of liquid diet patients lost an average of 3.6 kg (-1.4 kg/m^2) and then lost a further 4.3 kg (-1.4 kg/m^2) 1 week following DJBL implantation on the low-calorie diet. During the clamp, FPG concentrations and HGP were significantly reduced between visits 1 and 2 ($p < 0.05$) but values were not significantly reduced further between visits 2 and 3 (i.e. following the implantation of the DJBL). This study therefore concluded that the DJBL did not improve hepatic insulin sensitivity beyond the improvements achieved with caloric restriction. This study was, however, limited by its small number of participants and lack of a control group.

1.2.6.2.6 Durability of effects of DJBLs

The efficacy of the Endobarrier DJBL appears to be maximal within the first 9 – 12 months of treatment and plateaus thereafter. Quezada et al. are the only authors to report on implant durations up to three years and, in this study, 77 patients achieved a significant reduction in their weight by 3 months (EWL 35 $\pm$ 11%) with weight loss peaking at 12 months (EWL 43.6 $\pm$ 16%, n = 72).[248] Thereafter, a non-significant weight increase was observed (18 month EWL 40 $\pm$ 18%, n = 56; 2 year EWL 40 $\pm$ 23%, n = 43) and was then maintained in the 11 patients completing 3 year follow-up (EWL 39 $\pm$ 20%) (**Figure 1.8**). Similar observations were also made for improvements in glycaemic control, insulin resistance, blood pressure, total cholesterol and LDL-C. Therefore, for the first year of treatment with the Endobarrier device, patients achieved a significant reduction in their weight and improvements in several metabolic parameters with a low rate of adverse events. Thereafter, patients did not lose any more weight or improve their metabolic health further but were subject to considerably more serious adverse events. It was therefore recommended that treatment with the Endobarrier DJBL should not exceed 12 months.

More recently, Betzel et al. reported on the safety and efficacy of implant durations up to 2 years.[281] They similarly concluded that maximal weight loss with the Endobarrier occurred during the first 9 – 12 months of treatment and implantation beyond this time served only to maintain this. During the second year of implantation, over two-thirds of the patients experienced at least one adverse event and 45% of the patients required early removal of the DJBL due to AEs (including two liver abscesses). Twelve months following explantation there was a significant weight regain but, after 24 months, weight was still significantly lower when compared to baseline.

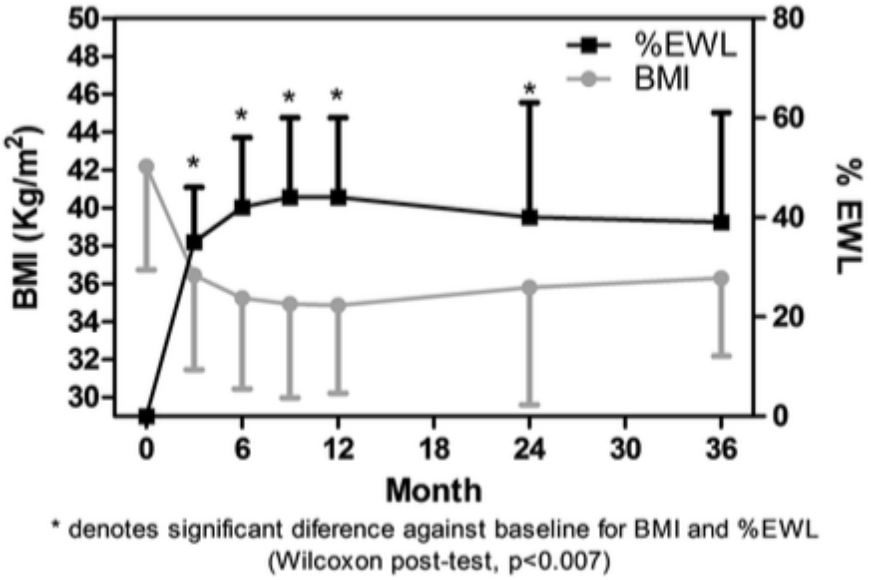


Figure 1.8 BMI and %EWL in patients treated with the Endobarrier for up to 3 years. Source: Quezada et al.[248]

The largest cohort of data of the effects on weight and metabolic profile following termination of treatment with the DJBL comes from the National German DJBL registry.[254] In a report of 77 patients with a follow-up duration of 0.04 to 3.85 years the authors concluded that 85% of patients maintained or increased their weight after removal of the DJBL and an increase in BMI of 2.1 kg/m^2 had to be expected (approximate 50% weight regain). A further sub-group analysis of 32 patients, who had follow-up data for at least one year, demonstrated an increase in BMI of 2.5 kg/m^2 but they also concluded that the weight/BMI at the follow-up visit remained significantly lower than at the baseline/implantation visit. Similar to this trend in weight, explantation of the DJBL was also associated with an increase in HbA_{1C} by 0.3% but a statistically significant reduction in HbA_{1C} of -0.9% was maintained when compared to the implantation visit. There was however a slight parallel increase (NS) in glucose-lowering medications within the same cohort, which may have contributed to this sustained improvement in glycaemic control. SBP, DBP, and LDL-C were also reduced during the implant period but returned to baseline levels (or higher) following removal of the device.

The largest RCT (to date) by Koehestanie et al. similarly reported a deterioration in treatment effect following device explantation (**Figure 1.9**).[225] At 6 months follow-up, patients in both groups regained weight but their BMI was still significantly lower than at the baseline visit and, importantly, the DJBL group maintained an EWL of 19.8% versus 11.7% in the control group ($p < 0.05$) (TBWL 5.8% Vs. 3.5%, $p < 0.05$). HbA_{1C} and fasting glucose levels also increased following removal of the Endobarrier device and by the end of the trial there was no statistically significant difference from the control group. Recently, the same group reported on the 4-year weight-loss

outcomes in 15 patients following device removal and observed that there was no significant difference in weight, BMI or TBWL when compared to baseline.[282]

This deterioration of effects following removal of the Endobarrier has also been presented in several other prospective cohort studies (**Figure 1.10**) and matches the experience of treatment decay observed following the cessation of other conservative and non-surgical treatments for obesity (e.g. the gastric balloon).[229,230,232,242,245,246,250] This should therefore be an important consideration when referring a patient for a temporary EBT and highlights the importance of implementing medical and lifestyle interventions alongside these therapies in order to maximise their efficacy and longevity.

As previously discussed, the mechanisms behind the effects of the Endobarrier device are not yet fully determined but several studies report a significant reduction or normalisation of glucagon, incretin hormone, bile acid and FGF-19 concentrations following removal of the Endobarrier device, which may explain the observed decay in treatment effect.[233,242] Despite this, there appear to be sustained improvements in weight and metabolic parameters beyond the treatment phase, albeit short-lived, and one reason for this could be a sustained improvement in beta cell function associated with an overall reduction in anabolic insulin action.[254] However, the sustained treatment effect may simply reflect ongoing benefits obtained from being followed up as part of a clinical study or the adoption and maintenance of behavioural, dietary and lifestyle changes by the patient during their treatment.

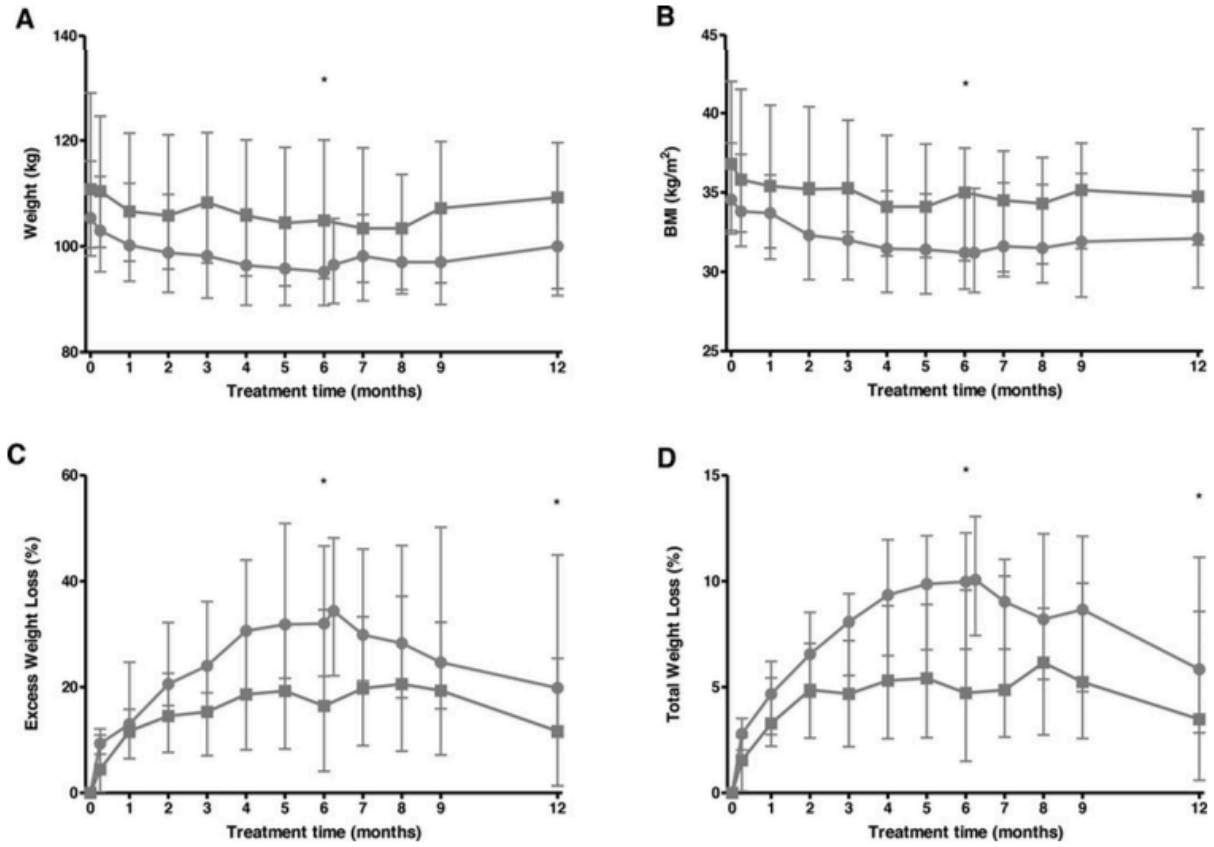


Figure 1.9 Loss of treatment effect following explantation of Endobarrier.
Evolution of (A) weight, (B) BMI, (C) %EWL and (D) %TWL in control (n = 35) and Endobarrier (n = 31) groups (6 months of intervention and 6 months of follow-up).
Modified from Koehestanie et al.[225]

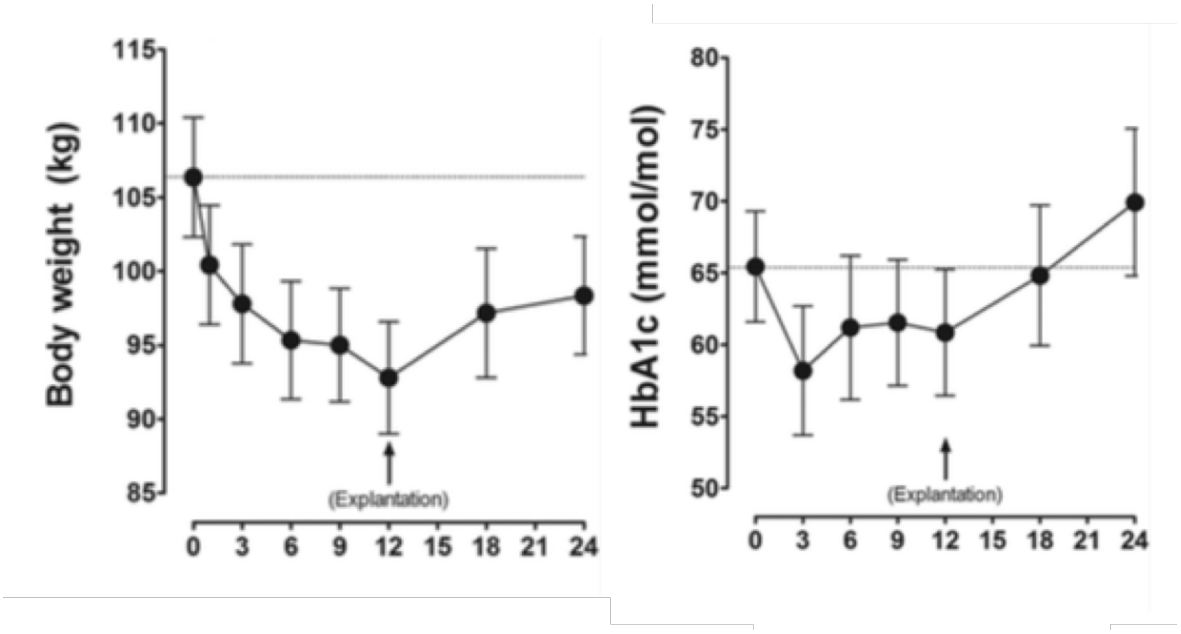


Figure 1.10 Evolution of body weight and HbA_{1C} following Endobarrier implantation.
Modified from Betzel et al.[245]

One solution to manage weight regain and the deterioration of glycaemic control following Endobarrier removal could be to reimplant the device, which was safely achieved in a limited number of patients (n = 5) by Koehestanie et al.[239] In this small cohort, the authors successfully reimplanted the DJBL for a further year in patients previously treated for 6 months and were able to demonstrate significant improvements in %EWL and BMI, beyond that achieved during the initial implantation period.

1.2.6.2.7 DJBL safety

Experience of 3717 EndoBarrier devices distributed worldwide has demonstrated a favourable risk-to-benefit ratio (GI Dynamics, February 2017) and their minimally invasive and reversible nature represents a very attractive treatment modality for patients with obesity and T2DM. In 2015, the ASGE Bariatric Endoscopy Task Force deemed that the DJBL met the $\leq$ 5% PIVI threshold of SAEs to indicate an acceptable safety profile. Additionally, there have been no deaths attributed to the EndoBarrier and all patients experiencing an SAE have recovered without long-term sequelae.

Nonetheless, it is reported that up to 100% of patients will experience a non-serious adverse event (predominantly abdominal discomfort and nausea immediately following implantation) and GiD actually report a 7.4% rate of SAEs (GI Dynamics safety reporting 2008 to March 2017). The exact nature of these events is summarised in **Table 1.7**. Furthermore, up to 40% of patients may require early explantation of the Endobarrier device and technical or anatomical factors preclude device implantation in some patients (implant failure rates of 0 – 16% have been reported).[257]

In a review of the National German DJBL registry (n = 235) it was reported that greater than 26% of patients experienced a mild to moderate adverse event, which were largely gastrointestinal symptoms in the immediate post-implantation period that resolved rapidly with symptomatic management only. Additionally, however, they also reported a SAE rate of 6.8%, including device dislocation/migration (2.6%), hepatic abscess (HA) (1.7%), GI bleeding (0.4%), sleeve obstruction (0.9%), perforated duodenal ulcer (0.4%), biliary colic (0.4%) and oesophageal laceration (0.4%).[256] Notably, the pivotal ENDO trial (ClinicalTrials.gov Identifier: NCT01728116) was a multi-centred, double-blinded RCT in the US where participants were randomised to receive either the Endobarrier device or sham treatment in order to assess the efficacy and safety of the device. The study opened in November 2012 but was terminated early by the FDA in 2015 after only 325 patients were randomised (216 EndoBarrier patients) due to a higher than expected HA rate of 3.5%. This is in comparison to reported HA rates of 0.73% globally, 1.2 % in Europe and 1.34% in the UK.

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017 (Jan-Mar)	TOTAL
Distributed (Denominator)	25	143	157	275	391	812	987	482	383	43	
Hepatic Abscess	0	0	0	0	1	8	12	9	4	2	36
Hepatic Abscess Rate											1.0%
Hepatic abscess with explant ≤ 12 months	0	0	0	0	1	5	9	7	1	2	25
Hepatic Abscess Rate											0.7%
Intolerance	0	5	4	12	5	10	11	4	18	0	69
Intolerance Rate											1.9%
Liner Obstruction	0	4	0	5	3	1	10	2	0	0	25
Liner Obstruction Rate											0.7%
GI Bleed	0	1	3	6	5	9	20	8	4	1	57
GI Bleed Rate											1.5%
Migration/ Movement	0	10	6	4	1	6	11	13	0	1	52
Migration/Movement Rate											1.4%
Pancreatitis	0	0	0	0	4	1	7	0	0	0	12
Pancreatitis Rate											0.3%
Perforation	0	0	1	0	0	5	2	2	1	0	11
Perforation Rate											0.3%
Surgical Removal	0	0	0	1	1	0	8	1	1	0	12
Surgical Removal Rate											0.3%
Total Incidences	0	20	14	28	20	40	81	39	28	4	274
Total Cumulative Rate											7.4%

Table 1.7 Serious adverse event rate for the Endobarrier device.
GI dynamics safety reporting data, 2008 to March 2017

1.2.7 Assessment of new surgical technologies: IDEAL framework

Surgery and invasive therapies, including EBTs, represent complex, multi-faceted interventions and, historically, studies into these therapies have often been poorly designed and have lacked standardisation.[283,284] This has frequently led to sub-optimal reporting of surgical therapies, heterogeneous study outcomes and poor inter-study correlation. Such research practices could lead to the potential adoption of sub-standard and/or harmful surgical practices or even the refusal of governing bodies to endorse and fund safe and efficacious surgical practices due to the lack of high level evidence supporting their implementation, thus leading to the slow adoption of innovations.

In 2009, the IDEAL collaboration sought to rectify this situation by introducing a standardised IDEAL framework, a proposed paradigm for the assessment and evaluation of surgical operations, invasive medical devices and other complex therapeutic interventions. In brief, this framework is composed of 5 distinct stages (Idea/Innovation, Development, Exploration, Assessment and Long-term study) as the progressive steps of evaluating any new surgical innovation with recommendations and methodological guidance being provided at each stage. The ultimate aim of this is to support the generation of robust, high-quality, standardised surgical research. The stages and a full description of the IDEAL framework are provided by Hirst et al.[285]

The quality of reporting in surgical studies has improved in recent years and the adoption of the IDEAL framework in surgical research is becoming more widely accepted.[286] However, to date, its implementation and correct usage remain limited and a recent review highlighted that, in those prospective studies that have used IDEAL in their design, the IDEAL-specific recommendations are often poorly adhered to.[287] This can partially be attributed to a lack of understanding and familiarity of researchers in implementing these recommendations and the developers of the framework have conceded that the original IDEAL publications lacked clarity on interpretation and implementation. As such, the framework has been updated in order to improve its usability and new supporting guidance has been produced.[285,288,289] Additionally, poor adherence to the guidance could be attributed to the absence of explicit and standardised reporting guidelines within the current framework, especially in IDEAL stages 2a and 2b (i.e. in the iterative modification and assessment of the intervention prior to a RCT). Stages 1 and 3 of the framework make specific reference to adhering to established reporting guidelines, such as: Surgical CAse REport (SCARE) Guidelines, Consolidated Standards Of Reporting Trials (CONSORT), Core Outcome Measures in Effectiveness Trials (COMET), Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Checklist, and the Template for Intervention Description and Replication (TIDieR) Checklist. However, work is currently underway by the IDEAL collaboration to

construct a specific set of reporting guidelines to be used at all stages of publishing an evaluation of a surgical innovation.[290] Finally, innovation reporting using IDEAL may be hindered by a clinician being unable to distinguish an innovative procedure from a modification in an established one, which in turn impacts on how such a procedure is evaluated and governed.[291]

The current version of the IDEAL framework continues to evolve and incremental evidence-based modifications are being made in response to analyses of its outcomes and impact. As researchers become increasingly familiar with the framework, one would envisage that it becomes universally adopted and more-appropriately implemented when designing, delivering and reporting prospective studies, such that the research is safe, has appropriate ethical oversight, and the data produced is robust and can accurately guide clinical decision making. Many examples of this are now beginning to emerge in the literature.[292]

Surgical technologies are continuously evolving with a large focus being placed on minimally-invasive and natural orifice interventions as a means of improving patient outcomes. EBTs represent an exciting, minimally-invasive therapeutic option to achieve weight-loss and treat obesity-related diseases by going beyond what can be achieved through medical and lifestyle interventions alone whilst limiting the potential morbidity and mortality associated with surgery. As such, new EBTs are continuously being conceived and these must be rigorously evaluated in order to avoid the adoption of potentially harmful practices and the other pitfalls discussed above. The IDEAL framework provides a means by which we can evaluate these new practices.

There is yet to be a study of the Endobarrier DJBL that directly references the IDEAL framework and it is yet to be applied to a novel endoscopic procedure of any kind. As discussed in **section 1.2.6.2**, there are numerous clinical considerations and safety concerns that need to be overcome before the Endobarrier can be widely adopted as part of routine clinical practice and the fact that the pivotal US ENDO trial was terminated due to an unacceptably high level of morbidity is testament to this. However, the Endobarrier continues to be studied and the results from two large RCTs, the Revise-Diabesity (NCT02055014) and Pivotal Trial (Rev B) (STEP-1) (NCT04101669), are awaited. This currently places the Endobarrier device in the Assessment stage (stage 3) of the IDEAL framework in which high-quality, RCT-evaluated, patient-centred clinical outcomes are expected. Although the quality of reporting of the Endobarrier to date has not been formally assessed, reviews of the early-stage development of other innovative procedures have identified significant deficiencies in their methodologies and reporting when assessing them against IDEAL stage recommendations.[293] In order to rigorously and transparently evaluate a surgical innovation, the validity, accuracy and safety of early stage studies must be established prior to performing an RCT. Failure to do so may result in a procedure being introduced without sufficient investigation, which has the potential to result in patient harm. Advanced endoscopic procedures,

such as the Endobarrier, would therefore benefit from standardised methods of evaluation and reporting through the application of the IDEAL framework.

Another example of an evolving EBT is the Endoscopic Sleeve Gastroplasty (ESG). This technique is developing a robust evidence-base in the form of multiple prospective observational studies, which have reported excellent short-term clinical and safety outcomes in a high number of patients. It lacks, however, high-level RCT data. This places it within Stage 2b of the IDEAL framework and is awaiting further assessment before it can be safely adopted as part of routine clinical practice. A recent systematic review by Currie et al (unpublished) of the innovation status of the ESG identified methodological and evaluation shortcomings in 28 studies when being compared to the IDEAL stage recommendations. This may limit the usefulness of some of the information obtained from this early-stage research and serves as another example of where robust and transparent research methodologies are required for evolving surgical interventions.

1.2.8 Endoscopic sleeve gastroplasty

1.2.8.1 Concept and technique

First described in 2008, the Endoscopic Sleeve Gastroplasty (ESG) is rapidly being established as an effective means of achieving significant weight loss via the transoral route.[294] The generally accepted technique of ESG utilises the OverStitch™ (Apollo Endosurgery Inc., Austin, TX, USA), which is a cap-based flexible endoscopic suturing system that is mounted onto a double-channel endoscope (**Figure 1.11a**).

Starting distally at the level of the incisura angularis and working proximally along the greater curvature towards the fundus, the OverStitch™ device is used to fashion multiple, interrupted triangular plications (**Figure 1.11b and c**). Each plication is formed using a tissue retraction screw (Helix; Apollo Endosurgery Inc., Austin, TX, USA) to draw the muscularis propria of the stomach wall into the jaws of the Overstitch device in order to take multiple full thickness bites. The bites of each plication are then clinched together to approximate the tissue and produce compressive occlusion of the greater curvature. This creates concentric compression along the greater curvature that results in the formation of a short tubular gastric lumen and a 70% reduction in stomach volume (**Figure 1.12**) without the need to amputate the greater curvature, as is required in LSG. Weight-loss is achieved by significantly restricting the volume of food consumed at each meal coupled with a delay in gastric emptying, which promotes early satiety.[295] This now widely-accepted technique has been fully described by Lopez-Nava et al.[296]

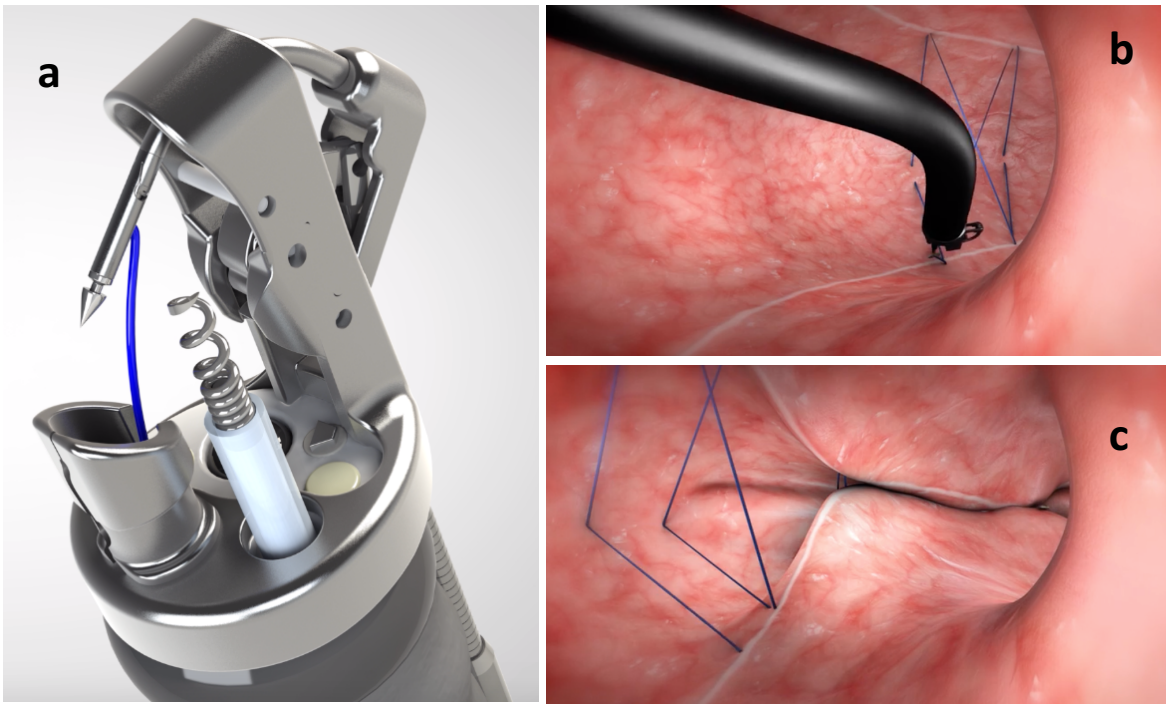


Figure 1.11 Apollo OverStitch™
a) The Apollo OverStitch™ endoscopic suturing system, b) the OverStitch™ device is used to fashion multiple, interrupted triangular plications along the greater curvature of the stomach, c) the bites of each plication are clinched together to produce compressive occlusion of the greater curvature. Images used with permission, copyright Apollo Endosurgery Inc.

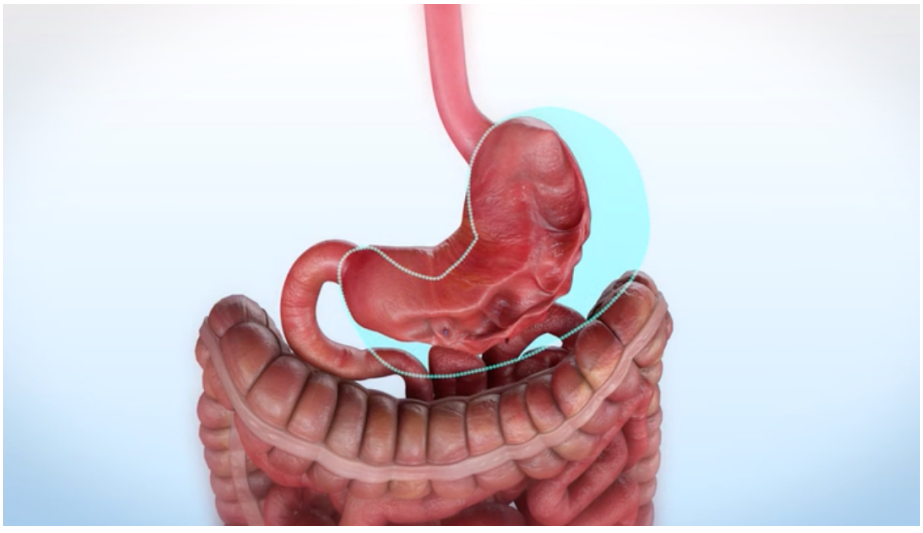


Figure 1.12 Schematic diagram demonstarting reduction in stomach volume following ESG. Image used with permission, copyright Apollo Endosurgery Inc.

1.2.8.2 Efficacy outcomes

ESG is rapidly being adopted worldwide, but evidence of its safety and efficacy currently only exists in the form of retrospective reports or several prospective observational studies. Long-term outcome data is still awaited and the ESG is yet to be examined in the context of a RCT.[295-311] The highest level evidence of the ESG currently comes from a systematic review and meta-analysis by Hedjoudje et al. in which they included data from 8 studies with 9 different cohorts (4 retrospective and 5 prospective), inclusive of 1772 patients.[312] At 6 months, the mean TBWL was 15.1%, the mean decrease in BMI was 5.65 kg/m^2 and the mean EWL was 57.7%. Weight loss was sustained at 12 months and 18-24 months with a %TBWL of 16.5% and 17.2% respectively. ESG therefore satisfies the ASGE/ASMBS weight-loss threshold for efficacy as a primary weight-loss intervention.[214] However, this meta-analysis is limited by the high proportion of retrospective studies included and the absence of any RCTs. The majority of the included studies have notable flaws in their methodology, substantial statistical heterogeneity and were vulnerable to bias; attrition bias, in particular, may have overestimated the treatment effect of the ESG. The fact that the majority of these studies were conducted in the United States and Europe, excluding the UK, also limits the generalisability of these results. However, given the impressive weight loss outcomes and reproducibility between independent centres, ESG appears to be an effective treatment for obesity and there is a call for it to be assessed as part of a larger, multicentre RCT with longer follow-up. Of note, Cheskin et al. have performed a comparative case-matched study of the ESG against high-intensity diet and lifestyle therapy and reported a TBWL of 20.6% at 12 months in the ESG cohort versus 14.3% in the dietary-matched patients ($p < 0.001$).[313]

1.2.8.3 Safety outcomes

Following ESG, patients report only mild-to-moderate, transient, post-operative symptoms and SAEs have been reported in selected studies at a rate of <3%.[295,297,299,300] In the 2019 systematic review, the pooled rate of SAEs was 2.2% (95% CI, 1.6%-3.1%), including pain or nausea requiring hospitalisation (n=18, 1.08%), upper gastrointestinal bleeding (n=9, 0.56%), and peri-gastric leak or fluid collection (n=8, 0.48%).[312] This sits very much below the ASGE/ASMBS safety threshold of 5% for the acceptable rate of SAEs associated with an EBT.[214]

1.3 Knowledge gaps

Metabolic surgery is an evolving concept as we are only just beginning to increase our understanding of the effects of these operations on GI physiology and human metabolism. As has been described within the previous sections, many of the underlying mechanisms and pathways that are modified by these interventions remain uncertain. Only by understanding these pathways can we optimise current therapies and design new, targeted treatments for patients with obesity and obesity-related diseases. An example of this are the observations made of the role of the foregut in improving glucose homeostasis and metabolic health following RYGB that led to the subsequent design of the Endobarrier DJBL.

EBTs, including the Endobarrier and ESG, represent innovative minimally-invasive therapeutic options as potential alternatives to metabolic surgery. However, these treatments remain in their relative infancy (IDEAL stages 2/3) and robust RCT evidence is lacking. As such, both of these interventions require ongoing research in order to: (1) provide robust, high-quality data on patient-centred clinical and safety outcomes, and (2) explore the mechanistic basis behind each of these new therapies.

Of particular interest is the effect of foregut exclusion using the Endobarrier on metabolic health. Despite extensive experimental data on the metabolic changes that occur following RYGB, physiological examination following DJBL implantation remains relatively unexplored in humans. The research described within the following chapters was therefore conducted in order to further examine some of the actions of the Endobarrier DJBL by investigating its effects on glycaemic control, tissue-specific insulin sensitivity, ß-cell function, lipid profile and fatty acid concentrations.

Furthermore, the technique of ESG continues to evolve and herein we describe the first UK experience of using ESG as a primary weight-loss treatment for patients with obesity using a modified suture pattern. I will present relevant clinical and safety outcomes, which will add new data to that which already exists in the literature with the future aim being to conduct the first UK RCT of this technique.

1.4 Aims

The aim of this research is to compare the effect of intensive lifestyle modification with and without the Endobarrier DJBL on weight loss and metabolic health in patients with obesity and T2DM. Furthermore, parallel work will evaluate the first UK experience of using ESG as a primary weight-loss treatment for patients with obesity using a modified suture pattern.

1.5 Hypotheses

The hypotheses being tested in the work that will be described in the following chapters are that:

- duodenal exclusion in patients with obesity and T2DM using the Endobarrier DJBL alongside intensive lifestyle modification:
    - results in significant weight loss that is greater than control patients with obesity and T2DM receiving intensive medical therapy alone during the implantation period of 12 months;
    - will result in significantly greater early improvements in hepatic insulin sensitivity, reduce hepatic glucose output and cause a rapid decline in fasting plasma glucose concentrations that are independent of weight-loss and calorie restriction when compared to control patients with obesity and T2DM receiving intensive medical therapy alone;
    - produces significantly greater weight-loss-dependent improvements in peripheral insulin sensitivity than in control patients treated with intensive medical therapy alone;
    - results in a rapid decline in total-body insulin resistance, an increase in peripheral glucose uptake and utilisation, and an improvement in β-cell function when compared to control participants with obesity and T2DM receiving intensive medical therapy alone;
    - results in significant improvements in blood lipid concentrations when compared to control participants with obesity and T2DM receiving intensive medical therapy alone;
    - depletes blood concentrations of long chain polyunsaturated fatty acids when compared to control participants with obesity and T2DM receiving intensive medical therapy alone.

- ESG using a modified suture pattern as a primary weight-loss treatment for patients with obesity:
    - results in no additional morbidity or increased procedural time when compared to ESGs being performed with standard suture patterns;
    - results in significant weight loss that is greater than that achieved by patients following ESG using standard suture patterns.

1.6 Objectives

The objectives of this research are:

- to present the study design, experimental methodology and recruitment results of: A randomized controlled trial of a duodenal sleeve bypass device (EndoBarrier®) compared with standard medical therapy for the management of obese subjects with type 2 diabetes (Trial registration number ISRCTN30845205, ClinicalTrials.gov Identifier NCT02459561);
- to evaluate the change in weight of participants with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for one year and compare it to the weight changes of control patients with obesity and T2DM receiving intensive medical therapy alone;
- to determine the changes in fasting plasma glucose concentrations and serum insulin concentrations of participants with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for one year and compare these changes to those of control patients with obesity and T2DM receiving intensive medical therapy alone;
- to evaluate the early (10 days) and late (6 months) changes in hepatic and peripheral insulin sensitivity following treatment with the Endobarrier DJBL using the gold standard HEC technique with inclusion of stable isotopes and compare these changes to those of control patients;
- to evaluate the overall changes in insulin sensitivity and ß-cell function in Endobarrier and control patients using model assessment methods in the basal state and in the stimulated state following a mixed meal tolerance test (HOMA-IR, HOMA-%ß, HOMA-%S, Matsuda Index, Insulinogenic Index, Disposition Index);
- to evaluate the changes in fasting blood lipid concentrations (total serum cholesterol, triglycerides, LDL-C and HDL-C) that occur in participants with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for one year and compare these changes to those that occur in control patients with obesity and T2DM receiving intensive medical therapy alone;
- to evaluate the changes in blood concentrations of long chain polyunsaturated fatty acids that occur in participants with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for one year and compare these changes to those of control patients with obesity and T2DM receiving intensive medical therapy alone;

- to compare procedural times and morbidity resulting from ESGs being performed with a modified suture pattern with ESGs being performed with a standard suture pattern;
- to evaluate the change in weight in patients with obesity 6 months following treatment with ESG using a modified suture pattern and compare it to the weight changes of patients with obesity treated with a standard ESG suture pattern.

Chapter 2:

Study design, participants' baseline characteristics and the effect of a duodenal-jejunal bypass liner (Endobarrier®) on body weight

2.1 Introduction

2.1.1 Rationale for present study

Obesity and obesity-related diseases are preventable conditions that represent a significant socioeconomic burden. In 2017, the World Health Organisation (WHO) estimated that 13% of the worlds adult population were obese and this incidence has tripled since 1975. In the UK alone, it has been projected that there will be an additional 11 million obese adults by 2030. Being overweight or obese accounts for 80-85% of the overall risk for developing insulin resistance and type 2 diabetes mellitus (T2DM) and, compared to the general population, patients with obesity and T2DM have significantly higher levels of morbidity and mortality from cardiometabolic disease and disease-related complications.[57,4] In a meta-analysis of 57 prospective studies and 900,000 adults, each increase in BMI > 25 kg/m^2 by 5 kg/m^2 was associated with a 30% increase in overall mortality.[5] This represents a significant socioeconomic burden for a largely preventable condition with combined healthcare costs for obesity and T2DM estimated to increase by up to 2 billion pounds each year in the UK.[6]

Metabolic surgery remains the most effective long-term means of treating these patients by producing often profound and sustained weight loss, as well as weight-loss-independent improvements in metabolic health, which consequently ameliorates, or even eliminates, associated co-morbidities and reduces mortality (**Section 1.2.5.2**). The safety profile of metabolic surgery has markedly improved in recent years with quoted mortality rates of 0.1-0.5% worldwide (0.11% in the UK) but with serious morbidity recorded in up to 6% of patients. Unfortunately, only 1% of eligible, obese patients currently undergo metabolic operations as there continue to be numerous, multifactorial barriers to surgery.[314]

Endoscopic Bariatric Therapies (EBTs) represent another treatment option in the armamentarium against obesity and obesity-related diseases. They provide a minimally-invasive, and potentially cost-effective, therapeutic option to achieve weight-loss and treat obesity-related diseases by going beyond what can be achieved through medical and lifestyle interventions alone whilst limiting the potential morbidity and mortality associated with surgery (**section 1.2.5.3**). Current evidence supports the Endobarrier duodenal-jejunal bypass liner (DJBL) as a safe and effective option for obese patients with diabetes by reducing weight, improving glycaemic control and potentially improving the associated features of metabolic syndrome (**section 1.2.6.2**). This has inherent cost-saving implications by reducing the burden of health and social care in the future. However, robust high-level evidence for the efficacy, safety and cost-effectiveness of DJBLs for implant periods of 1 year is lacking and the precise mechanism of action of the Endobarrier remains largely unknown (**section 1.2.6.2.4**). This therefore serves as the rationale for the herein-

described study, which is a well-powered, multi-centre, randomised controlled trial that has been designed with the intention of further investigating the efficacy, safety and cost-effectiveness of the Endobarrier DJBL as a potential alternative to metabolic surgery and existing medical therapies for the treatment of obesity and T2DM.

In advance of this trial, a multi-centre, non-randomised, pilot study was conducted at three investigational sites in London, Manchester and Southampton.[255] Forty-five patients were implanted with the Endobarrier device and 31 patients (69%) completed the 12 month study period. The mean reported weight loss was 15 kg, which was most rapid during the first 3 months, and BMI reduced significantly by 4.9 kg/m^2. HbA_{1c} concentrations also decreased significantly (-0.8%, $p < 0.05$) with maximal improvements being achieved after 9 months of treatment (-1.2%, $p < 0.00001$). Following device explantation, weight increased by 2.2 ± 5.1 kg and non-significant increases in BMI were observed up to 6 months. HbA_{1c} concentrations remained stable for 6 months after removal of the Endobarrier. During the implant period, 88.9% of patients reported 127 device-related adverse events but with no long-term sequelae and no reported deaths. At that time, this was the largest cohort of patients to be implanted with the Endobarrier for 12 months and effectively demonstrated the efficacy and safety of using this device within the UK. This study did not include a dietary control group but did provide the effect size of benefits to be expected in order for the herein-described RCT to be powered appropriately.

Within this chapter, the methodology for this study is described: A randomized controlled trial of a duodenal sleeve bypass device (EndoBarrier®) compared with standard medical therapy for the management of obese subjects with type 2 diabetes (Trial registration number ISRCTN30845205, ClinicalTrials.gov Identifier NCT02459561). The main outcomes from this study are reported elsewhere (unpublished). The research described within the following chapters was conducted as part of this trial and aims to further examine some of the mechanisms of action of the Endobarrier DJBL by investigating its effects on glycaemic control, tissue-specific insulin sensitivity, ß-cell function, lipid profile and fatty acid concentrations.

2.1.2 Aims

The aim of the present study is to compare the effect of intensive lifestyle modification with and without the Endobarrier DJBL on weight loss in patients with T2DM and obesity.

2.1.3 Hypotheses

The hypothesis being tested in the work that will be described in the following chapter are that duodenal exclusion in patients with obesity and T2DM using the Endobarrier DJBL alongside

intensive lifestyle modification for 12 months results in significant weight loss that is greater than control patients with obesity and T2DM receiving intensive medical therapy alone.

2.1.4 Objectives

The objectives of this chapter are:

- to present the study design, experimental methodology and recruitment results of: A randomized controlled trial of a duodenal sleeve bypass device (EndoBarrier®) compared with standard medical therapy for the management of obese subjects with type 2 diabetes (Trial registration number ISRCTN30845205, ClinicalTrials.gov Identifier NCT02459561);
- to evaluate the change in weight of participants with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for 12 months and compare it to the weight changes of control participants with obesity and T2DM receiving intensive medical therapy alone.

2.2 Methods

2.2.1 Study design

This study is a multicentre, randomised, controlled, non-blinded trial with two treatment arms. Men and women, aged 18–65 years with a BMI 30–50 kg/m^2 and a confirmed diagnosis of T2DM for at least 1 year, who have inadequate glycaemic control and are on oral anti-hyperglycaemic medications, were randomised to receive either the EndoBarrier DJBL for 12 months or conventional medical therapy, diet and exercise. Both treatments arms were followed-up for 2 years. Participants were recruited equally across two investigational sites in the United Kingdom: Imperial College Healthcare NHS Trust in London and University Hospital Southampton NHS Foundation Trust. The study groups and overall schema for the trial are summarised in **Table 2.1,** **Figure 2.1** and **Figure 2.2.**

Treatment Group	Number of participants	Treatment period 1	Follow-up period 2
EndoBarrier Device	80	12 months	12 months
Standard Medical Therapy	80	12 months	12 months
Total number of participants	**160**		

Table 2.1 Summary of treatment groups

In order to investigate the mechanisms of the effect of the EndoBarrier device, patients in both study arms were given the option of choosing to participate in one of three nested sub-groups, which had the following additional assessments during the course of the trial:

Sub-group 1: Functional MRI of food reward and addictive behaviours, eating behaviour assessment and post-meal gut hormones (Imperial College London only).

Sub-group 2: Hyperinsulinaemic euglycaemic clamps (total body and tissue-specific insulin resistance) (University Hospital Southampton only).

Sub-group 3: Assessment of taste and food preference, eating behaviour assessment and post-meal gut hormones.

Participants were therefore randomised to either arm of the main study and then optionally participated in one of these sub-groups. Alternatively, participants could decline participation within these nested studies and participate only in the main study assessments.

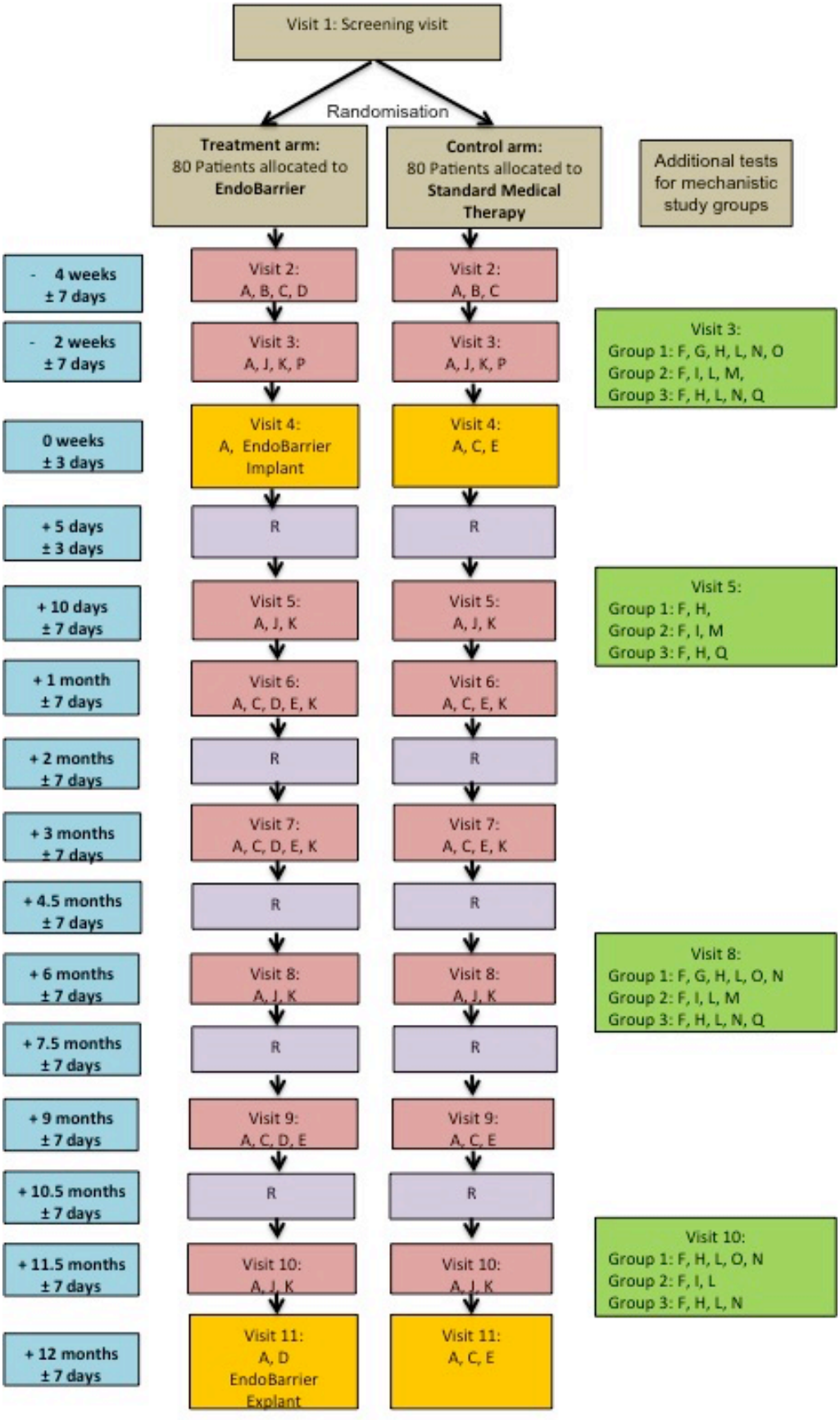
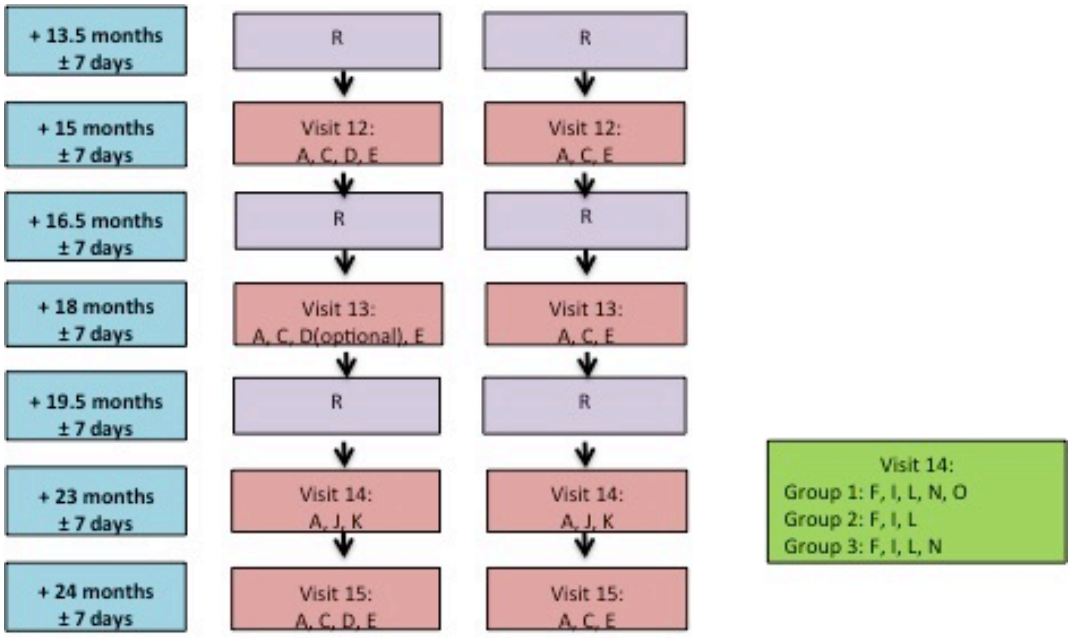


Figure 2.1 Study interventions and follow-up schedule (-4 weeks to +12 months)



Legend:
A = Weight, waist, blood pressure, routine bloods, adverse events, changes in medication/medical history
B = Dietary Counselling
C = Medical Therapy (Diabetologist/Endocrinologist)
D = Gastroenterologist
E = Dietitian follow up
F= Bioelectrical impedance
G= fMRI
H = Gut hormones(fasting and post-meal profile)
I= Gut hormones(fasting only)
J = Metabolomics
K = Health Economics questionnaires
L = Eating and behaviour questionnaires
M = Insulin clamps
N= Eating behaviour computerised tasks
O= Cognitive assessment tasks
P= DNA Sample
Q= Food preference and taste assessment
R= Telephone counselling

Figure 2.2 Study interventions and follow-up schedule (+13.5 months to +24 months)

2.2.2 Primary and secondary objectives of the Endobarrier trial

2.2.2.1 Primary Objective

The primary objective of this randomised controlled trial is to compare the EndoBarrier® with conventional medical therapy, diet and exercise for obesity-related T2DM and their effectiveness on metabolic state as defined by the IDF as a HbA1c reduction of 20% at 12 months.[315] This primary objective is reported elsewhere.

2.2.2.2 Secondary Objectives

The secondary objectives defined within the statistical analysis plan of the trial are:

(1) To compare the EndoBarrier with conventional medical therapy, diet and exercise for obesity-related T2DM and their effectiveness on:
    a. HbA1c < 42 mmol/mol
    b. Blood pressure < 135/85 mmHg
    c. Absolute weight loss > 15%
    d. Reduction in dose/number of medications
(2) To estimate the cost-effectiveness of the EndoBarrier device compared with conventional treatment.
(3) To evaluate the safety of the EndoBarrier® DJBL.

Furthermore, the mechanisms of the effect of the Endobarrier is evaluated within the nested sub-studies through exploratory analyses of changes in: gut hormone and bile acid concentrations; the microbiome; taste, appetite, food hedonics and brain reward systems; body fat content, blood lipid and fatty acid profile; food preference; hepatic and peripheral insulin sensitivity; and gene expression.

The majority of the secondary objectives and exploratory analyses are reported elsewhere. The outcomes that are reported within the following chapters of this thesis are: absolute weight loss; changes in hepatic and peripheral insulin sensitivity; and changes in blood lipid profile and fatty acid concentrations.

2.2.3 Inclusion and exclusion criteria

Inclusion criteria

1. Age 18–65 years (men and women)
2. Type 2 diabetes mellitus for at least one year
3. HbA_{1C} 58 – 97 mmol/mol
4. Using oral hypoglycaemic medications
5. BMI 30 – 50 kg/m^2

Exclusion criteria

1. Language barrier, mental incapacity, unwillingness or inability to understand and be able to complete questionnaires.
2. Non-compliance with eligibility criteria.
3. Women of childbearing potential who are pregnant, breast-feeding or intend to become pregnant or are not using adequate or reliable contraceptive methods.
4. Evidence of absolute insulin deficiency as indicated by clinical assessment, a long duration of T2DM and a fasting plasma C-peptide of <333 pmol/L.
5. Current use of insulin.
6. Previous diagnosis with type 1 DM or a history of ketoacidosis.
7. Requirement of NSAIDs (non-steroidal anti-inflammatory drugs) or prescription of anticoagulation therapy during the implant period.
8. Current iron deficiency and/or iron deficiency anaemia.
9. Symptomatic gallstones or kidney stones at the time of screening.
10. History of coagulopathy, upper gastro-intestinal bleeding conditions such as oesophageal or gastric varices, congenital or acquired intestinal telangiectasia.
11. Previous gastrointestinal surgery that could affect the ability to place the device or the function of the implant.
12. History or presence of active Helicobacter pylori (if individuals are randomised into the EndoBarrier arm and test positive for Helicobacter pylori at study visit 2 then they can receive appropriate eradication treatment and subsequently enrol into the study).
13. Family history of a known diagnosis or pre-existing symptoms of systemic lupus erythematosus, scleroderma or other autoimmune connective tissue disorder.
14. Severe liver impairment (i.e. AST, ALT or gGT >4 times upper limit of the reference range) or kidney impairment (i.e. estimated Glomerular Filtration Rate (GFR) < 45 ml/min/1.73 m^2).

15. Severe depression, unstable emotional or psychological characteristics (including Beck Depression Inventory II score >28).
16. Poor dentition and inability to adequately chew food.
17. Planned holidays up to three months following the EndoBarrier Implant.

2.2.4 Recruitment

Participants were identified from several areas across primary, secondary and tertiary healthcare and community settings:

1. Diabetes research registers (e.g. Diabetes Alliance for Research in England (DARE), REC 2002/7/118);
2. Hospital or General Practice (GP) patient databases (Participant Identification Centres);
3. Patients referred to diabetes and bariatric specialist clinics;
4. Other research studies within the Imperial College Healthcare NHS Trust and the Local Clinical Research Network (LCRN);
5. Study websites;
6. Local and national media – websites, radio, newspaper articles and adverts;
7. Posters;
8. Diabetes, obesity and other support groups;
9. Social media websites.

Potential patients, after reading a summary Patient Information Sheet (PIS) (See **A.1**), who would like to enter the trial gave their verbal consent for preliminary telephone screening to check basic inclusion and exclusion criteria. Written consent was then taken from the patient to allow the study team to contact their GP for the purpose of obtaining additional information on the patient's medical history, current medical therapies and to identify any other clinical reasons as to why the patient should not participate. Patients who appeared to meet eligibility criteria were provided with a full trial PIS and then invited to a formal screening visit at one of the study centres. At this stage, the participants were fully informed of the nature of the study and given relevant information about the objectives of the research, benefits and possible adverse events, verbally and in writing. The patient had the opportunity to ask questions about the trial and formal written consent was taken for the patient to participate in the main study (see **A.2**) ± additional consent for one of the three optional mechanistic sub-groups (see **A.3**). Once consent was obtained then the subject's full eligibility was checked against all inclusion and exclusion criteria (**Section 2.2.3**). Each patient was informed of their eligibility for the trial once all results were available (usually within one week from obtaining consent).

2.2.5 Randomisation

Eligible patients were randomised into one of the two trial arms using the InForm Integrated Trial Management system, a secure web-based data entry platform. This was programmed with a randomisation schedule by an independent statistician and was protected against bias in the randomisation process as group allocation was concealed and automatic. The randomisation was at a ratio of 1:1 and stratified by site and two BMI groups, 30–40 and 40–50 kg/m^2. Each patient was informed of their randomisation allocation and assigned a unique study identification number. Only the subject number and initials were recorded in the case report form (CRF) and all other patient-identifiable data were completely anonymised.

2.2.6 Trial interventions

2.2.6.1 EndoBarrier® Gastrointestinal Liner

The EndoBarrier Gastrointestinal liner device received CE Mark for 12 months implant duration on 11th December 2009 and is a single use, minimally invasive device, used to achieve weight loss and improve T2DM status in patients who are obese. The concept and design of the Endobarrier DJBL is fully described in **Section 1.2.6**.

At visit 2 (-4 weeks), participants who had been randomised to receive the EndoBarrier device were tested for the presence of *Helicobacter pylori*, either by faecal antigen or urea breath testing. Those patients testing positive were offered 1 week of triple-eradication therapy, as per guidance published within the British National Formulary, and then retested after a further 4 weeks to confirm complete eradication before continuing with implantation of the EndoBarrier device. Subsequently, all patients were prescribed a proton pump inhibitor (PPI) (Omeprazole 40 mg twice daily) and instructed to commence this three days prior to the implant procedure. This was continued for the duration of the implant period (12 months) and for a further two weeks following device removal.

At visit 4 (0 weeks), after an eight-hour fast, participants had the EndoBarrier device implanted under a general anaesthetic with endotracheal intubation and in the left lateral decubitus position. Implantations were performed by a single medical endoscopist at Imperial College London and a single surgical endoscopist at University Hospital Southampton. Both are experienced interventional endoscopists, proficient in general interventional techniques (endoscopic mucosal resection, submucosal dissection, endoscopic retrograde cholangiopancreatography (ERCP), and other EBTs (e.g. Primary Obesity Surgery Endoluminal (POSE)). Initial oesophagogastroduodenoscopy (OGD) was performed with a standard gastroscope

to evaluate the anatomy and inspect for any pathology or contraindications to implantation. Under direct vision, a hydrophilic guidewire was passed into the duodenum and, with fluoroscopic guidance, into the proximal jejunum. The encapsulated device was then delivered endoscopically over the guidewire on a custom catheter (**Figure 2.3**) and positioned just distal to the pylorus. Next, the 60cm fluoropolymer sleeve was unfurled by advancing an atraumatic ball from the tip of the catheter along the course of the intestine, deploying the liner behind itself. With the sleeve fully deployed and the ball detached, the nitinol anchor was positioned in the first part of the duodenum, approximately 0.5 cm distal to the pylorus (**Figure 2.4** and **Figure 2.5)**. The anchor is deployed collapsed but self-expands to a diameter of 25-50 mm and 10 barbs engage with the muscularis of the duodenal bulb wall in order to prevent migration. Final positioning plus patency of the DJBL was confirmed by assessing for the free flow of radio-opaque contrast and air boluses through the device. Videos and photos of the fluoroscopy images were recorded to help the investigators make treatment decisions. Participants were discharged from hospital the same day with an implant information card, which described the implant, identified who to call in the case of an emergency, and what symptoms to look for following the implant. If relevant, individuals would have their dose of sulphonylurea medication reduced by 50% at the time of EndoBarrier implantation in order to avoid potential hypoglycaemic episodes.

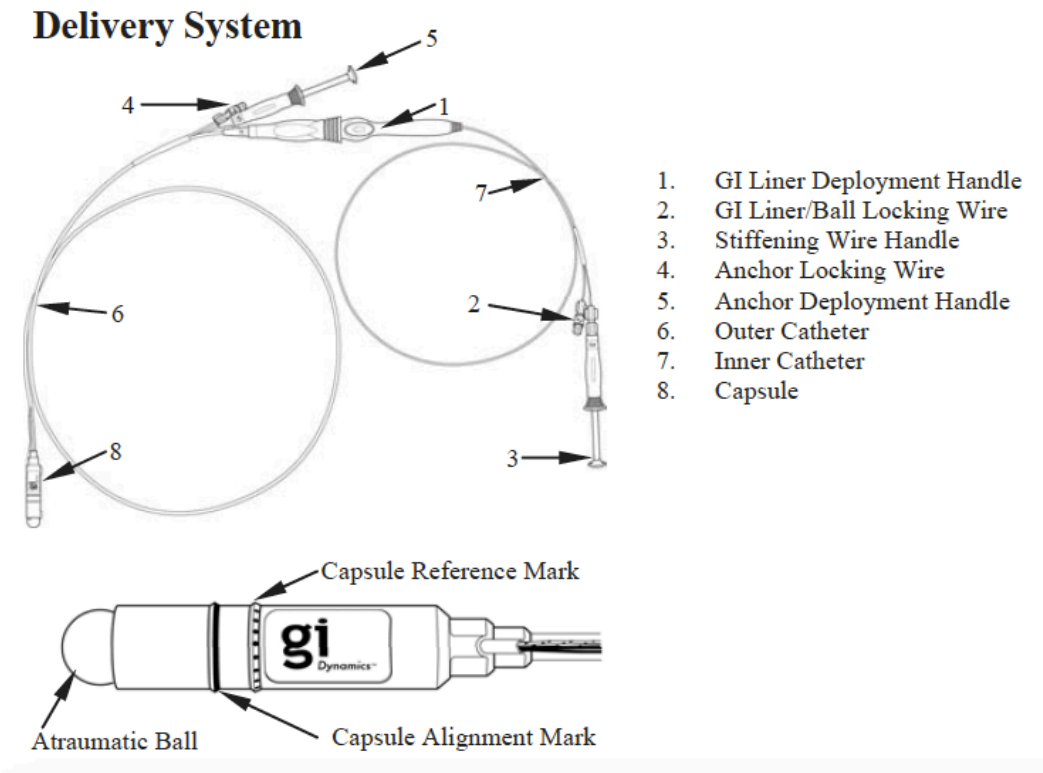


Figure 2.3 Endobarrier® endoscopic delivery system

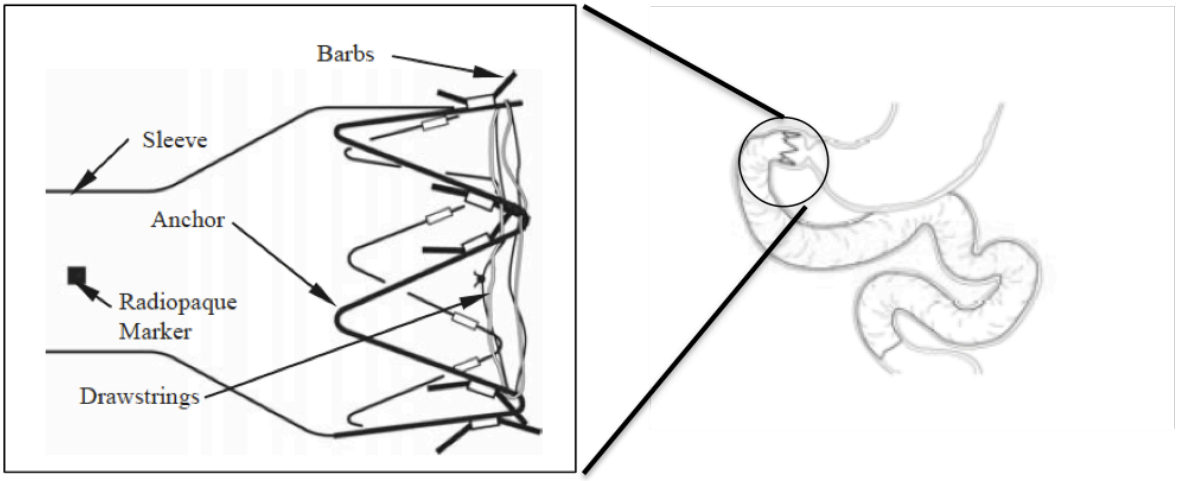


Figure 2.4 Endobarrier® anchor system within proximal duodenum

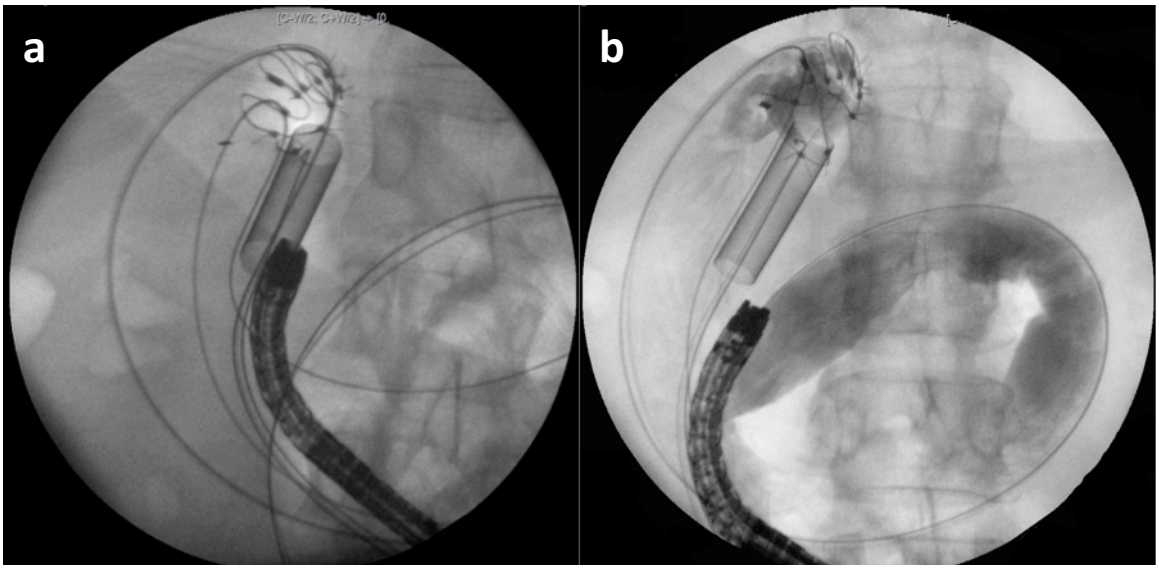


Figure 2.5 Fluoroscopic images of Endobarrier deployment.
(a) Fluoroscopic image of device and anchor system being deployed within the duodenal bulb and (b) confirming patency and position of the sleeve using gastrograffin contrast.

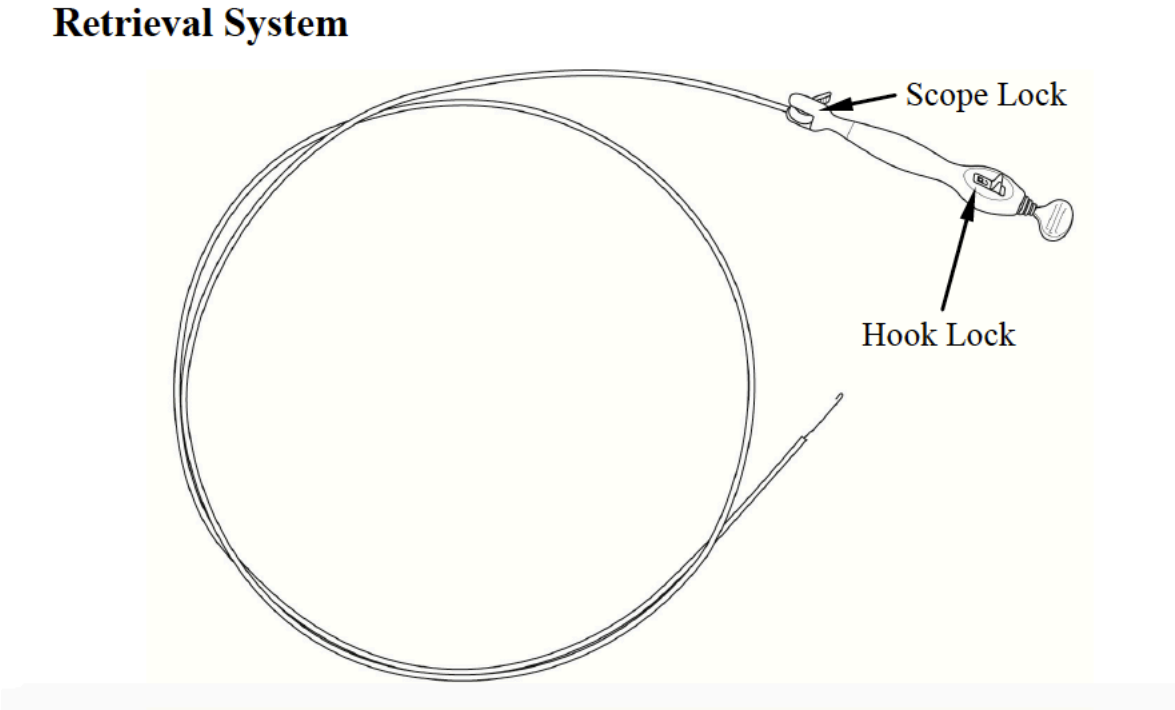


Figure 2.6 Endobarrier® endoscopic retrieval system

The device was removed at visit 11 (after 12 months) under sedation or general anaesthetic. The gastroscope, which was fitted with a foreign body retrieval hood, was used to locate the implant and a custom grasper (**Figure 2.6**) was passed through the working channel of the gastroscope to grab a polypropylene drawstring located on the proximal portion of the anchor. Pulling on this tether collapsed the proximal end of the anchor, which could then be pulled into the foreign body hood in order to incorporate the anchor and its barbs and avoid damage to the upper GI tract during withdrawal. Complete collapse of the anchor and containment within the foreign body hood was confirmed on x-ray before then removing the device by withdrawing the gastroscope through the subject's mouth. Following removal of the EndoBarrier® device, patients were followed up for a further 12 months.

2.2.6.2 Diabetes review

Participants in both arms of the trial had their T2DM managed in accordance with the guidelines of the American Diabetes Association (ADA).[98,316] These guidelines were chosen as they would adhere to the current best worldwide practice that would still be relevant when the results are published following study completion. Both treatment groups had a review of their T2DM by a suitably trained physician at visits 2, 6, 7, 9, 12, 13 and 15. Additionally, the standard care arm of the trial had an additional review at visits 4 and 11 in place of the EndoBarrier implant and

removal. Adjustments to a patient's oral anti-hyperglycaemic medications and escalation of therapy was at the investigators discretion and complied with general recommendations laid out by the ADA.[98,316]

2.2.6.3 Dietary counselling and physical activity

At visit 2, all patients historical and current eating behaviours were assessed by a qualified dietitian using the following information: anthropometry; biochemistry; co-morbidities; activity levels; eating habits including previous diets; lifestyle including smoking, drug and alcohol misuse; weight history; psychiatric history; family history of obesity, diabetes, mental illness or eating disorders; available support network; work status; readiness and motivation for change. Patients would then receive dietary and physical activity counseling in accordance with local standards with the intention of providing each participant with lifestyle/behavioural modification information and good eating practices. In addition, participants in the EndoBarrier arm would receive written information on how their diet would change after implantation of the device and they would receive specialist guidance for eating with their EndoBarrier.

All patients were reviewed by a specialist dietitian at visits 2, 6, 7, 9, 12, 13 and 15. In addition, participants in the standard care arm of the trial had an additional review at visits 4 and 11 in place of the EndoBarrier implant and removal. During the course of the trial, participants were recommended to consume 600 kcal less every day, depending on their age, sex, activity levels and body weight. Guidelines for daily amounts were between 1200 and 1500 kcal for women and between 1500 and 1800 kcal for men. In accordance with standard dietary practice, participants would be advised: to eat regularly every day (5 times per day); to control their portion sizes and intake of carbohydrates/starchy foods; to increase their intake of low glycaemic index and high protein foods, as well as vegetables; and to reduce their intake of foods high in fat and sugar, and alcohol. Participants were advised to include more physical activity in their daily routine and encouraged to do more activity in their leisure time. Their goal was to include 150 minutes a week of moderate intensity, and 75 minutes a week of vigorous intensity aerobic activity and muscle strengthening activities, on more than 2 days a week. Changes in physical activity level were monitored using the International Physical Activity Questionnaire (IPAQ).[317] In addition to routine follow-up visits, all patients received regular telephone counseling from a specialist dietitian to assess their wellbeing and motivation during the trial.

2.2.6.4 Liquid diet

To avoid disruption of the device in the immediate period following implantation, patients were prescribed a liquid diet for the 7 days before and 13 days (± 3 days) after the intervention visit

(visit 4). The liquid diet was guided by the specialist dietitian and comprised of 125 ml Fortisip Compact drinks (Nutricia, UK): 5 per day for men, 4 per day for women, containing per 100 mL: 240 kcal, 9.6g protein (16% total energy), 29.7 g carbohydrate (49%), 15 g sugars, 9.3 g fat (35%). Patients were also allowed to consume sugar-free squashes, smooth/clear soup (1 medium bowl per day), tea or coffee without sugar, or unsweetened puree. To standardise both therapy groups, all patients across both arms followed the liquid diet for this duration and period of the study.

2.2.7 Outcome measurements

Table 2.2 summarises the visit schedule, the data that were collected across both study arms and supplementary data that were collected from the three optional mechanistic sub-groups. Hereafter, I provide only the details of the experimental methodologies pertaining to the outcomes described in the following research chapters.

Table 2.2 Summary of study visit schedule

	Screening	Baseline	Treatment														Follow-up				
Activities	**V1**	**V2**	**V3**	**V4**	**T1**	**V5**	**V6**	**T2**	**V7**	**T3**	**V8**	**T4**	**V9**	**T5**	**V10**	**V11**	**T6**	**V12**	**T7**	**V13**	**T8**	**V14**	**V15**
Week/ Month/Day	-4w ±7d	-2w ±7d	-0w ±3d	+ 5d± 3d	+ 10d ±3	+ 1m ±7d	+ 2m ±7d	+ 3m ±7d	+ 4.5m ±7d	+ 6m ±7d	+ 7.5m ±7d	+ 9m ±7d	+ 10.5m ±7d	+ 11.5m ±7d	+ 12m ±7d	+ 13.5m ±7d	+ 15m ±7d	+ 16.5m ±7d	+ 18m ±7d	+ 19.5m ±7d	+ 23m ±7d	+ 24m ±7d
Informed consent (5.1)	X																						
Inclusion & exclusion criteria (4.2, 4.3)	X																						
Demographics (5.1)	X																						
Medical history (including meds) (5.1)	X																						
Physical examination (5.5.4)	X																						
ECG (5.5.5)	X																						
Vital signs (5.5.7)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body weight (5.5.8)	X	X	X	X		X	X		X		X		X		X	X		X	X	X	X	X	X
Height (5.5.8)	X																						
Waist circumference (5.5.9)	X	X	X	X		X	X		X		X		X		X	X		X	X	X	X	X	X
Routine blood tests (5.5.10)	X		X			X	X		X		X		X		X	X		X	X	X	X	X	X
Urine dipstick and female pregnancy test (5.5.6)	X																						
Changes in medical history/medication (5.5.4)		X	X	X		X	X		X		X		X		X	X		X	X	X	X	X	X
Randomisation (5.2)		X																					
Health Economic Questionnaires (5.5.12)			X	C		X	X		X		X				X							X	
Dietary counselling (5.5.3)		X		C																			
Dietitian follow up (5.5.3)						X	X		X				X		X	X		X		X			X
Urine albumin:creatinine ratio (5.5.11)			X			X					X				X							X	
Reporting of AEs (6)		X	X	X		X	X		X		X		X		X	X		X		X		X	X
DNA & RNA sampling (5.5.13)			X			X				X						X							X
Telephone counselling (5.5.3)					X			X		X		X		X			X		X		X		
Diabetologist Review (5.5.1 and 5.5.2)				C			X		X			X	X			C							X

	Screening	Baseline		Treatment													Follow-up					
Activities	**V1**	**V2**	**V3**	**V4**	**T1**	**V5**	**V6**	**T2**	**V7**	**T3**	**V8**	**T4**	**V9**	**T5**	**V10**	**V11**	**T6**	**V12**	**T7**	**V13**	**T8**	**V14**	**V15**
Week/ Month/Day		- 4w ±7d	- 2w ±7d	- 0w ±3d	+ 5d± 3d	+ 10d ±3	+ 1m ±7d	+ 2m ±7d	+ 3m ±7d	+ 4.5m ±7d	+ 6m ±7d	+ 7.5m ±7d	+ 9m ±7d	+ 10.5m ±7d	+ 11.5m ±7d	+ 12m ±7d	+ 13.5m ±7d	+ 15m ±7d	+ 16.5m ±7d	+ 18m ±7d	+ 19.5m ±7d	+ 23m ±7d	+ 24m ±7d
Metabolomics (5.5.14)			X			X					X				X							X	
Bioelectrical Impedance (5.5.15)			X			X					X				X							X	

EndoBarrier Group Only

PPI and H. Pylori test (5.5.16)		X																					
Distribution of Proton Pump Inhibitors (5.5.2)		T																					
EndoBarrier Implant (5.5.2)				T																			
Preparation for EndoBarrier removal (5.5.2)													T										
EndoBarrier removal (5.5.2)																T							
Biopsies during Implant and Explant (5.5.2)				T												T							
Gastroenterologist appointment (5.5.2)		T					T		T				T			T		T		T*			**T**

Sub-groups

Fixed/test meal and post-meal gut hormones and metabolites (Group 1 and 3) (5.6.4)			X			X					X				X								
Gut hormones and metabolites (Fasting only) (Group 1-3) (5.6.4)			X			X					X				X							X	
Food diaries (Groups 1-3) (5.6.2)			X			X					X				X							X	
Eating & Behaviour Questionnaires (Groups 1-3) (5.6.1)			X								X				X							X	
Appetite Visual Analogues Scales (Group 1-3) (5.6.3)			X			X					X				X							X	
Eating behaviour computerised tasks (Group 1 and 3) (5.6.5)			X								X				X							X	
Metal Check Form (Group 1) (5.1)	X																						
Handedness Questionnaire (Group 1) (5.1)	X																						
Additional pregnancy tests			F								F												

	Screening	Baseline		Treatment													Follow-up					
Activities	**V1**	**V2**	**V3**	**V4**	**T1**	**V5**	**V6**	**T2**	**V7**	**T3**	**V8**	**T4**	**V9**	**T5**	**V10**	**V11**	**T6**	**V12**	**T7**	**V13**	**T8**	**V14**	**V15**
Week/ Month/Day		- 4w ±7d	- 2w ±7d	- 0w ±3d	+ 5d± 3d	+ 10d ±3	+ 1m ±7d	+ 2m ±7d	+ 3m ±7d	+ 4.5m ±7d	+ 6m ±7d	+ 7.5m ±7d	+ 9m ±7d	+ 10.5m ±7d	+ 11.5m ±7d	+ 12m ±7d	+ 13.5m ±7d	+ 15m ±7d	+ 16.5m ±7d	+ 18m ±7d	+ 19.5m ±7d	+ 23m ±7d	+ 24m ±7d
DS-R disgust questionnaire (Group 1) (5.6.6)			X																				
Functional MRI (Group 1) (5.6.9)			X								X												
Insulin Clamps (Groups 2) (5.6.10)			X			X					X												
Cognitive assessment tasks (Group 1) (5.6.9)			X								X				X							X	
Food Preference / Taste Assessment(Group 3) (5.6.11)			X			X					X												
24hr Dietary Recall (Group 3) (5.6.11)			X			X					X				X							X	

X performed in all patients unless otherwise stated

F performed in Females only

C performed in Control arm (Standard medical therapy) only

T performed in Treatment arm (EndoBarrier) only

* optional (at request of the patient)

2.2.7.1 Anthropometry

2.2.7.1.1 Height and weight

Participant height was measured in centimetres, to the nearest mm, using a Marsden Leicester Height Measure. The participant was asked to remove their shoes and socks, stand upright against the measure with their head in a neutral position and to hold their breath in inspiration. Whilst the participant maintained this position, an adjustable headboard was positioned on the vertex, compressing their hair, and a height measurement was taken. This measurement was repeated three times and the average height recorded in the CRF.

Each participant was weighed barefoot and in light clothing using a Seca 861 electronic weighing scale for visits 1, 2, 4, 6, 7, 9, 11, 12, 13, 15 or using the Seca mBCA 515 (medical body composition analyser) for visits 3, 5, 8, 10, 14. Measurements were recorded in kilograms (kg) to the nearest 100 grams. Total Body Weight Loss (TBWL, kg), BMI (= weight (kg)/(height (m))^2), %TBWL (Total Body Weight Loss (%) = (TBWL (kg)/baseline weight (kg)) x 100), and %EWL (Excess Weight Loss (%) = (TBWL (kg)/(baseline weight (kg) – ideal body weight (kg)) x 100) were then calculated.

2.2.7.1.2 Waist circumference

The participant's waist circumference was measured using a non-stretchable tape-measure midway between the uppermost border of the iliac crest and the lower border of the costal margin. The tape was placed at this midway point and a reading taken when the tape was snug but did not compress the skin. This measurement was repeated three times and the average waist circumference was recorded, in cm to the nearest mm, in the CRF.

2.2.7.1.3 Body composition measurement

At visits 3, 5, 8, 10 and 14, all participants had their body composition measured by bioelectrical impedance using a Seca mBCA 515 (medical body composition analyser). This technique was selected as a means of rapidly and accurately measuring weight (kg), BMI (kg/m^2), fat mass (kg/%), and fat-free mass (kg/%) that was standardised across both study sites. This technique avoided the inter-rater variability and error rate associated with skinfold anthropometry and also negated the additional time and costs associated with dual energy x ray absorptiometry, densitometry (air displacement plethysmography), hydrometry, MRI assessment and other multicomponent models. The seca mBCA is validated against whole-body MRI (r^2 = 0.97), the 4-compartment model (r^2 = 0.98), sodium bromide (r^2 = 0.95) and deuterium dilution (r^2 = 0.98) methods, for different ethnicities, and also for obese individuals (> 30 kg/m²).[318,319]

During measurement, the participant was asked to remove their shoes, socks, heavy clothing and any exposed metal (e.g. jewellery) before then standing barefoot on the metal foot plates of the analyser with their heels on the posterior electrodes and their forefoot on the anterior electrodes. They were then asked to lightly grip the appropriate hand plate electrodes. The patient demographics, height and waist circumference were manually entered by the operator and the participant was asked to stand still and not talk whilst the bioelectrical impedance measurements were performed. Weight (kg), BMI (kg/m^2), fat mass (kg/%), and fat-free mass (kg/%) were recorded in the CRF.

2.2.7.2 Blood pressure measurement

Pulse rate and blood pressure were recorded at all visits in a sitting position and after the participant had rested for five minutes. Systolic and diastolic blood pressure were measured using an automated blood pressure device (ProBP 2400, Welch Allyn, Netherlands) and the average of three blood pressure measurements were recorded in the CRF in units of mmHg.

2.2.7.3 Blood sample collection and handling

An 18-20G cannula (B-D Insyte-W, Becton Dickinson, UK) was inserted into a large vein in the antecubital fossa in order to obtain baseline blood samples and perform regular blood sampling thereafter using the BD Vacutainer® system. The following steps were taken to standardise sampling across both study sites and reduce pre-analytical errors:

- sample collection, handling and processing were performed as per a standard operating procedure and staff were provided with training prior to commencement of the trial;
- samples were labelled at the bedside using a standardised, digital labelling system;
- where appropriate, study samples were placed on ice following collection and then centrifuged immediately at 4^{o}C for 10 minutes at 4,000 rpm;
- aliquots were labelled using a standardised, digital labelling system and then stabilised by deep-freezing at - 80 °C as soon as possible after collection;
- automated freezer sample logs were maintained at both study sites;
- routine haematology and chemistry samples were collected as per local protocols and processed by the local hospital laboratory using standard methods for routine tests (see below);
- all other research samples were transported on dry ice for central storage at Imperial College London;
- research samples for the main study and sub-studies were handled and analysed within the same laboratory.

2.2.7.4 Sample analysis

2.2.7.4.1 Measurement of plasma glucose, HbA_{1C} and serum insulin concentrations

Whole blood samples were collected into fluoride oxalate, K2 EDTA and plastic serum BD Vacutainer® Tubes and transferred immediately to the central chemical pathology laboratories at Southampton General Hospital and Imperial College London for the measurement of plasma glucose, HbA_{1C} and serum insulin concentrations, respectively. Plasma glucose concentrations were determined using an automated glucose analyser (AU600, Olympic Diagnostics, Southall, UK) with units being expressed as mmol/L. Serum insulin concentrations were measured using an automated enzyme-linked immunosorbent assay (ES700: Roche Diagnostics, Lewes, UK) with units being expressed as mU/L. HbA_{1C} was measured using capillary electrophoresis on a Sebia Flex 2 analyser (Sebia, Paris, France) with units being expressed as mmol/mol.

2.2.7.4.2 Measurement of serum lipid profile

Whole blood samples were collected into BD Vacutainer® SST Tubes and transferred immediately to the central chemical pathology laboratories at Southampton General Hospital and Imperial College London for the measurement of serum plasma lipid profile. Triglyceride, total serum cholesterol and HDL-C concentrations were measured and recorded using an automated lipid profile analyser (AU5800: Beckman Coulter, High Wycombe, UK). LDL-C concentrations were then extrapolated from these measurements. Units are expressed as mmol/L.

2.2.8 Sample size calculation

In June 2011, the International Diabetes Federation produced new guidelines for the conduct of studies in diabetes using metabolic surgery or devices with the aim of standardising outcomes and allowing comparisons between studies.[315] In line with this guidance, a 20% reduction in HbA_{1c} was chosen as the primary outcome measure.

The Steno study represents the best quality randomised study into the effect of best medical therapy published to date (80 patients in each arm).[132] It demonstrated that over an average of 7.8 years, there were significant reductions in HbA_{1c} amongst participants receiving intensive medical therapy from 8.4 +/- 1.6% to 7.7 +/- 1.2%, but with no change in HbA_{1c} amongst those participants continuing with standard medical therapy. This study defines the very best that could realistically be achieved in the control arm of our trial and it was estimated that 15% of patients were likely to reach the primary endpoint. This was a conservative figure and was likely to be an overestimate. Company data on the small number of patients who had reached a year with the Endobarrier device in place suggested that 40% would achieve this target.

According to the previously reported pilot study,[255] up to 30% of patients in the treatment group may have the device removed early. If these patients remain available for follow-up, in the worst-case scenario, the proportion reaching the primary endpoint is the same as in the control group, bringing the proportion estimate of the treatment group to 32.5%. However, most patients in keeping the device for some time would obtain some benefit. Based on this, it was plausible to assume that the estimate is higher than 32.5%. Therefore, within the 30% of patients who may have the device removed, we estimated that 1/3 would achieve the same effect as the control group (15% reaching the primary endpoint); 1/3 would achieve a marginally increased effect (23% reach the primary endpoint) and the final 1/3 would achieve a greater increase in effect (31% reach the primary endpoint). Overall, this provided a proportional estimate of 35% for the treatment group.

The treatment effect in achieving the primary endpoint of a 20% reduction in HbA1c at 12 months for the treatment arm vs. standard therapy arm was therefore diluted from 40% vs. 15% to 35% vs. 15%. 73 patients per group would give 80% power to detect a significant effect. Adding 10% for loss of follow-up, 2 arms of 80 patients would be sufficient to ensure potential demonstration of a significant effect and very conservatively allowed for; explant rates of up to 30%, a higher level of benefit in the control arm than predicted, and drop-out rates of 10%. Furthermore, the landmark Steno study which had, in all likelihood, a less effective intervention arm was sufficiently powered with 80 patients in each arm. To account for patients dropping out in the lead-in period from randomisation to intervention it was considered appropriate to randomise additional patients at each study site.

2.2.9 Statistical analysis

Data were analysed using SPSS® 25.0 software (SPSS, Chicago, IL, USA). For the main trial clinical outcomes, all statistical output was performed according to the intention to treat principle. Unless specified, any randomised patient was considered part of the analysis population. Patients lost to follow-up were subsequently approached at 12 and 24 months to provide data. Any data collected were included within the ITT analysis.

For baseline data of mechanistic groups, normally distributed variables are reported as means with standard deviation (SD) and for comparison of these variables the independent t-test was used. Non-normally distributed variables are reported as medians with the full range, and the Mann-Whitney U test was performed to compare such variables. Categorical variables were compared using the Chi-square test or the Fisher's exact test as appropriate. A p-value <0.05 was considered statistically significant.

For weight loss outcomes, normally distributed variables are reported as means with standard deviation (SD). Within group analysis was performed using the paired t-test (proc t-test). Between group analysis was performed using a mixed model approach with treatment, site, BMI subgroup, age and baseline weight as fixed effect with a random intercept assigned for each subject. No corrections were made for multiple testing.

Mixed model approach:

$$Yijk = \mu + \pi j + \tau i + CV1 + \ldots + CVr + (\pi j * \tau i) + bj(k) + eijk$$

In this model:

- *μ is the intercept of the model;*
- *τi is the ith fixed treatment, i = 0 (standard therapy) or = 1 (EndoBarrier®);*
- *πj is the fixed visit effect at j months where j = 1,...,12 ;*
- *CV1 +…+ CVr is the fixed effect of Covariates* 1 to r*
- *bj(k) is the random visit effect at the jth visit month of the kth subject;*
- *eijk is the random error associated with the kth subject receiving treatment i at visit j;*
- *bj(k) and eijk are independent for i = 2,1, j =1,...,5 , and k =1,..., n .*

**Covariates will include: Age, sex*

Where repeated measure variables are nested (for example, where multiple blood samples are taken per visit), an additional fixed effect (and subsequent interaction terms) were included to account for the nested variable. Due to the small size of the sub-groups, an additional random effect was not included in order to keep the nested model as simple as possible. An unstructured covariate structure was used. In the event where convergence was not met, an alternative structure was used as appropriate. Analysis is presented in the form of test results of fixed effects and estimates of model parameters. Post-hoc tests were performed on any model parameters with a p-value of < 0.05.

2.2.10 Trial management

The UK Clinical Research Collaboration-registered Imperial Clinical Trials Unit (ICTU) was responsible for trial management, quality assurance, trial statistics, development and

maintenance of the trial database, and data management. The ICTU core staff and the InForm team were supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London.

2.2.11 Trial sponsor

The sponsor of the trial was Imperial College London. Imperial College London signed a clinical trial agreement with each of the participating centres prior to the start of the trial.

2.2.12 Ethical considerations

The trial was conducted in accordance with the Declaration of Helsinki on research involving human subjects. The study protocol, patient information sheet (PIS) and informed consent form were submitted to Fulham Research Ethics Committee (REC) prior to the start of the study and a favourable opinion was obtained on 10 July 2014 (REC #14/LO/0871).

2.2.13 Research governance

The trial was carried out in accordance with the NHS Research Governance Framework and local NHS permission was granted by the research and development departments at each participating site prior to commencing recruitment.

2.2.14 Regulatory requirements

There was no need to obtain prior regulatory approval from the MHRA for this trial as the trial devices were CE marked and used within their intended purpose.

2.2.15 Trial registration

The trial was registered on the International Standard Randomised Controlled Trial Number (ISRCTN) clinical trial database with reference ISRCTN30845205.

2.2.16 Funding

This project was funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and National Institute for Health Research (NIHR) partnership. This trial presents independent research funded by the EME Programme and supported by the NIHR CRF and BRC at Imperial College Healthcare NHS Trust and University Hospital Southampton NHS Foundation Trust. The views expressed are those of the author(s) and not necessarily those of the EME Programme, the

NHS, the NIHR or the Department of Health. This study was executed with the support of GI Dynamics and with kind support of Nutricia Advanced Medical Nutrition for providing oral nutritional supplements.

2.3 Results

2.3.1 Recruitment outcomes

Between March 2015 and October 2016, 170 participants were recruited into the trial; 85 patients were randomised into the control arm and 85 were randomised into the Endobarrier arm. **Table 2.3** summarises the randomisation outcomes by site and also by optional subgroup. Nine patients withdrew from the trial in the Endobarrier group prior to device implantation and 11 patients withdrew from the control arm prior to the baseline visit. Endobarrier devices were successfully implanted in 75 of the 76 participants, with one device being unable to be implanted at the Imperial College site due to a short duodenal bulb precluding anchor placement. The mean time to complete implantation was 41 minutes. Twenty patients required early explantation of the Endobarrier device, 54 patients had the device successfully removed at 1 year follow-up (12 months $\pm$ 7 days) and 1 patient had the device removed late (13 months). The flow of patients through the trial and attrition rates is summarised in the consort diagram and flow diagram in **Figure 2.7** and **Figure 2.8**.

	Control					Treatment				
Site	Main Only	fMRI	Insulin Clamp	Food Pref.	**Total**	Main Only	fMRI	Insulin Clamp	Food Pref.	**Total**
Imperial College London	5	20	0	18	**43**	5	17	0	20	**42**
University Trust Southampton	8	0	24	10	**42**	10	0	20	13	**43**
All Sites	13	20	24	28	**85**	15	17	20	33	**85**

Table 2.3 Participant randomisation by site and subgroup

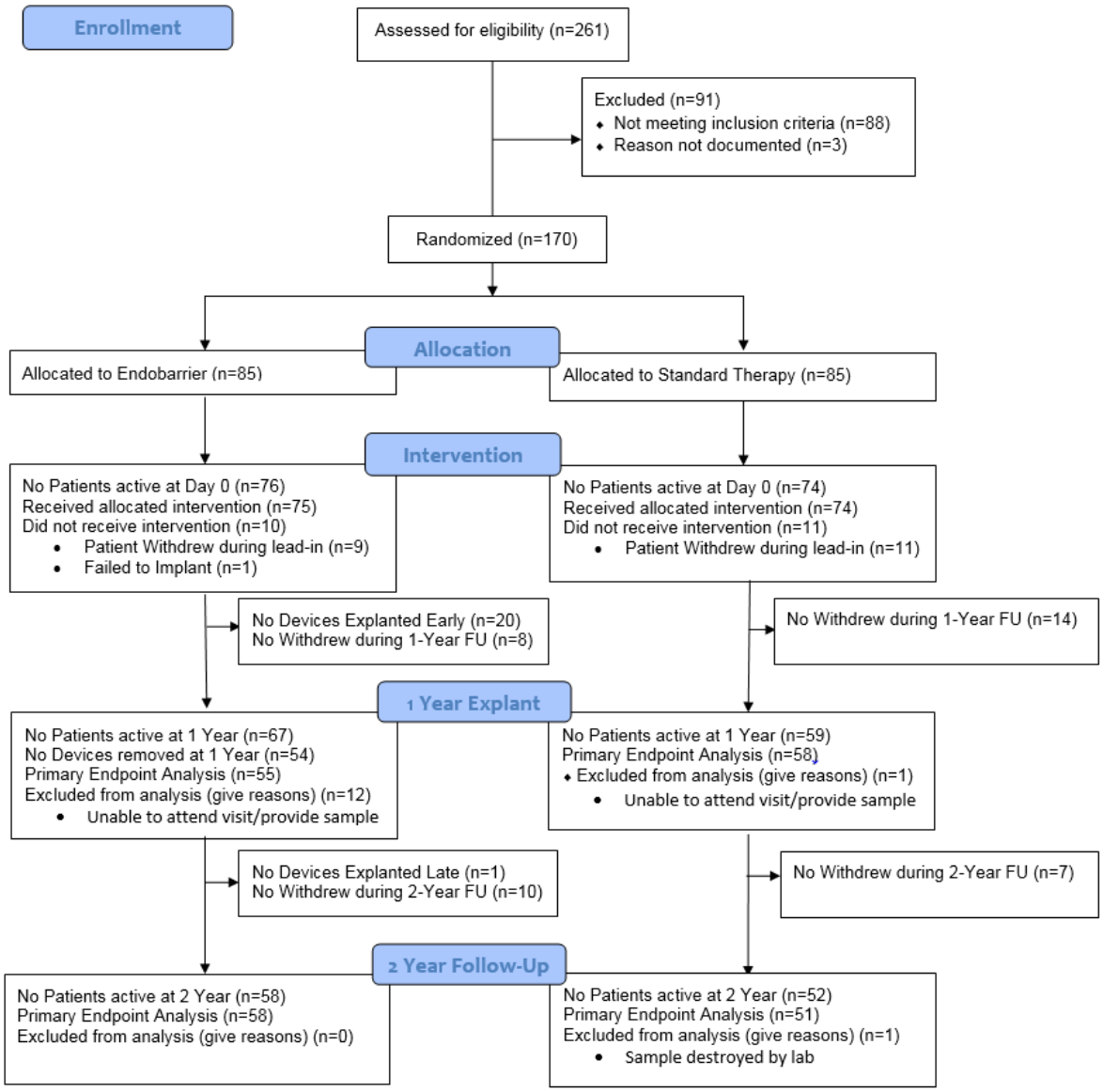
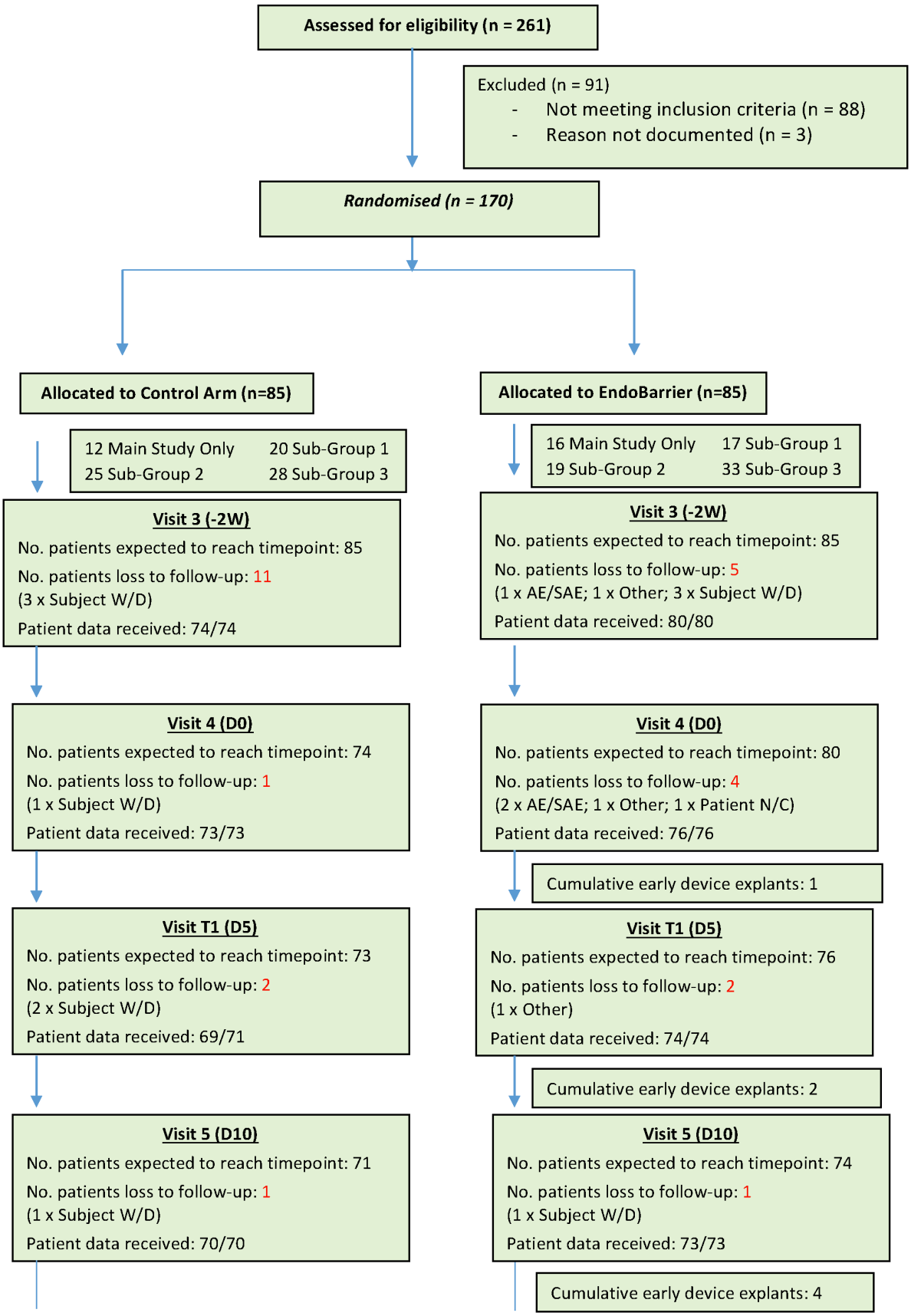
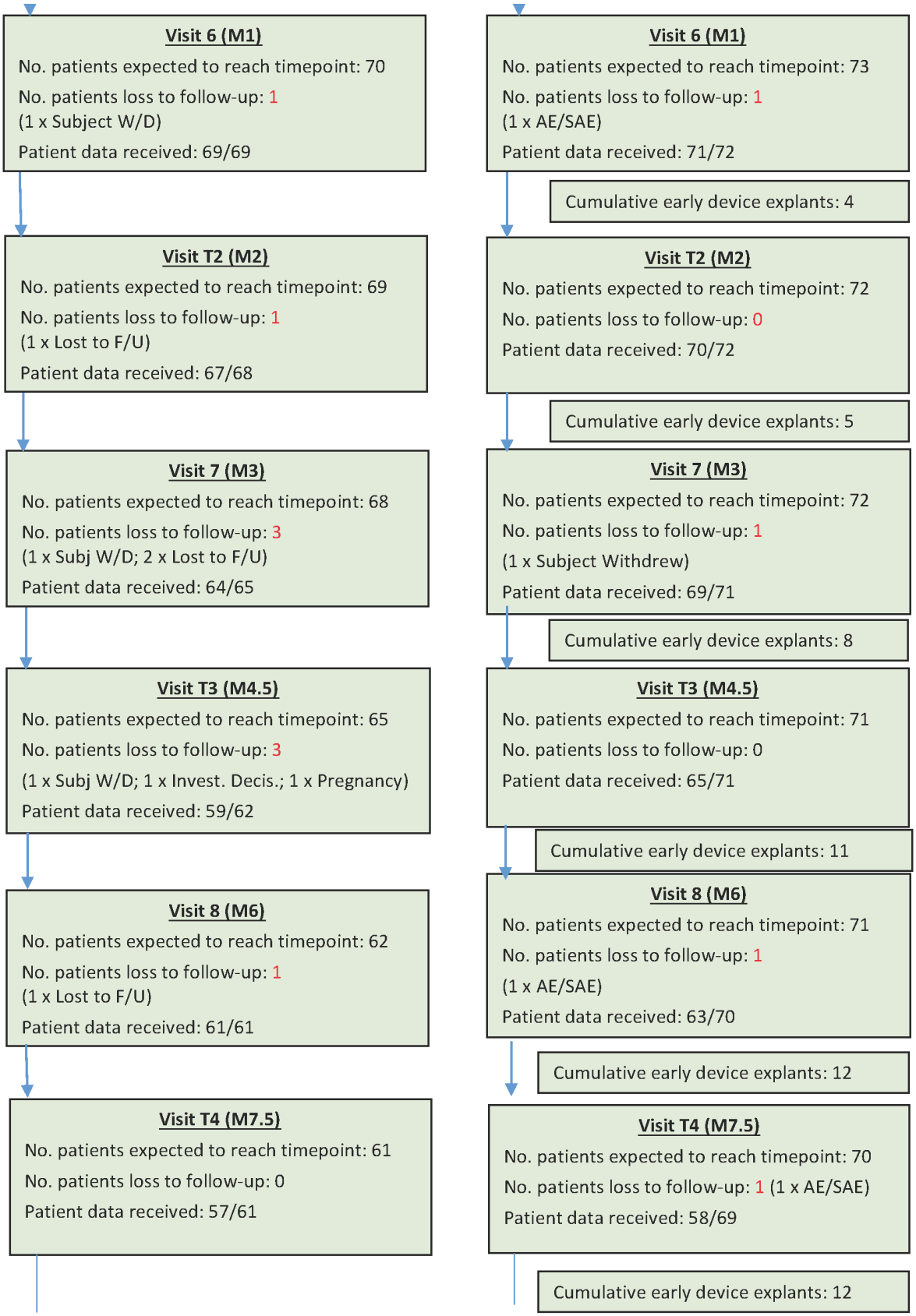
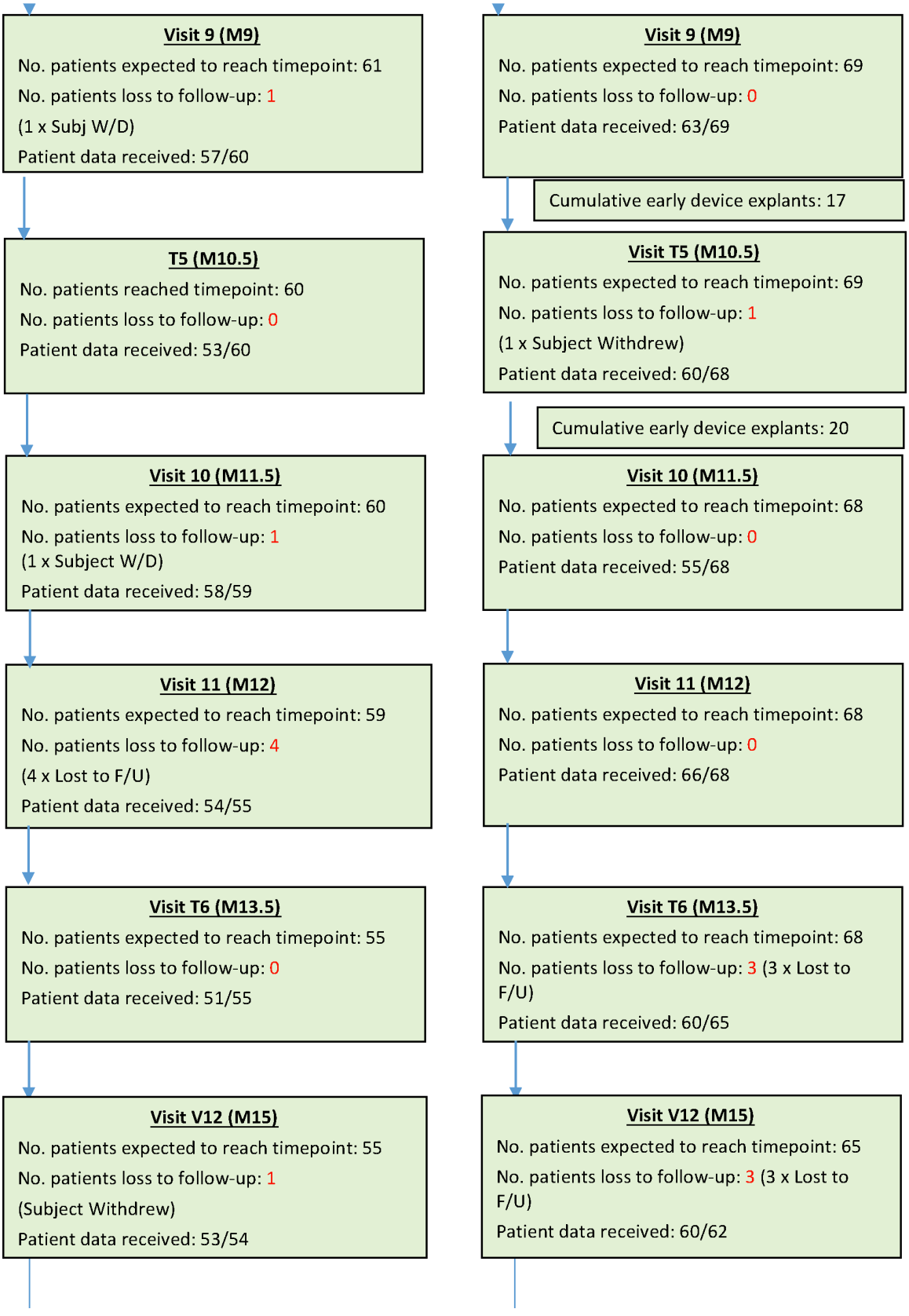


Figure 2.7 Trial flow diagram


Assessed for eligibility (n = 261)
Excluded (n = 91)
- Not meeting inclusion criteria (n = 88)
- Reason not documented (n = 3)
Randomised (n = 170)
Allocated to Control Arm (n=85)
12 Main Study Only
20 Sub-Group 1
25 Sub-Group 2
28 Sub-Group 3
Visit 3 (-2W)
No. patients expected to reach timepoint: 85
No. patients loss to follow-up: 11
(3 x Subject W/D)
Patient data received: 74/74
Visit 4 (D0)
No. patients expected to reach timepoint: 74
No. patients loss to follow-up: 1
(1 x Subject W/D)
Patient data received: 73/73
Visit T1 (D5)
No. patients expected to reach timepoint: 73
No. patients loss to follow-up: 2
(2 x Subject W/D)
Patient data received: 69/71
Visit 5 (D10)
No. patients expected to reach timepoint: 71
No. patients loss to follow-up: 1
(1 x Subject W/D)
Patient data received: 70/70
Allocated to EndoBarrier (n=85)
16 Main Study Only
17 Sub-Group 1
19 Sub-Group 2
33 Sub-Group 3
Visit 3 (-2W)
No. patients expected to reach timepoint: 85
No. patients loss to follow-up: 5
(1 x AE/SAE; 1 x Other; 3 x Subject W/D)
Patient data received: 80/80
Visit 4 (D0)
No. patients expected to reach timepoint: 80
No. patients loss to follow-up: 4
(2 x AE/SAE; 1 x Other; 1 x Patient N/C)
Patient data received: 76/76
Cumulative early device explants: 1
Visit T1 (D5)
No. patients expected to reach timepoint: 76
No. patients loss to follow-up: 2
(1 x Other)
Patient data received: 74/74
Cumulative early device explants: 2
Visit 5 (D10)
No. patients expected to reach timepoint: 74
No. patients loss to follow-up: 1
(1 x Subject W/D)
Patient data received: 73/73
Cumulative early device explants: 4


Visit 6 (M1)
No. patients expected to reach timepoint: 70
No. patients loss to follow-up: 1
(1 x Subject W/D)
Patient data received: 69/69
Visit T2 (M2)
No. patients expected to reach timepoint: 69
No. patients loss to follow-up: 1
(1 x Lost to F/U)
Patient data received: 67/68
Visit 7 (M3)
No. patients expected to reach timepoint: 68
No. patients loss to follow-up: 3
(1 x Subj W/D; 2 x Lost to F/U)
Patient data received: 64/65
Visit T3 (M4.5)
No. patients expected to reach timepoint: 65
No. patients loss to follow-up: 3
(1 x Subj W/D; 1 x Invest. Decis.; 1 x Pregnancy)
Patient data received: 59/62
Visit 8 (M6)
No. patients expected to reach timepoint: 62
No. patients loss to follow-up: 1
(1 x Lost to F/U)
Patient data received: 61/61
Visit T4 (M7.5)
No. patients expected to reach timepoint: 61
No. patients loss to follow-up: 0
Patient data received: 57/61
Visit 6 (M1)
No. patients expected to reach timepoint: 73
No. patients loss to follow-up: 1
(1 x AE/SAE)
Patient data received: 71/72
Cumulative early device explants: 4
Visit T2 (M2)
No. patients expected to reach timepoint: 72
No. patients loss to follow-up: 0
Patient data received: 70/72
Cumulative early device explants: 5
Visit 7 (M3)
No. patients expected to reach timepoint: 72
No. patients loss to follow-up: 1
(1 x Subject Withdrew)
Patient data received: 69/71
Cumulative early device explants: 8
Visit T3 (M4.5)
No. patients expected to reach timepoint: 71
No. patients loss to follow-up: 0
Patient data received: 65/71
Cumulative early device explants: 11
Visit 8 (M6)
No. patients expected to reach timepoint: 71
No. patients loss to follow-up: 1
(1 x AE/SAE)
Patient data received: 63/70
Cumulative early device explants: 12
Visit T4 (M7.5)
No. patients expected to reach timepoint: 70
No. patients loss to follow-up: 1 (1 x AE/SAE)
Patient data received: 58/69
Cumulative early device explants: 12


Visit 9 (M9)
No. patients expected to reach timepoint: 61
No. patients loss to follow-up: 1
(1 x Subj W/D)
Patient data received: 57/60
T5 (M10.5)
No. patients reached timepoint: 60
No. patients loss to follow-up: 0
Patient data received: 53/60
Visit 10 (M11.5)
No. patients expected to reach timepoint: 60
No. patients loss to follow-up: 1
(1 x Subject W/D)
Patient data received: 58/59
Visit 11 (M12)
No. patients expected to reach timepoint: 59
No. patients loss to follow-up: 4
(4 x Lost to F/U)
Patient data received: 54/55
Visit T6 (M13.5)
No. patients expected to reach timepoint: 55
No. patients loss to follow-up: 0
Patient data received: 51/55
Visit V12 (M15)
No. patients expected to reach timepoint: 55
No. patients loss to follow-up: 1
(Subject Withdrew)
Patient data received: 53/54
Visit 9 (M9)
No. patients expected to reach timepoint: 69
No. patients loss to follow-up: 0
Patient data received: 63/69
Cumulative early device explants: 17
Visit T5 (M10.5)
No. patients expected to reach timepoint: 69
No. patients loss to follow-up: 1
(1 x Subject Withdrew)
Patient data received: 60/68
Cumulative early device explants: 20
Visit 10 (M11.5)
No. patients expected to reach timepoint: 68
No. patients loss to follow-up: 0
Patient data received: 55/68
Visit 11 (M12)
No. patients expected to reach timepoint: 68
No. patients loss to follow-up: 0
Patient data received: 66/68
Visit T6 (M13.5)
No. patients expected to reach timepoint: 68
No. patients loss to follow-up: 3 (3 x Lost to F/U)
Patient data received: 60/65
Visit V12 (M15)
No. patients expected to reach timepoint: 65
No. patients loss to follow-up: 3 (3 x Lost to F/U)
Patient data received: 60/62

* One patient failed to successfully receive implant at V4

Figure 2.8 Patient flow and attrition rates by visit

2.3.2 Baseline characteristics

The baseline characteristics of all participants are summarised in **Table 2.4**. There were no statistically significant differences between study arms.

	Control (n=85) mean ± SD	Endobarrier (n=85) mean ± SD
Age, years	51.9 ± 8.46	51.6 ± 7.94
Ethnicity, n (%)		
Asian	9 (10.6)	11 (12.9)
Black	13 (15.3)	3 (3.5)
White	62 (72.9)	70 (82.4)
Mixed	1 (1.2)	1 (1.2)
Sex, n (%) Female	39 (45.9)	39 (45.9)
Weight, kg	104.24 ± 14.91	107.89 ± 17.06
BMI, kg/m^2	35.82 ± 4.22	36.82 ± 5.0
Systolic BP, mmHg	132.8 ± 15.33	130.3 ± 11.91
Diastolic BP, mmHg	83.2 ± 10.51	82.2 ± 9.69
HbA1C, mmol/mol	71 ± 10	74 ± 10

Table 2.4 Participant baseline characteristics

2.3.3 Weight loss outcomes and changes in BMI

The changes in absolute and percentage body weight of participants in each study arm through the trial are shown in **Table 2.5** and in **Figure 2.9**. Both treatment groups displayed a significant loss in weight over the 2-year follow-up period. In the control arm, there was an initial mean weight reduction of 4.19 kg (4.1%) during the liquid diet phase of the trial. TBWL then gradually increased over the first six months to peak at 6.30 kg (6.1%). Weight loss then remained relatively stable before then steadily declining to 4.82 kg (4.6%) by 24 months. In comparison, participants receiving the Endobarrier device achieved a significantly greater mean weight reduction of 10.82 kg (10.1%) at 6 months and this continued to increase to a peak reduction of 11.74 kg (10.9%) by 11.5 months. The Endobarrier produced significantly more weight loss at 12 months (Endobarrier -10.6 ± 6.2% vs. control -5.4 ± 5.8% (mean difference [95% CI] = -5.0% (-7.1, -2.9), p<0.0001)). After the device was explanted at 12 months, the mean weight reduction decreased in a consistent manner to 6.68 kg in the Endobarrier arm vs 4.51 kg in the control arm at 18 months (-6.2% vs. -4.3% (mean difference [95% CI] = -1.7% (-3.8, 0.2), p = 0.079) and 5.40 kg vs 4.82 kg at 24 months (-5.1% vs. -4.6% (mean difference [95% CI] = -0.4% (-2.4, 1.6), p = 0.70)). Weight loss at all visits in both groups was significant when compared to the baseline weight (p < 0.0001).

Changes in BMI correlated with weight loss outcomes and are summarised in **Table 2.6**. Patients receiving the Endobarrier device experienced a maximal BMI reduction of -4.06 ± 2.34 kg/m^2 after 11.5 months vs -2.11 ± 2.22 kg/m^2 for participants in the control group. The Endobarrier resulted

Total body weight loss							Adjusted difference in %TBWL between groups*
		Control group			Endobarrier group		
Visit	**n**	**kg**	**%**	**n**	**kg**	**%**	
10 days	70	- 4.19 ± 2.02	- 4.1 ± 1.9	73	- 6.06 ± 2.77	- 5.7 ± 2.5	-1.6 (-2.4, -0.9)
1 month	69	- 4.75 ± 3.03	- 4.6 ± 2.9	70	- 7.39 ± 2.91	- 6.9 ± 2.5	-2.3 (-3.2, -1.5)
3 months	64	- 5.61 ± 4.42	- 5.5 ± 4.3	69	- 9.33 ± 4.98	- 8.7 ± 4.3	-3.3 (-4.8, -1.9)
6 months	61	- 6.30 ± 5.53	- 6.1 ± 5.2	63	- 10.82 ± 5.39	- 10.1 ± 4.7	-4.0 (-5.7, -2.2)
9 months	57	- 5.82 ± 6.26	- 5.6 ± 5.7	63	- 11.02 ± 6.13	- 10.2 ± 5.2	-4.4 (-6.3, -2.5)
11.5 months	58	- 6.17 ± 6.39	- 5.9 ± 5.8	54	- 11.74 ± 6.84	- 10.9 ± 5.6	-4.6 (-6.7, -2.5)
12 months	54	- 5.57 ± 6.36	- 5.4 ± 5.8	66	- 11.43 ± 7.40	- 10.6 ± 6.2	-5.0 (-7.1, -2.9) [α]
15 months	53	- 5.14 ± 6.38	- 5.0 ± 5.8	60	- 8.44 ± 6.73	- 7.8 ± 5.7	-2.7 (-4.7, -0.6)
18 months	52	- 4.51 ± 6.46	- 4.3 ± 5.9	61	- 6.68 ± 5.70	- 6.2 ± 5.1	-1.7 (-3.8, 0.2) [α]
23 months	48	- 5.31 ± 5.66	- 5.1 ± 5.2	48	- 5.87 ± 5.74	- 5.6 ± 5.3	-0.4 (-2.4, 1.6)
24 months	51	- 4.82 ± 6.18	- 4.6 ± 5.7	58	- 5.40 ± 5.85	- 5.1 ± 5.4	-0.4 (-2.4, 1.6) [α]

Data represented as mean ± SD except for adjusted difference which is presented as mean (95% CI).

*Adjusted difference between groups is derived from the fixed effect for treatment group in a mixed-model analysing percentage weight loss at visit adjusting for fixed effect covariates; absolute weight at baseline, age, BMI group, site and a random effect for patient intercept.
[α] p-values from testing at 12 months, 18 months and 24 months yielded p-values of <0.0001, 0.079 and 0.70 respectively.

Weight loss at all visits in both groups was significant when compared to the baseline weight ($p < 0.0001$).

SD, standard deviation; kg, kilogram; CI, confidence interval.

Table 2.5 Change in weight over time in control and Endobarrier participants

in a significantly greater decrease in BMI at 12 months (Endobarrier - 3.95 ± 2.50 kg/m^2 vs. control -1.91 ± 2.23 kg/m^2 (mean difference [95% CI] = -1.75 (-2.56, -0.94), p<0.0001)). In line with weight loss, BMI increased following explantation of the Endobarrier device and was not statistically different from the control group at 18 months (-2.30 ± 1.93 kg/m^2 vs. - 1.55 ± 2.27 kg/m^2 (mean difference [95% CI] = -0.62 (-1.38, 0.15), p = 0.11)) or 24 months (- 1.89 ± 2.02 kg/m^2 vs. - 1.66 ± 2.18 kg/m^2 (mean difference [95% CI] = -0.10 (-0.87, 0.67), p = 0.80)).

At 12 months, 16 (24.2%) patients achieved a 15% weight reduction in the EndoBarrier arm compared to 2 (3.7%) in the standard therapy arm. Using logistic regression, adjusting for stratification variables of site and BMI Group; the odds-ratio estimate for achieving the target in the EndoBarrier arm compared to the Control arm was 8.3 (95% CI, 1.8, 39.0; p = 0.001). Following explantation of the DJBL, the number of patients achieving a 15% weight reduction reduced to 6 (10.0%) in the EndoBarrier arm and 1 (1.9%) in the standard therapy arm at 15 months (OR 5.7 95% CI, 0.7, 49.7; p = 0.12). Logistic regression also failed to return a significant difference between groups at 18 and 24 months.

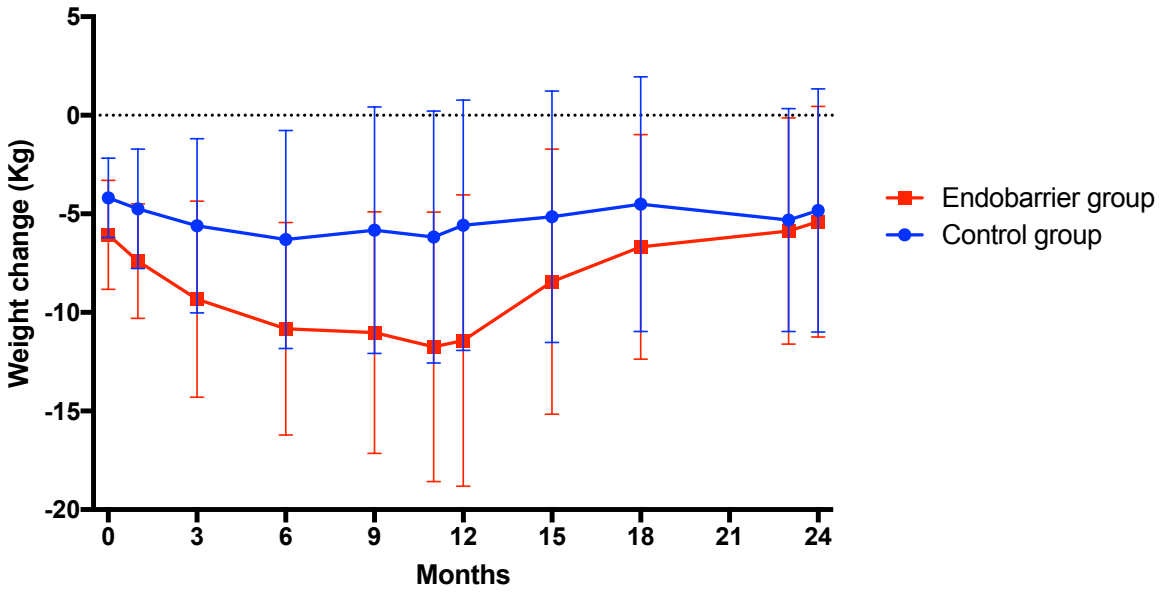


Figure 2.9 Change in weight over time in control and Endobarrier participants

Change in BMI					Adjusted difference between groups*
		Control Group		Endobarrier Group	
Visit	**n**	**Kg/m^2**	**n**	**Kg/m^2**	
10 days	70	- 1.43 ± 0.66	73	- 2.07 ± 0.92	-0.62 (-0.88, -0.36)
1 month	69	- 1.62 ± 0.99	70	- 2.54 ± 0.96	-0.87 (-1.20, -0.55)
3 months	64	- 1.92 ± 1.50	69	- 3.21 ± 1.67	-1.24 (-1.78, -0.70)
6 months	61	- 2.15 ± 1.90	63	- 3.75 ± 1.83	-1.48 (-2.13, -0.82)
9 months	57	- 2.00 ± 2.16	63	- 3.82 ± 2.08	-1.56 (-2.29, -0.83)
11.5 months	58	- 2.11 ± 2.22	54	- 4.06 ± 2.34	-1.63 (-2.44, -0.81)
12 months	54	- 1.91 ± 2.23	66	- 3.95 ± 2.50	-1.75 (-2.56, -0.94) $^{\alpha}$
15 months	53	- 1.76 ± 2.22	60	- 2.91 ± 2.25	-0.96 (-1.77, -0.16)
18 months	52	- 1.55 ± 2.27	61	- 2.30 ± 1.93	-0.62 (-1.38, 0.15) $^{\alpha}$
23 months	48	- 1.82 ± 2.00	48	- 2.06 ± 1.98	-0.11 (-0.89, 0.67)
24 months	51	- 1.66 ± 2.18	58	- 1.89 ± 2.02	-0.10 (-0.87, 0.67) $^{\alpha}$

Data represented as mean ± SD except for adjusted difference which is presented as mean (95% CI).

*Adjusted difference between groups is derived from the fixed effect for treatment group in a mixed-model analysing BMI at visit adjusting for fixed effect covariates; BMI at baseline, age, BMI group, site and a random effect for patient intercept.

$^{\alpha}$ p-values from testing at 12 months, 18 months and 24 months yielded p-values of <0.0001, 0.11 and 0.80 respectively.

Decrease in BMI at all visits in both groups was significant when compared to the baseline weight ($p < 0.0001$).

SD, standard deviation; kg, kilogram; CI, confidence interval.

Table 2.6 Change in BMI over time in control and Endobarrier participants

2.4 Discussion

Patients implanted with the Endobarrier DJBL for 12 months alongside specialist dietary and lifestyle guidance achieved statistically greater weight loss than patients managed with dietary and lifestyle interventions alone. This finding is in line with the best available systematic reviews and meta-analyses of this device.[257,259] For weight loss outcomes, Rohde et al conducted a random-effects meta-analysis of 4 RCTs, which included 151 patients, and showed that DJBL-treated patients experienced a mean of 5.1 kg greater weight loss compared with control patients [95% confidence interval (CI) −7.3, −3.0 kg; I^2 = 37%). This correlates closely to the 5.44 kg of additional weight loss achieved by Endobarrier-treated participants in this trial when compared to control participants. However, it must be noted that the median implant duration for the included studies in this analysis was only 12 weeks and our reported study represents the first RCT to examine weight loss outcomes over an implant duration of 1 year.

In this study, Endobarrier participants achieved a maximum absolute weight loss of 11.74 kg after an implant duration of 1 year and our observations support the notion that the efficacy of the Endobarrier DJBL appears to be maximal within the first 6 - 12 months of treatment and plateaus thereafter. Of the trials published to date there is a wide degree of inter-trial heterogeneity and implant durations vary between three and twelve months. However, individuals implanted with the Endobarrier for 12 months have been reported to lose anywhere between 5.9 and 22.4 kg in weight and %EWL of > 45% have been reported in several observational studies.[229,244,245,251,252] The national German DJBL registry receives data from over 30 German centres and is one of the largest cohorts of prospectively collected data on patients treated with the Endobarrier device. A presentation of this registry in 2018 (n = 235) reported a mean weight reduction of 14.7 $\pm$ 9.0 kg, a reduction in BMI by 5.0 $\pm$ 3.1 kg/m^2 and an EWL of 28.5 $\pm$ 9.0% after a mean implant duration of 47.5 $\pm$ 12.2 weeks. This was superior to matched controls receiving standard treatment.[256] Betzel et al. have published the largest prospective observational study of the Endobarrier to date, reporting on 185 patients implanted with a DJBL for a mean duration of 46 $\pm$ 15 weeks, and demonstrated that patients lost on average 12.8 $\pm$ 8.0 kg. This correlated to a %EWL of 46.3 $\pm$ 31.1% at the time of explantation ($p < 0.001$), a TBWL of 11.9 $\pm$ 6.9% ($p < 0.001$) and a reduction in BMI from 35.1 $\pm$ 4.3 kg/m^2 to 30.9 $\pm$ 4.3 kg/m^2 ($p < 0.001$).[244] Corroborating this, Forner et al. subsequently reported on 114 consecutive patients implanted with a DJBL for a mean duration of 51.1 weeks and demonstrated a mean weight loss of 12.0 $\pm$ 8.6 kg ($p < 0.001$), a reduction in BMI by 4.2 $\pm$ 3.2 kg/m^2 and a mean %TBWL of 10.5 $\pm$ 7.3% (range 8.4 – 40.2%).[246]

Following an implantation period of 12 months, the data also demonstrate that, when the device is removed, patients regained weight precipitously to achieve a weight loss of 5.4 kg at 24

months, which was only 0.6 kg greater than but not statistically different to participants in the control arm. The largest cohort of data for the effects on weight and metabolic profile following termination of treatment with the DJBL comes from the National German DJBL registry.[254] A report of 77 patients with a follow-up duration of 0.04 to 3.85 years concluded that 85% of patients maintained or increased their weight after removal of the DJBL and that an increase in BMI of 2.1 kg/m^2 had to be expected (approximate 50% weight regain). A further sub-group analysis of 32 patients, who had follow-up data for at least one year, demonstrated an increase in BMI of 2.5 kg/m^2 but they also concluded that the weight/BMI at the follow-up visit remained significantly lower than at the baseline visit, which shares the observations made within this study but this was not statistically different to control patients. In the RCT by Koehestanie et al. they similarly reported a deterioration in treatment effect following device explantation.[225] At 6 months follow-up, patients in both groups regained weight but their BMI was still significantly lower than at the baseline visit and, importantly, the DJBL group maintained an EWL of 19.8% versus 11.7% in the control group ($p < 0.05$) (TWL 5.8% Vs. 3.5%, $p < 0.05$). There is therefore a 'legacy effect' of the Endobarrier device beyond the treatment phase but the mechanisms behind this remain unclear. However, simply being followed up as part of a clinical study or the adoption and maintenance of behavioural, dietary and lifestyle changes by the patient during their treatment may account for this observation. More recent evidence suggests complete loss of this treatment effect after 4 years.[282]

The deterioration of effects following removal of the Endobarrier has also been presented in several other prospective cohort studies and matches the experience of treatment decay observed following the cessation of other conservative and non-surgical treatments for obesity (e.g. the gastric balloon).[229,230,232,242,245,246,250] This should therefore be an important consideration when referring a patient for a temporary EBT and highlights the importance of implementing medical and lifestyle interventions alongside these therapies in order to maximise their efficacy and longevity.

It has been recommended by a National Institute of Health consensus panel that patients who are obese with obesity-related co-morbidities should have personalised weight loss goals in order to meet targeted health outcomes. It is well evidenced that achieving a meaningful weight loss of 5, 10 or 15% improves cardiometabolic health, glycaemic control, quality of life, and reduces overall morbidity and mortality.[320,321] Patients with obesity and at least one severe obesity-related complication, should have weight loss targets of > 10-15%. As such, one of the current trials secondary outcome measures was the number of participants achieving a clinically meaningful weight loss of 15% or greater. The number of patients achieving this threshold was significantly higher in the Endobarrier group in comparison to the control group participants at 12 months (16

(24.2%) in the EndoBarrier® arm compared to 2 (3.7%) in the standard therapy arm; odds-ratio 8.3 (95% CI, 1.8, 39.0; p-value = 0.001)). The long-term effects of this observation are unknown. However, in a post hoc analysis in the Look AHEAD study, where individuals in either arm of the trial had lost at least 10% of their bodyweight in the first year, they had a 21% lower risk of the primary outcome of major cardiovascular events (p=0·034) and a 24% reduced risk of the secondary cardiovascular disease outcome (p=0·003) compared with individuals with stable weight or weight gain.[322]

The strengths of the trial are that it is a well-powered, randomised, multi-centre study with good short and medium term follow up for 2 years. It can be credited with the delivery of a truly intensive medical care programme by a multidisciplinary team and currently represents the largest RCT of the Endobarrier DJBL to date. The main limitation of the trial is the open-label design, which could serve as a source of bias.

2.5 Conclusion

In conclusion, 12 months of treatment with the Endobarrier DJBL resulted in superior weight loss when compared to control patients managed with intensive lifestyle and dietary interventions alone. The number of patients achieving a meaningful weight loss of > 15% was significantly greater within the first year of Endobarrier therapy, which could potentially have long-term effects on cardiometabolic morbidity and mortality. A degree of weight-loss is maintained one year following removal of the Endobarrier device but this was not statistically different from control patients.

Chapter 3:

Evaluating the effect of a duodenal-jejunal bypass liner (Endobarrier®) on hepatic and peripheral insulin sensitivity using hyperinsulinaemic euglycaemic clamps

3.1 Introduction

3.1.1 Tissue-specific insulin resistance

Insulin resistance is hallmarked by reduced suppression of hepatic glucose production, reduced peripheral clearance of glucose in skeletal muscle and increased rates of lipolysis in adipose tissue.[56] It is recognised that there is a stepwise development of insulin resistance in humans and that this will develop to differing degrees in different tissue compartments during the stages of 'pre-diabetes', but compensatory hyperinsulinaemia will act to increase glucose metabolism in other insulin-sensitive tissues (see **section 1.2.2.2**).[323] The earliest development of insulin resistance generally occurs in skeletal muscle resulting in the diversion of ingested carbohydrates from muscle glycogen storage to the liver. Combined with compensatory hyperinsulinaemia, this stimulates hepatic de novo lipogenesis and accumulation of lipid species, which ultimately results in hepatic insulin resistance.[56] Quantitative measurements of the determinants of fasting or postprandial glucose concentrations are fundamental to the research of disorders of glucose metabolism and to measuring the effect of therapeutic interventions on these disease states.

The hyperinsulinaemic euglycaemic clamp (HEC) is considered to be the gold standard direct method of assessing insulin sensitivity in vivo. It is the most sensitive measure of insulin action and can provide an accurate assessment of whole-body insulin sensitivity. Through the incorporation of stable isotopes, the clamp can be used to assess tissue-specific fluxes of glucose as a reflection of insulin sensitivity of individual organs and tissue compartments.

3.1.2 Measurement of compartment-specific insulin sensitivity: hyperinsulinaemic euglycaemic clamp

3.1.2.1 Dynamic isotope dilution technique

Isotopes are chemically identical elements with the same atomic number but differ in their atomic mass due to a differing number of neutrons, which do not affect the chemical properties of the atom. Those that do not decay spontaneously and are not a source of ionising radiation are termed stable isotopes and have been safely used in metabolic studies since the 1930s.[324] The substitution of a stable isotope for a common form within a molecule, known as 'labelling', creates a *tracer,* which is designed to be chemically indistinguishable from a metabolite of interest in the body (the *tracee*) but can be precisely identified with specific detection techniques, such as Gas Chromatography Mass Spectrometry (GC-MS). Tracers can therefore be used to study the kinetics of numerous physiological substrates using one of several different methodologies.[324]

The principle of the isotope dilution technique is that if a known amount of a tracer is added to a biological system then measurements of isotope dilution within body pools will yield an estimate of the size of that pool. To measure the flux between body pools then a *dynamic* method is used in which a tracer is infused at a constant rate into a single pool until a steady-state is reached. Assuming the pool concentrations of the tracee remain constant, once a steady-state is reached, the tracer is lost from the pool at the same rate as the tracee. The dilution of the tracer (or isotope) by the tracee at an isotopic steady-state is a measure of the rate of appearance (Ra) of the tracee into the pool, which is equal to the rate of disappearance (Rd) from the pool in a steady-state.

When a tracer infusion is first commenced at a constant rate into a single body pool, then the initial tracer concentration in the body pool will be relatively lower than in the infusion mixture and, hence, the tracer will leave the pool at a slower rate than the rate at which it appears. There will therefore be a progressive rise in the concentration of the tracer and, with time, the concentration of the tracer and tracee in the body pool will equal their respective concentrations in the infusate. This state is referred to as *isotonic* equilibrium in which there is a plateau in *enrichment* of the tracer in the body pool. A typical enrichment curve is shown in **Figure 3.1**. Once this stage is reached, the tracer will be lost at the same rate that it appears and there will be no further changes in the relative concentrations of the tracer and tracee in the body pool provided that the appearance/infusion of the tracee and tracer remain constant (**Figure 3.2**).

In a steady-state of isotopic equilibrium we can therefore calculate Ra and Rd:[325]

$$Ra = Rd$$

$$\frac{F}{Ra} = \frac{Tracer}{Tracee}$$

$$Ra = \frac{F}{(\frac{Tracer}{Tracee})}$$

Ra, rate of appearance of tracee. Rd, rate of disappearance. F, tracer infusion rate.

In order to make these calculations, certain assumptions need to be made: (1) the tracee must appear directly in the sampled compartment, (2) the tracer is treated physiologically in the exact same way as the tracee, (3) the tracer does not affect the kinetics of the tracee, (4) recycling of the tracer does not occur or is accounted for, and (5) sampling occurs from a single, homogenous and instantly mixing pool.

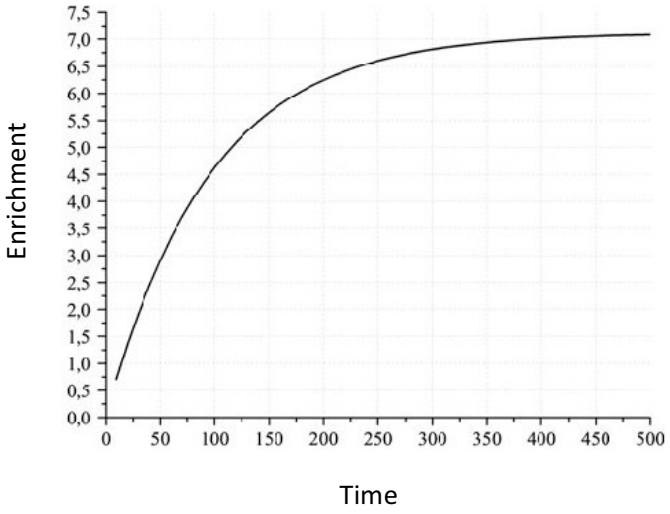


Figure 3.1 Exponential curve of enrichment of a single pool over time during a constant tracer infusion

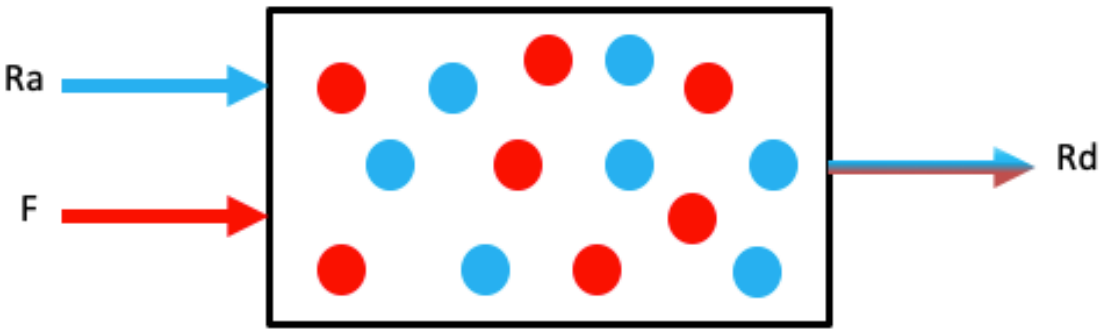


Figure 3.2 Schematic representation of isotope dilution technique in a single body pool Ra, rate of appearance of tracee. Rd, rate of disappearance. F, tracer infusion rate.

Some metabolic studies, such as the investigation of glucose metabolism (see **section 3.1.2.2**), must be investigated under non-steady-state conditions in which there are counter-regulatory mechanisms in operation that will serve to normalise the rate of glucose production and uptake. Examples of this include post-prandial studies, studies following exercise or during insulin/hormone infusions, which will all alter the rate of hepatic glucose production (HGP) and glucose efflux. In order to account for this, a plateau of enrichment must first be allowed to occur in steady-state conditions. Any subsequent perturbation of the system will then disrupt the

isotopic equilibrium and any subsequent acute changes in enrichment will be a reflection of the changes that have occurred in the plasma kinetics of the tracee. For example, if an insulin infusion was commenced, there will be a rapid decrease in HGP, thus decreasing concentrations of the glucose tracee within the circulation, and peripheral glucose disposal will increase to cause a perturbation to the steady-state isotopic equilibrium. In this 'non-steady-state', there will therefore be an imbalance between Ra and Rd but, from changes that occur in the tracer/tracee ratio from the steady-state with time, the changes in Ra with time can be calculated. Knowing this, changes in Rd with time can then also be calculated.[326] Extension of the constant-tracer infusion technique to a non-steady state situation and the equations required for this application were first described and are fully reported by Steele et al.[327]

3.1.2.2 Hyperinsulinaemic euglycaemic clamp

To make estimates of insulin sensitivity, glucose is the tracee of interest and flux is investigated by calculating Ra, which is the rate of appearance of glucose into plasma from the liver (i.e. HGP), and Rd, which is the rate of disappearance of glucose from the bloodstream into insulin-sensitive tissues (i.e. uptake and utilisation in adipose tissue and skeletal muscle). The tracer used in these studies is deuterium-labelled glucose as this tracer is not recycled within the Cori cycle.[324] Deuterium (^{2}H or D2) is a naturally occurring stable isotope of hydrogen (^{1}H), with one additional neutron, and is used to label glucose by replacing two hydrogen ions on the 6th carbon atom [6, 6-$^2H_2$]-glucose) (**Figure 3.3**).

During the HEC, weight-adjusted fixed rates of insulin are infused in order to create a hyperinsulinaemic plateau. Glucose is then infused intravenously at variable rates in order to maintain euglycaemia and, during the steady state, represents a crude index of insulin sensitivity - i.e. patients who are insulin resistant will have reduced suppression of HGP and decreased rates of peripheral glucose disposal and will therefore require a lower concentration of glucose to be infused to maintain euglycaemia under hyperinsulinaemic conditions. In contrast, those patients with improvements in insulin sensitivity will have increased suppression of HGP, increased rates of peripheral glucose disposal and will be more prone to hypoglycaemia during hyperinsulinaemia, thus necessitating the infusion of greater concentrations of glucose in order to maintain euglycaemia. Incorporation of [6, 6-$^2H_2$]-glucose as a tracer then allows differentiation between the relative contributions and changes in HGP (Ra) and peripheral glucose disposal (Rd), from which inferences of relative changes in insulin sensitivity in these two compartments can be made.

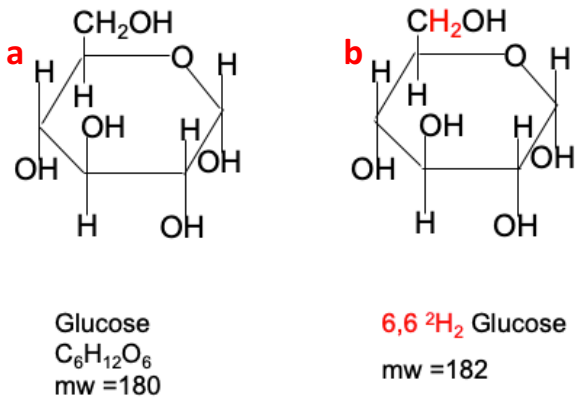


Figure 3.3 (a) Glucose tracee molecule and **(b)** deuterium-labelled glucose tracer

The experimental clamp protocol is started by first taking blood samples for determination of baseline glucose enrichment. The [6, 6-$^{2}H_2$]-glucose tracer is then introduced as a bolus followed by a continuous fixed-rate infusion for two hours in order to achieve isotopic equilibrium, which typically occurs within 100 mins. Further samples for the determination of glucose enrichment are taken at this stage. The blood glucose level at this point is recorded as the 'clamped' glucose (between 4.0 and 6.0 mmol/L) and a two-stage HEC is commenced. For the first stage (2 hours), insulin is infused at a low dose, during which time blood glucose concentrations are measured regularly and maintained around the 'clamped' glucose level ± 0.5 mmol/L using a variable rate infusion of intravenous glucose. Blood sampling occurs during the last 30 minutes of the stage, during steady-state, in order to measure the isotopic enrichment of glucose. For this first stage, measurements are to estimate hepatic insulin sensitivity by calculating Ra (HGP) as per the general principles discussed in **section 3.1.2.1**. For the second stage of the clamp, insulin is then infused at an increased fixed rate for a further 2 hours. During this stage, the high insulin concentrations will suppress HGP and, therefore, measurements are made of Rd (peripheral glucose disposal) and changes in peripheral insulin sensitivity can be determined. Using this technique, it is not possible to determine the relative contributions of different insulin-sensitive tissues to peripheral insulin sensitivity (i.e. skeletal muscle and adipose tissue) and inclusion of another tracer would be required to do this.

3.1.3 Effect of metabolic surgery and bypass of the foregut on tissue-specific insulin sensitivity

As previously discussed at length in **sections 1.2.5.2.2**, **1.2.6.2.4** and **1.2.6.2.5** there is significant evidence to support the notion that surgical or endoscopic bariatric therapies cause rapid improvements in hepatic insulin sensitivity, resulting in early reductions in hepatic glucose

production and improvements in FPG levels. Alongside this, most likely through weight-dependent and weight-independent mechanisms, total-body insulin resistance declines, peripheral glucose uptake and utilisation increases, and β-cell function improves.

Changes in tissue-specific insulin resistance following metabolic surgery using isotopic tracer clamp studies have been reported in several studies.[178,328-333] It is clear that significant weight loss 6-12 months following RYGB results in significant improvements in both peripheral and hepatic insulin sensitivity in obese patients with or without type 2 diabetes (T2DM). What remains unclear is whether improvements in insulin sensitivity play a role in the early weight loss-independent improvements in glucose homeostasis observed following RYGB. Multiple studies have reported improvements in HOMA-IR within the first 4 weeks of surgery but early changes in peripheral and hepatic insulin sensitivity, as measured by insulin clamp with tracer techniques, remain inconclusive.[178,188,329,334,335] It is also unclear as to whether any observed early improvements in hepatic insulin sensitivity are the result of peri-operative energy restriction or due to as yet unidentified weight loss-independent mechanisms related to surgery. In a recent study by Bojsen-Møller et al,[177] 20 patients were assessed using HEC combined with glucose tracer technique 1 week, 3 months and 1 year following RYGB. The authors concluded that hepatic insulin sensitivity increased as early as 1 week after surgery and that later improvements observed in peripheral insulin sensitivity at 3 months and 1 year were attributable to weight loss. However, it should be noted that patients included in this study were subject to a calorie-restricted liquid diet for 14-days post-operatively and that there was no non-operated control group to evaluate the differential effect of this on the insulin sensitivity results.

Only one study to date has evaluated glucose homeostasis following DJBL implantation using the gold-standard method of HEC. In this study by Miras et al,[247] 7 obese patients with a mean baseline BMI of 48.5 kg/m^2 underwent serial clamps in order to evaluate the early effects of DJBL implantation on insulin sensitivity and HGP, whilst controlling for the effects of caloric restriction in the peri-implantation period. Clamps were performed at three time-points: (1) at baseline, (2) 1 week after commencing a low-calorie liquid diet (Fortisip® Compact, 1500kcal d^{-1}), and (3) 1 week following DJBL implantation, whilst the patient continued on the liquid diet. 6 of the 7 patients had T2DM with a mean baseline HbA1C of 7.2% and were being treated with glucose-lowering tablets (n = 6), GLP-1 agonists (n = 2) or insulin (n = 3), which were unchanged during the study period in order to control for this as a possible confounder. Following the initial 1 week of liquid diet, patients lost an average of 3.6 kg (-1.4 kg/m^2) and then lost a further 4.3 kg (-1.4 kg/m^2) 1 week following DJBL implantation on the low-calorie diet. During the clamps, fasting plasma glucose and HGP were significantly reduced between visits 1 and 2 ($p < 0.05$) but values were not significantly reduced further between visits 2 and 3 (i.e. following the implantation of the DJBL).

This study therefore concluded that the DJBL did not improve hepatic insulin sensitivity beyond the improvements achieved with caloric restriction. However, this study was limited by its small number of participants and lack of a control group.

The herein reported study is therefore the first to evaluate changes in tissue-specific insulin sensitivity following DJBL implantation within a randomised controlled trial.

3.1.4 Aims

The aim of this research is to investigate the effects of duodenal exclusion using the Endobarrier DJBL on hepatic and peripheral insulin sensitivity.

3.1.5 Hypotheses

The hypotheses being tested in the work that will be described within this chapter are that duodenal exclusion in patients with obesity and T2DM using the Endobarrier DJBL alongside intensive lifestyle modification will result in:

- significantly greater weight loss than in control patients treated with intensive lifestyle modification alone;
- significantly greater reductions in fasting plasma glucose concentrations than in control patients treated with intensive lifestyle modification alone;
- significantly greater early improvements in hepatic insulin sensitivity than in control patients treated with intensive lifestyle modification alone that occur independently of weight-loss and calorie restriction;
- significantly greater weight-loss-dependent improvements in peripheral insulin sensitivity than in control patients treated with intensive lifestyle modification alone.

3.1.6 Objectives

The objectives of this research are:

- to evaluate the change in weight in patients with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for 6 months and compare it to the weight changes in control patients with obesity and T2DM receiving intensive medical therapy alone;
- to determine the changes in fasting plasma glucose and serum insulin concentrations in patients with obesity and T2DM treated with intensive medical therapy and implanted

with the Endobarrier DJBL for 6 months and compare it to the changes in control patients with obesity and T2DM receiving intensive medical therapy alone;

- to evaluate the early (10 days) and late (6 months) changes in hepatic insulin sensitivity following treatment with the Endobarrier DJBL using the gold standard HEC technique with inclusion of stable isotopes and compare these changes to those of control patients;
- to evaluate the early (10 days) and late (6 months) changes in peripheral insulin sensitivity following treatment with the Endobarrier DJBL using the gold standard HEC technique with inclusion of stable isotopes and compare these changes to those of control patients.

3.2 Methods

3.2.1 Patients and study design

All patients who were recruited into the Endobarrier RCT were given the option of participating in this nested sub-study. Those who agreed to participate provided additional informed written consent and underwent hyperinsulinaemic euglycaemic clamps at visits 3 (- 2 weeks ± 7 days), 5 (+ 10 days ± 7 days) and 8 (+ 6 months ± 7 days). The trial methodology, including patient selection, randomisation, trial interventions and follow-up schedule are described and discussed in detail in **Chapter 2**.

3.2.2 Medicines, reagents and materials

Reagents and materials used during the methodology are fully described in **Appendix B.**

3.2.3 Liquid diet

To avoid disruption of the device in the immediate period following implantation, patients were prescribed a liquid diet for the 7 days before and 13 days (± 3 days) after the intervention visit (visit 4). The liquid diet was guided by a specialist dietitian and comprised of 125 ml Fortisip Compact drinks (Nutricia, UK): 5 per day for men, 4 per day for women, containing per 100 mL: 240 kcal, 9.6 g protein (16% total energy), 29.7 g carbohydrate (49%), 15 g sugars, 9.3 g fat (35%). Patients were also allowed to consume sugar-free squashes, smooth/clear soup (1 medium bowl per day), tea or coffee without sugar, or unsweetened puree. To standardise both therapy groups, all patients across both arms followed the liquid diet for this duration and period of the study.

3.2.4 Hyperinsulinaemic euglycaemic clamp

At visits 3 (- 2 weeks ± 7 days), 5 (+ 10 days ± 7 days) and 8 (+ 6 months ± 7 days) participants in sub-group 2 underwent a HEC. All glucose lowering medications were discontinued for one day prior to the clamp study visit i.e. participants were advised to take their usual oral glucose-lowering medications the morning of the day before the study visit and then not again until after the clamp was completed. Participants were also told to avoid alcohol or strenuous exercise in the 24 hours prior to the visit and on the evening prior to the clamp all participants consumed a fixed meal of 2 x 125 ml Fortisip Compact drinks, containing per 100 mL: 240 kcal, 9.6g protein (16% of total energy), 29.7 g carbohydrate (49%), 15g sugars, 9.3g fat (35%). They then remained fasted until after the clamp was completed.

Participants attended the Wellcome Trust Clinical Research Facility at Southampton General Hospital for their visit. On arrival in the morning, the patient had a clinical review in which their general wellbeing was assessed and any adverse events or changes to concomitant medications were recorded. They then had their blood pressure, pulse rate, weight, waist circumference and body composition measured as described in **section 2.2.7.1.** Each participant was seated in an infusion chair in a semi-recumbent position with their arms placed horizontally on armrests at chest height. An 18-20G cannula (B-D Insyte-W, Becton Dickinson, UK) was placed into a large vein in each antecubital fossa with one cannula being used for blood sampling and the other for administering multiple infusions via two 3-way taps. During cannulation, baseline blood samples were taken (see **Table 3.1** and **Table 3.2**).

The participant was then commenced on a variable rate insulin infusion: 50 international units (iu) of Actrapid® insulin diluted in 48 ml of 0.9% normal saline and 2 ml of the patient's own blood in a 50 ml luer-lock syringe. The infusion rate was adjusted in response to the patients' blood glucose level, which was measured at the bedside every 5-15 minutes using 0.5 ml of whole blood by the glucose oxidase method with a YSI biochemistry analyser (YSI Life Sciences, Yellow Springs, Ohio) until a stable blood glucose level of 4.0-6.0 mmol/L was achieved. During this phase, the [6, 6-$^{2}H_{2}$]-glucose tracer was prepared: 8.7 ml of [6, 6-$^{2}H_{2}$]-glucose (100 mg/ml) was diluted with 41.3 ml of normal saline in a 50 ml luer-lock syringe. Two 0.5 ml samples of this were taken for the measurement of [6, 6-$^{2}H_{2}$]-glucose concentration in the infusate.

Time (mins)	Glucose	Serum Insulin	Glucagon	NEFA	C-Peptide	FBC	Biochemistry	Gut hormones	DNA/RNA	Plasma metabolomics
Baseline	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
-120	✓	✓	✓	✓	✓					
-20	✓	✓								
-15	✓	✓								
-10	✓	✓								
-5	✓	✓								
0	✓	✓	✓	✓	✓					
+30	✓	✓		✓	✓					
+60	✓	✓								
+90	✓	✓	✓	✓	✓					
+100	✓	✓								
+110	✓	✓								
+120	✓	✓	✓	✓	✓					
+150	✓	✓		✓	✓					
+180	✓	✓								
+210	✓	✓	✓	✓	✓					
+220	✓	✓								
+230	✓	✓								
+240	✓	✓	✓	✓	✓					

Table 3.1 Schedule for blood sampling during hyperinsulinaemic euglycaemic clamp

Code	Name	Tube	Sample Type	No. aliquots	Aliquot vol.	Assay
Collected at each mechanistic visit:						
T	Trasylol Plasma	10mL Orange plastic	Li heparin plasma AEBSF Trasylol	4	≥1mL	PYY, GLP1
A	Acidified Plasma	10mL Orange plastic	Li heparin AEBSF Trasylol 0.05M HCl	4	160mcL	Ghrelin
U	Urine		none	4	1.8mL	Metabonomics
F	Faeces		none	4	1.8mL	Metabonomics
P	Plasma	6mL Green	Li heparin plasma	4	0.75mL	Metabonomics
DNA	DNA	10ml Purple	EDTA whole blood	1	tube	DNA extraction
RNA	RNA	2.5mL Dark orange	Paxgene whole blood	1	tube	RNA lymphocytes
Collected only during insulin clamps:						
S	Serum	6mL Red	none	2	1.5mL	Insulin, C-peptide
G	Glucose	2mL Grey	Flouride oxalate plasma	2	0.5mL	Glucose
H	Heparinised plasma	6mL Green	Li heparin plasma	1	1.8mL	Glucagon
N	NEFA	3mL Purple	EDTA plasma	1	1.5mL	NEFA
IG	Infusion labelled Glucose			2	1.8mL	
II	Infusion Insulin			2	1.8mL	
ID	Infusion Dextrose			2	1.8mL	

Table 3.2 Details of samples obtained during hyperinsulinaemic euglycaemic clamp

Once a stable blood glucose was achieved, at T = -120 mins, whole blood samples were taken for determination of baseline glucose enrichment followed immediately by a primed continuous infusion of the [6, 6-$^2H_2$]-glucose tracer (170 mg priming bolus followed by a 1.7 mg/min continuous infusion) which was infused at a constant rate for the duration of the clamp experiment. After approximately 100 minutes, a steady state of enrichment with the stable isotope was achieved and was measured by taking five baseline samples every 5 minutes between T = -20 and 0 min.

At T = 0 min, the blood glucose level was recorded as the 'clamped' glucose and a two-step HEC was initiated. For the first stage (T = 0 min to +120 min), Actrapid insulin was infused at a fixed rate of 0.5 mU/kg/min ('low dose') and blood glucose concentrations were measured every 5-10 minutes using the YSI analyser. Euglycaemia was maintained around the 'clamped' glucose level ± 0.5 mmol/L using a variable rate infusion of 20% dextrose (200 mg/ml) spiked with 8mg of [6, 6-$^2H_2$]-glucose per gram of dextrose. 500 ml of 20% dextrose is 100 g glucose and, therefore, for this first stage of the clamp the 500 ml bag of dextrose was mixed with 8 ml of [6, 6-$^2H_2$]-glucose (100 mg/ml) in order to prevent dilution of the tracer pool during the course of the study. Two 0.5 ml samples of the dextrose infusion were taken before and after spiking with [6, 6-$^2H_2$]-glucose for GC-MS analysis. The approximate rate of the variable dextrose infusion was calculated knowing the weight of the study participant in a range of 0.25 – 10 mg/kg/min. This was then converted to ml/hr in order to adjust rates easily using standard infusion pumps that operate with these units. Blood samples were taken every 30 minutes for the first 90 minutes and then every 10 minutes

for the final 30 minutes to measure the isotopic enrichment of glucose (plus serum insulin, glucagon, c-peptide and NEFA – see **Table 3.1**).

At T = +120 min, the second stage of the clamp was commenced by infusing Actrapid insulin at an increased fixed rate of 1.5 mU/kg/min ('high dose') for a further 2 hours. For this stage, the variable rate infusion of 20% dextrose was spiked with a further 2 mg/g of [6, 6-$^{2}H_{2}$]-glucose and euglycaemia was maintained as described above. After spiking, a further two 0.5 ml samples of the dextrose infusion were taken for GC-MS analysis. As the clamp proceeded, blood samples were obtained as per the schedule in **Table 3.1**.

Following completion of the clamp at +240 min, the insulin and tracer infusions were stopped but the 20% dextrose infusion was continued for a further 30 minutes and the participant was given a meal in order to avoid hypoglycaemia. After 30 minutes, the dextrose infusion was terminated and a further blood sample was taken to ensure that the patient's blood glucose concentration was back to normal before removing the dextrose lines. This was repeated again after a further 10 minutes before allowing the patient to be discharged home.

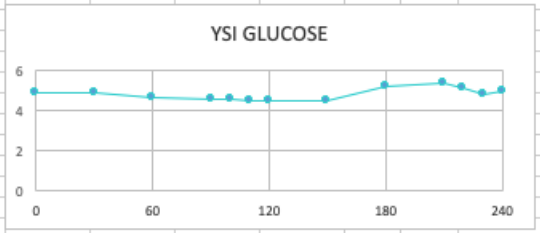
During the clamp, blood glucose concentrations were recorded in a data table. The time point at which the variable infusion was changed was recorded precisely in order to maintain an accurate chronological record of the actions taken during the clamp. Following the clamp, data were transcribed into an excel spreadsheet and precise infusion rates were calculated (**Figure 3.4**). As the majority of glucose uptake occurs in skeletal muscle and only a small proportion occurs in adipose tissue, the dextrose infusion rate could overestimate insulin sensitivity in obese subjects. Therefore, infusion rates were normalised to FFM.

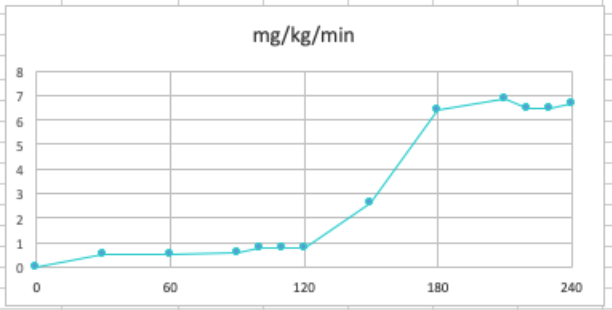
3.2.5 Sample collection and analysis

3.2.5.1 Routine biochemistry and haematology

Routine biochemical and haematological samples collected at baseline were transferred immediately to the central chemical pathology laboratory at Southampton General Hospital. Whole blood samples were collected into fluoride oxalate, K2 EDTA and plastic serum BD Vacutainer® Tubes for the measurement of plasma glucose, HbA_{1C} and serum insulin concentrations, respectively. The precise methods used for analysing these samples are fully described in **Section 2.2.7.4.1**.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1				Weight (kg)	78.6	41.6	ffm	DEX Conc	200	mg/ml							
2																	
3	start	end	Time	ml/hr	ml	ml/min	mg/kg/min	µmol/kg/min		Time	ml/min	mg/kg/min	µmol/kg/mi	µmol/ffmkg/min			whole blood
4	**0**	**30**	30	12	6	**0.2**	**0.508906**	**2.827255**		0	0	0	0			**TIME**	**YSI GLUCOSE**
5	**30**	**60**	30	12	6	**0.2**	**0.508906**	**2.827255**		**30**	**0.2**	**0.508906**	**2.827255**	5.34188		0	4.88
6	**60**	82	22	12	4.4					**60**	**0.2**	**0.508906**	**2.827255**	5.34188		**30**	4.83
7	82	**90**	8	18	2.4	**0.226667**	**0.57676**	**3.204222**		**90**	**0.226667**	**0.57676**	**3.204222**	6.054131		**60**	4.65
8	**90**	**100**	10	18	3	**0.3**	**0.763359**	**4.240882**		**100**	**0.3**	**0.763359**	**4.240882**	8.012821		**90**	4.56
9	**100**	**110**	10	18	3	**0.3**	**0.763359**	**4.240882**		**110**	**0.3**	**0.763359**	**4.240882**	8.012821		**100**	4.57
10	**110**	**120**	10	18	3	**0.3**	**0.763359**	**4.240882**		**120**	**0.3**	**0.763359**	**4.240882**	8.012821		**110**	4.45
11	**120**	134	14	35	8.166667					**150**	**1.031667**	**2.625106**	**14.58392**	27.5552		**120**	4.44
12	134	141	7	59	6.883333					**180**	**2.507222**	**6.3797**	**35.44278**	66.96641		**150**	4.42
13	141	**150**	9	106	15.9	**1.031667**	**2.625106**	**14.58392**		**210**	**2.683333**	**6.82782**	**37.93233**	71.67023		**180**	5.14
14	**150**	157	7	106	12.36667					**220**	**2.55**	**6.48855**	**36.0475**	68.10897		**210**	5.31
15	157	166	9	153	22.95					**230**	**2.55**	**6.48855**	**36.0475**	68.10897		**220**	5.11
16	166	173	7	189	22.05					**240**	**2.61**	**6.641221**	**36.89567**	69.71154		**230**	4.79
17	173	**180**	7	153	17.85	**2.507222**	**6.3797**	**35.44278**								**240**	4.9
18	**180**	182	2	153	5.1												
19	182	202	20	165	55												
20	202	**210**	8	153	20.4	**2.683333**	**6.82782**	**37.93233**									
21	**210**	**220**	10	153	25.5	**2.55**	**6.48855**	**36.0475**									
22	**220**	**230**	10	153	25.5	**2.55**	**6.48855**	**36.0475**									
23	**230**	231	1	153	2.55												
24	**231**	**240**	9	157	23.55	**2.61**	**6.641221**	**36.89567**									
25																	
26																	
27																	
28																	
29																	
30																	





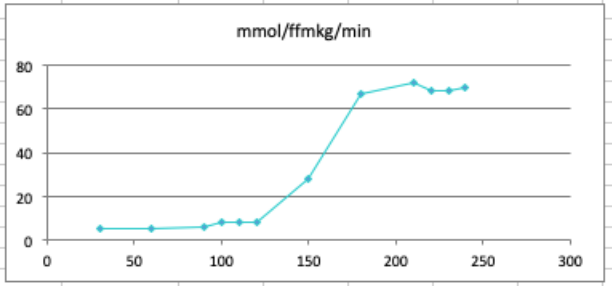


Figure 3.4 Example of clamp summary spreadsheet

3.2.5.2 Infusate samples

Infusate samples ([6, 6-$^{2}H_{2}$]-glucose infusion, insulin infusion, infusion dextrose, infusion dextrose post-spiking (first stage) and infusion dextrose post-spiking (second stage)) were stored in 0.5 ml aliquots at - 80°C until analysis was performed.

3.2.5.3 Clamp serum insulin samples

6 ml of whole blood was collected into red plastic serum BD Vacutainer® tubes for measurement of serum insulin concentrations. Samples were left to clot for 10 minutes at room temperature before then centrifuging at 4°C for 10 minutes at 4000 rpm. 1.5 ml of serum was aliquoted into a cryotube and stabilised by deep-freezing at - 80°C until analysis was performed. Analysis of all insulin samples was performed at the Diabetes Complications Research Centre, University College Dublin, under the supervision of Professor Carel Le Roux with an ARCHITECT Insulin assay (Abbott Laboratories, IL, USA) (**Appendix C**).

3.2.5.4 Clamp plasma glucose samples

2 ml of whole blood was collected into fluoride oxalate BD Vacutainer® tubes for the estimation of the isotopic enrichment of plasma glucose by GC-MS and also for determination of plasma glucose concentrations. After collection, samples were immediately placed on ice and centrifuged at 4°C for 10 minutes at 4000 rpm. 0.5 ml of plasma was aliquoted into two separate cryotubes and stabilised by deep-freezing at - 80°C until analysis was performed. Plasma glucose samples obtained from the clamp studies were analysed at the Wolfson Centre for Translational Research, University of Surrey, by myself, Nicola Jackson and Lucy Coppin.

3.2.5.5 Quantitative in-vitro determination of glucose PAP in plasma by colorimetry

Plasma glucose concentrations were measured on a Mira autoanalyser using the glucose PAP assay (Horiba) with units being expressed as mmol/L.

3.2.5.6 Quantitative measurement of the isotopic enrichment of plasma glucose by gas chromatography-mass spectrometry

3.2.5.6.1 Principles of gas chromatography

Gas chromatography (GC) is a reproducible and reliable method for high-resolution separation of compounds for subsequent measurement by one of several methods, such as mass spectrometry (MS) or flame ionisation detection (FID). To be separated by GC, a sample, or its derivatives, must enter a volatile state at temperatures below 300°C and be chemically inert and thermally stable

during this process. Polar hydroxyl groups particularly reduce the volatility of biological substrates by generating strong intermolecular hydrogen bonds. As such, in order to increase volatility, these compounds need to go through a process of derivatisation in which the hydrogen atoms of this group are replaced through alkylation, acylation or silylation.

A schematic diagram of the GC-MS system is shown in **Figure 3.5.** The components of a sample can be quantitatively measured as they partition between two phases; the stationary phase and the gas phase. In brief, the sample to be analysed is vaporised (typically at 250°C) in the heated injection port and carried into an oven-heated capillary column by an inert carrier gas (i.e. the gas phase), such as helium or hydrogen. The stationary phase can either be an inert solid or non-volatile liquid that lines the capillary column. At low temperatures, components of a sample will condense rapidly onto the column lining/stationary phase and then, as the column is heated, each component will dissociate from the stationary phase back into the gas phase as their boiling point is reached. Each component of the sample will therefore separate differentially and exit the column at different times depending on its biochemical structure, relative affinity for the stationary phase and boiling point. Thus, temperature is used as a means of improving compound separation through a capillary column, or by the use of an alternative stationary phase.

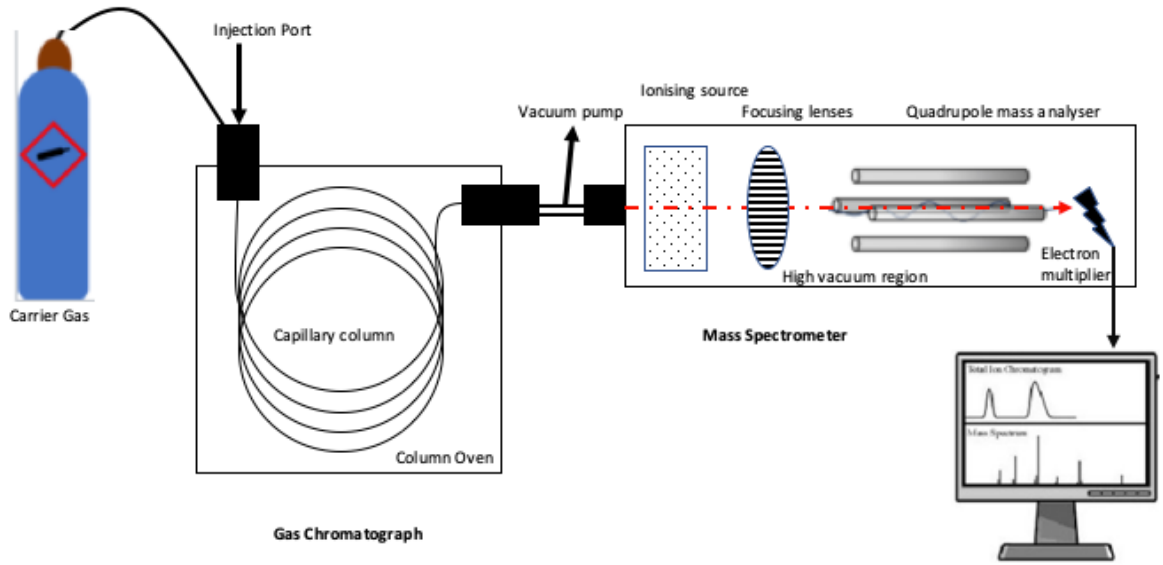


Figure 3.5 Schematic diagram of gas chromatograph and mass spectrometer

At any one time, the different compounds in any given sample will present partially in the stationary phase and partially in the gas phase, in accordance with their vapour pressure. The ratio of weight of solute per ml in the stationary phase to weight of solute per ml in the gas phase is the partition coefficient (K). Compounds with a high vapour pressure will have a low K and will present to a larger extent in the gas phase and leave the column (elute) at a quicker rate. Compared to compounds with a low vapour pressure that will have a relatively higher K and will present predominantly in the stationary phase, therefore leaving the column at a slower rate. Differences in the partition coefficient therefore allow for the chromatographic separation, which is identified and recorded electronically. To measure a sample with an unknown concentration, a sample with a known concentration is also injected into the instrument to act as a standard sample peak retention time and area to which all test samples can be compared and calculated.

3.2.5.6.2 Principles of mass spectrometry and flame ionisation detection

GC proceeds to detection and measurement as the eluting compounds exit the capillary column and enter the mass spectrometer. The basic principle of MS is that the differential masses of charged ions are measured by passing them through an electromagnetic field, presenting each component of an analyte as a peak on a mass spectrum. Relative and absolute abundancies of ionic components are therefore measured relative to their mass/charge ratio (m/z).

A separator sits at the interface between the GC and MS in order to reduce the pressure and flow rate of the carrier gas whilst simultaneously increasing the relative amount of the sample entering the spectrometer. First, charged ions are created by passing the sample molecules through a beam of electrons (electron impact ionisation) or an ionised reagent gas (chemical ionisation) within an ionisation chamber. These ions then pass through to the mass analyser. The quadrupole analyser consists of four parallel circular rods that radiate an electromagnetic field with both DC and radiofrequency (RF) components. At a specified RF, ions entering the field will oscillate and drift between the electrodes and, therefore, the mass filter will only allow ions of a certain mass to pass through. A range of ionic masses or a single selected ion mass, depending on the magnitude of the field, are allowed to pass through the filter whilst all other masses are removed. Each ion then exits the mass analyser and strikes an electron multiplier, which causes electrons to be ejected in an exponential fashion that is proportional to the mass (m) and charge (z) of the incident ion and the voltage applied to the electron multiplier. This is then detected and the data processed, analysed and interpreted. Data may be represented as a total ion chromatogram (TIC), which demonstrates the total ionic abundances over time, or as a mass spectrum, which is a bar graph representing the relative abundancies of all of the ionic fragments against mass to charge ratio (m/z) that result from the impact of ionisation on a molecule. The fragmentation of any

compound under certain MS conditions is highly distinctive and the resultant spectrum can be used for identification purposes. Peak areas on the TIC are used to quantify ionic abundances.

FID is an alternative technique of quantifying molecular abundancies. In this process, a hydrogen flame is used to combust the elutes of GC, which creates an ion current proportional to the amount of molecule passing through it. This can then be recorded and the resulting chromatogram will contain a number of peaks corresponding to each molecule detected. The area under the curve of each peak is proportional to the mass of the molecule injected onto the column.

3.2.5.6.3 Derivatisation of glucose

Prior to quantitative measurement by GC-MS, plasma glucose samples obtained from the clamp study were processed in order to prepare methyloxime penta-O-trimethylsilyl derivatives (MOX-TMS; penta-O-trimethylsilyl-D-glucose-O-methyloxime) of glucose (tracee) and [6, 6-$^{2}H_{2}$]-glucose (tracer) (**Figure 3.6**).

Plasma samples and spiked dextrose infusates, which were used as quality controls, were first fully thawed and mixed before then centrifuging them at 4°C at 2500 rpm for 10 minutes in order to remove any proteins. 0.5 ml ethyl alcohol was added to a 50 µl aliquot of each plasma sample/QC in a glass tube, vortexed and then centrifuged at 4°C at 2500 rpm for 10 minutes. The supernatant was then transferred to a ½ dram vial and dried under oxygen-free nitrogen (OFN) at 50°C for 25 minutes before then adding 100 µl methylhydroxamine hydrochloride freshly made in pyridine 2% (0.02 g/ml). Tubes were then capped, vortexed and heated at 90°C for 120 minutes. 50 µl of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane was subsequently added and heated at 120°C for 15 minutes and samples were then completely dried under OFN at room temperature for approximately 30 minutes. Samples were reconstituted in 500 µl of decane before then transferring 50 µl of each sample plus 500 µl of decane into an autosampler vial.

Dextrose infusate samples were first diluted in distilled water (10 µl dextrose in 3 ml di$H_{2}O$) before then taking 50 µl aliquots and adding 500 µl of ethyl alcohol to each. Samples were then prepared for MS according to the methodology for plasma samples described above.

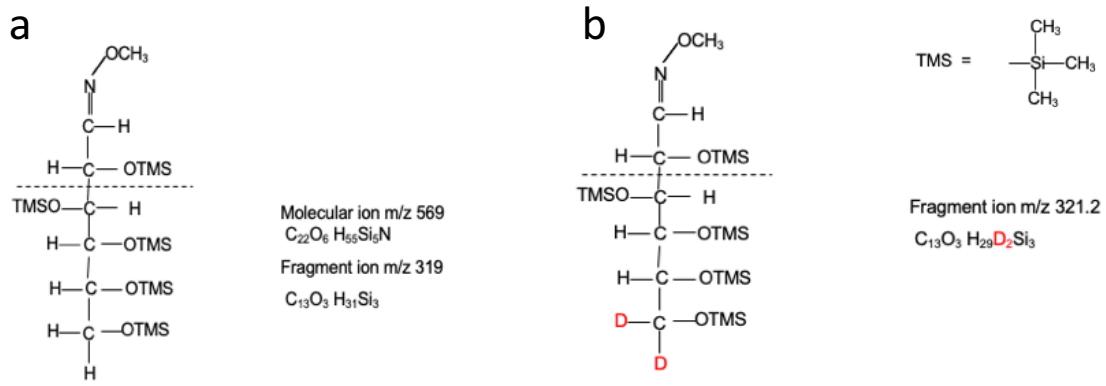


Figure 3.6 methyloxime penta-O-trimethylsilyl derivatives of (a) glucose, and (b) [6, 6-$^{2}H_2$] glucose Note that the H of each hydroxyl group is replaced with a O-trimethylsilyl (OTMS) group. The dotted line denotes the point of division between ion fragments (C3-C6 fragments are 319.2 m/z and 321.2 m/z respectively).

3.2.5.6.4 Quantitative measurement of MOX-TMS derivatives of glucose and [6, 6-2H_2]-glucose by gas chromatography-mass spectrometry

MOX-TMS derivatives were analysed by GC-MS on an Agilent Technologies 5975C inert Xl EI/CI MSD system with 7890A GC and 7683B autosampler. Separation was performed on an Rxi-1 ms (cross-linked dimethyl polysiloxane) capillary column with dimensions of 30 m x 0.25 mm ID 0.25 um DF. The injector port was set at 250°C with a helium carrier gas at a flow rate of 1.2 ml/min. Samples (1 µl) were injected into the autosampler in the splitless mode. To condense analytes, the column oven was held at an initial temperature of 120°C for 2 minutes, then increasing by 20°C min^{-1} up to 300°C, where it was then held for 1 min. The ion source of the MS was heated indirectly to 230°C by the GC-MS connecting line, which was set at 280°C. The electron ionisation energy was 70 eV with electron impact (EI) ionisation. Glucose and [6, 6-$^{2}H_2$]-glucose derivatives were analysed in a single GC-MS run in the EI mode to obtain mass spectra from m/z 50 – 650 (**Figure 3.7**). In the selected ion monitoring mode, the ions measured were m/z 319.2 ± 0.3 and 321 ± 0.3 with a dwell time of 25 ms for MOX-TMS derivatives of glucose and [6, 6-$^{2}H_2$]-glucose respectively (**Figure 3.8**).

TICs were analysed with Agilent Chemstation software and peak areas were used to quantify total ionic abundances. Peaks were identified automatically and, following peak integration, the area under each peak was used in order to calculate isotopic enrichment:

$$\frac{Area\ of\ peak\ at\ m/z\ 321}{Area\ of\ peak\ at\ m/z\ 319} = \frac{tracer}{tracee}$$

3.2.5.7 Determination of Ra and Rd

Tracer and tracee concentrations obtained from GC-MS were used to calculate rates of glucose appearance/HGP (Ra) and rates of glucose disappearance/peripheral glucose disposal (Rd) from plasma as described in **section 3.1.2.1**. Calculations were performed by Professor Anne Margot Umpleby at the Wolfson Centre for Translational Research, University of Surrey, using non-steady-state equations proposed by Steele and modified for stable isotopes.[327] Steady-state Ra and Rd values during the basal, low-dose and high-dose phases of the clamp were corrected for the mean insulin concentration during the respective steady-state phase.

3.2.6 Statistical analysis

This sub-study performs exploratory analyses as part of the main RCT and, as such, no formal power calculation was performed for these outcomes. For baseline data, normally distributed variables are reported as means with standard deviation (SD) and for comparison of these variables the independent t-test was used. Non-normally distributed variables are reported as medians with the interquartile range, and the Mann-Whitney U test was performed to compare such variables. Categorical variables were compared using the Chi-square test or the Fisher's exact test as appropriate. A p-value <0.05 was considered statistically significant. Analysis was performed using SPSS® 25.0 software (SPSS, Chicago, IL, USA) and mechanistic data were assessed using a mixed model approach as described in **Section 2.2.9**. The Pearson product-moment correlation coefficient was used on normally distributed datasets to evaluate the relationship between variables of interest.

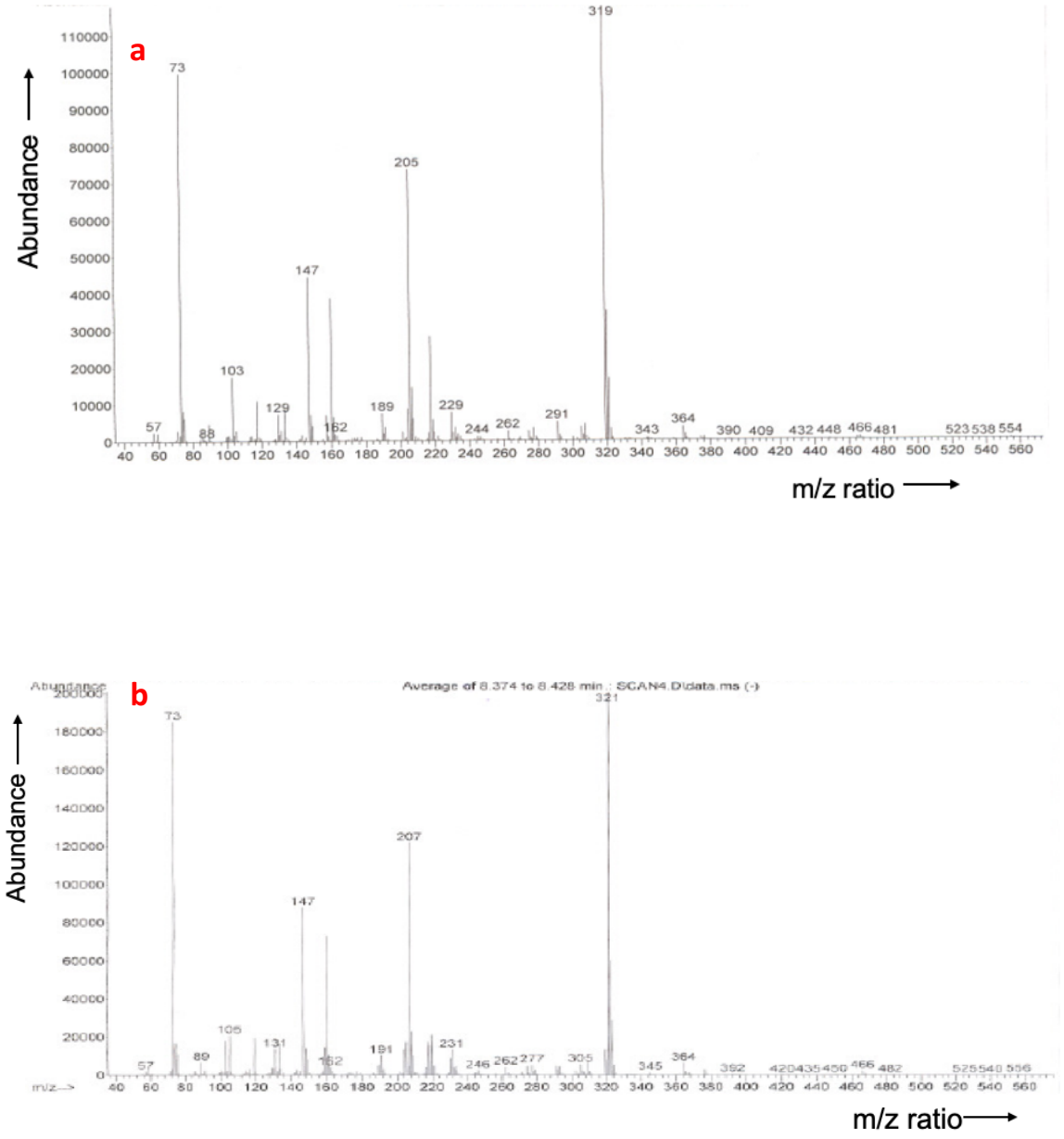


Figure 3.7 Mass spectrum for MOX-TMS derivatives of (a) glucose and (b) [6, 6-2H_2] glucose

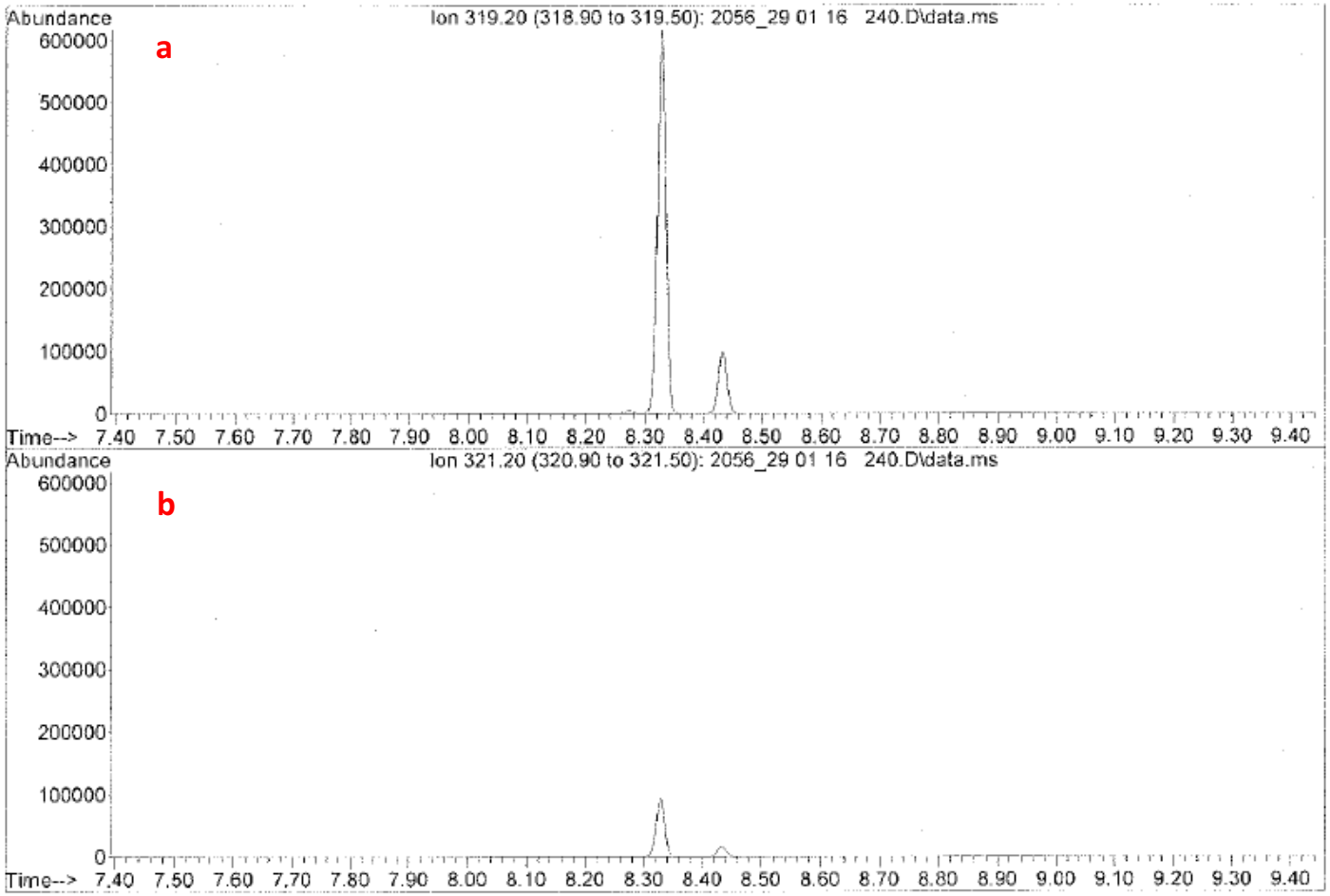


Figure 3.8 Total ion chromatogram obtained from GC-MS analysis of MOX-TMS derivatives of (a) glucose and (b) [6, 6-2H_2] glucose

3.3 Results

3.3.1 Patient characteristics and baseline data

Figure 3.9 summarises the flow of participants through the trial that were included in the insulin clamp sub-group at study visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), and 8 (+6 months ± 7 days). For a full description of the recruitment outcomes of the Endobarrier trial please refer to **section 2.3.1** and the consort diagram in **Figure 2.8**, in which the reasons for patient attrition/non-inclusion are fully detailed. Missing or insufficient samples account for all other unavailable data. For the purposes of this sub-group analysis, visit 3 (-2 weeks ± 7 days, i.e. pre-intervention) served as the baseline visit. No serious adverse events occurred during the clamp procedures.

Thirty-five patients provided additional consent to take part in the insulin clamp sub-group of the Endobarrier trial. Two patients in the Endobarrier arm and 1 patient in the control arm of the study withdrew from the sub-group following the first clamp and were not included within the final analysis. The baseline characteristics of the two groups are presented in **Table 3.3** and were representative of the whole study population (**Table 2.4**)**.** Of note, the control group within this sub-study had a significantly lower baseline BMI compared to the Endobarrier group (33.9 $\pm$ 3.3 vs. 36.8 $\pm$ 5.0, p = 0.029). Groups were otherwise comparable at their baseline visit.

	Controls (n=17) mean ± SD	**Endobarrier (n=15) mean ± SD**	**p-value**
Age, years	53 ± 9	52 ± 6	0.804
Sex, n (%) Female	4 (24)	7(47)	0.169
Weight, kg	104.4 ± 14.6	107.6 ± 17.4	0.531
BMI, kg/m^2	33.9 ± 3.3	36.8 ± 5.0	0.029
Fasting Glucose, mmol/L	10.4 ± 2.7	11.0 ± 2.9	0.529
Fasting Insulin, mU/L	11.7 ± 5.6	11.5 ± 4.2	0.978
HbA1C, mmol/mol	71 ± 7	72 ± 9	0.841
Basal Ra, μmol/kg/min	10.5 ± 1.2	10.2 ± 1.2	0.601
Basal Rd, μmol/kg/min	10.6 ± 1.2	10.3 ± 1.2	0.600

Table 3.3 Baseline characteristics of participants included in insulin clamp sub-study. SD, standard deviation; kg, kilograms; BMI, body mass index.

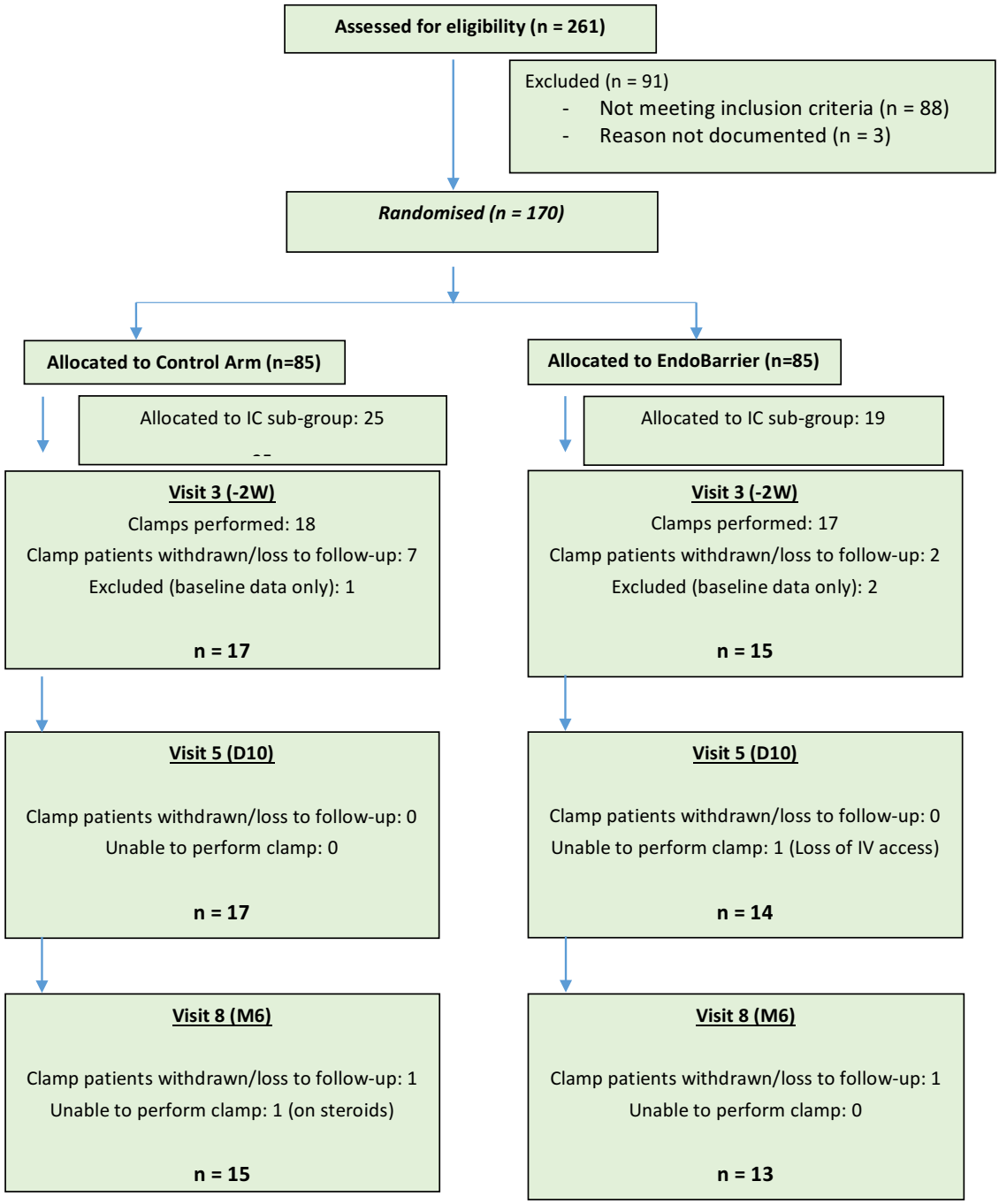


Figure 3.9 Consort flow chart of participants included in insulin clamp sub-group analysis.

3.3.2 Anthropometric outcomes

Anthropometric outcomes are summarised in **Table 3.5.** Both groups significantly reduced their weight between their baseline clamp visit and their second visit at + 10 days. Between + 10 days and + 6 months, the weight in the control group plateaued whereas the weight in the Endobarrier cohort continued to reduce significantly. Absolute and total % weight loss was significantly greater in the DJBL group when compared to the control group at 10 days (7.2 $\pm$ 3.3 kg versus 4.4 $\pm$ 2.1 kg (mean difference [95% CI] = 3.21 kg [0.13, 6.30], p = 0.042)); 6.6 $\pm$ 2.4% versus 4.2 $\pm$ 1.7% (2.7% [0.3, 5.1], p = 0.031)) and 6 months (13.3 $\pm$ 6.3 kg versus 5.7 $\pm$ 5.0 kg (7.99 kg [4.80, 11.17], p < 0.001); 12.3 $\pm$ 4.8% vs 5.2 $\pm$ 4.3% (7.3% [4.8, 9.8], p < 0.001)).

3.3.3 Glycaemic control outcomes

Glycaemic control outcomes are summarised in **Table 3.6.** In the Endobarrier arm there was a significant reduction in fasting plasma glucose concentrations from 11.0 $\pm$ 2.9 mmol/L at baseline to 8.5 $\pm$ 3.0 mmol/L at 10 days, which was then maintained at 6 months (8.7 $\pm$ 2.4 mmol/L). In the control group, there was also a significant reduction in fasting glucose from 10.4 $\pm$ 2.7 mmol/L at baseline to 7.6 $\pm$ 1.8 mmol/L at 10 days but this increased significantly again by 6 months to 9.2 $\pm$ 2.6 mmol/L. Fasting insulin levels followed the same trend. There were no significant differences in fasting glucose or insulin values between groups at baseline, + 10 days or 6 months. Overall glycaemic control, as demonstrated by a decrease in HbA_{1C}, significantly improved in both groups at + 10 days. In the Endobarrier group, HbA_{1C} significantly decreased further between + 10 days and + 6 months with a total decrease in HbA_{1C} of 17 mmol/mol from baseline. In the control group, HbA_{1C} levels plateaued between 10 days and 6 months with a total decrease in HbA_{1C} of 14 mmol/mol from baseline. There was no significant difference in HbA_{1C} between groups at any of the follow-up visits. There was a reduction in the median number of oral glucose-lowering medications taken by patients in the Endobarrier group but this was not statistically significant in comparison to the control group (**Table 3.4**).

Medications	**Endobarrier n = 15** Median (range)	**Controls n = 17** Median (range)	**p-value**
Baseline	2 (1-3)	2 (1-3)	1.000
10 days	2 (1-3)	2 (1-3)	0.637
6 Months	1 (1-2)	2 (1-3)	0.121

Table 3.4 Change in number of glucose-lowering medications

	Control		Endobarrier		Mixed Model Analysis	
	n	**Mean** ± SD	**n**	**Mean** ± SD	**Effect**	**p-value**
		Weight (kg)				
Baseline	17	104.4 ± 14.6	15	107.6 ± 17.4	Group	0.911
10 days	17	100.0 ± 13.5*	14	98.7 ± 14.8*	Visit	<0.001
6 months	15	100.2 ± 13.2*	13	91.9 ± 13.5*§	Group*Visit	<0.001
		BMI (kg/m^2)				
Baseline	17	33.9 ± 3.3†	15	36.8 ± 5.0†	Group	0.210
10 days	17	32.4 ± 3.0*	14	33.7 ± 3.8*	Visit	<0.001
6 months	15	32.5 ± 3.1*	13	32.3 ± 4.2*§	Group*Visit	<0.001
		Total Body Weight Loss (kg)				
Baseline	17	-	15	-	Group	<0.001
10 days	17	4.4 ± 2.1†	14	7.2 ± 3.3†	Visit	<0.001
6 months	15	5.7 ± 5.0$^{\Delta}$	13	13.3 ± 6.3$^{\S\Delta}$	Group*Visit	0.003
		Total Body Weight Loss (%)				
Baseline	17	-	15	-	Group	<0.001
10 days	17	4.2 ± 1.7†	14	6.6 ± 2.4†	Visit	<0.001
6 months	15	5.2 ± 4.3$^{\Delta}$	13	12.3 ± 4.8$^{\S\Delta}$	Group*Visit	0.002

Table 3.5 Anthropometric outcomes in sub-group 2

*denotes $p < 0.001$ compared to baseline within the same group, § denotes $p < 0.001$ compared to 10 days within the same group, † denotes $p < 0.05$ between groups, Δ denotes $p < 0.001$ between groups

3.3.4 Insulin sensitivity Outcomes

Insulin sensitivity outcomes are summarised in **Table 3.7** and **Table 3.8.** There were significant reductions in Ra within both groups at 10 days compared to baseline. This was maintained at 6 months in the Endobarrier group but returned to levels similar to baseline in the control group. There were no significant differences between treatment arms (**Figure 3.10**). Rd increased significantly at 10 days within both groups but there were no significant differences between groups. Rd continued to increase significantly only in the Endobarrier group at 6 months, but returned to levels similar to baseline in the control group. Rd was significantly higher in the Endobarrier group compared to the control group at the 6 months follow-up visit (**Figure 3.11**).

	Control		Endobarrier		Mixed Model Analysis	
	n	**Mean** ± SD	**n**	**Mean** ± SD	**Effect**	**p-value**
		Fasting Glucose (mmol/L)				
Baseline	17	10.4 ± 2.7	15	11.0 ± 2.9	Group	0.807
10 days	17	7.6 ± 1.8*	14	8.5 ± 3.0*	Visit	< 0.001
6 months	15	9.2 ± 2.6**$^{\alpha}$	13	8.7 ± 2.4*	Group*Visit	0.053
		Fasting Insulin (mU/L)				
Baseline	17	11.7 ± 5.6	14	11.5 ± 4.2	Group	0.643
10 days	16	8.7 ± 5.1***	14	9.8 ± 4.3	Visit	0.002
6 months	15	11.4 ± 5.5$^{\alpha}$	13	8.5 ± 2.8*	Group*Visit	0.020
		HbA1C (mmol/mol)				
Baseline	17	71 ± 7	15	72 ± 9	Group	0.784
10 days	17	58 ± 8*	14	63 ± 17***	Visit	< 0.001
6 months	15	57 ± 15*	13	55 ± 16*$^{\alpha}$	Group*Visit	0.266

Table 3.6 Glycaemic control outcomes in sub-group 2

* denotes p < 0.001 compared to baseline within the same group, ** denotes p < 0.05 compared to baseline within the same group, *** denotes p < 0.01 compared to baseline within the same group, $^{\alpha}$ denotes p < 0.05 compared to 10 days within the same group.

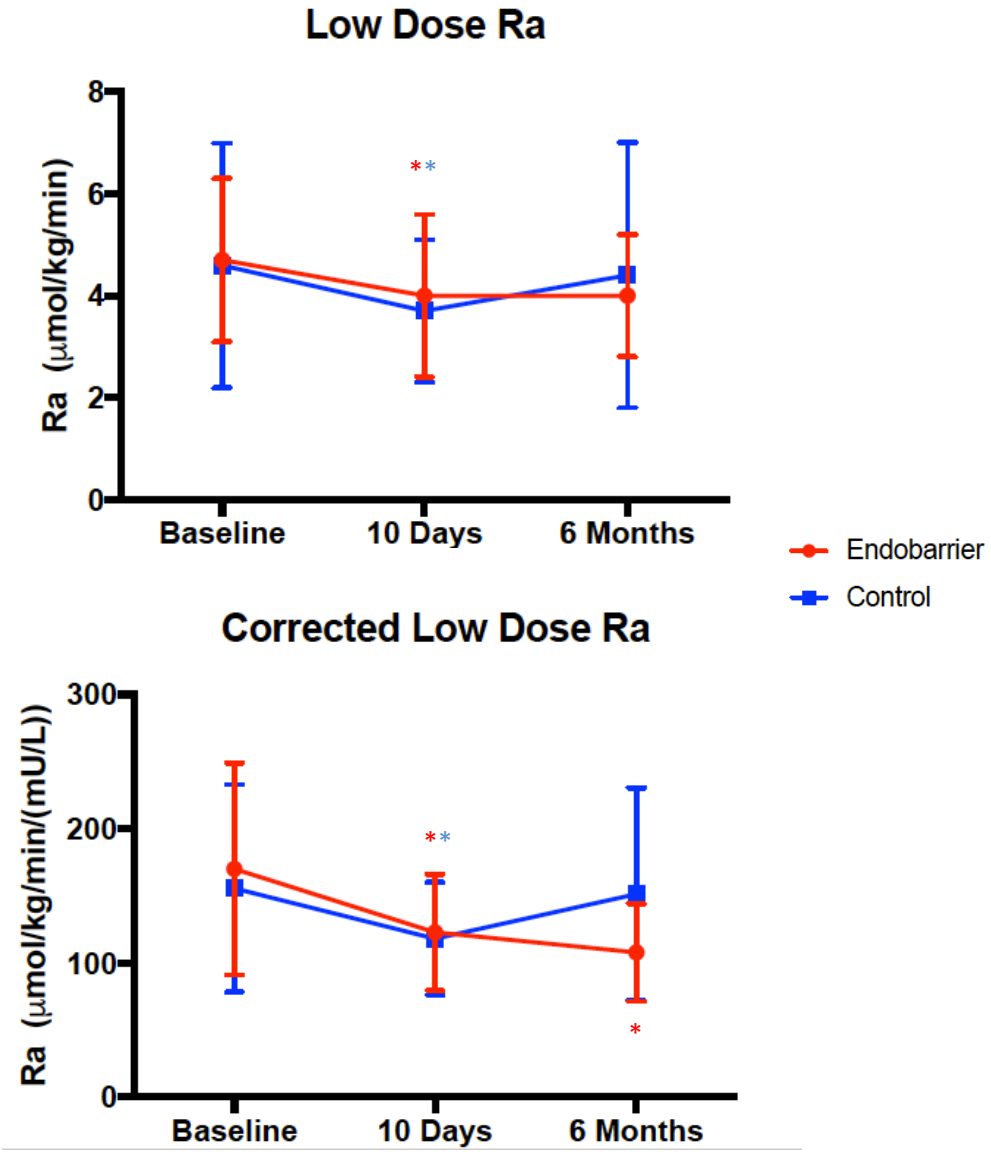


Figure 3.10 Rate of appearance of glucose (Ra) during hyperinsulinaemic euglycaemic clamp with corrections for prevailing insulin concentration.
* denotes $p < 0.05$ compared to baseline within the same group

	Control		Endobarrier		Mixed Model Analysis	
	n	**Mean** ± SD	**n**	**Mean** ± SD	**Effect**	**p-value**
		Low Dose Ra (μmol/kg/min)				
Baseline	17	4.6 ± 2.4	15	4.7 ± 1.6	Group	0.986
10 days	17	3.7 ± 1.4**	14	4.0 ± 1.6**	Visit	0.009
6 months	15	4.4 ± 2.6	13	4.0 ± 1.2	Group*Visit	0.440
		Corrected Low Dose Ra (μmol/kg/min/(mU/L))				
Baseline	17	155.6 ± 77.3	15	170.0 ± 79.0	Group	0.690
10 days	17	118.0 ± 42.1**	14	122.9 ± 43.3**	Visit	0.004
6 months	15	151.3 ± 79.2	13	107.9 ± 36.6***	Group*Visit	0.110
		High Dose Ra (μmol/kg/min)				
Baseline	17	0.91 ± 1.30	14	1.18 ± 1.18	Group	0.759
10 days	17	0.36 ± 1.56	14	0.80 ± 1.16	Visit	0.285
6 months	15	1.03 ± 2.25	12	0.03 ± 1.37**	Group*Visit	0.055
		Corrected High Dose Ra (μmol/kg/min/(mU/L))				
Baseline	17	91.6 ± 165.4	14	158.7 ± 164.6	Group	0.531
10 days	17	27.8 ± 181.1	14	126.4 ± 91.5	Visit	0.365
6 months	15	103.6 ± 262.1	12	31.2 ± 147.7**	Group*Visit	0.059

Table 3.7 Rate of appearance of glucose (Ra) during two step hyperinsulinaemic euglycaemic clamp with correction for prevailing insulin concentration.
* denotes p < 0.001 compared to baseline within the same group, ** denotes p < 0.05 compared to baseline within the same group, *** denotes p < 0.01 compared to baseline within the same group, § denotes p < 0.001 compared to 10 days within the same group, α denotes p < 0.05 compared to 10 days within the same group, † denotes p < 0.05 between groups.

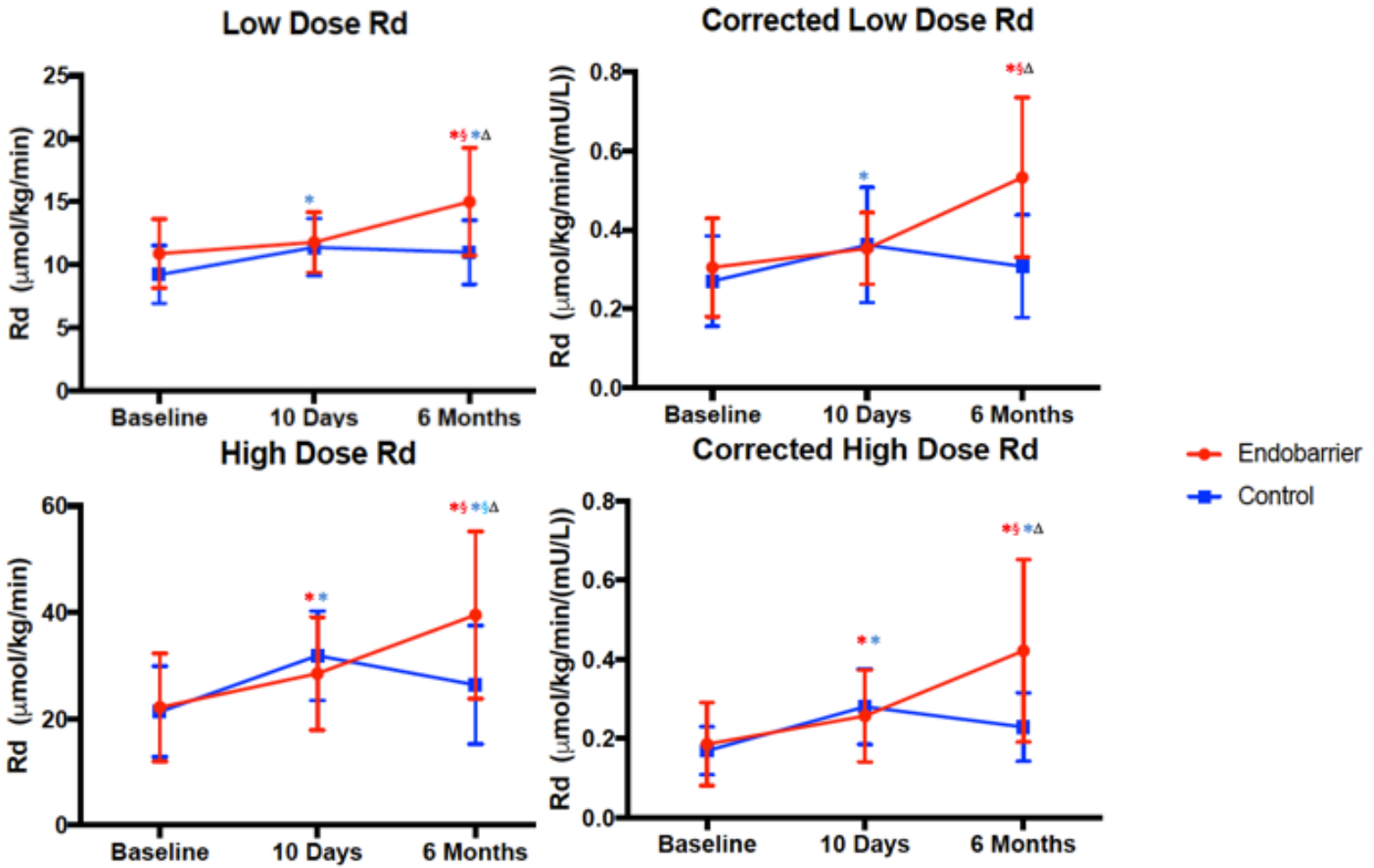


Figure 3.11 Rate of disappearance of glucose from plasma (Rd) during two step hyperinsulinaemic euglycaemic clamp with corrections for prevailing insulin concentration. * denotes $p < 0.05$ compared to baseline within the same group, § denotes $p < 0.05$ compared to 10 days within the same group, Δ denotes $p < 0.05$ between groups.

	Control		Endobarrier		Mixed Model Analysis	
	n	**Mean** ± SD	**n**	**Mean** ± SD	**Effect**	**p-value**
		Low Dose Rd (μmol/kg/min)				
Baseline	15	9.24 ± 2.29	15	10.9 ± 2.75	Group	0.023
10 days	15	11.4 ± 2.28***	14	11.8 ± 2.40	Visit	< 0.001
6 months	14	11.0 ± 2.54**$^{\Delta}$	13	15.0 ± 4.27*$^{\S\Delta}$	Group*Visit	0.002
		Corrected Low Dose Rd (μmol/kg/min/(mU/L))				
Baseline	15	0.270 ± 0.115	15	0.305 ± 0.125	Group	0.050
10 days	15	0.362 ± 0.146***	14	0.353 ± 0.091	Visit	< 0.001
6 months	14	0.308 ± 0.130$^{\Delta}$	13	0.533 ± 0.202*$^{\S\Delta}$	Group*Visit	< 0.001
		High Dose Rd (μmol/kg/min)				
Baseline	15	21.3 ± 8.51	14	22.2 ± 10.1	Group	0.313
10 days	15	31.9 ± 8.42*	14	28.5 ± 10.6**	Visit	< 0.001
6 months	14	26.4 ± 11.1**$^{\alpha\dagger}$	13	39.5 ± 15.8*§†	Group*Visit	< 0.001
		Corrected High Dose Rd (μmol/kg/min/(mU/L))				
Baseline	15	0.169 ± 0.061	14	0.186 ± 0.105	Group	0.124
10 days	15	0.280 ± 0.096*	14	0.257 ± 0.116**	Visit	< 0.001
6 months	14	0.229 ± 0.087**$^{\Delta}$	13	0.422 ± 0.231*$^{\S\Delta}$	Group*Visit	< 0.001

Table 3.8 Rate of disappearance of glucose from plasma (Rd) during two step hyperinsulinaemic euglycaemic clamp with correction for prevailing insulin concentration

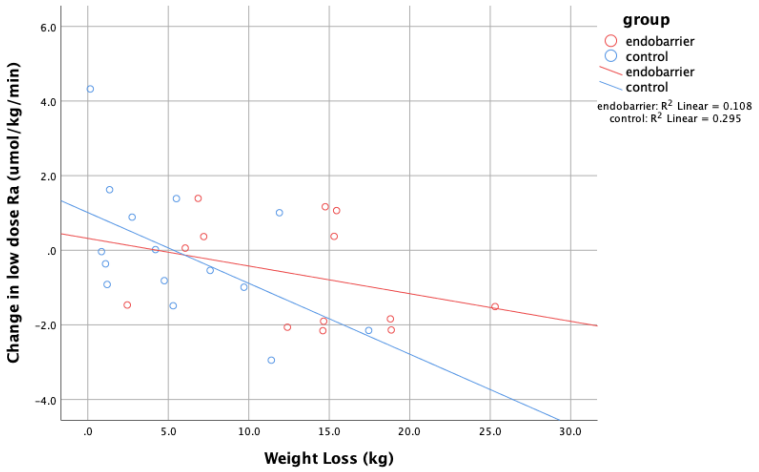
* denotes p < 0.001 compared to baseline within the same group, ** denotes p < 0.05 compared to baseline within the same group, *** denotes p < 0.01 compared to baseline within the same group, § denotes p < 0.001 compared to 10 days within the same group, $^{\alpha}$ denotes p < 0.05 compared to 10 days within the same group, † denotes p < 0.05 between groups, Δ denotes p < 0.001 between groups.

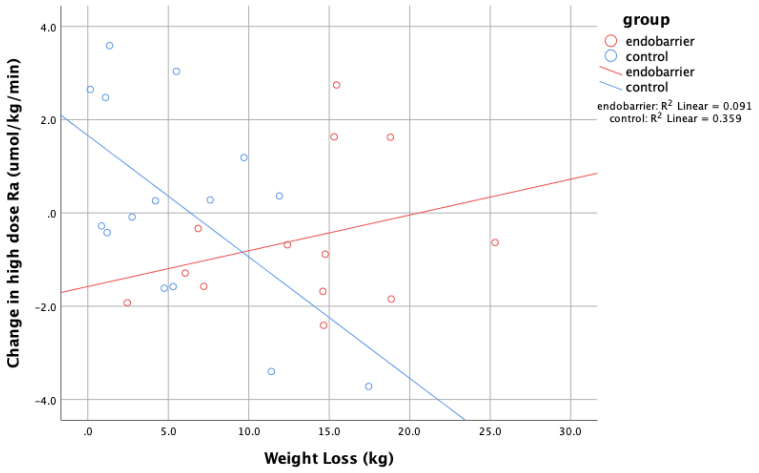
3.3.5 Correlations

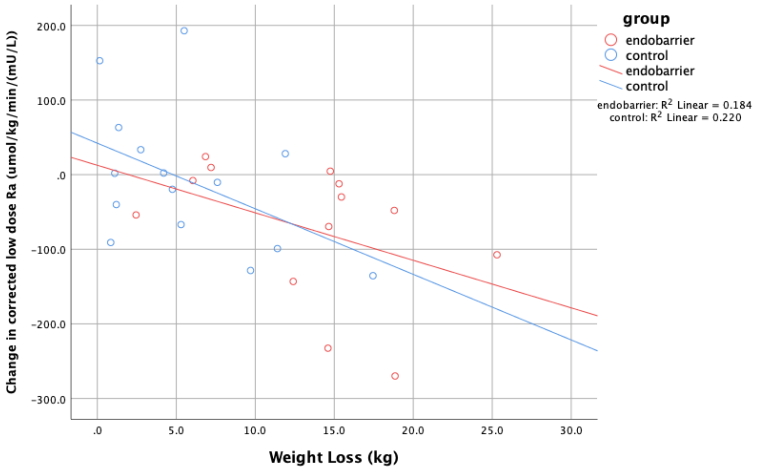
Pearson product-moment correlations were run to determine the relationship between changes in weight, HbA1c and clamp indices of peripheral and hepatic insulin sensitivity in patients completing 6 months of follow-up (**Table 3.9**). In the control group, there was a strong negative correlation between increasing weight loss and changes in Ra (low dose Ra, r = -0.543, p = 0.036; high dose Ra, r = -0.599, p = 0.018; corrected low dose Ra, r = -0.469, p = 0.078; corrected high dose Ra, r = -0.561, p = 0.030) and a strong, positive correlation between increasing weight loss and changes in Rd (low dose Rd, r = 0.759, p = 0.001; high dose Rd, r = 0.782, p = 0.001; corrected low dose Rd, r = 0.273, p = 0.345; corrected high dose Rd, r = 0.874, p = < 0.001). There were no statistically significant correlations between weight loss and either Ra or Rd in the Endobarrier participants. Scatterplots showing the relationship between weight loss and changes in Ra and Rd are presented in **Figure 3.12** and **Figure 3.13**. In both the Endobarrier and control groups, decreases in HbA1c concentrations were positively correlated with changes in Ra and negatively correlated with changes in Rd but these changes did not reach statistical significance.

Correlations at 6 months		Endobarrier (n=13)		Controls (n=15)	
		Correlation Coefficient	Significance	Correlation Coefficient	Significance
Change in HbA1c	Low Dose Ra	-0.210	0.491	0.481	0.070
	High Dose Ra	0.086	0.780	0.505	0.055
	Corrected Low Dose Ra	-0.072	0.815	0.479	0.071
	Corrected High Dose Ra	0.069	0.823	0.485	0.067
	Low Dose Rd	-0.245	0.421	-0.379	0.164
	High Dose Rd	-0.312	0.299	-0.444	0.098
	Corrected Low Dose Rd	-0.179	0.558	-0.251	0.387
	Corrected High Dose Rd	-0.251	0.407	-0.471	0.089
Weight loss	Low Dose Ra	-0.329	0.273	-0.543	0.036
	High Dose Ra	0.301	0.317	-0.599	0.018
	Corrected Low Dose Ra	-0.429	0.143	-0.469	0.078
	Corrected High Dose Ra	0.130	0.671	-0.561	0.030
	Low Dose Rd	0.172	0.575	0.759	0.001
	High Dose Rd	0.138	0.653	0.782	0.001
	Corrected Low Dose Rd	0.328	0.273	0.273	0.345
	Corrected High Dose Rd	0.255	0.401	0.874	<0.001

Table 3.9 Correlations between changes in HbA1c, weight loss and clamp indices of hepatic glucose output (Ra) and peripheral glucose disposal (Rd) in sub-group 2 participants completing 6 months of follow-up







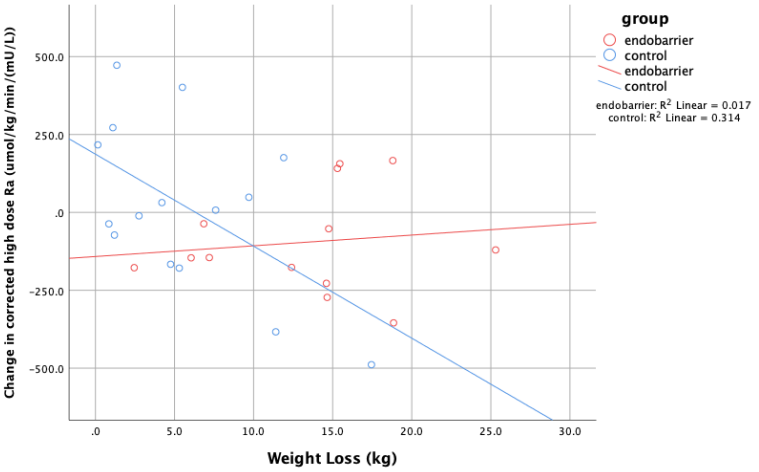
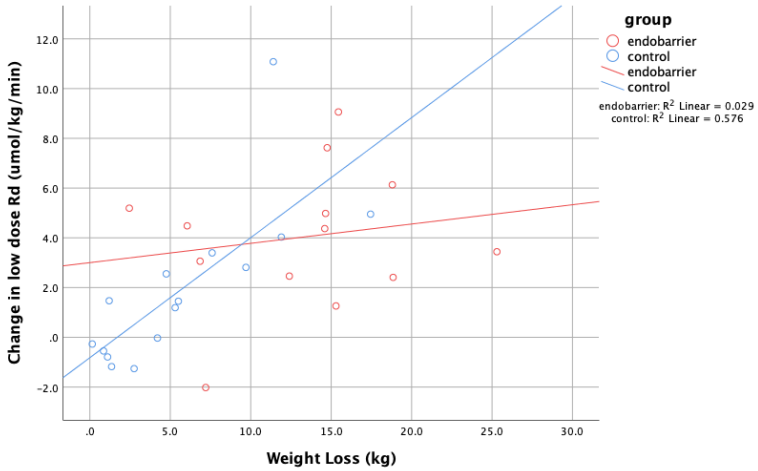
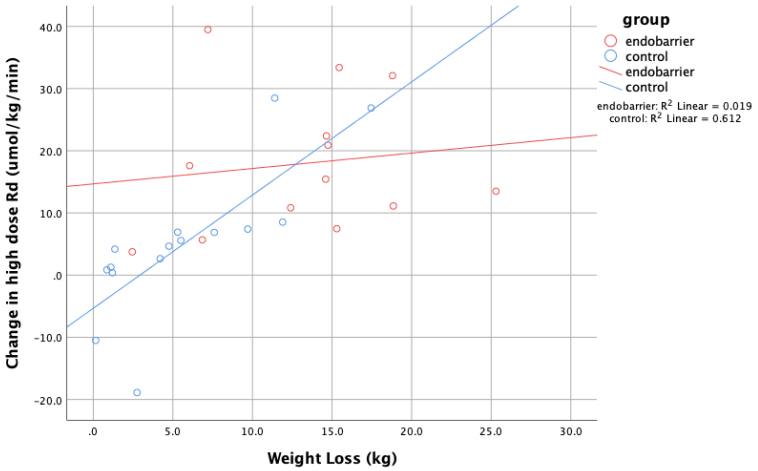
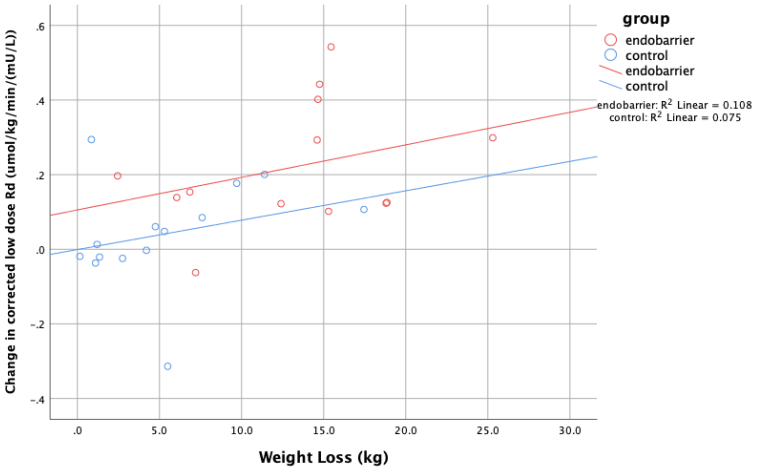


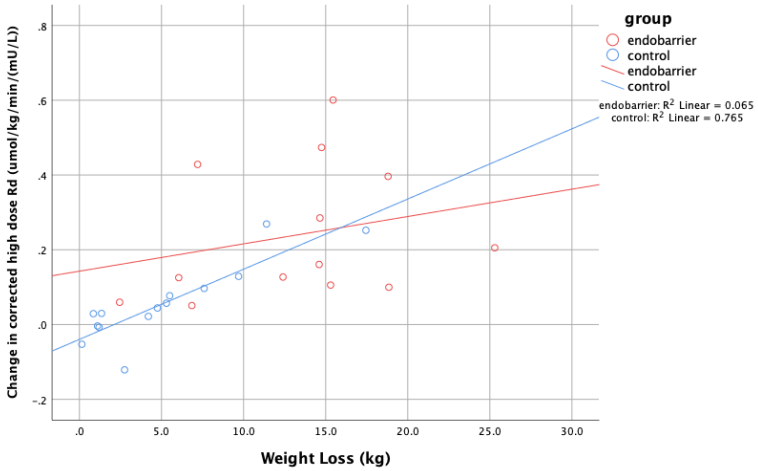
Figure 3.12 Scatterplots showing the relationship between weight loss and changes in Ra in sub-group 2 participants completing 6 months of follow-up

Figure 3.13 Scatterplots showing the relationship between weight loss and changes in Rd in sub-group 2 participants completing 6 months of follow-up.









3.4 Discussion

This is only the second study to investigate changes in hepatic and peripheral insulin sensitivity following Endobarrier implantation using HEC with addition of stable isotopes. Using this technique, we have demonstrated a significant reduction in the rate of appearance of glucose from the liver (HGP/Ra) and a significant increase in peripheral glucose uptake (Rd) at 10-days. However, similar observations were also made for participants in the control group and there were no significant differences in Ra and Rd between the two treatment arms at 10 days. As HGP is the main determinant of FPG, it seems likely that the significant reductions in FPG concentrations that we also observed at + 10 days may be as a consequence of reductions in Ra.

Of note, following the baseline visit at – 2 weeks, patients were prescribed a liquid diet for the 7 days before and 13 days after the intervention visit (0 weeks). The clamps performed in both study groups at 10 days were therefore performed whilst patients were equally calorie-restricted. It is hypothesised that bypass of the foregut may result in early improvements in glycaemic indices through weight loss-independent mechanisms, as has been observed following RYGB. This data, however, does not support this notion and demonstrates that a duodenal-jejunal bypass liner results in no additional early improvements in glucose homeostasis above those achieved through calorie restriction alone. Between 10 days and 6 months there was no further decrease in Ra in the Endobarrier arm and in the control group Ra values increased back up to baseline levels, which further supports the observation. FPG concentrations followed the same trend by remaining stable in the Endobarrier group between 10 days and 6 months and increasing back up to baseline levels in the control arm.

These findings are consistent with the previously published clamp study by Miras et al. and also support the work of several studies that have suggested that it is energy restriction and not anatomical/physiological derangement induced by duodenal exclusion that is responsible for the early improvements in glucose homeostasis observed following metabolic procedures (**see sections 1.2.6.2.4 and 1.2.6.2.5**).[150,178,247,336] However, in contrast to this, there is also convincing evidence in support of superior early improvements in HOMA-IR and insulin sensitivity following RYGB above that of calorie restriction alone, for which several weight-loss-independent mechanisms have been proposed (see **sections 1.2.5.2.2** and **1.2.6.2.4**). [27,178,334,337] The evidence therefore remains conflicting as to the differential effects of duodenal exclusion and caloric restriction on early changes in peripheral and hepatic insulin sensitivity.

Participants in both study arms of this sub-group achieved significant weight loss at 10 days follow-up (7.2 kg in the Endobarrier group vs 4.4 kg in the control group), which is representative of the weight loss outcomes achieved by all participants within the main trial analysis (see

Chapter 2). Patients implanted with the Endobarrier device in this sub-group lost significantly more weight than those patients receiving standard medical therapy, which was maintained at 6 months (TBWL 13.3 kg vs 5.7 kg; %TWL 12.3% vs 5.2%, $p < 0.001$). This finding is in keeping with the best available systematic review and meta-analysis in the field and other published literature on the Endobarrier device.[225,227,237,244,246,251,257] Notably, weight loss was significantly greater in the Endobarrier group at the early 10 day follow-up visit. As the diet was standardised between both groups at this point, we can reasonably assume that weight loss was achieved through mechanisms, not yet fully understood, which go beyond calorie restriction alone. However, quantitative caloric data were unavailable in this sub-group of patients to validate this observation.

Possibly as a result of superior weight loss in participants receiving the Endobarrier DJBL, there was a significantly higher rate of peripheral glucose uptake and utilisation (Rd) at 6 months when compared to the control group. This is the first study to report on this outcome following treatment with an endoscopic bariatric therapy and correlates well with substantiated observations of improvements in peripheral insulin sensitivity following weight-loss by any means.

To the contrary, however, no significant relationship was identified to exist between weight reduction in the Endobarrier group and changes in either rate of glucose appearance or peripheral insulin sensitivity using Pearson correlation coefficients. From this we can propose that the reductions in hepatic glucose output and increases in peripheral glucose disposal/utilisation seen following DJBL implantation have occurred independently of weight-loss and other factors related to the Endobarrier may have contributed to changes in glucose homeostasis. In contrast, in the control group, there were strong correlations between increasing weight loss and changes in Ra and Rd, suggesting that it was indeed weight-loss that was responsible for improved glucose homeostasis in patients not treated with the DJBL. Currently, very little evidence exists in the literature to explain the mechanisms behind the actions of the Endobarrier but, as the device has been designed to mimic the foregut-bypass element of the RYGB, we can presume that similar neurohormonal pathways in the foregut and hindgut may be involved. Changes to incretin/anti-incretin concentrations, bile flow modulation, nutrient sensing and/or alterations to the gut microbiota have all been implicated as potential mediators of these metabolic changes (see **section 1.2.5.2.2 and 1.2.6.2.4**).[338] Each of these factors have been studied as part of the main Endobarrier trial and are reported elsewhere, but further work to examine for correlations between these outcomes and the clamp data reported in this chapter is justified and will be of great significance.

Despite the observed improvements in Rd and sustained reductions in Ra, there were no further improvements in FPG beyond the 10 days follow-up visit. However, we can see that overall glycaemic control improved, as reflected by HbA_{1C} concentrations, despite no significant changes to glucose-lowering medications. Endobarrier participants achieved a decrease in HbA_{1C} of 17.3 mmol/mol vs 13.9 mmol/mol in the control group at 6 months but there was no significant difference between groups at any of the follow-up visits. This is in line with conclusions from the systematic review and meta-analysis by Rohde et al. in which DJBL implantation was associated with a non-significant reduction in HbA_{1C} by -0.9% and FPG by -3.7 mmol/L when compared with diet alone.[257] This must be interpreted in light of the fact that the median implant duration for the five RCTs published to date is only 12 weeks and there were only 2 trials and 24 patients included in this meta-analysis. More recently, a case control study from the National German DJBL registry (DJBL, n = 111 vs matched controls receiving standard treatment, n = 222) demonstrated superior reductions in HbA_{1C} in the DJBL group (-1.37 $\pm$ 1.54% vs -0.51 $\pm$ 1.83%, $p < 0.0001$) and was associated with significantly greater reductions in glucose-lowering medications.[256]

The HEC with addition of stable isotopes is considered to be the gold standard and most sensitive method of assessing insulin resistance in vivo. Due to the fact that clamps are labour-intensive, time-consuming and costly, they are not routinely used in metabolic studies of bariatric surgery. Paradigm models (e.g. HOMA-IR) are more commonly adopted due to their relative ease of application and acceptable correlation with results derived from euglycaemic clamps ($Rs = 0.88$, $p < 0.0001$;[130] $Rs = 0.85$, $P < 0.0001$).[128] The euglycaemic clamp has good intra-subject reproducibility but coefficients of variation (CV) between patients have been reported as high as 21% in normal volunteers, rising to 46% in patients with T2DM. This compares to a CV of 7.8 – 11.7% reported using paradigm models.[339] The current study is limited by the fact that there were no parallel assessments of insulin sensitivity and β-cell function using paradigm models derived from fasting or oral tolerance tests in this sub-group and it is therefore difficult to draw direct comparisons between our findings and other reports of changes in insulin sensitivity following DJBL implantation. However, this study is the first to examine changes in tissue-specific insulin sensitivity related to the Endobarrier DJBL within a RCT using the 'gold-standard' HEC.

3.5 Conclusion

In conclusion, six months of therapy with the Endobarrier DJBL in patients with obesity and type 2 diabetes resulted in superior weight loss when compared to dietary modification and lifestyle advice alone. Through the use of hyperinsulinaemic euglycaemic clamps with addition of stable isotopes it was shown that patients implanted with the Endobarrier device demonstrate superior improvements in peripheral insulin sensitivity, which might have occurred independently of

weight loss. Early reductions in fasting plasma glucose may be as a consequence of improvements in hepatic insulin sensitivity but the data show that a duodenal-jejunal bypass liner provided no additional improvements to early changes in glucose homeostasis above that achieved through calorie restriction alone.

Chapter 4:

Evaluating the effect of a duodenal-jejunal bypass liner (Endobarrier®) on model assessments and indices of insulin sensitivity and beta cell function

4.1 Introduction

4.1.1 Measurements of insulin sensitivity and β-cell function

Normal glucose homeostasis is determined by the delicate balance between insulin secretion (β-cell function) and insulin sensitivity. The progressive impairment of either or both of these will result in glucose intolerance, impaired fasting glycaemia and eventually type 2 diabetes mellitus (T2DM). Quantitative measurements of insulin sensitivity and β-cell function are therefore fundamental to the research of disorders of glucose metabolism and allows reliable evaluation of the efficacy of therapeutic interventions. Both can be quantified through several techniques by measuring changes in glucose metabolism that vary widely in their sensitivity, limitations and complexity (**Section 1.2.4**). Measurements of tissue-specific insulin sensitivity using the hyperinsulinaemic euglycaemic clamp (HEC) has been fully described in **Chapter 3**.

Several model assessments have been designed to quantify insulin sensitivity and β-cell function and are broadly categorised into two groups: (1) basal indices that are extrapolated from *fasting* plasma levels of insulin, glucose, and triglycerides (e.g. HOMA, QUICKI index and McAuley index), or (2) those extrapolated from plasma concentrations of glucose and insulin obtained during an oral glucose tolerance test (OGTT) or standardised mixed meal tolerance test (MMTT) (E.g. Matsuda, Belfiore, Cederholm, Avignon and Stumvoll index).[340]

4.1.2 Basal assessment: Homeostasis Model Assessment

In non-diabetic individuals, the reciprocal of fasting insulin is a reasonable proxy measure for insulin resistance. However, fasting insulin concentrations are not normally distributed and, thus, linear correlations between 1/(fasting insulin) and estimates of insulin sensitivity from HEC are weak. In addition, this simple measurement does not take account of inadequate insulin secretion and impaired beta cell function accompanying hyperglycaemia observed in subjects with diabetes or glucose-intolerance. As such, using 1/(fasting insulin) as a measurement of insulin resistance in individuals with diminished pancreatic reserve leads to erroneous results.[35,129]

To account for this, the Homeostasis Model Assessment (HOMA) approach uses a mathematical model to estimate *basal* insulin resistance (HOMA-IR) and β-cell function (HOMA-β) from fasting insulin/c-peptide and glucose concentrations. It is derived from models made with human experimental data and makes predictions for a wide range of possible combinations of insulin resistance and β-cell function, giving results as percentages of a normal reference population.[130] The model relies on the principle that, in the fasting/basal state, the relationship between glucose and insulin reflects the balance between hepatic glucose production, insulin secretion and

peripheral glucose uptake, which is maintained by a negative feedback loop between pancreatic β-cells and hepatocytes. In its calculation, the current HOMA model (HOMA2 available from http://www.dtu.ox.ac.uk./homa) also takes account of other physiological determinants of glucose flux, including: (1) variations in hepatic and peripheral glucose resistance, (2) the reduction of peripheral glucose-stimulated glucose uptake, (3) increases in insulin secretion in response to a plasma glucose concentrations above 10 mmol/l, (4) contributions made by circulating pro-insulin, and (5) renal glucose losses, thus allowing its use in hyperglycaemic patients. Notably, the results yielded from this model make no distinction between hepatic and peripheral insulin sensitivity but, as the liver is primarily responsible for maintaining basal glucose homeostasis, HOMA-IR is generally regarded as a measure of hepatic insulin resistance.[341]

The formulas used to make linear approximations of HOMA-%B and HOMA-IR (the reciprocal of HOMA-%S) are as follows:

$$\text{HOMA1\_IR} = (\text{FPI [mU/L]} \times \text{FPG [mmol/L]})/22.5$$

$$\text{HOMA1\_\%B} = (20 \times \text{FPI [mU/L]})/(\text{FPG [mmol/L]} - 3.5)$$

FPI, fasting plasma insulin. FPG, fasting plasma glucose. 22.5, a constant to scale HOMA-IR.

Of importance, ß-cell function should always be reported alongside its corresponding insulin sensitivity calculation as a normal, healthy participant may have normal glucose tolerance with a HOMA2-%B of 50% and HOMA2-%S of 200%. However, if a HOMA2-%B of 50% was to be reported in isolation then it could be misconstrued as a reduction in ß-cell function.

HOMA-IR has an inverse, curvilinear correlation with clamp-measured insulin sensitivity and the strength of this relationship is variably reported in the literature.[341-344] However, HOMA-IR values are not equivalent to insulin sensitivity measurements derived from HEC; HOMA yields steady-state values of sensitivity in the basal state whereas HEC measures steady-state insulin sensitivity in the maximally-stimulated range of the insulin dose-response curve.[131,344] Over a wide range of insulin sensitivity/resistance, logarithmic transformation of HOMA-IR (i.e. 1/log(HOMA-IR)) linearizes the skewed distribution of fasting insulin values to determine a much stronger correlation with clamp estimates of insulin sensitivity.[36] Therefore, log (HOMA-IR) is a much more useful tool for evaluating insulin resistance in individuals with glucose intolerance, mild to moderate diabetes, and other insulin-resistant conditions.[35,129,341] The validity of HOMA-IR measurements is reduced in subjects with severely impaired or absent beta cell function, in subjects with lower BMIs, and in the presence of hyperglycaemia. In these individuals, HOMA-IR can give falsely low estimates of insulin resistance and as such, HOMA-IR measurements in a population with beta-cell dysfunction should be interpreted with caution.[345]

Due to variations in specific assays used for glucose and insulin, there remains no absolute 'normal' ranges for HOMA indices. and goes some way to explain the wide coefficients of variation reported for HOMA.[128,339] HOMA results can also vary according to the number of fasting samples obtained and are also particularly vulnerable to errors in measurements.[346] As such, HOMA is preferably used in studies involving a high number of subjects in which such errors are minimised; several publications have confirmed the usefulness of HOMA in estimating insulin sensitivity in large epidemiological studies.[347,348] However, as the HOMA results reported in different studies may have been derived using differing assays and analysis techniques, comparisons between such studies should be done so with caution. It is well recognised that HOMA is inferior to HEC in terms of accuracy of evaluating insulin resistance, but due to its relative simplicity, low cost, strong correlation coefficients with HEC in people with diabetes (without significant hyperglycaemia), and ability to simultaneously estimate insulin sensitivity and secretory function, HOMA has been widely adopted in research as a quick and robust means of measuring changes in overall insulin resistance over time.[128,130,131,339]

4.1.3 Stimulated assessment: OGTT/MMTT

During an OGTT/MMTT, a pre-determined oral glucose load results in the stimulation of both insulin secretion and glucose disposal. Relative changes in the ratio of glucose to insulin concentrations are measured and several mathematical models or indices can then be used to make estimates of insulin sensitivity and/or β-cell function in the non-steady-state. Compared to intravenous tolerance tests, oral-stimulated tests provide a more complete assessment of ß-cell function as there is also activation of entero-insular pathways (e.g. incretin hormone stimulation) so giving a more 'physiological' assessment of glucose tolerance.[343] However, using these models, individual contributions from each of these determinants of glucose homeostasis cannot be differentiated.

4.1.3.1 Assessment of insulin sensitivity

The Matsuda index is a measure of whole body insulin sensitivity derived from measurements of plasma glucose and insulin in the fasting state and then during an OGTT:[126]

$$ISI_{Matsuda} = 10{,}000 / \sqrt{G_0 I_0 G_{mean} I_{mean}}$$

ISI, insulin sensitivity index. G_0, fasting glucose concentration. I_0, fasting insulin concentration. G_{mean}, mean glucose concentration during OGTT. I_{mean}, mean insulin concentration during OGTT. √, correction of the non-linear values distribution.

This validated model was first described by Matsuda and DeFronzo in 1999 and has been found to correlate well with the rate of whole-body glucose disposal calculated during a HEC ($r = 0.73$, $p < 0.0001$).[126]

4.1.3.2 Assessment of ß-cell function

The assessment of ß-cell function is difficult as several factors must be taken into account: (1) insulin secretion is biphasic, (2) the interplay of multiple inhibitory and potentiating phenomena (e.g. incretin hormones), (3) stimulation of insulin secretion by non-glucose substrates, and (4) ß-cells adapt to insulin resistance and, therefore, ß-cell function is dependent on overall insulin sensitivity. As a consequence, there is no gold standard for measuring ß-cell function and multiple indices exist to reflect different ß-cell processes.[344] Several methods focus on deconvolution analysis of c-peptide concentrations as a means of calculating pre-hepatic insulin secretion, which are fully described elsewhere and will not be analysed within this study.[342,349]

Other indices are obtained from empirical and model-based descriptions of insulin secretion corrected for circulating glucose concentrations. The most common one is the insulinogenic index, which is a ratio of increments of insulin and glucose during the first 30 minutes of an OGTT as a measure of first-phase insulin secretion. It is calculated as follows:[344]

$$IGI = \frac{I_{30} - I_0}{G_{30} - G_0}$$

IGI, insulinogenic index. I, insulin concentration. G, glucose concentration.

This is a composite index of insulin secretion and does not discriminate between changes in different ß-cell processes.

4.1.3.3 Relationship between insulin sensitivity and ß-cell function

It is known that β-cell function is inversely related to the prevailing insulin sensitivity and changes in one domain will induce alterations in the other. Therefore, in order to accurately evaluate the determinants of glucose tolerance, simultaneous assessment of both β-cell function and insulin sensitivity is required. The disposition index accounts for this by normalising β-cell function to insulin sensitivity and is a product of the ISI (Matsuda) and IGI. It is calculated as follows:[343]

$$DI = ISI_{Matsuda} \times IGI$$

DI, disposition index. ISI, insulin sensitivity index. IGI, insulinogenic index.

Typically, patients with T2DM will have a lower insulin sensitivity and blunted first-phase insulin response, thus resulting in a decreased DI.

4.1.4 Model assessments of insulin sensitivity and ß-cell function following DJBL implantation

Alterations in insulin sensitivity and ß-cell function have been extensively evaluated following metabolic surgery (especially RYGB) using a range of quantitative assessment methods and models.[27,176-178] To date, the majority of studies looking at the Endobarrier DJBL have reported clinical improvements in glycaemic control on the basis of decreases in fasting plasma glucose (FPG) and HbA1c concentrations. Model assessments of insulin sensitivity and ß-cell function have only been used in a small number of non-randomised human studies.[229,230,233,235,242,243,248] The majority of these studies have reported values of HOMA-IR in isolation but generally describe significant reductions in insulin resistance following DJBL implantation. These improvements occurred as early as 1 week following implantation in two studies and Quezada et al reported on sustained improvements in HOMA-IR following implant durations of up to 3 years; HOMA-IR decreased from 6.02 $\pm$ 3 at baseline to 2.3 $\pm$ 1 at 1 year and 3.84 $\pm$ 2 at 3 years.[233,235,248]

Cohen et al. is the only report to evaluate changes in insulin sensitivity and ß-cell function using several different model assessments in patients implanted with the Endobarrier DJBL. In this prospective observational study, 16 patients, with a mean baseline BMI of 30 kg/m^2, were implanted with the Endobarrier for 1 year. Weight decreased by 1.3 kg after 1 week and by 2.4 kg after 1 year ($p < 0.001$). Mean HbA1c concentrations reduced from 71.3 mmol/mol to 58.1 mmol/mol and FPG reduced from 203.3 mg/dl to 155.1 mg/dl (both $p < 0.001$). At each visit, patients were evaluated with a MMTT over 120 minutes following a fixed meal of Ensure Plus (525 kcal). Insulin sensitivity improved by >50% as early as 1 week after implantation as measured by the Matsuda index and HOMA-IR ($P < 0.001$) and was maintained until 1 year follow-up. There was a trend towards deterioration 26 weeks after explantation. Fasting and stimulated total insulin secretion rates were calculated using deconvolution of C-peptide concentrations and application of population-based variables or C-peptide kinetics. They demonstrated a trend towards decreasing but this was not significantly changed during the implantation period or following explantation for both fasting values ($p = 0.051$) and AUC analysis ($p = 0.28$). There were also non-significant changes in the insulinogenic index.[230]

The herein reported study will be the first to evaluate changes in insulin sensitivity and β-cell function using model assessments in patients implanted with the Endobarrier DJBL for 1 year within a randomised controlled trial.

4.1.5 Aims

The aim of this research is to evaluate the effects of duodenal exclusion using the Endobarrier DJBL on ß-cell function and insulin sensitivity.

4.1.6 Hypotheses

The hypotheses being tested in the work that will be described within this chapter are that duodenal exclusion in patients with obesity and T2DM using the Endobarrier DJBL alongside intensive lifestyle modification:

- results in significantly greater weight loss than in control patients with obesity and T2DM treated with intensive lifestyle modification alone;
- rapidly reduces fasting plasma glucose concentrations to a greater extent than in control patients with obesity and T2DM treated with intensive lifestyle modification alone;
- results in early improvements in overall insulin sensitivity when compared to control patients with obesity and T2DM treated with intensive lifestyle modification alone;
- results in enhanced insulin secretion and improvements in ß-cell function when compared to control patients with obesity and T2DM treated with intensive lifestyle modification alone.

4.1.7 Objectives

The objectives of this research are:

- to evaluate the change in weight of patients with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for 12 months and compare it to the weight changes of control patients with obesity and T2DM receiving intensive medical therapy alone;
- to determine the changes in fasting plasma glucose and serum insulin concentrations during one year of treatment with the Endobarrier DJBL and compare these changes to those that occur in control patients;
- to determine the changes in fasting plasma glucose and serum insulin concentrations following explantation of the Endobarrier DJBL and compare these changes to those of control patients;
- to evaluate the changes in basal insulin sensitivity and ß-cell function during one year of treatment with the Endobarrier DJBL using the HOMA method and compare these changes to those that occur in control patients;

- to evaluate the changes in basal insulin sensitivity and ß-cell function following explantation of the Endobarrier DJBL using the HOMA method and compare these changes to those that occur in control patients;
- to evaluate the changes in basal and stimulated insulin sensitivity following treatment with the Endobarrier DJBL for 1 year using model assessments following a MMTT and compare these changes to those in control patients;
- to evaluate the changes in basal and stimulated ß-cell function following treatment with the Endobarrier DJBL for 1 year using model assessments following a MMTT and compare these changes to those in control patients.

4.2 Methods

4.2.1 Patients and study design

This chapter reports on the basal model-assessment outcomes of all patients recruited into the Endobarrier RCT. The trial methodology, including patient selection, randomisation, trial interventions and follow-up schedule, is described and discussed in detail in **Chapter 2**. In addition, all patients who were recruited into the trial were also given the option of participating in this nested mechanistic sub-study. Those who agreed to participate provided additional informed written consent and also underwent a MMTT at visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), 8 (+6 months ± 7 days), and 10 (+11.5 months ± 7 days).

All participants across both study arms attended the Wellcome Trust Clinical Research Facility at Southampton General Hospital or the Imperial Clinical Trials Unit at Imperial College London. Patients arrived fasted for these visits (at least 8 hours without food and drink, except for water). Patients were advised to take their usual glucose-lowering medications as normal. On arrival, the patient had a clinical review in which their general wellbeing was assessed and any adverse events or changes to concomitant medications were recorded. Basic anthropometric measurements were taken and fasting blood samples were obtained and analysed in accordance with the methods described in detail in **section 2.2.7.** Those patients who provided additional consent to take part in sub-group 3 (food preference) at visits 3 (- 2 weeks ± 7 days), 5 (+ 10 days ± 7 days), 8 (+ 6 months ± 7 days) and 10 (+11.5 months ± 7 days) then underwent a MMTT. The outcome measurements relevant to this chapter that were recorded at these visits are summarised in **Table 4.1**.

	Sample	Analysis
Anthropometric measurements	Weight (kg) Height (m)	BMI (kg/m^2) TBWL (kg) %TBWL
Fasting blood samples	Serum	Insulin (mU/L)
	Plasma	Glucose (mmol/L)
Mixed Meal Tolerance Test	Serum	Insulin (mU/L)
	Plasma	Glucose (mmol/L)

Table 4.1 Summary of relevant outcome measurements taken at visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), 8 (+6 months ± 7 days) and 10 (+11.5 months ± 7 days)

4.2.2 Medicines, reagents and materials

Reagents and materials used during the methodology are fully described in **Appendix B**.

4.2.3 Mixed Meal Tolerance Test

Each participant was seated in an infusion chair in a semi-recumbent position with their arms placed horizontally on armrests at chest height. An 18-20G cannula (B-D Insyte-W, Becton Dickinson, UK) was inserted into a large vein in the antecubital fossa in order to obtain baseline blood samples and perform regular blood sampling thereafter using the BD Vacutainer® system.

At the beginning of the MMTT, the first blood pressure reading, heart rate, and blood samples were taken as per the protocol in **Table 4.2**. Each patient was then asked to consume 2 x 125 ml Fortisip Compact drinks, containing per 100 mL: 240 kcal, 9.6 g protein (16% of total energy), 29.7 g carbohydrate (49%), 15 g sugars, 9.3 g fat (35%). Fortisip drinks needed to be consumed within 10 minutes and then a timer was started. Blood pressure readings, heart rate and blood samples were then taken at T = +15 min, T = +30 min, T = +60 min, T = +120 min and T = +180 min as per protocol.

Time	Sample collected	Action
0	1 Yellow (SST) 5 mL (Insulin) 1 grey (FO) 2 mL (Glucose)	Take Blood Pressure and pulse Collect blood samples GIVE FORTISIP. 10 MINS TO CONSUME Start timer when the drink is finished
+ 15 min	1 Yellow (SST) 5 mL (Insulin) 1 grey (FO) 2 mL (Glucose)	Take Blood Pressure and pulse Collect blood samples
+ 30 min	1 Yellow (SST) 5 mL (Insulin) 1 grey (FO) 2 mL (Glucose)	Take Blood Pressure and pulse Collect blood samples
+ 60 min	1 Yellow (SST) 5 mL (Insulin) 1 grey (FO) 2 mL (Glucose)	Take Blood Pressure and pulse Collect blood samples
+ 120 min	1 Yellow (SST) 5 mL (Insulin) 1 grey (FO) 2 mL (Glucose)	Take Blood Pressure and pulse Collect blood samples
+ 180 min	1 Yellow (SST) 5 mL (Insulin) 1 grey (FO) 2 mL (Glucose)	Take Blood Pressure and pulse Collect blood samples Remove Cannula and apply dressing

Table 4.2 Protocol for mixed meal tolerance test.
SST, spray-coated silica; FO, fluoride oxalate.

4.2.4 Sample analysis

Whole blood samples were collected into fluoride oxalate and SST BD Vacutainer® Tubes and transferred immediately to the central chemical pathology laboratories at Southampton General Hospital and Imperial College London for the measurement of plasma glucose and serum insulin concentrations respectively. Plasma glucose concentrations were determined using an automated glucose analyser (AU600, Olympic Diagnostics, Southall, UK) with units being expressed as mmol/L. Serum insulin concentrations were measured using an automated enzyme-linked immunosorbent assay (ES700: Roche Diagnostics, Lewes, UK) with units being expressed as mU/L.

4.2.5 Model assessments of β-cell function and insulin sensitivity

Model-derived estimates of basal steady-state ß-cell function (HOMA2-%B), insulin sensitivity (HOMA2-%S) and insulin resistance (HOMA2-IR) were made using the HOMA2 calculator available from https://www.dtu.ox.ac.uk/homacalculator. This computer model provides values expressed as a % of a normal reference population (where 100% is normal). A description of the basic formulation used by this model is provided in **section 4.1.2**.

The Matsuda index was used to estimate non-steady-state insulin sensitivity from glucose and insulin samples obtained from the MMTT. The Insulinogenic index and disposition index were calculated to determine estimates of ß-cell function and first-phase insulin response. All calculations were made using a calculator available from Matsuda at http://mmatsuda.diabetes-smc.jp/EnglishHPMM.html. The formulas used to make these calculations are described in **section 4.1.3.**

4.2.6 Statistical analysis

For baseline data, normally distributed variables are reported as means with standard deviation (SD) and for comparison of these variables the independent t-test was used. Non-normally distributed variables are reported as medians with the interquartile range, and the Mann-Whitney U test was used to compare such variables. Categorical variables were compared using the Chi-square test or the Fisher's exact test as appropriate. A *p*-value <0.05 was considered statistically significant. Due to the skewed distribution of MMTT data, a normally distributed dataset was obtained by logarithmically transforming (Log10) the data prior to statistical analysis. This analysis was performed using SPSS® 25.0 software (SPSS, Chicago, IL, USA) and mechanistic

data were assessed using a mixed model approach as described in **Section 2.2.9**. The Spearman rank-order correlation coefficient was used on non-parametric datasets to evaluate the relationship between variables of interest.

4.3 Results

4.3.1 Patient characteristics and baseline data

For a full description of the recruitment outcomes of all patients within the Endobarrier trial please refer to **section 2.3.1** and the consort diagram in **Figure 2.8**, in which the reasons for patient attrition/non-inclusion are fully detailed. The baseline characteristics for all trial patients that were included in the baseline HOMA analyses are shown in **Table 2.4**.

Figure 4.1 summarises the flow of participants through the trial who provided additional consent to take part in sub-group 3 (food preference) and underwent a MMTT at study visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), 8 (+6 months ± 7 days) and 10 (+11.5 months ± 7 days). Missing or insufficient blood samples account for any unavailable data. For the assessments made in this chapter, visit 3 (-2 weeks ± 7 days, i.e. pre-intervention) served as the baseline visit and the patient characteristics of each of the study arms are summarised in **Table 4.3.** In total, 18 patients in the control arm and 23 patients in the EndoBarrier underwent a MMTT and were included in the final analysis. There were a significantly greater number of women in the control arm of this sub-group and the mean weight was statistically higher in the Endobarrier group at baseline. However, there was no significant difference in BMI between treatment arms at baseline.

	Control (n=18) mean ± SD	**Endobarrier (n=23) mean ± SD**	
Age, years	53.7 ± 5.8	51.5 ± 7.9	p = 0.330
Sex, n (%) Female	11 (61.1)	6 (26.0)	**p = 0.031**
Weight, kg	100.9 ± 14.8	112.2 ± 19.0	**p = 0.034**
BMI, kg/m^2	36.0 ± 4.2	36.6 ± 5.4	p = 0.688

Table 4.3 Baseline characteristics of participants undergoing mixed meal tolerance test
SD, standard deviation; kg, kilograms; BMI, body mass index.

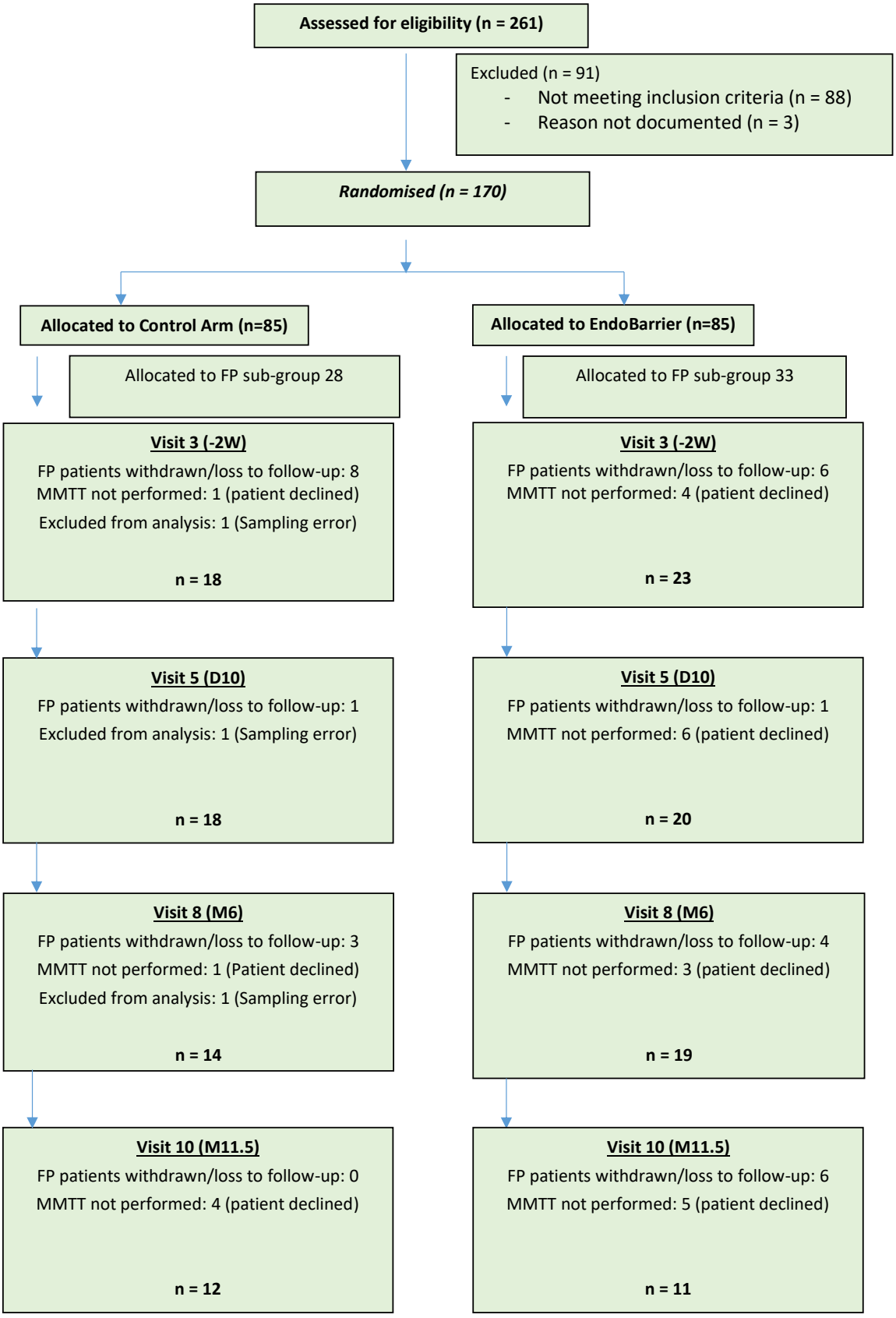


Figure 4.1 Consort flow chart of participants undergoing mixed meal tolerance test. MMTT, mixed meal tolerance test; FP, Food Preference.

4.3.2 Anthropometric outcomes

The anthropometric outcomes for all trial patients in both study arms are fully reported in **section 2.3.3**. Anthropometric outcomes for sub-group 3 patients are summarised in **Table 4.4**. In sub-group 3, participants in both arms of the trial significantly reduced their weight by 10 days and continued to lose a significant amount of weight between 10 days and 6 months. Weight-loss then appeared to plateau with no further significant weight loss being achieved in either group between 6 months and 11.5 months follow-up. A peak weight-loss of 10.5 $\pm$ 6.5 kg (9.5 $\pm$ 4.9%) was achieved in the Endobarrier arm of the group versus 9.2 $\pm$ 10.5 kg (8.5 $\pm$ 9.0%) in the control arm. Similarly, BMI reduced from 36.6 kg/m^2 to 32.6 kg/m^2 and from 36.0 kg/m^2 to 32.8 kg/m^2 in each group respectively. There were no significant differences between groups at any of the follow-up visits.

	Control		Endobarrier		Mixed Model Analysis	
Visit	n	Mean ± SD	n	Mean ± SD	Effect	p-value
Weight (kg)						
Baseline	18	100.9 ± 14.8†	23	112.2 ± 19.0†	Group	0.052
10 days	18	96.6 ± 14.2*	20	108.1 ± 18.6*	Visit	<0.001
6 months	14	91.6 ± 13.7*φ	19	100.6 ± 19.1*§	Group*Visit	0.754
11.5 months	12	94.6 ± 11.7*φ	11	97.9 ± 12.7*§		
BMI (kg/m^2)						
Baseline	18	36.0 ± 4.2	23	36.6 ± 5.4	Group	0.790
10 days	18	34.4 ± 4.2*	20	35.1 ± 5.4*	Visit	<0.001
6 months	14	32.5 ± 3.3*φ	19	33.4 ± 5.9*§	Group*Visit	0.952
11.5 months	12	32.8 ± 3.8*§	11	32.6 ± 4.3*φ		
Total body Weight Loss (kg)						
Baseline	18	-	23	-	Group	0.291
10 days	18	4.3 ± 1.2	20	5.5 ± 2.3	Visit	<0.001
6 months	14	8.4 ± 7.0φ	19	10.0 ± 4.2§	Group*Visit	0.951
11.5 months	12	9.2 ± 10.5φ	11	10.5 ± 6.5φ		
Total body Weight Loss (%)						
Baseline	18	-	23	-	Group	0.430
10 days	18	4.3 ± 1.1	20	4.9 ± 2.0	Visit	<0.001
6 months	14	8.1 ± 5.9φ	19	9.1 ± 3.4φ	Group*Visit	0.961
11.5 months	12	8.5 ± 9.0φ	11	9.5 ± 4.9φ		

Table 4.4 Anthropometric outcomes for sub-group 3 patients.
*denotes p < 0.001 compared to baseline within the same group, § denotes p < 0.001 compared to 10 days within the same group, φ denotes p < 0.01 compared to 10 days within the same group, † denotes p < 0.05 between groups.

4.3.3 Outcomes in all trial patients

4.3.3.1 Changes in fasting glucose and insulin concentrations

Changes in fasting glucose and insulin concentrations in all trial participants are summarised in **Table 4.5** and **Figure 4.2**. FPG concentrations decreased significantly in both treatment arms. In patients implanted with the Endobarrier DJBL, FPG concentrations decreased significantly in the first 10 days following device implantation and then remained stable until the device was removed at 1 year. Following device explantation, between 11.5 and 23 months, FPG concentrations increased significantly again but remained statistically lower than at baseline. In the control arm, FPG concentrations also decreased significantly within the first 10 days, during the low-calorie liquid diet phase of the trial, but then subsequently increased again and were then stable for the remainder of the study. FPG concentrations were significantly lower in the control group at 10 days when compared to the Endobarrier group but were not statistically different at 6, 11.5 or 23 months follow-up.

Fasting serum insulin concentrations followed a similar trend to FPG concentrations in patients implanted with the Endobarrier device; levels significantly reduced within the first 6 months and then remained stable until device explantation. Concentrations then increased significantly but remained statistically lower than baseline levels. In the control arm, insulin concentrations were significantly reduced within the first 10 days and were then stable for the remainder of the study. Fasting serum insulin concentrations were significantly lower in patients implanted with the Endobarrier device at 11.5 months in comparison to control group participants at the same time-point.

4.3.3.2 All patients: Insulin sensitivity outcomes

Model-derived estimates of basal steady-state insulin sensitivity (HOMA2-%S) and, its reciprocal, insulin resistance (HOMA2-IR) are summarised in **Table 4.6** and **Figure 4.2**. HOMA2-%S and HOMA2-IR values significantly improved for the first 6 months following Endobarrier implantation and then remained stable between 6 and 11.5 months. Following removal of the device, indices of sensitivity reduced but remained significantly improved from baseline levels at 23 months follow-up. In the control group, insulin sensitivity significantly improved within the first 10 days and then remained statistically unchanged for the remainder of the study. HOMA2-%S values were significantly higher (and HOMA2-IR values were significantly lower) in patients treated with the Endobarrier DJBL at 11.5 months when compared to control participants at the same time-

	Control		Endobarrier		Mixed Model Analysis	
Visit	n	Median (Interquartile range)	n	Median (Interquartile range)	Effect	p-value
Fasting plasma glucose mmol/L						
Baseline	72	9.8 (8.3 – 12.0)	79	11.1 (9.2 – 13.4)	Group	0.081
10 days	69	6.5 (5.9 – 8.3)*Δ	73	7.9 (6.8 – 9.3)*Δ	Visit	<0.001
6 months	59	7.7 (6.2 – 9.6)*§	62	7.9 (6.6 – 9.7)*	Group*Visit	0.016
11.5 months	57	7.2 (6.2 – 9.6)*§	54	7.0 (6.4 – 8.3)*		
23 months	46	8.3 (6.7 – 10.1)*§	48	8.3 (6.9 – 10.0)*¥		
Fasting serum insulin mU/L						
Baseline	69	10.3 (8.0 – 16.3)	76	10.8 (8.1 – 15.7)	Group	0.604
10 days	64	9.0 (6.1 – 12.6)*	72	9.4 (7.5 – 13.5)*	Visit	<0.001
6 months	59	9.4 (6.2 – 14.4)*	60	7.6 (5.5 – 10.5)*§	Group*Visit	<0.001
11.5 months	54	10.0 (6.4 – 13.9)*†	55	7.8 (6.0 – 10.2)*§†		
23 months	45	9.9 (6.2 – 12.8)*	48	10.1 (7.2 – 14.2)*φ¥		

Table 4.5 Changes in fasting glucose and insulin concentrations in all trial participants.
* denotes p < 0.05 compared to baseline within the same group, § denotes p < 0.05 compared to 10 days within the same group, φ denotes p < 0.05 compared to 6 months within the same group, ¥ denotes p < 0.05 compared to 11.5 months within the same group, † denotes p < 0.05 between groups, Δ denotes p < 0.001 between groups. Data expressed as median + interquartile range. Mixed model analysis performed on Log10 transformed data.

point. There were no differences between treatment arms at 23 months follow-up, following device removal.

4.3.3.3 All patients: ß-cell function outcomes

Model-derived estimates of basal steady-state ß-cell function (HOMA2-%B) are summarised in **Table 4.6** and **Figure 4.2**. In both study arms, HOMA2-%B values increased significantly within the first 10 days of the study and were significantly greater in control arm participants at 10 days when compared to patients implanted with the Endobarrier device. After this time-point, values decreased significantly in the Endobarrier group and remained largely stable but statistically improved from baseline for the remainder of the study. In the control arm, HOMA2-%B values precipitously declined but remained significantly improved from baseline values at 23 months follow-up. There were no significant differences in HOMA2-%B values between groups at 6, 11.5, and 23 months follow-up.

	Control		Endobarrier		Mixed Model Analysis	
Visit	n	Median (Interquartile range)	n	Median (Interquartile range)	Effect	p-value
		HOMA2-%B				
Baseline	69	34.2 (22.4 – 49.6)	76	29.5 (20.2 – 43.6)	Group	0.060
10 days	64	62.4 (39.7 – 76.9)*†	72	46.5 (34.0 – 68.2)*†	Visit	<0.001
6 months	58	43.7 (30.3 – 62.9)*§	59	40.8 (30.4 – 56.4)*§	Group*Visit	0.149
11.5 months	54	46.7 (31.2 – 63.8)*§	54	44.1 (30.9 – 59.7)*		
23 months	45	38.3 (26.9 – 58.0)*§¥	48	43.5 (29.5 – 63.3)*		
		HOMA2-%S				
Baseline	69	61.0 (38.2 – 84.3)	76	59.7 (39.8 – 81.1)	Group	0.833
10 days	64	81.9 (56.9 – 122.1)*	72	72.8 (50.2 – 95.0)*	Visit	<0.001
6 months	58	74.1 (48.5 – 114.1)*	59	94.7 (64.6 – 131.7)*§	Group*Visit	<0.001
11.5 months	54	66.6 (50.4 -106.6)*†	54	90.4 (70.6 – 114.0)*§†		
23 months	45	67.6 (50.4 – 116.8)*	48	69.7 (49.0 – 94.1)*φ¥		
		HOMA2-IR				
Baseline	69	1.64 (1.19 – 2.62)	76	1.68 (1.23 – 2.51)	Group	0.832
10 days	64	1.22 (0.82 – 1.76)*	72	1.37 (1.05 – 1.99)*	Visit	<0.001
6 months	58	1.35 (0.88 – 2.06)*	59	1.06 (0.76 – 1.55)*§	Group*Visit	<0.001
11.5 months	54	1.50 (0.94 – 1.99)*†	54	1.11 (0.88 – 1.42)*§†		
23 months	45	1.48 (0.86 – 1.98)*	48	1.44 (1.06 – 2.04)*φ¥		

Table 4.6 Insulin sensitivity and ß-cell function outcomes for all trial participants

* denotes p < 0.05 compared to baseline within the same group, § denotes p < 0.05 compared to 10 days within the same group, φ denotes p < 0.05 compared to 6 months within the same group, ¥ denotes p < 0.05 compared to 11.5 months within the same group, † denotes p < 0.05 between groups, Δ denotes p < 0.001 between groups. Data expressed as median + interquartile range. Mixed model analysis performed on Log10 transformed data.

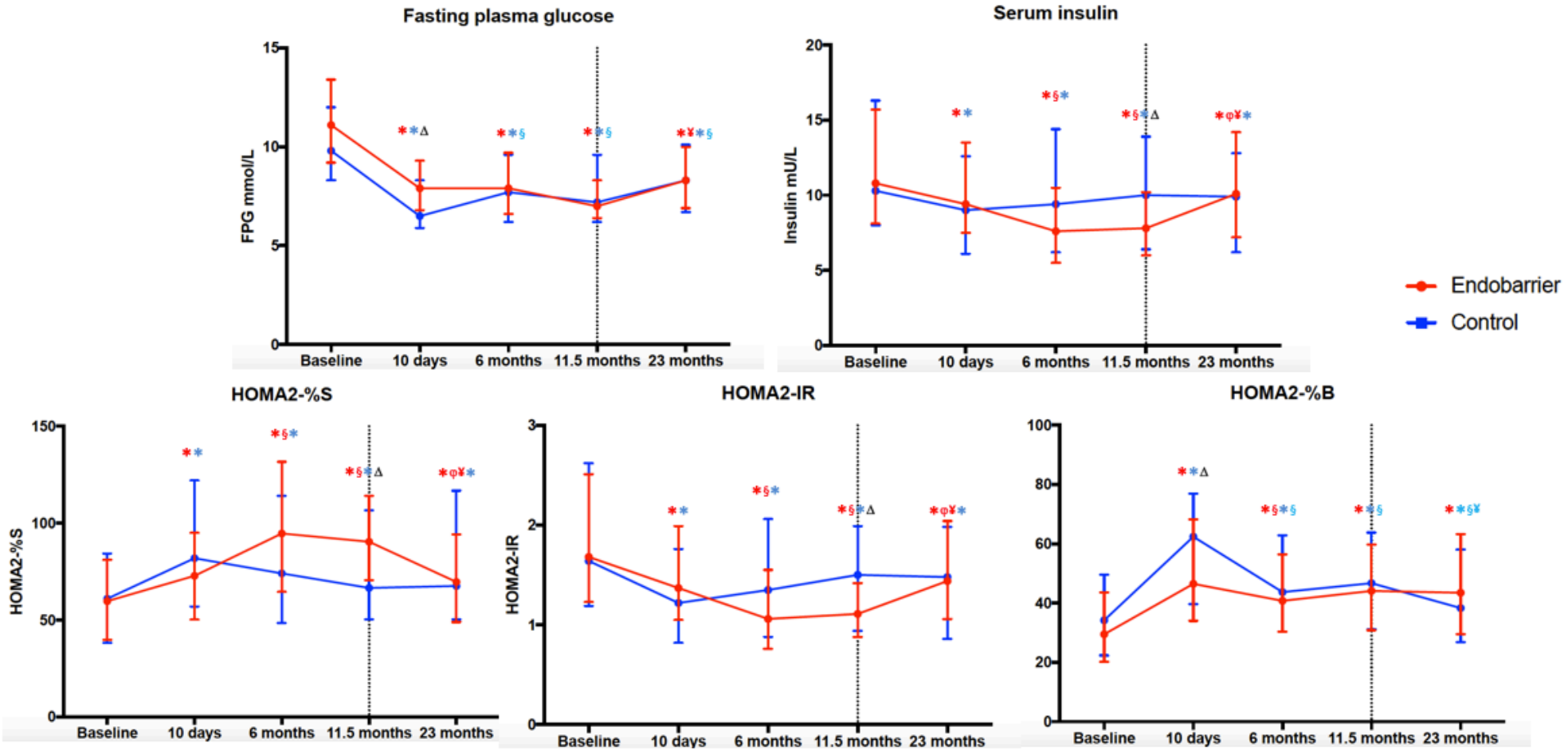


Figure 4.2 Changes in fasted model assessment outcomes in all Endobarrier and Control group participants

* denotes $p < 0.05$ compared to baseline within the same group, § denotes $p < 0.05$ compared to 10 days within the same group, φ denotes $p < 0.05$ compared to 6 months within the same group, ¥ denotes $p < 0.05$ compared to 11.5 months within the same group, Δ denotes $p < 0.05$ between groups. Dotted line indicates device explantation.

4.3.3.4 All patients: correlations

A Spearman's rank-order correlation was run to determine the relationship between weight loss and the fasted model assessment outcomes in trial participants completing 1 year of follow-up (**Table 4.7**). There was strong, positive relationship between weight loss and changes in insulin sensitivity (HOMA2-%S), which was statistically significant in both the Endobarrier group (r_s = 0.333, p = 0.014) and the control group (r_s = 0.268, p = 0.042) (**Figure 4.4**). There were no statistically significant correlations between weight loss and HOMA2-%B or HOMA2-IR in either treatment group.

Correlations at 1 year		Endobarrier (n=54)		Controls (n=58)	
		Correlation Coefficient	Significance	Correlation Coefficient	Significance
Weight loss	Change in HOMA2-%B	-0.031	0.824	0.218	0.101
	Change in HOMA2-%S	0.333	0.014	0.268	0.042
	Change in HOMA2-IR	-0.180	0.192	-0.211	0.150

Table 4.7 Correlations between weight loss and fasted model assessment outcomes in trial participants completing 1 year of follow-up

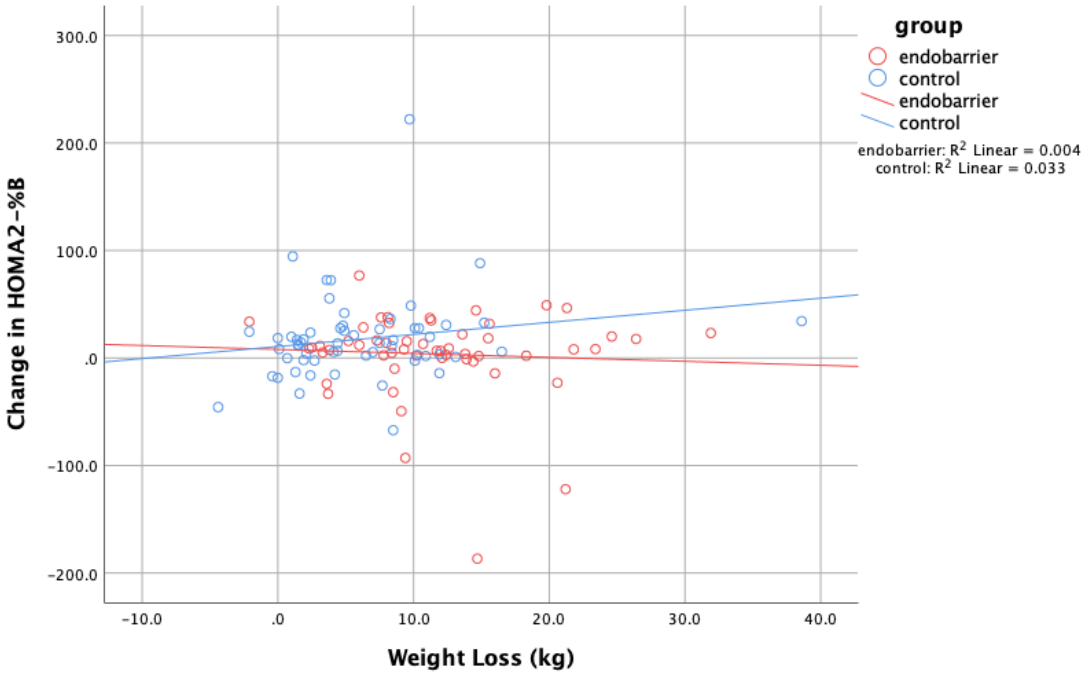


Figure 4.3 Scatterplot showing the relationship between weight loss and HOMA2-%B in trial participants completing 1 year of follow-up

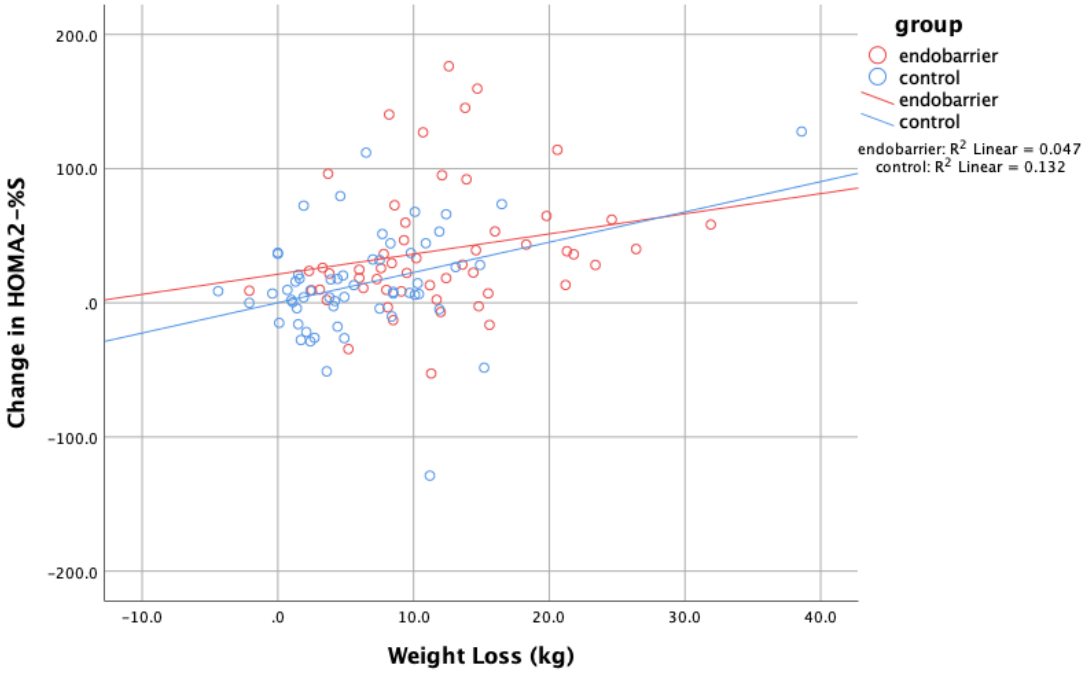


Figure 4.4 Scatterplot showing the relationship between weight loss and HOMA2-%S in trial participants completing 1 year of follow-up

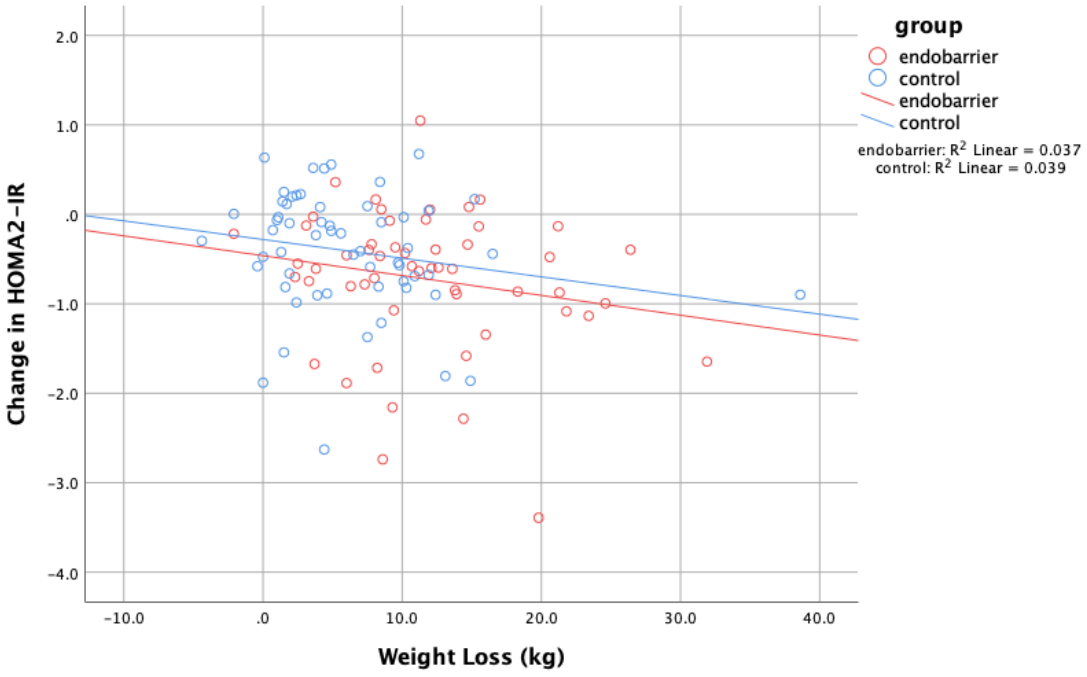


Figure 4.5 Scatterplot showing the relationship between weight loss and HOMA2-IR in trial participants completing 1 year of follow-up

4.3.4 Sub-group 3 outcomes

4.3.4.1 Sub-group 3: fasting glucose and insulin concentrations

Changes in fasting glucose and insulin concentrations in sub-group 3 participants are summarised in **Table 4.8**. FPG concentrations decreased significantly from baseline to 10 days across both treatment groups. In participants implanted with the Endobarrier device, levels decreased from 10.1 to 8.1 mmol/L and then plateaued, remaining significantly reduced at 7.6 mmol/L at the 11.5 months follow-up visit. In the control arm, levels initially decreased from 9.2 to 6.3 mmol/L but then significantly increased back up to baseline levels of 8.3 mmol/L at the 11.5 months follow-up visit. FPG concentrations were significantly lower in the control arm participants after 10 days but were not significantly different between treatment arms at 6 or 11.5 months follow-up.

Fasting serum insulin concentrations significantly decreased only in control arm participants; levels reduced from 11.8 to 9.1 mU/L after 10 days and then plateaued, remaining significantly reduced at 8.5 mU/L at the 11.5 months follow-up visit. Fasting insulin levels showed a downward trend in the Endobarrier group but this failed to reach statistical significance ($p = 0.225$). There were no significant differences between treatment arms at any of the follow-up visits.

4.3.4.2 Sub-group 3: Insulin sensitivity outcomes

Model-derived estimates of basal steady-state insulin sensitivity (HOMA2-%S) and, its reciprocal, insulin resistance (HOMA-IR), plus index assessments of glucose-stimulated insulin sensitivity (Matsuda index) are summarised in **Table 4.9**. HOMA2-%S and Matsuda index values significantly increased and HOMA-IR values significantly decreased across both study groups. In the case of the Matsuda index and HOMA-IR results, these significant changes occurred within 10 days and then remained statistically unchanged between 10 days and 11.5 months follow-up, but remained improved from baseline levels. For HOMA2-%S, values increased significantly at 10 days in the control group and then remained statistically unchanged but improved from baseline values between 10 days and 11.5 months follow-up. In the Endobarrier group, HOMA2-%S values trended upwards across all follow-up visits but only reached statistical significance at 11.5 months. There were no significant differences in insulin sensitivity outcomes between treatment arms at any of the follow-up visits in this sub-group.

	Control		Endobarrier		Mixed Model Analysis	
Visit	n	**Mean** ± SD	n	**Mean** ± SD	Effect	p-value
		Fasting Glucose (mmol/L)				
Baseline	18	9.2 ± 2.3	22	10.1 ± 2.5	Group	0.231
10 days	18	6.3 ± 1.4*†	20	8.1 ± 3.1*†	Visit	<0.001
6 months	14	7.2 ± 2.2**	19	7.9 ± 1.9*	Group*Visit	0.236
11.5 months	12	8.3 ± 3.5φ	11	7.6 ± 1.4*		
		Fasting Insulin (mU/L)				
Baseline	18	11.8 ± 7.2	21	10.7 ± 5.6	Group	0.577
10 days	18	9.1 ± 4.4**	20	10.3 ± 6.2	Visit	0.005
6 months	14	7.5 ± 4.0*	19	9.1 ± 5.1	Group*Visit	0.262
11.5 months	12	8.5 ± 4.6**	11	8.3 ± 4.5		

Table 4.8 Changes in fasting glucose and insulin concentrations in sub-group 3 participants

*denotes $p < 0.001$ compared to baseline within the same group, **denotes $p < 0.05$ compared to baseline within the same group, φ denotes $p < 0.05$ compared to 10 days within the same group, † denotes $p < 0.05$ between groups. Data expressed as means ± standard deviation.

4.3.4.3 Sub-group 3: ß-cell function outcomes

Model-derived estimates of basal steady-state ß-cell function (HOMA2-%B) plus index assessments of glucose-stimulated insulin secretion (insulinogenic and disposition indexes) are summarised in **Table 4.10.** HOMA2-%B and Disposition Index values significantly increased by 10 days within both study groups. In participants implanted with the Endobarrier DJBL, values then plateaued and remained statistically unchanged but significantly improved from baseline values between 10 days and 11.5 months. In comparison, values for control group participants decreased significantly again and were not significantly different to baseline levels at 6 and 11.5 months follow-up. There were no significant differences in ß-cell function outcomes between treatment arms at any of the follow-up visits in this sub-group. There were no significant changes in the insulinogenic index in either treatment arm at any of the follow-up visits in this sub-group.

	Control		Endobarrier		Mixed Model Analysis	
		Median (Interquartile range)	n	**Median** (Interquartile range)		
		HOMA2-%S				
Baseline	18	71.0 (42.7 – 114.3)	21	71.6 (49.4 – 93.0)	Group	0.404
10 days	18	91.9 (53.0 – 148.4)**	20	75.0 (52.7 – 123.2)	Visit	<0.001
6 months	14	105.2 (68.8 – 171.5)*	19	95.4 (60.9 – 128.5)	Group*Visit	0.309
11.5 months	12	89.7 (59.4 – 171.1)**	11	103.5 (72.2 – 131.4)**		
		HOMA-IR				
Baseline	18	3.76 (1.97 – 7.20)	21	4.17 (3.15 – 5.59)	Group	0.256
10 days	18	1.81 (1.39 – 3.68)*	20	2.76 (1.86 – 4.81)**	Visit	<0.001
6 months	14	1.95 (1.04 – 3.50)*	19	2.53 (2.19 – 3.71)**	Group*Visit	0.393
11.5 months	12	2.08 (1.34 – 5.19)*	11	2.24 (1.45 – 3.08)**		
		Matsuda Index				
Baseline	16	4.27 (2.47 – 5.90)	20	2.86 (2.41 – 3.69)	Group	0.517
10 days	16	6.20 (3.71 – 9.14)*	18	4.87 (3.60 – 7.55)*	Visit	<0.001
6 months	13	5.23 (2.90 – 8.54)**	18	4.95 (3.68 – 6.67)*	Group*Visit	0.833
11.5 months	11	4.31 (2.03 – 7.96)*	10	5.76 (3.17 – 7.35)**		

Table 4.9 Insulin sensitivity outcomes for sub-group 3 participants

*denotes $p < 0.001$ compared to baseline within the same group, **denotes $p < 0.05$ compared to baseline within the same group, $^{\varphi}$ denotes $p < 0.05$ compared to 10 days within the same group, † denotes $p < 0.05$ between groups. Data expressed as medians and interquartile range. Mixed model analysis performed on Log10 transformed data.

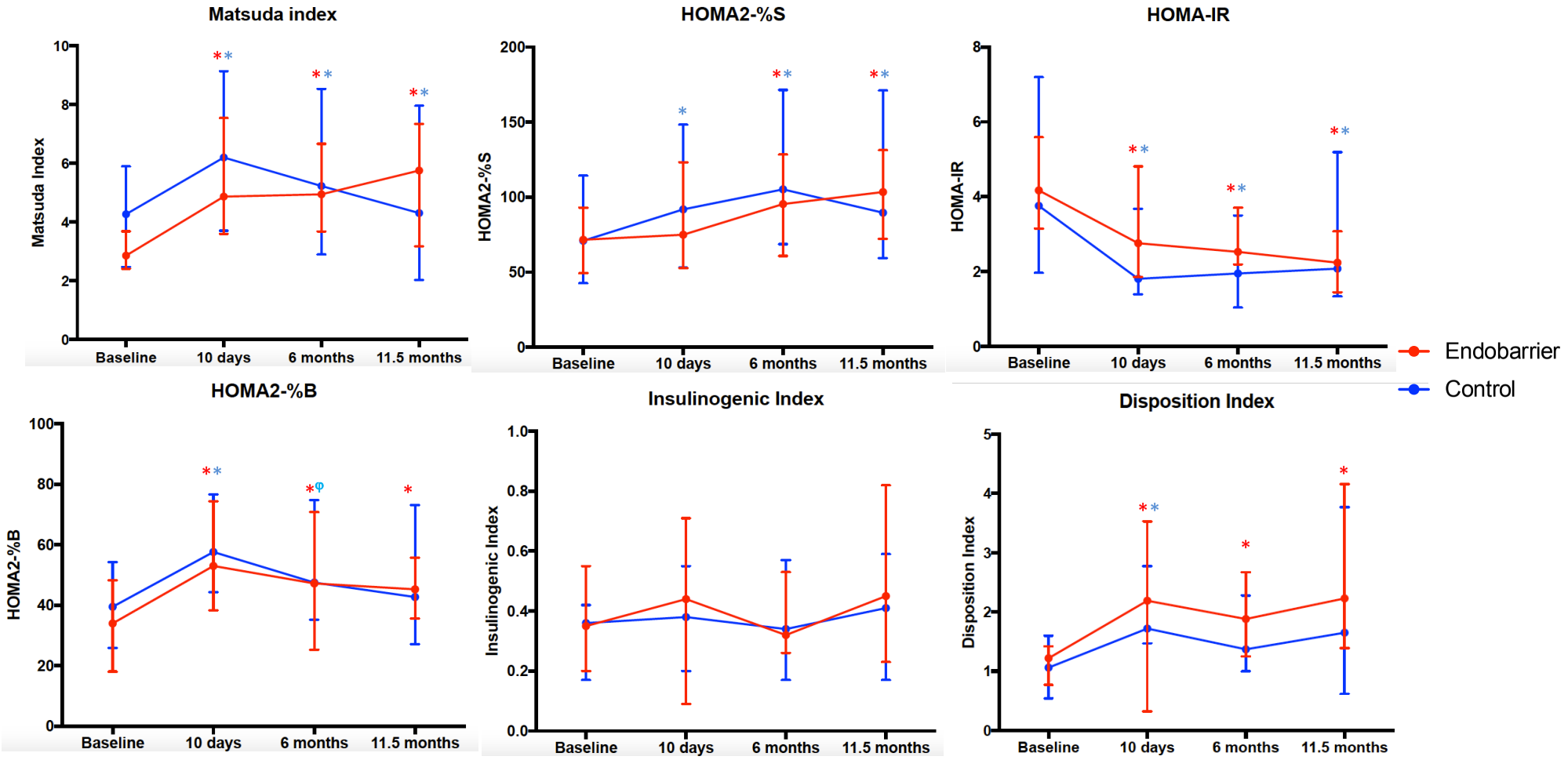


Figure 4.6 Changes in model assessment outcomes in Endobarrier and Control group participants in sub-group 3
* denotes $p < 0.05$ compared to baseline within the same group, § denotes $p < 0.05$ compared to 10 days within the same group, φ denotes $p < 0.05$ compared to 6 months within the same group, Δ denotes $p < 0.05$ between groups.

	Control		Endobarrier		Mixed Model Analysis	
Visit	n	Median (Interquartile range)	n	Median (Interquartile range)	Effect	p-value
HOMA2-%B						
Baseline	18	39.5 (25.9 – 54.2)	21	34.0 (18.1 – 48.2)	Group	0.390
10 days	18	57.6 (44.4 – 76.7)*	20	53.0 (38.4 – 74.3)*	Visit	<0.001
6 months	14	47.5 (35.2 – 74.8)$^{\varphi}$	19	47.2 (25.3 – 70.8)*	Group*Visit	0.464
11.5 months	12	42.7 (27.1 – 73.2)	11	45.3 (35.6 – 55.8)**		
Insulinogenic Index						
Baseline	17	0.36 (0.17 – 0.42)	21	0.35 (0.20 – 0.55)	Group	0.379
10 days	17	0.38 (0.20 – 0.55)	18	0.44 (0.09 – 0.71)	Visit	0.221
6 months	13	0.34 (0.17 – 0.57)	18	0.32 (0.26 – 0.53)	Group*Visit	0.800
11.5 months	12	0.41 (0.17 – 0.59)	10	0.45 (0.23 – 0.82)		
Disposition Index						
Baseline	16	1.06 (0.54 – 1.60)	20	1.22 (0.77 – 1.42)	Group	0.550
10 days	16	1.72 (1.47 – 2.77)**	18	2.19 (0.32 – 3.53)**	Visit	0.002
6 months	13	1.37 (1.00 – 2.28)	18	1.88 (1.25 – 2.67)**	Group*Visit	0.543
11.5 months	11	1.65 (0.62 – 3.77)	10	2.23 (1.39 – 4.16)**		

Table 4.10 ß-cell function outcomes for sub-group 3 participants

*denotes $p < 0.001$ compared to baseline within the same group, **denotes $p < 0.05$ compared to baseline within the same group, $^{\varphi}$ denotes $p < 0.05$ compared to 10 days within the same group. Data expressed as median + interquartile range. Mixed model analysis performed on Log10 transformed data.

4.4 Discussion

This study is the first to evaluate changes in insulin sensitivity and β-cell function using model assessments in patients implanted with the Endobarrier DJBL for 1 year within a randomised controlled trial. The main trial outcomes have shown that 12 months of treatment with the Endobarrier DJBL alongside intensive lifestyle and dietary guidance resulted in superior weight loss and reductions in BMI when compared to control participants managed with intensive lifestyle and dietary interventions alone. Notably, although participants in both study arms achieved significant weight loss and reductions in their BMI, there were no statistically significant differences in anthropometric outcomes between groups in the MMTT sub-group of patients (sub-group 3), suggesting that this sub-group was underpowered to show this effect.

In the MMTT sub-group of patients implanted with the Endobarrier DJBL, overall insulin sensitivity as estimated by HOMA2-%S (and its reciprocal HOMA-IR) and the Matsuda index improved within the first 10 days of treatment, during the low-calorie liquid diet phase of the trial. There were no significant differences in insulin sensitivity between the treatment arms and, in line with the reported weight-loss outcomes, insulin sensitivity values in both groups remained statistically unchanged between 10 days and 11.5 months follow-up, but remained improved overall in comparison to baseline levels. It is hypothesised that bypass of the foregut may result in early improvements in glycaemic indices through weight loss-independent mechanisms, as has been observed following RYGB. The current data, however, do not support this notion and the Endobarrier produced no additional improvements to early changes in insulin sensitivity above that achieved through weight loss and calorie restriction alone.

Evaluating all trial participants, in which control arm patients achieved significantly less weight loss than Endobarrier participants, insulin sensitivity outcomes initially followed a similar trend to the sub-group 3 patients in that they improved rapidly during the liquid diet phase of the trial and were not statistically different between treatment arms. In line with weight loss outcomes (**Table 2.5**), HOMA2-%S and HOMA2-IR values then plateaued in the control arm and were statistically unchanged between 10 days and 11.5 months follow-up. In contrast, however, HOMA2-%S and HOMA2-IR values continued to improve in participants treated with the Endobarrier device as their weight-loss increased and were significantly greater than control arm participants at 11.5 months follow-up ($p < 0.05$). This association was confirmed by Spearman rank-order correlation which showed a strong, positive relationship between weight loss and changes in insulin sensitivity (HOMA2-%S), which was statistically significant in both the Endobarrier group ($r_s = 0.333$, $p = 0.014$) and the control group ($r_s = 0.268$, $p = 0.042$). This is similar to results reported by

Munoz et al. who observed a strong negative correlation between %EBWL and HOMA-IR after 1 year of Endobarrier treatment on univariate regression analysis (r_s = -0457, p = < 0.019).[252]

In the control group, these correlations support those made from the HEC data reported in **section 3.3.5** in which weight loss was strongly correlated with changes in hepatic (Ra) and peripheral (Rd) insulin sensitivity. Unlike the correlations reported in this chapter, however, no such correlation existed in the Endobarrier participants, suggesting that early changes in insulin sensitivity may be as a consequence of weight-loss-independent mechanisms. Notably, the correlations performed as part of sub-group 2 were on significantly fewer patients and only for patients completing 6 months of follow-up. It is reported in the literature that improvements in peripheral insulin sensitivity occur over a longer time period and, from our knowledge of the changes that occur following RYGB, peripheral insulin sensitivity increases significantly after 4 weeks in proportion to ensuing weight loss and peaks after approximately 2 years.[27,178,179,350] The correlations reported in these chapters therefore support the notion that Endobarrier implantation may initially result in early changes in insulin sensitivity that occur secondary to weight-loss-independent mechanisms, such as the action of caloric restriction, incretins/anti-incretins, bile flow modulation, nutrient sensing and/or alterations to the gut microbiota, and that the later improvements in insulin sensitivity are the consequence of weight reduction.[27,338]

Similar to the trend observed for overall insulin sensitivity, markers of enhanced insulin secretion and β-cell function also significantly improved during the low-calorie diet phase of the trial; in both Endobarrier and control patients, HOMA2-%B and disposition index values increased significantly within the first 10 days with no significant differences between study groups. The effect of a low-calorie diet in acutely improving β-cell function is well documented in the literature and early reductions in pancreatic fat content have been implicated in restoring the first-phase insulin response.[150,350] Studies suggest that it may take up to 8 weeks to completely restore β-cell function but this effect may go some way to explain the current observations. Notably, however, the insulinogenic index, which is an estimate of first-phase insulin response, was unchanged in either group in this study. Another explanation may be due to an acute reduction in FPG levels and reduced levels of glucolipotoxicity.[27,176,192] Lim et al. demonstrated that in patients with T2DM who underwent a low-calorie diet or RYGB, hepatic fat content rapidly decreased in parallel to improvements in hepatic insulin sensitivity and FPG levels within 7 days.[150] This has been observed in the current study whereby FPG concentrations reduced significantly within the first 10 days of the trial, during the low-calorie diet phase, and this mirrors the improvements observed in both HOMA2-%B and disposition index values. Furthermore, following the resumption of *ad libitum* eating in control group participants in sub-group 3, FPG levels increased significantly back to baseline levels alongside a significant decrease in β-cell

indices (and decrease in insulin sensitivity). Conversely, in the sub-group 3 Endobarrier group, FPG concentrations remained significantly reduced between 10 days and 11.5 months and this was mirrored by a sustained improvement in insulin sensitivity and β-cell function. This trend is also seen in the HOMA2-%B analysis from all trial participants in which sustained reductions in FPG after 10 days were matched by sustained improvements in β-cell function in both study groups. Notably, there were no significant differences in β-cell indices between study arms and, from this, we can state that the Endobarrier resulted in no additional improvements in β-cell function above that achieved through calorie restriction alone. Certainly, increased weight loss in the Endobarrier group had no influence on improvements in β-cell function, as demonstrated by the Spearman correlations (Endobarrier group: r_s = -0.031, p = 0.824; control group: r_s = 0.218, p = 0.101), and highlights the likely contribution of acute caloric restriction, or some other weight-loss-independent factor, as discussed above.

Cohen et al. is the only other report to evaluate changes in insulin sensitivity and ß-cell function using several different model assessments in patients implanted with the Endobarrier DJBL and their outcomes closely match those reported within the current study.[230] Insulin sensitivity, as measured by them using HOMA-IR and Matusuda Index, improved by > 50% as early as 1 week following Endobarrier implantation. These values then remained improved but static between follow-up visits at week 1 and week 52. However, this prospective observational study lacked a control group as a comparator and the authors themselves conclude that any improvements in glycaemic control could be attributable to the inclusion of patients in the study and greater medical contact. Unlike our study, Cohen at al. evaluated insulin secretion rates using deconvolution of c-peptide concentrations and reported no significant changes during an Endobarrier implantation period of 1 year or following explantation of the device. They also reported no changes in the insulinogenic index, which correlates with the results of our sub-group analysis.

Following an implantation period of 12 months, our data demonstrate that, when the Endobarrier device was removed, patients regained weight precipitously to achieve a weight loss of 5.4 kg at 24 months, which was only 0.6 kg greater than and not statistically different to participants in the control arm (see **section 2.3.3**). In parallel to this, basal steady-state insulin sensitivity (HOMA2-%S) decreased significantly and insulin resistance (HOMA2-IR), FPG and insulin concentrations increased significantly in the 11 months following device removal. At this time-point, all values remained significantly improved from baseline levels but were not statistically different from control arm participants. β-cell function (HOMA2-%B) was seemingly unaffected by removal of the Endobarrier device and remained significantly improved from baseline levels but was not statistically different from the 10 days follow-up visit or from control arm participants at 23

months. This treatment decay and the observable deterioration in insulin sensitivity and glucose homeostasis that occurs following removal of the Endobarrier device matches what has been presented in several other prospective cohort studies.[229,230,242] This should therefore be an important consideration when referring a patient for a temporary EBT and highlights the importance of implementing medical and lifestyle interventions alongside these therapies in order to maximise their efficacy and longevity.

Limitations to this examination of sub-group 3 patients include the low participant numbers and high attrition rate. As such, the treatment effect of the Endobarrier device is likely to be underestimated within this cohort of patients. We are also lacking dynamic assessments of changes in insulin sensitivity and β-cell function derived from a MMTT following device explantation. Of note, the HOMA calculations performed in this study were based on single baseline measurements of plasma glucose and serum insulin concentrations. As insulin secretion is pulsatile, it is recommended that calculations be made based on the mean of three results taken at 5 minute intervals and this has been shown to yield HOMA2-%B and HOMA2-%S results with lower coefficients of variation.[131] However, in research practice, it has become acceptable for a single sample to be taken and this produces similar results in large datasets. In fact, HOMA2-%B and HOMA2-%S data obtained from 30 patients with diet-controlled T2DM that was computed from the mean of three basal samples at 5-min intervals correlated well with a single basal sample.[351]

In **Chapter 3** we have examined changes in tissue-specific insulin sensitivity using the 'gold standard' HEC. As discussed by Wallace et al.,[131] combining different assessments of insulin sensitivity and ß-cell function gives information across the dose-response curve; HOMA and HEC assessments give steady-state measurements in the basal and maximally-stimulated states, whereas the MMTT will yield measurements of dynamic (i.e. non-steady-state) insulin secretion and sensitivity over the mid-portion of the physiological range. In this trial, the MMTT and HECs were conducted on different cohorts of patients and we are therefore unable to draw direct correlations between the two study groups.

Despite these limitations, this is the first RCT to examine changes in insulin sensitivity and ß-cell function following Endobarrier implantation and makes an interesting and valuable contribution to this expanding field. More studies with greater participant numbers are justified in order to further investigate the changes in glucose homeostasis that occur following endoscopic bariatric therapies. Of interest, the Endobarrier System Pivotal Trial (Rev B) (STEP-1) is a randomised, multi-centre, double-blinded, sham-controlled trial that has commenced recruitment in the US. This trial is similar in design to our current study and aims to randomise 240 patients to receive

either the Endobarrier device plus moderate intensity lifestyle and dietary counselling or a sham control group receiving moderate intensity lifestyle and dietary counselling alone. One of the trials secondary outcome measures is to evaluate changes in HOMA-IR during the 1 year implant period and then for 1 year following device explantation, which will provide us the highest-level evidence of the effect of the Endobarrier on insulin resistance to date. The estimated study completion date is April 2024.

4.5 Conclusion

In conclusion, 12 months of treatment with the Endobarrier DJBL in patients with obesity and type 2 diabetes resulted in superior weight loss when compared to control patients managed with intensive lifestyle and dietary interventions alone. The DJBL produced no additional early improvements in insulin sensitivity or ß-cell function above that achieved through calorie restriction. However, increased weight loss in Endobarrier-treated patients was associated with significantly greater improvements in insulin sensitivity after 12 months of therapy. These treatment effects dissipated following explantation of the device.

Chapter 5:

The effect of a duodenal-jejunal bypass liner (Endobarrier®) on lipid profile and blood concentrations of long chain polyunsaturated fatty acids

5.1 Introduction

5.1.1 Background

5.1.1.1 Fatty acid classification and nomenclature

Lipids are a heterogeneous group of physiological substrates characterised by their relative insolubility in water and their solubility in non-polar solvents. Broadly, lipids consist of fatty acids (FAs), triglycerides (TAGs), phospholipids, steroids, eicosanoids, fat soluble vitamins and lipoproteins.[352] These all have metabolic and functional roles in the body. For example, phospholipids are major components of all cell membranes, FAs are important substrates for energy production and some lipids have important roles in cell signalling (e.g. eicosanoids). Intake of FAs, and other energy-yielding nutrients, that is in excess of requirements results in storage in the form of TAGs, mainly in adipose tissue but also in ectopic sites, such as in skeletal muscle and the liver. Hence, lipids contribute the bulk of fat mass.[353-355]

FAs are hydrocarbon chains of various lengths with a terminal carboxylate group and are classified according to the presence, number and position of double bonds, which also determine their physical and biochemical characteristics. Saturated FAs have no double bonds in the hydrocarbon chain, while monounsaturated FAs (MUFAs) contain one, and polyunsaturated FAs (PUFAs) contain more than one. In unsaturated FAs, geometric isomers exist depending on the orientation of the hydrocarbon chain around the axes of double bonds, which do not allow for rotation. Cis isomers exist when acyl chains are on the same side of the double bond whereas trans isomers form when they are on opposite sides.[352] The most commonly occurring FAs have double bonds in the cis configuration.[353]

FAs are often referred to by their common names, which often relate to their origin (such as oleic acid being derived from olive oil and palmitic acid being from palm oil). There is also a systematic nomenclature from the International Union of Pure and Applied Chemistry which names FAs according to the hydrocarbon chain length and the number of double bonds with the absence or presence of double bonds being indicated by the suffix *-anoic* and *–enoic*. The unsaturation position(s) is then denoted with a number before the FA name, which reflects the number of carbon atoms in the hydrocarbon chain. The double bond isomerism is then designated as either cis-trans or E/Z and is written before each unsaturation position number. Another naming system used to locate the unsaturation position is counting the number of carbon atoms from the omega/ω (methyl carbon) end of the chain and can further classify FAs into ω3, ω6 and ω9 families.[352] Finally, FAs can also be referred to by their shorthand name in which the number of carbon atoms is followed by the number of double bonds and their carbon atom number away

from the methyl carbon group.[353,355] Applying shorthand nomenclature assumes cis isomerism and cannot be used for trans configurations. **Table 5.1** lists the different nomenclatures for several common fatty acids.

5.1.1.2 Essential fatty acids, elongation and propagation

Humans can synthesise most FAs de novo from precursors such as glucose and, as such, these are termed *non-essential fatty acids*. In this process, acetyl-CoA is formed from glucose via the oxidation of pyruvate and, in turn, acetyl-CoA is converted into malonyl-CoA and subsequently into palmitic acid (16:0) through the action of fatty acid synthase. The pathway then elongates saturated and unsaturated fatty acyl-CoAs by using two carbons donated from malonyl-CoA via a microsomal fatty acid elongase system of enzymes.[356] Unsaturated FAs are synthesised by the introduction of a double bond in the Δ^9 position by the Δ^9 desaturase enzyme system. As the first double bond is inserted at the Δ^9 position, humans and other animals are able to completely synthesise the ω9 family of unsaturated FAs. In contrast, humans and other animals are not able to endogenously synthesise the parent members of the ω3 and ω6 families, linoleic and α-linolenic acid, respectively. This requires Δ^{12} and Δ^{15} desaturation enzymes, which are lacking in animals. Therefore, linoleic and α-linolenic acids are termed *essential fatty acids* and must be supplied in the diet, largely from plant foods such as nuts, seeds and oils. Other ω3 and ω6 PUFAs can then be synthesised from these two essential FAs through a well-recognised chain of elongation and desaturation (**Figure 5.1**).[357]

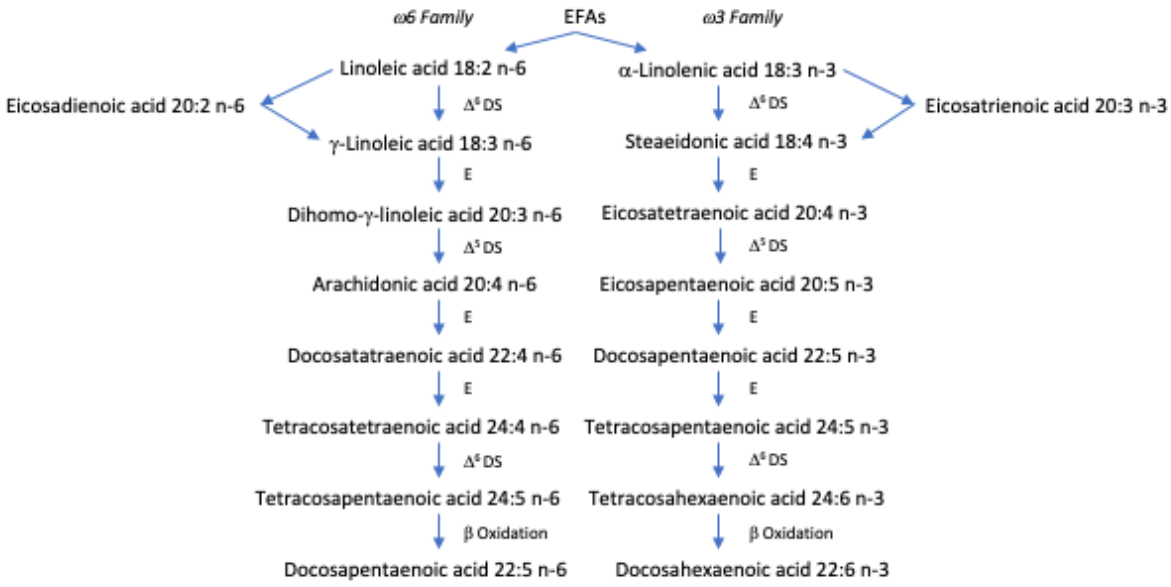


Figure 5.1 Biosynthesis of the ω3 and ω6 polyunsaturated fatty acids from linoleic and α-linolenic acid.
DS, desaturase; E, elongase; EFA, essential fatty acid.

Common Name	Family	Shorthand Name	IUPAC	Structure
Oleic acid (OA)	ω9	18:1n-9	9z-octadecenoic acid	$C_8H_{17}CH=CH(CH_2)_7COOH$
Linoleic acid (LA)	ω6	18:2n-6	(9*Z*,12*Z*)-octadeca-9,12-dienoic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
α-Linolenic acid (ALA)	ω3	18:3n-3	(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2=CH=CH(CH_2)_7COOH$
Arachidonic acid (AA)	ω6	20:4n-6	(5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$
Eicosapentaenoic acid (EPA)	ω3	20:5n-3	(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenoic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$
Docosahexaenoic acid (DHA)	ω3	22:6n-3	(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2COOH$
Palmitic acid (PA)		16:0	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$

Table 5.1 Fatty acid structure and nomenclature

5.1.1.3 Polyunsaturated fatty acids, inflammation and obesity

There is substantial evidence that very long chain omega-3 (ω3 or n-3) PUFAs have an essential protective role against cardiovascular and cerebrovascular disease, specifically α-linolenic acid (ALA; 18:3ω-3), eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3).[358,359] Furthermore, these FAs are associated with improvements in cognitive health, better visual and neurological development in infants, and resolution of inflammatory processes in chronic inflammatory diseases, such as asthma and arthritis.[354,358] As such, the WHO strongly recommend the weekly consumption of 1 – 2 servings of oily fish, which is the equivalent to 200 – 500 mg of EPA and DHA daily, or adequate intake of plant sources of α-linolenic acid in vegetarians.[360]

ALA consumption varies widely in Western diets, between 0.5 and 2.3 g/day.[358] Along with other long-chain FAs, ALA absorption occurs in the small intestine following hydrolysis of TAGs carrying FAs, emulsification and micellisation. Micelles containing the monoglycerides and FAs that result from TAG hydrolysis are then absorbed across the apical surfaces of epithelial cells, within which they are esterified back into TAGs and, together with other products of lipid digestion, are secreted as chylomicrons into the lymphatic system. Chylomicron TAGs ultimately enter the bloodstream via the thoracic duct and upon entering capillary beds are hydrolysed on the endothelial surface by lipoprotein lipase activity. The FAs that are released by this process then diffuse into hepatocytes, myocytes and adipocytes in which they enter one of several different biochemical pathways depending on their ultimate fate, which may include incorporation into TAGs and storage, inclusion within cell membranes as components of phospholipids, β-oxidation and energy production, or desaturation and elongation into other related PUFAs (**Figure 5.1**).

As discussed in **section 1.2.2.4**, obesity is characterised by pro-inflammatory adipose tissue, which plays a pivotal role in the development of the insulin resistant state and the metabolic syndrome. FA elongase and desaturase activity is known to be regulated by insulin and, in obese patients, insulin resistance results in alterations in endogenous fatty acid metabolism.[361] Specifically, it has been demonstrated that there is attenuated Δ^5-/Δ^6-desaturase and elongase activity, resulting in lower concentrations of arachidonic acid (AA), EPA and DHA when compared to normal weight controls.[362,363] The precise correlation of this to metabolic health outcomes and the exact anti-inflammatory properties of ω-3 PUFAs remain unclear but can be explained by several mechanisms. Firstly, EPA and DHA are substrates for the synthesis of resolvins and protectins and are ligands for GPCR120, which act to resolve inflammation and inhibit inflammatory signalling pathways through decreased production of chemoattractants, growth factors, adhesion molecules, eicosanoids and cytokines.[364] Furthermore, they have also been shown to decrease

TAG concentrations, blood pressure, thrombus formation and cardiac arrhythmias, and increase atherosclerotic plaque stability, nitric oxide production and endothelial relaxation.[358,359,365,366] Lastly, eicosanoids (prostaglandins, thromboxanes and leukotrienes) produced from ω-3 PUFAs are less inflammatory than those from the ω-6 counterpart, AA.[359,365] A reduction in endogenous ω-3 PUFAs therefore potentiates a pro-inflammatory state and may contribute to the morbidity and mortality observed in those with obesity and metabolic syndrome.

5.1.1.4 Effect of weight loss surgery on long chain PUFAs

The impact of bariatric surgery on cardiovascular risk factors is well documented with numerous randomised and prospective observational studies collectively demonstrating the superiority of metabolic surgery over medical treatment in the short- and medium-term in achieving a reduction in weight, diabetes and cardiovascular medications, improving TAG, LDL and HDL cholesterol concentrations, ameliorating or resolving hypertension, and improving metabolic syndrome.[182] It has been proposed that the effects of surgery and weight loss on endogenous FA metabolism and PUFA concentrations may contribute to these effects.[367,368] However, data on the precise impact of bariatric surgery and the differing effects of purely restrictive procedures (e.g. LAGB) compared to primarily malabsorptive procedures (e.g. RYGB and BPD-DS) on FA pools remains limited.[357,361,363,367,369-378]

Those studies that have examined changes in FA profile following weight loss surgery support the observation that, at baseline, patients with obesity have significantly higher concentrations of MUFAs and lower concentrations of both ω-6 and ω-3 PUFAs when compared to healthy weight controls.[363,373] Those undergoing calorie restriction for the purpose of weight loss, through any means, typically have a low intake of EFAs and, consequently, endogenous stores of LA and ALA are also depleted through β-oxidation.[371] However, it is evidenced that obese individuals who lose weight have increased circulating concentrations of EPA and DHA as a result of restored desaturase and elongase activity.[361,362,371] Several studies therefore support the notion that bariatric surgery increases EFAs, ω-3 and/or ω-6 PUFAs to levels that are comparable to normal weight controls (i.e. reversing the pathological FA profile seen in obese individuals).[361,367,369,371,373] These improvements have also been shown to be superior in RYGB[361,371] when compared to LAGB.[371] However, there is also evidence to support EFA and PUFA deficiency following malabsorptive bariatric operations (i.e. RYGB and BPD-DS) and the preliminary recommendations are that patients should be supplemented with ω-3 PUFAs following such procedures.[357,363,367]

5.1.1.5 Effect of EBTs on long chain PUFAs

As discussed in **section 1.2.5.3,** Endoscopic Bariatric Therapies (EBTs) provide a minimally-invasive therapeutic option to achieve weight-loss and treat obesity-related diseases by going beyond what can be achieved through medical and lifestyle interventions alone whilst limiting the potential morbidity and mortality associated with surgery (**Figure 1.5**). EBTs include intragastric balloons, duodenal-jejunal bypass liners (DJBLs), transoral gastroplasty procedures (e.g. POSE, ESG), and duodenal mucosal resurfacing. There is an increasing body of evidence to support these interventions as effective treatments for weight loss and metabolic disease.[213-215,379-384] However, to date, no studies have examined the effect of these interventions on FA metabolism. It is also unknown whether any changes seen in lipid profile and FA metabolism are simply proportional to weight loss or may be secondary to relative nutritional malabsorption or yet some unidentified factor in the foregut that may alter lipid metabolism.

5.1.2 Aims

The aim of this research is to investigate the effects of duodenal exclusion using the Endobarrier DJBL on blood lipid profile and long chain polyunsaturated fatty acid concentrations.

5.1.3 Hypotheses

The hypotheses being tested in the work that will be described within this chapter are that duodenal exclusion in patients with obesity and T2DM using the Endobarrier DJBL alongside intensive lifestyle modification:

- results in greater improvements in blood lipid profile than in control patients with obesity and T2DM treated with diet and intensive lifestyle modification alone;
- significantly depletes blood concentrations of long chain PUFAs to a greater extent than in control patients with obesity and T2DM treated with diet and intensive lifestyle modification alone.

5.1.4 Objectives

The objectives of this research are:

- to evaluate the changes in lipid profile (total serum cholesterol, triglycerides, LDL-C and HDL-C) in patients with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for 12 months and compare these changes to those

in control patients with obesity and T2DM receiving diet and intensive medical therapy alone;

- to evaluate the changes in blood concentrations of long chain PUFAs in patients with obesity and T2DM treated with intensive medical therapy and implanted with the Endobarrier DJBL for 12 months and compare these changes to those in control patients with obesity and T2DM receiving diet and intensive medical therapy alone.

5.2 Methods

5.2.1 Patients and study design

The results reported in this chapter describe the changes in lipid profile and blood concentrations of long chain PUFAs for all patients recruited into the EndoBarrier trial. The trial methodology, including patient selection, randomisation, trial interventions and follow-up schedule, is described and discussed in detail in **Chapter 2**.

At visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), 8 (+6 months ± 7 days) and 10 (+11.5 months ± 7 days) all participants across both study arms attended the Wellcome Trust Clinical Research Facility at Southampton General Hospital or Imperial Clinical Trials Unit at Imperial College London. Patients arrived fasted for these visits (at least 8 hours without food and drink, except for water). On arrival, basic anthropometric measurements were taken and fasting blood samples were obtained and analysed in accordance with the methods described in detail in **section 2.2.7.** The outcome measurements relevant to this chapter that were recorded at these visits are summarised in **Table 5.2**.

	Sample	Analysis
Anthropometric measurements	Weight (kg) Height (m)	BMI (kg/m^2) TBWL (kg) %TBWL %EWL
Biological samples	Serum	Total cholesterol (mmol/L) LDL cholesterol (mmol/L) HDL cholesterol (mmol/L) Triglycerides (mmol/L)
	Plasma	Fatty acids (ug/ml)
Food preference data	Epic Food Frequency 24-hour dietary recall 3-day food diary	Total caloric intake Macronutrient composition

Table 5.2 Summary of outcome measurements at visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), 8 (+6 months ± 7 days) and 10 (+11.5 months ± 7 days)

5.2.2 Nutritional assessment

In order to evaluate for differences in dietary habits between the two study arms as a possible alternative explanation for changes in blood PUFA concentrations, nutritional assessments were completed at each study visit. There are inherent limitations to the relying on verbal reports from patients, especially in a trial that is not double-blinded. The preferred method of measuring food intake would be using a buffet meal or a 24-hour residential stay. The problem of under-reporting of food intake among patients with obesity is common when using most dietary assessment methods.[385] As an attempt to reduce the variability in data collection and dietary analysis, these were carried out by the same two dietitians throughout the trial period. Additionally, participants in both groups received the same behavioural and dietary modification instructions from a single dietitian throughout the whole trial period. Changes in food intake and food choices after any obesity intervention incorporate several alterations in the components of the ingestive behaviour. As a result, having a single method to assess changes in eating behaviour is considered inadequate to understand the impact of any weight loss approach on food intake and preference. As such, in this trial, several validated measures were used concurrently to assess the effect of using the DJBL device on food intake and food preferences.

Participants who gave additional consent to take part in the Food Preference sub-group of the trial (sub-group 3) were asked to complete The European Prospective Investigation of Cancer Food Frequency Questionnaire (EPIC FFQ) and a trained dietitian performed a detailed 24-hour dietary recall assessment.[386] Furthermore, patients were asked to complete a food diary, detailing all food and drink consumed in the 72 hours immediately prior to this visit. Information gathered from food diaries and the 24-hour dietary recall were entered and analysed using Dietplan7 software (Forestfield Software Ltd, UK) to obtain total calories (kcal) and macronutrients (carbohydrates, protein, and fat). Macronutrients are expressed as the percentage contribution of total calories per day. Data obtained from the EPIC FFQ were evaluated using the FETA system, provided from the same group, which converts frequency codes into average daily nutrient intake for an individual from 130 listed foods. A specific code was used if any data were missing. Missing data for more than ten items was considered high and required the participant to be excluded from the analysis. The analysis provides results for total calories and macronutrients.

5.2.3 Measurement of serum lipid profile

Whole blood samples were collected into BD Vacutainer® SST Tubes from an 18-20G cannula (B-D Insyte-W, Becton Dickinson, UK) sited in the antecubital fossa. Samples were transferred immediately to the central chemical pathology laboratories at Southampton General Hospital and

Imperial College London for the measurement of serum plasma lipid profile. TAG, total serum cholesterol and HDL-C concentrations were measured and recorded using an automated lipid profile analyser (AU5800: Beckman Coulter, High Wycombe, UK). LDL-C concentrations were then extrapolated from these measurements. Units are expressed as mmol/L.

5.2.4 Collection and storage of plasma samples

Whole blood samples were collected into 6 ml Sodium Heparinised BD Vacutainer® Tubes. Samples were gently mixed in order to prevent coagulation and, within 30 minutes of collection, were centrifuged at 4°C for 15 minutes at 1600 g to generate plasma from whole blood. The plasma was collected and aliquoted into cryotubes and stored at -80°C until analysis.

5.2.5 Analysis of fatty acids by gas chromatography

5.2.5.1 Reagents and materials

Reagents and materials used for all experiments are described in **Appendix B**.

5.2.5.2 Principles of fatty acid analysis by GC

The general principles of GC are explained at length in **section 3.2.5.6.1**. FA chain length and the number, position and configuration of any double bonds will determine the temperature at which they become volatile, the degree of affinity to the silica capillary column (stationary phase) and, as a consequence, the order in which fatty acids elute. Increasing chain length increases the temperature at which FAs enter the gas phase and increases FA-silica affinity, whereas double bonds lower the boiling point and reduce the affinity to the stationary phase. In order to improve column separation of FAs at lower temperatures, especially long chain FAs, they are derivatised into fatty acid methyl esters (FAMEs) by adding a methyl group to the carboxylic acid terminal through a reaction with a methyl donor (e.g. methanol) in the presence of a catalyst (e.g. sulphuric acid).

FAs are insoluble in aqueous solution and in plasma will either be esterified to form more complex lipids (such as TAGs, phospholipids or cholesteryl esters) or will be bound to albumin in the form of non-esterified fatty acids (NEFAs). In addition to improving the volatility of fatty acids, the acid present in the methylation reaction will also liberate FAs from TAGs, phospholipids and cholesteryl esters.

5.2.5.3 Total lipid extraction

The most widely used method of total lipid extraction from plasma is described by Folch et al.[387] Plasma samples were thawed and mixed by vortexing and centrifuged at 13,000 rpm for 5 minutes to remove any denatured protein. Plasma (0.4 ml) was mixed with 0.1 ml of C15 phosphatidylcholine internal standard, dissolved in 1 ml of dry chloroform:methanol (2:1, vol:vol) containing butylated hydroxytoluene (BHT; 50 mg/l) as an anti-oxidant. A further 5.0 ml dry chloroform:methanol (2:1, vol:vol) containing BHT (50 mg/l) and 1.0 ml 1 M NaCl was added and mixed. Samples were centrifuged at 2000 rpm for 10 minutes (low brake at room temperature) and the lower phase lipid layer was collected and dried under nitrogen at 40°C.

5.2.5.4 Preparation of fatty acid methyl esters

0.5 ml of dry toluene was added to the purified lipid fraction and mixed, followed by 1.0 ml of methylation reagent (dry methanol with 2% H_2SO_4). Samples were mixed gently by inversion and then incubated for 2 hours at 50°C. Once cool, 1.0 ml of neutralising solution (0.25 M $KHCO_3$ (25.03 g/l)), 0.5M K_2CO_3 (69.10 g/l) and 1.0 ml dry hexane were added and mixed by vortexing. Samples were then centrifuged at 1000 rpm for 2 minutes (low brake at room temperature) and the upper phase containing the FAMEs was collected and dried under nitrogen at 40°C. 75 µl of dry hexane was subsequently added to each sample, vortex mixed and transferred to a GC auto sampler vial. This final step was then repeated, at which time the samples could be capped and stored at -20°C for up to a month.

5.2.5.5 GC-FID analysis

FAMEs were analysed on a Hewlett-Packard 6890 GC using a SGE BPX-70 fused silica capillary column (30m x 0.25µm x 0.25µm) with temperature control and a flame ionising detector. Split ratio was programmed at 25:1. The injector port was set at 300°C with a helium carrier gas (flow rate 1.0, pressure 14.6 and velocity 29). To condense FAMEs, the oven was held at 115°C for 2 mins, then increasing by 10°C min^{-1} up to 200°C, where it was then held for 18.5 mins. For the next cycle, temperature was increased at a rate of 60°C min^{-1} up to 245°C, where it was held for 4 mins. The flame ionisation detector was held at 300°C and FAME chromatograms were produced.

5.2.5.6 Analysis of chromatograms

FAME chromatograms were analysed with Agilent Chemstation software (**Figure 5.2**). Peaks from each fatty acid were identified automatically and, following peak integration, the area under each peak was calculated and compared to the internal standard in order to quantify absolute fatty

acid concentrations. Absolute concentrations of EFAs (LA and ALA) and their derivatives were recorded. The following formula was used:

$$\mu g/ml = \frac{Ap}{Ai} \times mi \times ms$$

Ap = area under the peak of interest, Ai = area under the internal standard, mi = mass of internal standard, ms = mass or volume of sample extracted.

5.2.6 Statistical analysis

For baseline data, normally distributed variables are reported as means with standard deviation (SD) and for comparison of these variables the independent t-test was used. Non-normally distributed variables are reported as medians with the interquartile range, and the Mann-Whitney U test was used to compare such variables. Categorical variables were compared using the Chi-square test or the Fisher's exact test as appropriate. A *p*-value <0.05 was considered statistically significant. Due to the skewed distribution of fatty acid data, a normally distributed dataset was obtained by logarithmically transforming (Log10) the data prior to statistical analysis. This analysis was performed using SPSS® 25.0 software (SPSS, Chicago, IL, USA) and mechanistic data was assessed using a mixed model approach as described in **Section 2.2.9**.

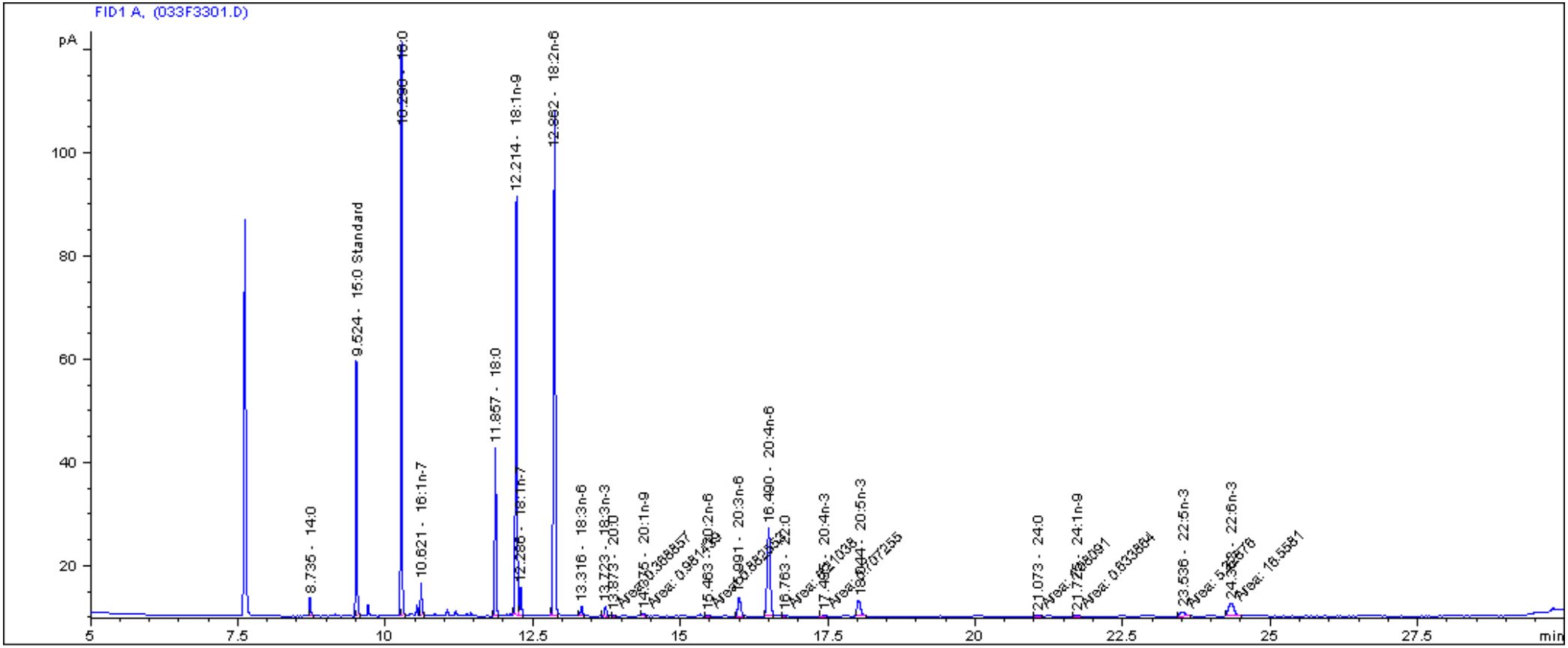


Figure 5.2 Example chromatogram from GC-FID analysis of plasma total lipid and C15 Phosphatidylcholine standard.

pA, picoamperes

5.3 Results

5.3.1 Patient characteristics and baseline data

Figure 5.3 summarises the flow of participants through the trial that were included in fatty acid analysis at study visits 3 (-2 weeks ± 7 days), 5 (+10 days ± 7 days), 8 (+6 months ± 7 days) and 10 (+11.5 months ± 7 days). For a full description of the recruitment outcomes of the Endobarrier trial please refer to **section 2.3** and the consort diagram in **Figure 2.8**, in which the reasons for patient attrition/non-inclusion are fully detailed. Missing or insufficient plasma samples account for all other unavailable data.

For the purposes of fatty acid analysis, visit 3 (-2 weeks ± 7 days, i.e. pre-intervention) served as the baseline visit and the patient characteristics of each study arm are summarised in **Table 5.3.** In total, data were analysed from 70 participants in each arm of the trial, who were matched with regard to age, gender and weight. An investigative independent samples t-test produced a significant difference in BMI between the groups at baseline (p = 0.033)..

	Control (n=70) mean ± SD	**Endobarrier (n=70) mean ± SD**	
Age, years	52.3 ± 8.3	51.6 ± 7.8	p = 0.585
Sex, n (%) Female	31 (44.3)	32 (45.7)	p = 0.266
Weight, kg	103.6 ± 13.9	107.8 ± 17.1	p = 0.109
BMI, kg/m^2	35.4 ± 3.7	37.0 ± 5.0	p = 0.033

Table 5.3 Baseline characteristics of participants included in fatty acid analysis.
SD, standard deviation; kg, kilograms; BMI, body mass index.

5.3.2 Anthropometric outcomes

Anthropometric outcomes are summarised in **Table 5.4**. Weight and BMI significantly reduced in both groups from baseline to 6 months and then plateaued between 6 months and 11.5 months. There were no statistically significant differences in weight or BMI between groups at any of the follow-up visits. TBWL (kg and %) significantly increased in both groups up until 6 months and then plateaued. TBWL was significantly greater in the Endobarrier group compared to the control group at all time points: 10 days = 5.7 ± 2.5 % versus 4.3 ± 1.7 % (mean difference [95% CI] = 1.42 % [0.02, 2.82], p = 0.047), 6 months = 10.4 ± 4.4 % versus 6.1 ± 5.1 % (4.35 % [2.88, 5.82], p <0.001) and 11.5 months = 11.3 ± 5.3 % versus 6.0 ± 5.7 % (5.27 % [3.75, 6.80], p <0.001).

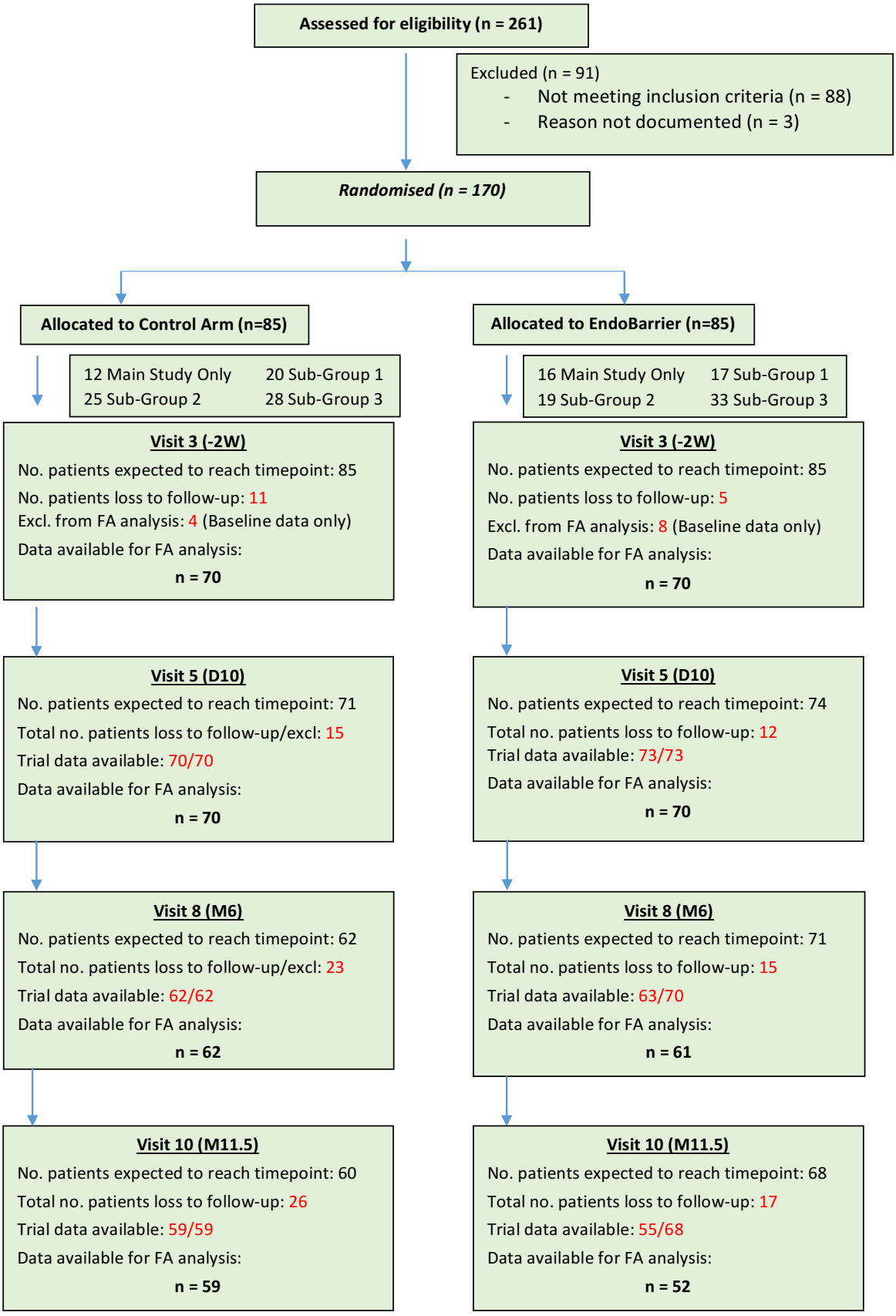


Figure 5.3 Consort flow chart of participants included in fatty acid (FA) analysis

5.3.3 Nutritional assessment outcomes

Data gathered from the EPIC food frequency questionnaires, detailed 24-hour dietary recall assessment and 3-day food diaries were analysed at Imperial College London by Dr Madhawi Aldwayan. The results from this analysis are summarised in **Appendix D**.

Forty-seven participants took part in this food preference sub-group of the trial. There was a significant reduction in the absolute weight within each group at 10 days, 6, and 12 months compared to baseline ($p < 0.001$). There was a significant reduction in % weight loss within each group at 10 days, 6 and 12 months compared to baseline ($p < 0.001$) but no significant differences between the groups.

5.3.4 Total caloric intake using 24-hour dietary recall and food diaries

Total caloric intake per day obtained from both the 24-hour dietary recall and 3-day food diaries was significantly reduced within both groups at all-time points compared to baseline ($p \leq 0.001$), but there were no significant differences between the groups.

5.3.5 Food preferences using 24-hour dietary recall and food diaries

There was a significant reduction in the % contribution from carbohydrates ($p \leq 0.001$) and a significant increase in the % contribution from protein to daily caloric intake within both groups ($p \leq 0.001$). However, there were no significant differences between the groups. There was no significant reduction in the % contribution from fat either within or between groups.

5.3.6 Food preferences using food frequency questionnaires (FFQs)

There was a significant reduction in the total calories consumed within the Endobarrier group at all-time points compared to baseline ($p \leq 0.001$). Also, there was a significant difference between groups at baseline ($p < 0.05$). There was a significant reduction in the consumption of carbohydrates within the Endobarrier group at all-time points compared to baseline ($p \leq 0.001$) and there was a significant difference between groups at baseline ($p < 0.05$).

There was a significant reduction in the consumption of fat within the Endobarrier group at all-time points compared to baseline ($p \leq 0.001$), and at one year in the control group ($p < 0.05$). Also, there was a significant difference between groups at baseline ($p < 0.05$). At 12 months, consumption of fats and oils was significantly greater in the Endobarrier group than in the control group (**Table 5.5**).

	Control		Endobarrier		Mixed Model Analysis	
Visit	n	Mean ± SD	n	Mean ± SD	Effect	p-value
		Weight (kg)				
Baseline	70	103.6 ± 13.9	70	107.8 ± 17.1	Group	0.687
10 days	70	99.2 ± 13.3*	70	101.7 ± 16.8*	Visit	<0.001
6 months	62	97.8 ± 14.7*$^{\varphi}$	61	96.2 ± 16.7*§	Group*Visit	<0.001
11.5 months	59	97.2 ± 14.6*$^{\varphi}$	52	94.9 ± 14.9*§		
		BMI (kg/m^2)				
Baseline	70	35.4 ± 3.7	70	37.0 ± 5.0	Group	0.485
10 days	70	33.9 ± 3.7*	70	34.9 ± 5.0*	Visit	<0.001
6 months	62	33.3 ± 4.0*$^{\varphi}$	61	33.3 ± 5.0*§	Group*Visit	<0.001
11.5 months	59	33.2 ± 4.0*$^{\varphi}$	52	32.7 ± 4.3*§		
		Total body weight Loss (kg)				
Baseline	70	-	70	-	Group	<0.001
10 days	70	4.4 ± 1.9†	70	6.1 ± 2.8†	Visit	<0.001
6 months	62	6.3 ± 5.4$^{\varphi\Delta}$	61	11.1 ± 5.2$^{\S\Delta}$	Group*Visit	<0.001
11.5 months	59	6.2 ± 6.3$^{\varphi\Delta}$	52	12.2 ± 6.6$^{\S\Delta}$		
		Total body weight Loss (%)				
Baseline	70	-	70	-	Group	<0.001
10 days	70	4.3 ± 1.7†	70	5.7 ± 2.5†	Visit	<0.001
6 months	62	6.1 ± 5.1$^{\varphi\Delta}$	61	10.4 ± 4.4$^{\S\Delta}$	Group*Visit	<0.001
11.5 months	59	6.0 ± 5.7$^{\varphi\Delta}$	52	11.3 ± 5.3$^{\S\Delta}$		

Table 5.4 Anthropometric outcomes for patients included in fatty acid analysis

*denotes $p < 0.001$ compared to baseline within the same group, § denotes $p < 0.001$ compared to 10 days within the same group, $^{\varphi}$ denotes $p < 0.01$ compared to 10 days within the same group, † denotes $p < 0.05$ between groups, Δ denotes $p < 0.001$ between groups.

There was a significant reduction in the consumption of nuts and seeds within the Endobarrier group at six months only (p = 0.02), but not in the control group. There was also a significant reduction in the consumption of meat and meat products within the Endobarrier group at one year compared to baseline (p < 0.05), but not in the control group.

The following food groups were not significantly different between groups or within groups at any time point; protein, alcohol, cereal and cereal products, eggs and eggs dishes, fish and fish products, fruit, milk and milk products, non-alcoholic beverages, potatoes, soups and sauces, sugars; preserves and snacks, and vegetables.

	Group								Mixed Model Analysis	
	Endobarrier				Control				Effect	*P value*
Fats and oils group (g)										
Baseline	22	27	±	20	15	16	±	13 †	Group	0.07
6 months	22	15	±	12***	15	11	±	9	Time	0.001
12 months	16	21	±	26	15	8	±	7*†	Group x Time	0.06
24 months	14	13	±	10**	15	13	±	9		

Results presented as mean ± SD
* P <0.05 compared to baseline within the same group
** P <0.01 compared to baseline within the same group
*** P <0.001 compared to baseline within the same group
† P <0.05 Between groups

Table 5.5 Changes in fat and oil consumption determined from EPIC Food Frequency Questionnaire.

5.3.7 Changes in blood lipid profile

Blood lipid profile outcomes are summarised in **Table 5.6**. TAG concentrations significantly reduced in both groups between baseline and 10 days. In the Endobarrier group, concentrations then remained stable at 11.5 months follow-up whereas, in the control group, concentrations increased again from 6 months to 11.5 months back to baseline levels. There was no statistically significant difference between treatment groups at any of the follow-up visits.

Total serum cholesterol concentrations were significantly reduced in the Endobarrier group at +10 days and then increased again but remained significantly lower than baseline levels at 11.5 months follow-up. In the control group, levels significantly decreased by +10 days and then increased back up to baseline levels by the 6 months follow-up visit. Total serum cholesterol concentrations were significantly lower in the Endobarrier arm compared to the control arm at 6 months (3.99 $\pm$ 0.89 mmol/L versus 4.53 $\pm$ 1.36 mmol/L; mean difference [95% CI] = 0.64 [0.28, 0.99], p = 0.001) and 11.5 months (4.10 $\pm$ 0.96 mmol/L versus 4.36 $\pm$ 0.96 mmol/L; mean difference [95% CI] = 0.40 [0.03, 0.76], p = 0.035).

	Control		Endobarrier		Mixed Model Analysis	
	n	Mean ± SD	n	Mean ± SD	Effect	p-value
Triglycerides (mmol/L)						
Baseline	68	1.95 ± 0.85	70	2.01 ± 1.14	Group	0.888
10 days	68	1.48 ± 0.59*	70	1.68 ± 0.66**	Visit	<0.001
6 months	61	1.69 ± 0.95***	59	1.56 ± 0.64*	Group*Visit	0.138
11.5 months	59	1.86 ± 1.52$^{\varphi}$	52	1.71 ± 0.79 ***		
Total Serum Cholesterol (mmol/L)						
Baseline	69	4.50 ± 1.04	70	4.55 ± 0.93	Group	0.159
10 days	68	3.60 ± 1.07*	70	3.73 ± 1.10*	Visit	<0.001
6 months	62	4.53 ± 1.36§†	61	3.99 ± 0.89*†	Group*Visit	<0.001
11.5 months	59	4.36 ± 0.96§†	53	4.10 ± 0.96*$^{\varphi\dagger}$		
LDL-C (mmol/L)						
Baseline	67	2.45 ± 0.94	68	2.49 ± 0.86	Group	0.450
10 days	68	1.82 ± 0.95*	69	1.96 ± 0.97*	Visit	<0.001
6 months	61	2.44 ± 1.00§†	59	2.19 ± 0.71*$^{\alpha\dagger}$	Group*Visit	0.003
11.5 months	56	2.32 ± 0.88§	52	2.25 ± 0.76**$^{\alpha}$		
HDL-C (mmol/L)						
Baseline	69	1.16 ± 0.31	70	1.16 ± 0.27	Group	0.051
10 days	68	1.12 ± 0.25***	70	1.03 ± 0.26*	Visit	<0.001
6 months	62	1.29 ± 0.32*§†	61	1.16 ± 0.29§†	Group*Visit	<0.001
11.5 months	59	1.29 ± 0.32*§†	53	1.15 ± 0.30§†		

Table 5.6 Lipid profile outcomes

* denotes p < 0.001 compared to baseline within the same group, ** denotes p < 0.01 compared to baseline within the same group, *** denotes p < 0.05 compared to baseline within the same group, § denotes p < 0.001 compared to 10 days within the same group, $^{\varphi}$ denotes p < 0.01 compared to 10 days within the same group, α denotes p < 0.05 compared to 10 days within the same group, † denotes p < 0.05 between groups, Δ denotes p < 0.001 between groups.

LDL-C concentrations were significantly reduced in the Endobarrier group at +10 days and then increased again but remained significantly lower than baseline levels at 11.5 months follow-up. In the control group, levels significantly decreased by +10 days and then increased back up to baseline levels at 6 months. LDL-C concentrations were significantly lower in the Endobarrier arm compared to the control arm at 6 months (2.19 $\pm$ 0.71 mmol/L versus 2.44 $\pm$ 1.00 mmol/L; mean difference [95% CI] = 0.34 [0.03, 0.65], p = 0.031) but no difference was seen between groups at 11.5 months.

HDL-C concentrations were significantly reduced in the Endobarrier group at +10 days and then returned to baseline levels by 6 months and were maintained at 1 year follow-up. In the control group, levels were also significantly decreased by +10 days but then increased to be significantly greater than baseline levels by 6 months follow-up and was maintained at 1 year. HDL-C concentrations were significantly higher in the control arm than in the Endobarrier arm at 6 months (1.16 $\pm$ 0.29 mmol/L versus 1.29 $\pm$ 0.32 mmol/L; mean difference [95% CI] = 0.13 [0.03, 0.23], p = 0.008) and 11.5 months (1.15 $\pm$ 0.30 mmol/L versus 1.29 $\pm$ 0.32 mmol/L; mean difference [95% CI] = 0.14 [0.05, 0.24], p = 0.004).

5.3.8 Changes in long chain PUFA concentrations

Changes to fatty acid concentrations in the control and Endobarrier arms of the trial are summarised in **Table 5.7, Table 5.8** and in **Figure 5.4.**

5.3.8.1 Essential fatty acids: ALA and LA

Absolute EFA concentrations decreased significantly between the baseline visit and 10 days follow-up visit in both treatment arms (i.e. following the intervention and calorie-controlled liquid-diet phase of the trial). There were no significant differences between groups for either ALA or LA for either of these visits. In the control group, ALA and LA concentrations significantly increased between 10 days and 11.5 months back to baseline levels. In the Endobarrier group, concentrations also increased but remained significantly lower than baseline levels at the 11.5 months follow-up visit. ALA concentrations were significantly lower in the Endobarrier group than in the control group at both 6 and 11.5 months. Similarly, LA concentrations were also significantly lower in the Endobarrier group at 6 months but were not significantly different to the control arm at 11.5 months.

	Control		Endobarrier		Mixed Model Analysis	
	n	Median (Interquartile range)	n	Median (Interquartile range)	Effect	p-value
		α-Linolenic acid (ALA; 18:3n-3) - µg/ml				
Baseline	67	14.92 (11.48 – 22.02)	68	13.81 (11.01 – 19.76)	Group	0.030
10 days	68	11.47 (7.95 – 14.97)*	70	10.87 (8.02 – 13.54)*	Visit	<0.001
6 months	60	13.06 (8.96 – 19.90)**$^{\alpha}$†	60	11.07 (15.16 – 9.02)*†	Group*Visit	0.564
11.5 months	55	12.51 (10.20 – 19.15)$^{\alpha}$†	52	12.42 (8.54 – 16.54)*$^{\alpha}$†		
		Eicosapentaenoic acid (EPA; 20:5n-3) - µg/ml				
Baseline	67	17.41 (12.83 – 25.96)	70	17.26 (11.60 – 25.65)	Group	0.020
10 days	69	9.31 (6.43 – 13.64)*	70	8.12 (5.92 – 12.71)*	Visit	<0.001
6 months	61	18.52 (12.58 – 24.70)§Δ	60	14.22 (9.69 – 19.34)*§Δ	Group*Visit	0.024
11.5 months	56	19.05 (12.87 – 23.09)§†	52	14.35 (10.39 – 20.35)*§†		
		Docosapentaenoic acid (DPA; 22:5n-3) - µg/ml				
Baseline	67	11.46 (9.34 – 15.40)	70	11.53 (8.85 – 15.19)	Group	0.018
10 days	68	8.25 (7.08 – 10.74)*	70	8.45 (6.44 – 10.68)*	Visit	<0.001
6 months	60	10.78 (8.90 – 14.71)§†	60	9.65 (6.79 – 11.87)*$^{\alpha}$†	Group*Visit	0.008
11.5 months	55	11.72 (9.57 – 14.39)§†	52	9.92 (7.62 – 13.25)*§†		
		Docosahexaenoic acid (DHA; 22:6n-3) - µg/ml				
Baseline	68	40.88 (30.26 – 53.03)	70	36.30 (26.21 – 48.34)	Group	0.017
10 days	69	33.60 (27.43 – 41.50)*	70	30.25 (23.20 – 38.26)*	Visit	<0.001
6 months	61	41.40 (30.38 – 49.43)§†	60	31.71 (26.20 – 44.64)**†	Group*Visit	0.056
11.5 months	56	42.60 (31.73 – 50.74)§†	52	34.05 (26.27 – 40.45)**†		

Table 5.7 Changes in Omega 3 long chain PUFAs.

* denotes p < 0.001 compared to baseline within the same group, ** denotes p < 0.05 compared to baseline within the same group, § denotes p < 0.001 compared to 10 days within the same group, α denotes p < 0.05 compared to 10 days within the same group, φ denotes p < 0.05 compared to 6 months within the same group † denotes p < 0.05 between groups, Δ denotes p < 0.001 between groups. Data expressed as median + interquartile range. Mixed model analysis performed on Log10 transformed data.

5.3.8.2 Omega-3 long chain PUFAs: EPA, DPA and DHA

As seen with the essential fatty acids, concentrations of EPA, DPA and DHA decreased significantly between the baseline visit and 10 days follow-up visit in both treatment arms and were not significantly different between groups. In the control arm, all three of these PUFAs returned to baseline levels by 6 months and remained stable at 11.5 months follow-up. In the Endobarrier group, these ω-3 PUFAs also increased between 10 days and 6 months but then plateaued and remained significantly lower than baseline levels at 11.5 months. EPA, DPA and DHA concentrations were all significantly lower in participants with the Endobarrier when compared to controls at 6 and 11.5 months.

5.3.8.3 Omega-6 long chain PUFAs: AA

AA followed a similar trend to EPA, DPA and DHA in both treatment arms: concentrations decreased significantly between the baseline visit and 10 days follow-up visit and were not significantly different between groups. In the control arm, AA concentrations then returned to baseline levels by 6 months and remained stable at 11.5 months follow-up. In the Endobarrier group, AA concentrations also increased between 10 days and 6 months but then plateaued and remained statistically lower than baseline at 11.5 months. AA concentrations were significantly lower in participants with the Endobarrier when compared to controls at 6 and 11.5 months.

	Control		Endobarrier		Mixed Model Analysis	
	n	Median (Interquartile range)	n	Median (Interquartile range)	Effect	p-value
Linoleic acid (LA; 18:2n-6) - µg/ml						
Baseline	64	500.47 (406.02 – 615.61)	70	567.88 (432.08 – 654.45)	Group	0.388
10 days	65	397.45 (303.37 – 509.78)*	70	389.05 (295.09 – 515.56)*	Visit	<0.001
6 months	58	485.06 (407.03 – 591.80)§†	60	429.72 (345.56 – 502.61)*$^{\alpha}$†	Group*Visit	<0.001
11.5 months	53	489.91 (392.06 – 603.53)§	52	470.38 (354.76 – 558.50)*§		
Arachidonic acid (AA; 20:4n-6) - µg/ml						
Baseline	65	149.77 (129.25 – 189.81)	70	146.83 (113.91 – 187.49)	Group	0.098
10 days	67	136.61 (115.68 – 165.02)*	70	131.41 (110.01 – 160.52)*	Visit	<0.001
6 months	59	148.16 (134.64 – 188.50)§†	60	134.89 (104.77 – 161.47)*†	Group*Visit	0.006
11.5 months	54	159.89 (128.56 – 188.84)§†	52	151.92 (111.65 – 174.57)**$^{\alpha}$†		

Table 5.8 Changes in Omega 6 long chain PUFAs.

* denotes p < 0.001 compared to baseline within the same group, ** denotes p < 0.05 compared to baseline within the same group, § denotes p < 0.001 compared to 10 days within the same group, α denotes p < 0.05 compared to 10 days within the same group, φ denotes p < 0.05 compared to 6 months within the same group † denotes p < 0.05 between groups, Δ denotes p < 0.001 between groups. Data expressed as median + interquartile range. Mixed model analysis performed on Log10 transformed data.

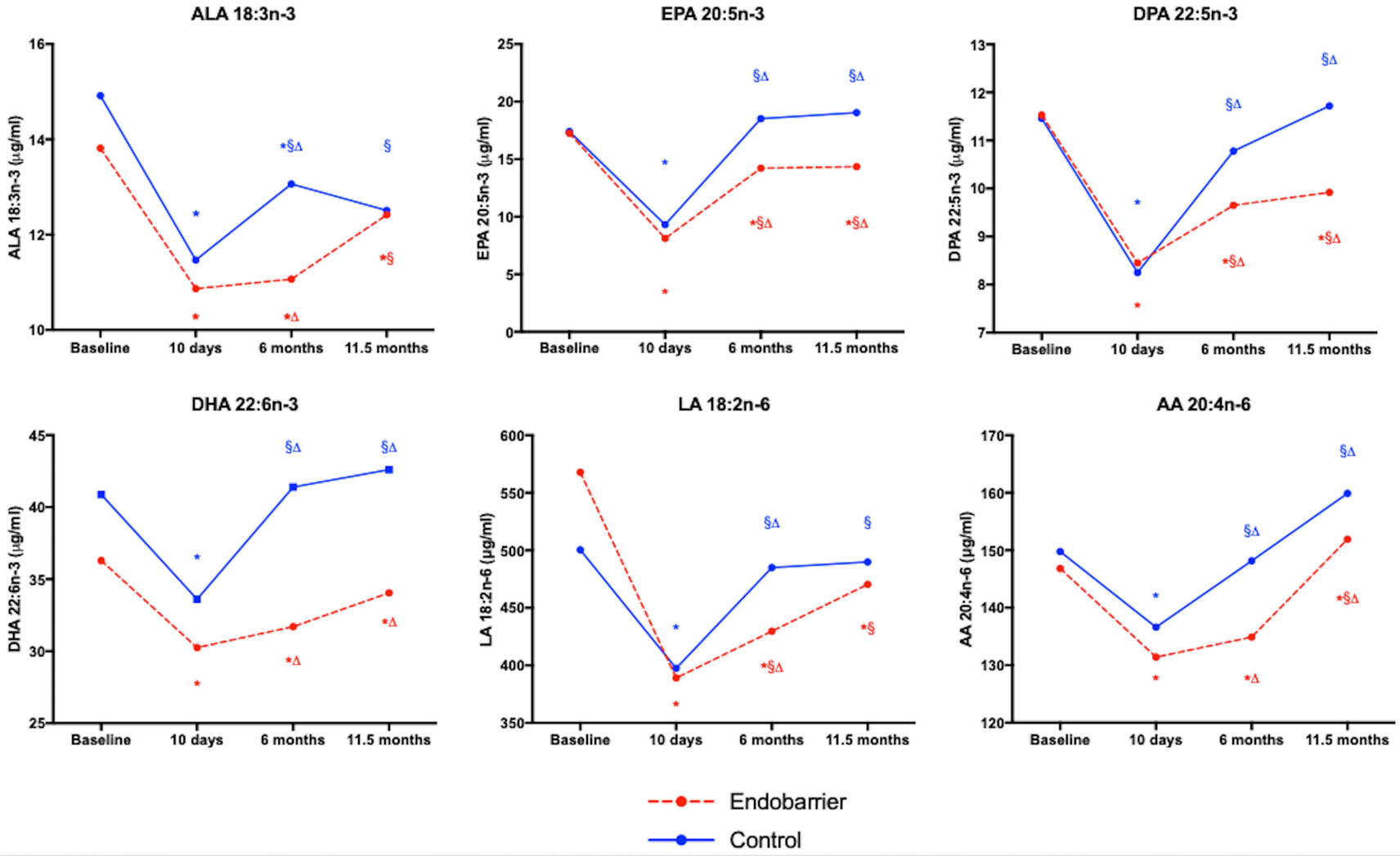


Figure 5.4 Changes in long chain PUFA concentrations in Endobarrier and Control group participants

* denotes $p < 0.05$ compared to baseline within the same group, § denotes $p < 0.05$ compared to 10 days within the same group, φ denotes $p < 0.05$ compared to 6 months within the same group, Δ denotes $p < 0.05$ between groups.

5.4 Discussion

In this study cohort, weight loss was superior in participants treated with the Endobarrier DJBL when compared to medically-treated control participants at all follow-up visits with a %TBWL of 11.3% and 6.0% achieved in each group respectively at 11.5 months. This finding is in keeping with the best available systematic review and meta-analysis in the field and other published literature of the Endobarrier device.[225,227,237,244,246,251,257] In the food-preference sub-group of patients, we were able to demonstrate that there were no significant differences in caloric intake between study arms and, beyond the baseline visit, food preferences and caloric intake were matched between groups except for a significantly greater intake of fats and oils in the Endobarrier group at 12 months when compared to the control group. We can therefore reasonably assume that weight loss is achieved through mechanisms, not yet fully understood, which go beyond calorie restriction alone.

In line with weight-loss, we have also been able to demonstrate positive changes in the blood lipid profile of those patients implanted with the Endobarrier device for 1 year compared to control participants. Notably, total serum cholesterol concentrations were significantly lower in the Endobarrier arm after 11.5 months and LDL-C concentrations were significantly lower at 6 months follow-up, but this difference was not maintained at 1 year. Although TAG concentrations significantly reduced in both arms, there were no significant differences between groups. Again, these findings match the best available literature, which support overall reductions in total serum cholesterol and LDL-C concentrations with variable reports of changes in TAG concentrations. In this study, HDL-C concentrations were unchanged in the Endobarrier arm at 1 year follow-up, which also agrees with outcomes reported elsewhere.[225,229,242,246,248,253,257] In the control arm, however, HDL-C concentrations significantly increased to above baseline levels that were statistically greater than Endobarrier patients. This is a unique report which cannot be explained by our weight loss or nutritional assessment outcomes.

This is the first study to report on the effect of an endoscopic bariatric therapy on fatty acid concentrations. Common to both study groups is that calorie restriction with a liquid diet for 20 days resulted in a significant decrease in all measured FA concentrations. In this phase of the trial, all participants were advised to consume 125 ml Fortisip Compact drinks (Nutricia, UK) (5 per day for men, 4 per day for women), with each containing 3.4 g of polyunsaturated fats. Following this period, patients resumed ab libitum eating and, thereafter, there were no significant differences in dietary habits between treatment arms, except for a greater intake of fats and oils in the Endobarrier group, as evaluated from food diaries, 24-hour dietary recall and the EPIC food frequency questionnaire. Correspondingly, between 10 days and 11.5 months all FA

concentrations trended upwards in both groups, most likely as dietary fat intake increased. However, for both omega-3 and -6 long-chain PUFAs, concentrations were significantly lower in Endobarrier patients at 6 and 11.5 months follow-up when compared to control patients, except LA for which there was no difference at 11.5 months. There was also an observed reduction in the EPA:AA ratio in the Endobarrier arm (0.12 at baseline vs 0.09 at 11.5 months), which remained unchanged in the control arm (0.12 vs 0.12). This therefore supports the notion that the Endobarrier device depletes absolute serum concentrations of EFAs and their PUFA derivatives. Lower EFAs could result from reduced dietary intake and/or reduced absorption. For the longer chain derivative PUFAs there could be reduced dietary intake, reduced absorption or reduced endogenous synthesis because of less availability of the EFA substrates.

If we look at the literature for metabolic surgery, it is well documented that RYGB, BPD-DS and other bariatric operations result in significant changes in FA pool concentrations. These reports, however, do conflict in the changes observed. Our findings match those studies that report an overall decrease in serum long chains PUFAs following weight-loss surgery.[357,363,367] This is possibly a consequence of reduced dietary intake coupled with increased liberation from adipose stores and increased FA oxidation during significant weight loss.[363,388] Furthermore, it has been observed that fat malabsorption occurs with limb lengths of 150 cm.[389,390] Although fat absorption was not formally measured in this study, bypass of the duodenum with the Endobarrier is analogous with a 60 cm biliopancreatic limb and we can assume that weight loss and fat malabsorption likely contributes to the observed changes in FA concentrations. Garla et al.[357] also propose that decreased intestinal expression of the Fatty Acid Desaturase-1 (FADS1) gene, which is responsible for the desaturase activity converting ALA to EPA within intestinal cells, may also be responsible, in part, for the observed reduction in this specific PUFA.

Several studies have reported on proportional changes in ω-3 and ω-6 PUFAs (as opposed to absolute concentrations) and observed that, although absolute concentrations of these FAs decreased, relative proportions were actually increased following metabolic surgery, thus creating a more favourable lipid profile.[357,361,373] In the study by Walle et al., however, participants were instructed to consume 3 teaspoons of rapeseed oil and 6 tea spoons of mainly rapeseed oil based spreads daily for at least 1–2 years after the obesity surgery and were instructed to consume fish 2–3 times a week. It is therefore unclear whether the observed changes are as a result of increased dietary intake following metabolic surgery or occur due to enhanced elongase and desaturase activity secondary to weight loss.[361]

In view of the mounting evidence that very long chain ω-3 PUFAs have an essential protective role against cardiovascular and cerebrovascular disease, it seems prudent to identify whether or not

participants undergoing these metabolic procedures, such as Endobarrier implantation, are at risk of FA deficiency. The evidence we have so far, taken from metabolic surgery, suggests that these patients are indeed at risk of nutritional deficiency but that this can be offset by maintaining an adequate dietary intake of ω-3 PUFAs or by adequately supplementing the diet. Given the clear findings in this study that absolute concentrations of ω-3 and ω-6 PUFAs decline following Endobarrier implantation, dietary supplementation should be advised. Of note, omega 3 supplementation prior to metabolic surgery has been examined in one RCT and resulted in greater perioperative weight loss and reduced post-operative pain and CRP levels.[378]

Unlike RYGB and BPD-DS, Endobarrier is a temporary measure and implantation is currently limited to 12 months. Literature on the Endobarrier to date suggests that there is weight regain following explantation of the device but several studies have reported sustained weight loss up to one year after explant. It can be postulated, given the general increase in absolute ω-3 and ω-6 PUFA concentrations observed in this study following initial calorie restriction, that the reductions in PUFA concentrations are unlikely to be maintained following explantation, but this is unknown.

We recognise some limitations to this study, including the lack of healthy weight controls as a comparator and also the lack of correlation of our findings with quantitative measurements of fat absorption or intestinal gene expression. We have also limited our examination to polyunsaturated fatty acids in the serum and further examination of the effect of EBTs on FA metabolism should look at all fatty acid pools and correlations with other potential confounding factors, such as changes in lipid lowering medications. Finally, FA analysis was limited to only the implant period and the change to serum FA concentrations following removal of the device remains unknown.

Despite this, the current study is a well-powered, randomised controlled trial in which we have provided the first convincing evidence that duodenal bypass with the Endobarrier DJBL results in significant reductions in serum concentrations of long chain PUFAs when compared to dietary modification and lifestyle advice alone.

5.5 Conclusion

In conclusion, one year of therapy with the Endobarrier DJBL in patients with obesity and T2DM resulted in superior weight loss and significantly greater reductions in total serum cholesterol and LDL concentrations when compared to dietary modification and lifestyle advice alone. Furthermore, absolute blood concentrations of EFAs and their long chain ω-3/-6 PUFA derivatives were significantly lower in those participants receiving one year of Endobarrier therapy and, in

view of this, dietary supplementation with ω-3 and ω-6 PUFAs should be advised in these patients.

Chapter 6:

Endoscopic Sleeve Gastroplasty: a modified technique with greater curvature compression sutures

6.1 Introduction

Obesity and obesity-related diseases are preventable conditions that represent a significant socioeconomic burden. In 2017, the World Health Organisation (WHO) estimated that 13% of the worlds adult population were obese (clinically defined as a body mass index (BMI) of ≥ 30 kg/m^2) and this incidence has tripled since 1975.[391] In the UK alone, it has been projected that there will be an additional 11 million obese adults by 2030. Metabolic surgery remains the most effective long-term means of treating these patients by producing often profound and sustained weight loss, as well as weight-loss independent improvements in metabolic health, which consequently ameliorates, or even eliminates, associated co-morbidities and reduces mortality.[169,171,173] The safety profile of metabolic surgery has markedly improved in recent years with quoted mortality rates of 0.1 - 0.5% worldwide (0.11% in the UK) but with serious morbidity recorded in up to 6% of patients.[155,185] Unfortunately, only 1% of eligible, obese patients currently undergo metabolic operations as there continue to be numerous, multifactorial barriers to surgery.[314]

Endoscopic Bariatric Therapies (EBTs) provide a minimally-invasive, and potentially cost-effective, therapeutic option to achieve weight-loss and treat obesity-related diseases by going beyond what can be achieved through medical and lifestyle interventions alone whilst limiting the potential morbidity and mortality associated with surgery.

First described in 2008, the Endoscopic Sleeve Gastroplasty (ESG) is rapidly being established as an effective means of achieving significant weight loss via the transoral route.[294] The generally accepted technique of ESG utilises the OverStitch™ (Apollo Endosurgery Inc., Austin, TX, USA), cap-based flexible endoscopic suturing system which is mounted onto a double-channel endoscope. Starting distally at the level of the incisura angularis and working proximally along the greater curvature towards the fundus, the OverStitch™ device is used to fashion multiple, interrupted triangular plications. This creates concentric compression along the greater curvature that results in the formation of a short tubular gastric lumen and a 70% reduction in stomach volume without the need to amputate the greater curvature, as is required in Laparoscopic Sleeve Gastrectomy. Weight-loss is achieved by significantly restricting the volume of food consumed at each meal coupled with a delay in gastric emptying, which promotes early satiety.[295] This now widely-accepted technique, as described by Lopez-Nava et al,[296] has excellent short-term safety and efficacy outcomes reported in numerous prospective observational studies.[295-311] After 2 years, patients can expect to achieve a total body weight loss (TBWL) of 15-20% and two studies have reported associated improvements in several metabolic parameters.[295,299,392] Following ESG, patients report only mild-to-moderate, transient, post-operative symptoms and significant adverse events have been reported in selected studies at a rate of <3%.[295,297,299,300]

Multiple ESG suture patterns already exist that are based broadly around a triangular configuration of plications whereby tension on these plications acts to reduce the volume of the gastric cavity by concentrically compressing the greater curvature with some additional shortening of the sleeve (**Figure 6.1**). More recently, several different "Z"-shaped running suture patterns have also been trialled.[298,300] Graus Morales et al. demonstrated comparable short-term results using this technique but failed to demonstrate superiority of this suture pattern over the 'conventional' triangular pattern in terms of weight-loss outcomes after 18 months.

In our centre, a modified suture pattern was designed in order to optimise the biomechanical compressive forces acting along the greater curvature. Through the addition of longitudinal compression sutures, tension is more equally distributed across each stitch and produces maximal, uniform compression in both anterior-posterior and craniocaudal dimensions. With better, more homogenous compression and greater volume reduction in this manner, we hypothesize that this will translate to earlier satiety and superior weight loss outcomes.

Herein are reported the 6 month outcomes of consecutive patients undergoing ESG by a single operator in our centre, comparing the previously described triangular configuration of plications (**Figure 6.1**) against a modified suture configuration that incorporates longitudinal compression sutures along the greater curvature (**Figure 6.2**).

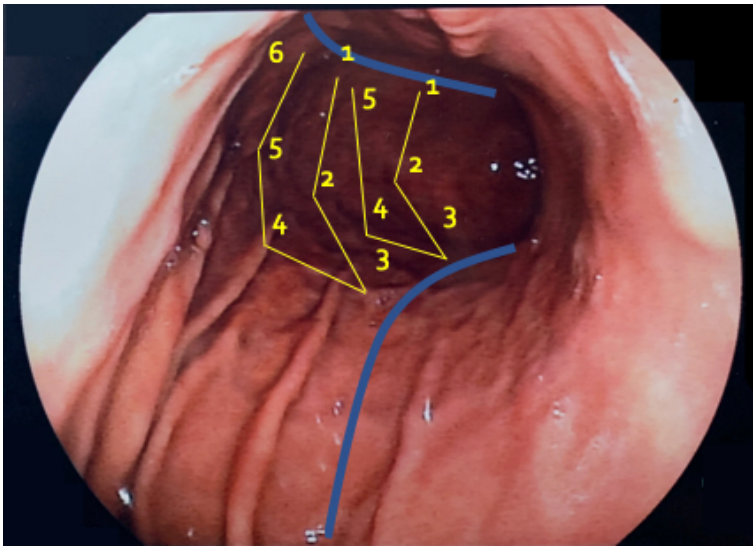


Figure 6.1 Schematic representation of the conventional triangular suture pattern utilised by Lopez-Nava et al. (2015) (No longitudinal Compression).

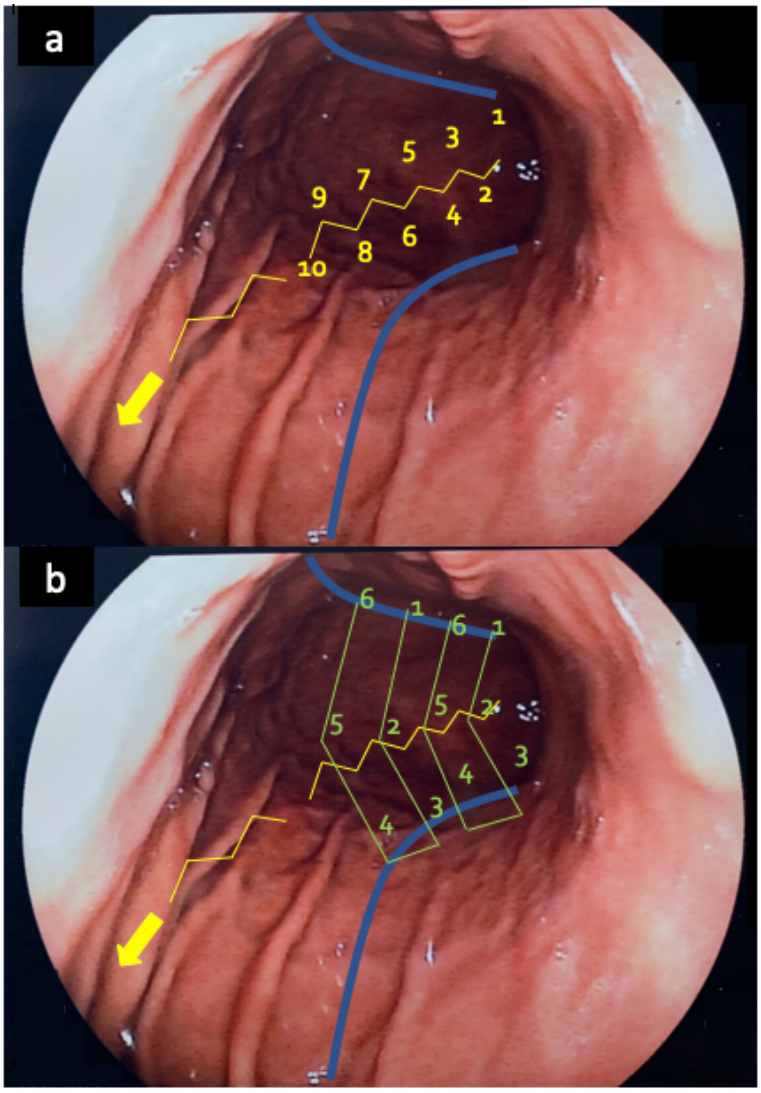


Figure 6.2 Schematic illustration of modified suture pattern
a) initial placement of longitudinal compression sutures, followed by b) a second parallel layer of 'U'-shaped plications.

6.1.1 Aims

The aim of this retrospective study is to identify the effects of a modified suture pattern on weight loss outcomes in patients undergoing ESG in a single UK centre.

6.1.2 Hypotheses

The hypotheses being tested in the work that will be described within this chapter are that performing ESG in patients with obesity using a modified suture pattern resulted in:

- no additional morbidity or increased procedural time when compared to ESGs being performed with standard suture patterns;
- significant weight loss that was greater than that achieved by patients following ESG using standard suture patterns.

6.1.3 Objectives

The objectives are:

- to compare procedural times and morbidity resulting from ESGs being performed with a modified suture pattern with ESGs being performed with a standard suture pattern;
- to evaluate the change in weight in patients with obesity 6 months following treatment with ESG using a modified suture pattern and compare it to the weight changes of patients with obesity treated with a standard ESG suture pattern.

6.2 Methods

6.2.1 Patient selection and study design

This is a retrospective analysis of all patients undergoing ESG by a surgical endoscopist in a single centre (Spire Healthcare, Southampton, UK) between January 2016 and December 2017. The surgeon conducting the procedures is established as an experienced interventional endoscopist, proficient in general interventional techniques (endoscopic mucosal resection, submucosal dissection, ERCP and endoscopic suturing using the OverStitch™ device (e.g. transoral gastric outlet reduction)) and other EBTs (Endobarrier and Primary Obesity Surgery Endoluminal (POSE)). Prior to performing ESG, they had attended a two-day dedicated training course followed by four proctored ESG cases.

This work does not report on primary research and all procedures, inclusive of the pre-, peri- and post-operative care practices, were conducted as part of routine treatment within this institution. Procedures were only performed after necessary approvals were sought and granted by the local Medical Advisory Committee and Institutional Review Board. Privately-funded patients with obesity were referred for consideration of ESG and were deemed eligible if they were: $\geq$ 18 years old, had a BMI $\geq$ 30 kg/m^2 and had declined or were not deemed eligible for weight-loss surgery. All patients had already participated in a medical weight-loss management programme and a comprehensive surgical, anaesthetic and psychiatric pre-assessment was performed in all cases. Nutritional assessment and education was delivered by a specialist dietitian. Every potential patient received comprehensive verbal and written information about the ESG procedure, including: general care advice; technical details about ESG; local experience of ESG; potential risks and benefits associated with the procedure based on currently available evidence; and possible treatment alternatives. Furthermore, all patients were informed of the novelty of this procedure and there was full transparency with regards to its development and implementation within the UK. Each patient provided informed, written consent prior to the ESG being performed.

Data were obtained from the retrospective review of patient records that were compiled as part of routine hospital treatment. Data are completely anonymised and as this analysis was performed as a service evaluation, formal ethical approval was not required. This evaluation was performed with full conformity to local clinical governance policies and with the permission of the hospital governance manager. No funding was obtained for this project.

6.2.2 ESG technique

Between January 2016 and June 2016, consecutive patients underwent ESG with the previously described triangular configuration of plications, henceforth referred to as pattern (1): 'no longitudinal compression'. Thereafter, between June 2016 and December 2017, consecutive cases were performed implementing our adapted suture pattern, hereafter referred to as pattern (2): 'longitudinal compression'.

6.2.2.1 Suture pattern (1): No longitudinal compression

This technique has been well described previously.[296] In brief, full thickness bites are taken from the anterior stomach wall, followed by the greater curvature and then the posterior wall before then repeating the pattern in the opposite direction (**Figure 6.1**). Each triangular-shaped plication suture generally consists of 3 – 9 full-thickness bites that, when clinched together, produce circumferential compression (and, to some degree, shortening) of the greater curvature. 6 – 8 plications are typically used to create the gastric sleeve.

6.2.2.2 Suture pattern (2): Longitudinal compression

Using this modified technique, the ESG is started by siting 2 longitudinal compression sutures along the greater curvature of the stomach; the first suture commences in the proximal antrum and terminates in the mid-body along the greater curve, and the second suture commences in the mid-body and progresses into the fundus along the greater curve (**Figure 6.2a**). Each linear compression suture consists of approximately 10 bites sited 1.0 - 1.5cm apart so that when each suture is clinched together the compressive force is distributed evenly along the long-axis of the stomach. The greater curvature is therefore maximally compressed in a concertina-type pattern. Supplementary to this, a second parallel layer of 3-6 interrupted 'U'-shaped plications are sited, with each plication being formed of 5-9 bites of the Overstitch™ (**Figure 6.2b**). This second layer acts to produce supplementary compression and volume reduction.

As per the institutions ESG protocol, patients were instructed to consume only a clear liquid diet one day prior to admission and, on the morning of the ESG, a standard oral premedication regime of a proton pump inhibitor (PPI) (Lansoprazole 30 mg), analgesia (Paracetamol 1 g), an anti-inflammatory (Etoricoxib 60 mg) and an antiemetic (Aprepitant 80 mg) were prescribed.

All procedures were carried out after an 8 hour fast in the operating theatre under a general anaesthetic with the patient in the left-lateral decubitus position with endotracheal intubation. At the time of induction of anaesthetic, a prophylactic intravenous dose of Cefuroxime 1.5 g and Metronidazole 500 mg were administered. An initial gastroscopy was performed using standard

CO_2 insufflation in order to evaluate the anatomy and ensure that there were no contraindications to performing the ESG, such as gastric ulceration, erosive duodenitis, the presence of a hiatus hernia > 5 cm or any malignant or pre-malignant gastric lesions. A full length oesophageal overtube was then sited and the OverStitch™ device, mounted onto a double-channel endoscope (GIF-2T240; Olympus Medical Systems Corp., Tokyo, Japan), was delivered into the stomach. Starting distally at the level of the incisura angularis and working proximally along the greater curvature to the fundus of the stomach, each plication was formed using a tissue retraction screw (Helix; Apollo Endosurgery Inc., Austin, TX, USA) to draw the muscularis propria of the stomach wall into the jaws of the Overstitch™ device in order to take multiple full thickness bites. The bites of each plication were then clinched together to approximate the tissue and produce compressive occlusion of the greater curvature.

Patients were admitted overnight and received regular analgesia, a PPI (Lansoprazole Fast Tabs 30 mg BD), two further doses of intravenous antibiotics, and regular anti-emesis, with strong emphasis placed on avoidance of retching. Patients were permitted to drink water immediately following the procedure and were subsequently progressed to a clear liquid diet for 3 days, full liquid diet for 2 weeks, pureed food for 2 weeks, and then soft diet for a further 2 weeks before then returning to ab libitum eating. Patients were advised to consume a low fat, low carbohydrate diet with approximately 60 g of protein per day and were strongly advised to eat small volumes with cessation of eating immediately at the point of satiety. Dietary follow-up was closely supervised by a specialist dietitian.

6.2.3 Outcome measures and follow-up

The complete medical records of all patients undergoing ESG within this period were reviewed and data was collected using a standardised pro forma. Procedural details and outcomes were obtained by scrutinising the hand-written operative and anaesthetic records. Appointments with the multidisciplinary team were offered to patients at baseline and then at 1, 3, 6, 9 and 12 months, with annual clinical reviews thereafter. During each visit, wellbeing was reviewed by the operating consultant and full dietary counselling was performed by a specialist dietitian. Basic anthropometric measurements were recorded in the clinical notes during visits, including weight (kg) and height (m), from which Total Body Weight Loss (TBWL, Kg), BMI (= weight (kg)/ (height (m))^2), %TBWL (Total Body Weight Loss (%) = (TBWL (kg)/baseline weight (Kg)) x 100), and %EWL (Excess Weight Loss (%) = (TBWL (Kg)/(baseline weight (Kg) – ideal body weight (Kg)) x 100) were extrapolated. All other clinically relevant qualitative data reported in the notes during the visits were also recorded in the pro forma, including the nature and frequency of adverse events and the subjective impact of ESG on portion sizes, appetite and satiety.

6.2.4 Statistical Analysis

Normally distributed variables are reported as means with standard deviation (SD) and for comparison of these variables the independent t-test was used. Non-normally distributed variables are reported as medians with the full range and the Mann-Whitney U test was used to compare such variables. Categorical variables were compared using the Chi-square test or the Fisher's exact test as appropriate. A *p*-value < 0.05 was considered statistically significant. Data were analysed using SPSS® 24.0 software (SPSS, Chicago, IL, USA).

6.3 Results

6.3.1 Patient characteristics and baseline data

The medical records of thirty-two patients undergoing ESG between January 2016 and June 2016 were reviewed; the first n = 9 consecutive cases were completed utilising suture pattern 1 (no longitudinal compression) and the subsequent n = 23 consecutive cases were completed using suture pattern 2 (longitudinal compression). In the no compression and compression groups the mean ages were 45 $\pm$ 12 years and 43 $\pm$ 10 years, the median baseline weights were 113.6 kg (range 82.0 -156.4) and 107 kg (range 74.0 – 136.0), and the median baseline BMIs were 35.9 kg/m^2 (range 30.9 – 43.8) and 36.5 kg/m^2 (range 29.8 – 42.9) respectively. 56% (n = 5) were women in the no compression group versus 78% (n = 18) in the longitudinal compression group. **Table 6.1** summarises the baseline patient characteristics. Procedural and baseline anthropometric data were available for all patients and follow up data were available for n = 6 and n = 12 at 1 month for each group respectively, n = 4 and n = 20 at 3 months and n = 5 and n = 7 at 6 months. The high attrition rate and loss of data at sequential visits was accounted for by patients not attending review appointments and being lost to follow-up.

	No longitudinal compression (n=9)	**Longitudinal compression (n=23)**
Age, years, mean ± SD	45 ± 12	43 ± 10
Sex, n (%)		
Female	5 (56)	18 (78)
Weight, kg (range)	113.6 (82 – 156.4)	107 (74 – 136)
BMI, kg/m^2 (range)	35.9 (30.9 – 43.8)	36.5 (29.8 – 42.9)
Theatre time, min (range)	135 (123 – 204)	138 (90 – 185)
Procedural time, min (range)	96 (85 – 135)	105 (65 – 139)
LoS, days (range)	1 (1 – 4)	1 (1 – 2)

Table 6.1 Baseline patient characteristics and ESG technical data
SD, standard deviation; BMI, body mass index; LoS, length of stay.

6.3.2 Procedural outcomes

In the no longitudinal compression group, a median of 6 (range, 5 – 8) plications were used to fashion the endoscopic gastric sleeve, with each plication being created from 4 – 8 full thickness bites. In the second group, 2 longitudinal compression plications were created in the first instance, each with 10 bites, followed by construction of the second layer of 'U'-shaped plications with a median of 4 (range, 3 – 6) sutures, each made up of 5 – 9 full-thickness bites. There was no significant difference in procedural time (i.e. the time elapsed between commencing and terminating general anaesthesia) between the two cohorts with a median procedural time in the no compression group of 96 minutes (range, 85 – 135 minutes) versus 105 minutes (range, 65 – 139 minutes) in the longitudinal compression group. All patients were admitted to hospital for observation following their ESG with a median length of stay in both groups of 1 day.

The majority of patients experienced self-limiting symptoms post-operatively, including nausea or vomiting in 71.8% and epigastric discomfort in 62.5%, which all resolved within 48 hours. These symptoms were reported as mild-to-moderate and were readily controlled with medications. There was no significant difference in post-operative symptoms between the two ESG suture patterns and no serious adverse events were reported in this series.

6.3.3 Weight-loss outcomes

Weight loss outcomes are summarised in **Table 6.2** and in **Figure 6.3**. After 6 months, body weight had decreased by 21.1 kg (range, 12.2 – 34.0 kg) in the longitudinal compression group versus 10.8 kg (range, 7.0 – 25.8 kg) in the no longitudinal compression group. Correspondingly, BMI decreased by 7.8 kg/m^2 (range, 4.9 – 11.2) and 4.1 kg/m^2 (range, 2.6 – 7.2) in each group respectively. At 6 months, %TBWL in the longitudinal compression group was 19.5% (range, 12.9 – 30.4%) versus 13.2% (range, 6.2 – 17.1%) in the no longitudinal compression group and %EWL was 52.6% (37.2 -120.2%) versus 35.5% (15.1 – 77.8%) in each group respectively.

During follow up visits, subjective reports on appetite, satiety and portion sizes were recorded for 81% (n = 26) of the patients. Of these, 100% reported significant early satiety and portion sizes were reported as being decreased by 50-75%. A significant number of patients also reported an appreciable reduction in their appetite. Two patients in the 'no longitudinal compression' group reported an initial good response to their ESG but at 3 months their weight plateaued and the restrictive effects of the ESG declined. Notably, the first of these patients was hospitalised elsewhere with gastroenteritis and violent vomiting. A follow-up endoscopy demonstrated complete loss of 4 plications, presumed to be a consequence of excessive vomiting. Despite this, the first patient achieved a TBWL of 9.0% and the second achieved 13.2%.

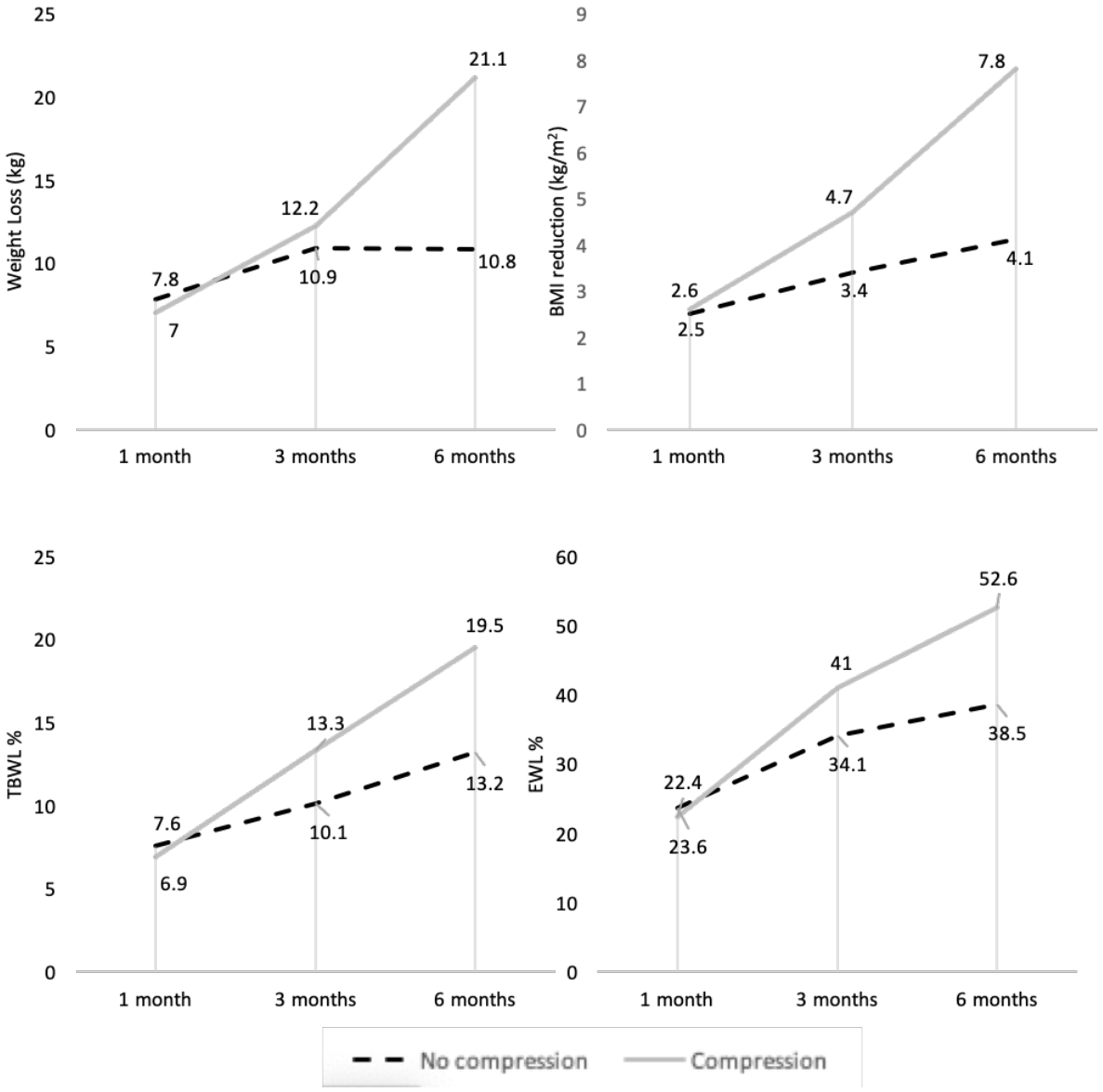


Figure 6.3 Weight loss (kg), BMI reduction (kg/m^2), %TBWL and %EWL at 1, 3 and 6 months following ESG with and without longitudinal compression sutures.

	1 month		3 months		6 months	
	No longitudinal compression (n = 6)	**Longitudinal compression (n = 12)**	**No longitudinal compression (n = 4)**	**Longitudinal compression (n = 20)**	**No longitudinal compression (n = 5)**	**Longitudinal compression (n = 7)**
Weight, kg	104.6 (75.0–139.3)	98.3 (70.6–130.6)	102.2 (95.0–132.0)	90 (65.0–120.7)	106.6 (71.2 – 130.6)	86.2 (76.8–108.9)
BMI, kg/m^2	33.6 (28.2–39.0)	33.6 (28.7–40.9)	34.7 (28.7–39.4)	31.5 (25.7–37.8)	33.1 (26.6 – 39.6)	31.8 (23.3–33.3)
BMI reduction, kg/m^2	2.5 (2.0–4.8)	2.6 (1.7–3.7)	3.4 (2.8–6.8)	4.7 (3.0–7.5)	4.1 (2.6 – 7.2)	7.8 (4.9–11.2)
TBWL, kg	7.8 (6.0–17.1)	7.0 (5.4–12.0)	10.9 (7.6–24.4)	12.2 (9.0–23.0)	10.8 (7.0 – 25.8)	21.1 (12.2–34)
%TBWL	7.6 (5.5–10.9)	6.9 (4.0–10.7)	10.1 (6.7–15.6)	13.3 (8.6–20.5)	13.2 (6.2 – 17.1)	19.5 (12.9–30.4)
%EWL	23.6 (18.0–46.6)	22.4 (9.6–44.2)	34.1 (16.4–47.9)	41 (23.2–86.3)	38.5 (15.1 – 77.8)	52.6 (37.2–120.2)

Data reported as median (range)

Table 6.2 Weight loss outcomes at 1, 3 and 6 months following ESG, with and without longitudinal compression sutures.

6.4 Discussion

The findings of this study further support ESG as a safe and effective means of achieving significant weight loss in obese individuals. Alqahtani et al. have reported on the largest prospective series of ESG patients (n = 1000) to date with a follow-up of 18 months.[393] This group utilised the same suture pattern as the 'no longitudinal compression' group in our study, and reported a TBWL of 13.7 $\pm$ 6.8%, 15.0 $\pm$ 7.7% and 14.8 $\pm$ 8.5% and EWL of 64.3 $\pm$ 56.2%, 67.5 $\pm$ 52.3% and 64.7 $\pm$ 55.4% at 6, 12 and 18 months respectively. At 6 months, the median TBWL in our comparative group was 13.2% (range, 6.2 - 17.1) and thus comparable to this series. In contrast, TBWL achieved by patients undergoing ESG using our unique suture pattern was 19.5% (range, 12.9 – 30.4) with a median EWL of 52.6% (range, 37.2 – 120.2). This is greater than the no compression group within our own series and also superior to the 6 month %TBWL outcomes reported by Alqahtani. Our 6 month %TBWL outcomes in this group are also superior to the 6 month outcomes and comparable to the 12 and 24 month outcomes reported in a prospective series by Lopez-Nava et al,[392] which currently represents the best weight-loss outcomes reported in any study of ESG to date. We await our longer-term follow-up data for direct comparison. However, we postulate that the longitudinal compression sutures create superior 'concertina-like' compression of the greater curvature, further reducing the volume of the gastroplasty and therefore potentially further supporting the remaining sutures. As a result, satiety increases and portion sizes reduce, as evidenced by our qualitative data.

Adopting this new suture pattern resulted in no additional morbidity or mortality and can be considered as safe practice. Additionally, fashioning of these longitudinal compression sutures is technically straightforward and did not result in any significant increase in procedural time. The transient post-procedural symptoms of nausea and abdominal discomfort are in line with other reported studies and there was no demonstrable difference in symptoms between suture patterns. Unlike other studies that have reported cases of peri-gastric collections, upper gastrointestinal bleeds and perforation, we are yet to report any serious adverse events following ESG.

There are some important limitations in this report, including its retrospective nature, the short duration of follow-up data available and the small number of patients included. Additionally, all procedures were conducted by a single surgeon, which limits the generalisability of the findings, and the operator learning curve of this procedure may be considered as a potential confounding factor. Saumoy et al.[300] reviewed the learning curve of a single operator performing ESG and concluded that efficiency was attained after performing 38 cases, with mastery after 55 cases.

They also concluded that there were no significant differences in weight-loss outcomes before and after reaching the status of efficiency. It is therefore unlikely that the operator learning curve of the 32 patients reported in this series contributed to the differences in weight-loss observed between the two suture patterns.

Nonetheless, these outcomes provide preliminary evidence that additional longitudinal compression plications along the greater curvature of the stomach during ESG may result in superior weight loss and, therefore, further research into this finding in the form of a prospective randomised controlled trial is justified. Using the 6-month follow-up data, using %TBWL as the primary outcome measure, a sample size of n = 29 (90% power with a 0.05 significance level and accounting for 20% loss to follow-up) would be required.

6.5 Conclusion

In conclusion, ESG is now established as a safe and efficacious minimally-invasive treatment modality in the armamentarium against obesity and obesity-related diseases. The technique of ESG is evolving and outcomes from EBTs continue to improve. We provide preliminary evidence of superior weight loss achieved through a modified gastroplasty suture pattern but further prospective data are required to validate these findings.

Chapter 7:

Final Discussion and Conclusions

7.1 Endobarrier duodenal-jejunal bypass liner

7.1.1 Discussion of main outcomes

Exclusion of the foregut following roux-en-Y gastric bypass (RYGB) surgery results in rapid metabolic changes and this observation has led to the development of endoscopically-delivered duodenal-jejunal bypass liners (DJBLs), such as the Endobarrier. The precise mechanisms underlying how the Endobarrier device exerts its positive effects remain unclear and so we draw much of our mechanistic understanding of it from trials examining patients post-RYGB surgery. The present study, however, represents the largest randomised controlled trial (RCT) of the Endobarrier DJBL to date and provides a wealth of new clinical outcome data, device safety information, cost-effectiveness analyses and a vast amount of new mechanistic data (reported herein and also elsewhere).

As was hypothesised, 12 months of treatment with the Endobarrier DJBL in the current study resulted in superior weight loss when compared to control participants managed with intensive lifestyle and dietary interventions alone (**see section 2.3.3).** In the control arm, total body weight loss (TBWL) gradually increased over the first six months to peak at 6.30 kg. Weight loss then remained relatively stable before then steadily declining to 4.82 kg at 24 months. In comparison, participants receiving the Endobarrier device achieved a significantly greater mean weight reduction of 10.82 kg at 6 months and this continued to increase to a peak reduction of 11.74 kg by 11.5 months. Patients receiving the Endobarrier device experienced a maximal BMI reduction of 4.06 ± 2.34 kg/m^2 after 11.5 months vs 2.11 ± 2.22 kg/m^2 for participants in the control group. These findings are in line with the best available systematic reviews and meta-analyses of the device,[257,259] the largest prospective observational studies of the Endobarrier and are also in keeping with the results presented from national and international DJBL registries.[244,256,257,394]

The primary outcome measure of this RCT was improvements in glycaemic control as determined by decreases in HbA_{1C} concentrations and the number of patients achieving a reduction in HbA_{1c} of ≥ 20%. This primary objective will be reported elsewhere (unpublished) but the current work reports improvements in glycaemic control on the basis of changes in fasting plasma glucose (FPG) concentrations. FPG concentrations decreased significantly from 11.1 mmol/L at baseline to 7.9 mmol/L 10 days following DJBL implantation and then remained significantly reduced until the device was removed at 12 months (7.0 mmol/L). FPG concentrations in control arm participants, however, were significantly lower than in Endobarrier participants at 10 days (during the low-calorie liquid diet phase of the study) and were not statistically different from the Endobarrier group at 6 months (7.9 mmol/L in Endobarrier group vs 7.7 mmol/L in the control group) or 11.5

months (7.0 mmol/L in Endobarrier group vs 7.2 mmol/L in the control group). On reviewing the current literature, many studies report rapid improvements in glucose homeostasis and type 2 diabetes (T2DM) following implantation with the Endobarrier, although there continues to be conflicting evidence as to its efficacy in this regard (**see section 1.2.6.2.2**). Following an implantation period of 3 – 12 months, reductions in HbA_{1c} have been reported in the range of 3 – 27 mmol/mol alongside improvements in FPG and reductions in post-prandial glucose excursions. However, a systematic review and meta-analysis by Rohde et al. concluded that DJBL implantation was associated with a non-significant reduction in HbA_{1C} by -0.9% and FPG by -3.7 mmol/L when compared with diet alone.[257] Indeed, several studies have failed to observe any statistically significant improvements in either HbA_{1C} or FPG following DJBL implantation.[224,236,239,246,249] The outcomes of the current study, therefore, corroborate those made by Rohde et al. since in the current work implantation with the DJBL did not produce any additional improvement in FPG above that achieved through calorie restriction alone. Dose-reductions or discontinuation of anti-diabetic medications have also been reported in many studies and a systematic review concluded that the relative risk of reducing or discontinuing antidiabetic medications was 3.28 and 1.13 in DJBL and dietary control groups, respectively.[221,225,227-231,233,237,238,240,242,244,245,248,251,256,257] In the current study, after 12 months of Endobarrier therapy, 13 (19.4%) patients recorded a decrease in the number of medications taken whilst 19 (28.4%) recorded an increase. In comparison, within the control arm, 10 (17.2%) patients recorded a decrease whilst another 10 (17.2%) patients recorded an increase. 34 (50.8%) patients in the treatment arm recorded no difference in the number of medications in comparison to 38 (65.5%) in the control arm. Chi-squared testing revealed no significant difference between the two treatment arms.

The DJBL is analogous with the bypass element of a RYGB for 60 cm and there is convincing evidence that the Endobarrier may moderate glucose homeostasis through similar mechanisms **(see section 1.2.6.2.4)**.[229,230,233,235,242,243,248,263,264] The current work therefore sought to be the first to investigate changes in insulin sensitivity and β-cell function in patients implanted with the Endobarrier DJBL for 12 months within a RCT and it was hypothesised that similar outcomes would be reported. The data from this study show that markers of enhanced insulin secretion and β-cell function significantly improved within the first 10 days of the trial i.e. during the low-calorie liquid-diet phase of the study. However, there were no significant differences between treatment arms. In patients implanted with the Endobarrier, there were sustained improvements in the disposition index up to one year but, again, these were not significantly different from control group participants at any of the follow-up visits. The DJBL, therefore, did not produce any additional improvement in β-cell function above that achieved through calorie restriction alone. Furthermore, the greater weight loss achieved in the Endobarrier group at 11.5 months did not

correlate with any further improvements in β-cell function. These observations agree with the findings made by Cohen et al., which is the only other study to examine insulin secretion rates and first phase insulin response during treatment with the Endobarrier for a period of 1 year.[230] In comparison, following RYGB, there are significant improvements in a patient's metabolic state with improvements in their glycaemic control, a reduction in medications or even resolution of T2DM. Oral glucose tolerance tests performed after RYGB demonstrate early and exaggerated post-prandial rises in insulin concentrations and a more rapid return of insulin concentrations to fasting levels, consistent with parallel improvements in insulin sensitivity.[178,395-398] Several studies have reported early improvements in the insulinogenic index, as a measure of improvements in post-prandial first-phase insulin response, and these improvements have also been observed following intravenous glucose tolerance tests in patients with T2DM following RYGB.[396,399,400] Significant increases in the Disposition Index are also reported and there is convincing evidence that these changes occur independently of acute caloric restriction.[399,401-403] From the current data it can be deduced that the Endobarrier differs significantly from RYGB in terms of reduced efficacy and it is probable that the mechanisms by which these two procedures exert their metabolic effects are different, either in terms of the magnitude of shared mechanisms or through different mechanisms entirely (see below).

It is well documented that, following RYGB, there are significant improvements in insulin sensitivity; hepatic insulin resistance (and hence hepatic glucose production) decreases in the immediate week(s) following surgery and, after approximately a month, peripheral insulin sensitivity significantly increases in proportion to ensuing weight loss and peaks after approximately 2 years.[176-179] To date, a small number of non-randomised human studies have reported significant improvements in insulin sensitivity following DJBL implantation, as demonstrated by reductions in HOMA-IR.[229,230,233,235,242,243,248] The current study is the first to examine changes in insulin sensitivity following 12 months of Endobarrier treatment within a well-powered RCT. There were early, rapid improvements during the low-calorie liquid-diet phase of the trial but these improvements were not superior to the those achieved by participants in the control arm. However, insulin sensitivity continued to improve in participants treated with the Endobarrier device as their weight-loss increased and was significantly greater than control arm participants at 11.5 months follow-up. This association was confirmed by Spearman rank-order correlation which showed a strong, positive relationship between weight loss and changes in insulin sensitivity (HOMA2-%S) in both treatment arms. This is in keeping with the results reported by Munoz et al. who observed a strong negative correlation between %EBWL and HOMA-IR after 1 year of Endobarrier treatment on univariate regression analysis (r_s = -0457, p = < 0.019).[252] Therefore, as hypothesised, Endobarrier treatment resulted in significantly greater

weight loss when compared to treatment with intensive lifestyle and dietary interventions alone and this correlated with superior improvements in insulin sensitivity after 11.5 months of treatment. Of note, however, these improvements were not reflected by superior improvements in glycaemic control as determined by FPG concentrations.

Changes in tissue-specific insulin sensitivity following RYGB have been reported in several studies and It is clear that significant weight loss produces considerable improvements in both peripheral and hepatic insulin sensitivity after approximately 6-12 months.[178,328-333] However, it remains contested as to whether or not the early improvements (< 4 weeks) in glucose homeostasis are a consequence of weight-loss-independent improvements in tissue-specific insulin sensitivity (**see Chapters 1 and 3**) or are the consequence of acute caloric restriction. In the case of Endobarrier therapy, Miras et al. concluded that the DJBL did not improve hepatic insulin sensitivity beyond the improvements achieved with caloric restriction.[247] The current study is only the second to investigate changes in hepatic and peripheral insulin sensitivity following Endobarrier implantation using hyperinsulinaemic euglycaemic clamps (HECs) with addition of stable isotopes. This technique revealed a significant reduction in the rate of appearance of glucose from the liver (HGP/Ra) and a significant increase in peripheral glucose uptake (Rd) at 10-days. As HGP is the main determinant of FPG, it seems likely that the significant reductions in FPG concentrations observed at + 10 days may be as a consequence of reductions in Ra. These changes, however, were not statistically different from control arm patients at 10 days and this supports the conclusions made by Miras et al. that the Endobarrier liner produces no additional early improvements in glucose homeostasis above those achieved through calorie restriction alone.

After 6 months, there was a significantly higher rate of peripheral glucose uptake and utilisation (Rd) in participants treated with the Endobarrier. We hypothesised that these improvements in peripheral insulin sensitivity would be a consequence of superior weight loss in the Endobarrier group. However, no such relationship between weight loss and Rd (or Ra) was identified through Pearson correlations at 10 days or 6 months follow-up. As stated above, however, patients completing one year of Endobarrier therapy demonstrated significantly greater improvements in whole-body insulin sensitivity (as estimated by HOMA2-%S) that was strongly associated with greater degrees of weight loss. This suggests that Endobarrier implantation results in: (1) early, weight-loss-independent improvements in hepatic and peripheral insulin sensitivity as a consequence of calorie-restriction, and (2) superior reductions in weight that peak between 9 and 12 months leading to weight-loss-dependent improvements in insulin sensitivity.

The early and late changes in insulin sensitivity that have been observed following 12 months of Endobarrier treatment are similar to, but less pronounced than, improvements that occur

following RYGB. Following RYGB, profound and sustained weight loss is, in part, achieved through caloric restriction as the GI anatomy is manipulated to reduce the capacity and reservoir function of the stomach and to limit the surface area of the intestine in contact with ingested nutrients, thus resulting in relative malabsorption. DJBL differs in that no resection of the stomach takes place and exclusion of the foregut is limited to 60 cm (from the duodenal bulb), as opposed to the biliopancreatic limbs lengths of 50 – 200 cm (from the ligament of Treitz) used in RYGB. It has also been suggested that the radial force of the Endobarrier anchor and the presence of chyme within the foregut may reduce the rate of gastric emptying by distending and irritating the duodenal bulb, thus activating mechanoreceptors in this region and setting up local neuro-hormonal enterogastric feedback loops.[238,263,264] This is in contrast to the RYGB in which this region of the foregut is bypassed of all stimuli. Decreased gastric emptying would subsequently reduce the rate of flow of nutrients along the GI tract, promote early satiety and restrict caloric intake over time. As caloric intake decreases following Endobarrier implantation, the mass of adipose tissue declines, adipokine concentrations are moderated, insulin-receptor concentration and signalling is enhanced, glucose and fatty acid metabolism increases, glucolipotoxicity reduces and intramuscular and visceral lipid concentrations are depleted. Hepatic and peripheral insulin sensitivity therefore increases proportionally +/- associated improvements in β-cell function and mass.[27,177,178] In the current study, however, these later improvements in insulin sensitivity failed to translate to overall improvements in glycaemic control above that of patients managed with dietary and lifestyle interventions alone.

The early improvements in β-cell function and insulin sensitivity that occurred equally in both treatment arms are likely to have occurred as a result of acute calorie restriction during the liquid-diet phase of the trial. The effect of a low-calorie diet in acutely improving β-cell function is well documented in the literature and early reductions in pancreatic fat content have been implicated in restoring the first-phase insulin response.[150,350] Another explanation may be due to an acute reduction in FPG levels and reduced levels of glucolipotoxicity.[27,176,192] Lim et al. demonstrated that in patients with T2DM who underwent a low-calorie diet or RYGB, hepatic fat content rapidly decreased in parallel to improvements in hepatic insulin sensitivity and FPG levels within 7 days, which could equally explain the outcomes in our treatment groups.[150] Other weight-loss-independent mechanisms related to Endobarrier implantation may also be involved and there is some evidence to support the role of incretins/anti-incretins, bile flow modulation, nutrient sensing and/or alterations to the gut microbiota, which are fully discussed in **sections 1.2.5.2.2 and 1.2.6.2.4**. However, the current study failed to demonstrate any additional early improvements in insulin sensitivity and glycaemic control above that of control arm participants and that were independent of calorie restriction or weight-loss.

In addition to early changes in glucose homeostasis, early and sustained improvements in cholesterol and triglyceride concentrations following Endobarrier implantation are also reported here. Acute calorie restriction resulted in rapid, significant improvements in total serum cholesterol (TSC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TAG) concentrations equally in both study arms. Notably, however, TSC concentrations were significantly lower in the Endobarrier arm after 11.5 months and LDL-C concentrations were significantly lower at 6 months follow-up, but this difference was not maintained at 1 year. These findings are likely to be explained by superior weight loss in the Endobarrier cohort and match the best available evidence in the Endobarrier literature, which support overall reductions in TSC and LDL-C concentrations with variable reports of changes in TAG concentrations. In this study, HDL-C concentrations were unchanged in the Endobarrier arm at 1 year follow-up, which also agrees with outcomes reported elsewhere.[225,229,242,246,248,253,257] Alongside obesity, hyperlipidaemia is a hallmark of metabolic syndrome and is a major risk factor for cardiovascular morbidity and mortality. Studies of the Endobarrier to date have been limited to short follow-up durations and, as such, no conclusions can be drawn as to the overall effect of the Endobarrier device on long-term cardiovascular health. However, the data reported in this study support the notion that the Endobarrier device induces significantly greater weight loss and improvements in lipid profile above those achieved through dietary and lifestyle guidance alone. These improvements most likely occur as a consequence of caloric restriction, subsequent reductions in visceral and subcutaneous adipose tissue, and improvements in lipid metabolism as a consequence of improved insulin sensitivity (**see section 1.2.2.4**). The impact of this on medium- and long-term health outcomes is yet to be determined.

There is substantial evidence that very long chain omega-3 (ω3) polyunsaturated fatty acids (PUFAs) have an essential protective role against cardiovascular and cerebrovascular disease and are associated with improvements in cognitive health, better visual and neurological development in infants, and resolution of inflammatory processes in chronic diseases such as asthma and arthritis.[354,358,359] It has been proposed that the effects of metabolic surgery and weight loss on endogenous fatty acid (FA) metabolism and PUFA concentrations may contribute to these effects.[367,368] However, data on the precise impact of bariatric surgery on FA pools remains limited.[357,361,363,367,369-378] Several studies support the notion that bariatric surgery increases ω-3 and/or ω-6 PUFA concentrations to levels that are comparable to normal weight controls (i.e. reversing the pathological FA profile seen in obese individuals)[361,367,369,371,373] but there are also studies that report essential fatty acid (EFA) and PUFA deficiency following malabsorptive bariatric operations. This is the first study to report on the effect of an endoscopic bariatric therapy on circulating FA levels. Common to both study groups is that acute calorie restriction

with a liquid diet resulted in a significant decrease in all measured FA concentrations. However, despite there being no differences in subsequent dietary or caloric intake, ω-3 and -6 long-chain PUFA concentrations were significantly lower in Endobarrier patients at 6 and 11.5 months follow-up when compared to control patients, except LA for which there was no difference at 11.5 months. The Endobarrier device therefore depleted absolute serum concentrations of EFAs and their PUFA derivatives, which is in line with conclusions made by several studies following weight loss surgery.[357,363,367] It has been observed that fat malabsorption occurs following RYGB and, as bypass of the duodenum with the Endobarrier is analogous with a 60 cm biliopancreatic limb, this is also likely to impair FA absorption in the proximal small bowel. This, coupled with reduced dietary intake of EFAs and other long chain PUFAs, will result in reduced circulating FA concentrations.[389,390] Furthermore, this will reduce the bioavailability of EFAs available as substrates for endogenous synthesis of their PUFA derivatives and FA oxidation will increase during significant weight loss, thus depleting concentrations even further.[363,388] It has been reported in the literature that these FA deficiencies that arise following metabolic surgery can be offset by maintaining an adequate dietary intake of ω-3/-6 PUFAs or by adequately supplementing the diet, which seems prudent given their essential protective role against cardiovascular and cerebrovascular disease.

Unlike RYGB, Endobarrier is a temporary measure and implantation is currently limited to 12 months. After this time, data from the current study demonstrate that when the Endobarrier device was removed patients regained weight precipitously to achieve a weight loss of 5.4 kg at 24 months, which was only 0.6 kg greater than, and not statistically different from, participants in the control arm. In parallel to this, basal steady-state insulin sensitivity decreased significantly and insulin resistance, FPG and insulin concentrations increased significantly in the 11 months following device removal. At this time-point, all values remained significantly improved from baseline levels but were not statistically different from control arm participants. β-cell function was seemingly unaffected by removal of the Endobarrier device and remained significantly improved from baseline levels but was not statistically different from the 10 days follow-up visit or from control arm participants at 23 months. This treatment decay and the observable deterioration in insulin sensitivity and glucose homeostasis that occurs following removal of the Endobarrier device matches what has been reported elsewhere (**see section 1.2.6.2.6**).[229,230,242] During this study we did not make FA measurements following device explantation. However, given the increasing trend of absolute ω-3 and ω-6 PUFA concentrations following initial calorie restriction, it is unlikely that PUFA deficiency continues following device removal, but this is unknown.

7.1.2 Current status of the Endobarrier

The Endobarrier DJBL provides a minimally-invasive therapeutic option to achieve weight-loss and treat obesity-related diseases that is superior to medical and lifestyle interventions and avoids the potential morbidity and mortality associated with surgery. It is clear that isolated foregut exclusion with an Endobarrier device is inferior, in terms of efficacy, when compared to metabolic surgery but represents a therapeutic option in certain sub-groups of patients. Notably, of the millions of patients with obesity who are eligible for metabolic surgery, only 1% will undergo these operations due to multifactorial barriers to surgery.[301,314] The Endobarrier could therefore be used as a primary weight-loss or metabolic intervention in many of these patients. Alternatively, it may be utilised as an early intervention in patients with class I obesity and those not eligible for metabolic surgery in order to achieve modest weight loss and improve their metabolic risk profile; Vilarrasa et al. have published good outcomes in such a cohort of patients.[251] Finally, the Endobarrier has been shown to be effective as a bridging therapy, intended to promote weight loss and reduce a patient's risk from a subsequent planned intervention, such as metabolic surgery or another operation what would benefit from weight- and risk-reduction (e.g. cardiac surgery, orthopaedic surgery or organ transplantation). This has been evaluated within two RCTs prior to bariatric surgery and pre-operative weight loss in the order of 5-10% has been shown to be associated with a reduced inpatient stay, decreased blood loss, shorter operating times, reduced perioperative morbidity and improved post-operative weight-loss outcomes.[223,224,404]

Currently, the Endobarrier is only licensed to be implanted for 1 year and the deterioration in treatment effects following removal of the device is clearly reported within this study and elsewhere in the literature. One solution to manage weight regain and the deterioration of glycaemic control following Endobarrier removal could be to re-implant the device, which was safely achieved in a limited number of patients by Koehestanie et al.[239] In this small cohort, the authors successfully reimplanted the DJBL for a further year in patients previously treated for 6 months and were able to demonstrate significant improvements in EWL and BMI, beyond what was achieved during the initial implantation period. Another strategy to enhance the efficacy of the Endobarrier could be to combine DJBL therapy with other medical therapies, such as injectable glucagon-like peptide-1 (GLP-1) receptor agonists that will improve glycaemic control and reduce weight. This is currently being evaluated as part of the REVISE-Diabesity trial (ClinicalTrials.gov Identifier: NCT02055014) and outcomes are awaited.

An ideal solution would be the creation of a device which could remain in situ for longer, thus providing a more permanent solution for these patients. Quezada et al. are the only authors to

report on implant durations up to three years and, in this study, 77 patients achieved a significant reduction in their weight by 3 months with weight loss peaking at 12 months.[248] Thereafter, a non-significant weight increase was observed and was then maintained in 11 patients completing the 3 year follow-up. Similar observations were also made for improvements in glycaemic control, insulin resistance, blood pressure, TSC and LDL-C. Therefore, for the first year of treatment with the Endobarrier device, patients achieved a significant reduction in their weight and improvements in several metabolic parameters with a low rate of adverse events. Thereafter, patients were able to maintain the improvements in weight and metabolic health but were subjected to considerably more serious adverse events. It was therefore recommended that treatment with the Endobarrier DJBL should not exceed 12 months. Of significance, the results of this trial were compared with the 4-year follow-up results obtained from intensive medical treatment in the look AHEAD study and it was shown that Endobarrier treatment achieved greater weight loss at the mid-term evaluation (Delta BMI -2 vs. -6 kg/m^2).[9]

The pivotal ENDO trial (ClinicalTrials.gov Identifier: NCT01728116) was a multi-centred, double-blinded RCT in the US where individuals were randomised to receive either the Endobarrier device or sham treatment in order to assess the efficacy and safety of the device. The study opened in November 2012 but was terminated early by the FDA in 2015 after only 325 patients were randomised (216 EndoBarrier patients) due to a higher than expected hepatic abscess (HA) rate of 3.5%. This is in comparison to reported HA rates of 0.73% globally, 1.2 % in Europe and 1.34% in the UK. Due to non-conformances related its quality management system, GI Dynamics Inc. (Lexington, MA) voluntarily withdrew its CE certificate of conformity on 12th November 2017, rendering the company unable to affix the CE mark and sell the Endobarrier device in EU countries. At the time of writing, this CE mark is yet to be re-issued but GI Dynamics Inc. confirm that the Endobarrier conforms to the requirements of the European Medical Device Directive and is awaiting device recertification.

In August 2018, Endobarrier was approved by the FDA for a second trial in the US and recruitment into the Endobarrier System Pivotal Trial (Rev B) (STEP-1) commenced on 9th September 2019. This is a randomised, multi-centre, double-blinded, sham-controlled trial and aims to randomise 240 patients to receive either the Endobarrier device plus moderate intensity lifestyle and dietary counselling or a sham control group receiving moderate intensity lifestyle and dietary counselling alone. The estimated study completion date is April 2024 and this will become the largest RCT of the Endobarrier device.

The Endobarrier DJBL continues to attain high level trial data, with clinically-relevant and essential safety outcomes. This is in line with stage 3 of the IDEAL framework for the assessment and

evaluation of new surgical technologies (see **section 1.2.7**).[285] The device now enters stage 4 of evaluation as longer-term outcomes are reported and assurances of quality are made. In 2013, the nationwide German DJBL registry was established for the purpose of evaluating the efficacy and long-term safety of DJBL therapy. The outcomes from this registry are discussed in **section 1.2.6.2**.[256] Even more recently, in 2017, the Association of British Clinical Diabetologists have established the Endobarrier Worldwide Registry. The first risk-benefit data from this registry were published in July 2018, obtained from 403 patients from 4 centers across 13 countries.[394] This data demonstrate that Endobarrier therapy resulted in weight reductions of 14.5 ± 10.3 kg (n = 265 p<0.001), improvements in HbA1c by 1.4 ± 1.6% (n = 195, p<0.001), and decreases in systolic blood pressure from 138.5 ± 18.1 to 130.0 ± 17.2 mmHg (n = 149, <0.001). The reported rate of SAEs from this registry was 5.7%, which sits above the recommended ASGE/ASMBS safety threshold but it was concluded that the benefits of Endobarrier therapy are likely to reduce the complications of diabetes and suggested that the benefits of the Endobarrier far outweigh the risks.

7.1.3 Study strengths, limitations and future work

The strengths of the main trial are that it is a well-powered, randomised, multi-centre study with good short and medium term follow up for 2 years. It can be credited with the delivery of a truly intensive medical care programme by a multidisciplinary team and currently represents the largest RCT of the Endobarrier DJBL. It has generated a wealth of new clinical outcome data, device safety information, cost-effectiveness estimations and a vast amount of new mechanistic data. The open-label design serves as a significant source of bias and, as such, the results of the double-blinded, sham-controlled Endobarrier System Pivotal Trial are eagerly awaited.

This is the first study to investigate changes in tissue-specific insulin sensitivity following Endobarrier implantation within a RCT using the gold-standard method of HEC with addition of stable isotopes. This provides novel insights into the changes in insulin sensitivity that occur following acute calorie restriction and foregut exclusion. However, the examinations in this work were limited to the first 6 months of therapy and any future work would benefit from also performing a clamp at 1 year and following removal of the device, in order to examine for later changes in tissue-specific insulin sensitivity. Furthermore, very little evidence exists in the literature to explain the mechanisms behind the actions of the Endobarrier but it is presumed that it mimics the foregut-bypass element of the RYGB and may have metabolic effects by activating similar neurohormonal pathways along the entero-insular axis in the foregut and hindgut. Changes to incretin concentrations, bile flow modulation, nutrient sensing and alterations to the gut microbiota have all been implicated as potential mediators of these metabolic changes and

have all been studied as part of the main Endobarrier trial. Further work is therefore required in order to evaluate if changes in any of these parameters correlate with early and late changes in hepatic and peripheral insulin sensitivity. Similarly, correlation of these outcomes with changes in β-cell function would also be of great significance.

The finding of superior weight loss in the EndoBarrier group should be further investigated in order to expand the understanding of the mechanisms through which the device exerts its metabolic effects and reduces energy intake. Future studies with larger sample sizes and direct measurements of both energy intake and energy expenditure could provide this important information. The aim would be to identify the mediators of these processes and use the knowledge to develop targets for obesity pharmacotherapy.

An interesting prospect is the potential of achieving sustained weight loss and metabolic improvements through acute caloric restriction alone. In the recent Diabetes Remission Clinical Trial (DiRECT),[120] Lean et al. highlighted the feasibility of achieving sustained weight loss and remission of T2DM in the primary care setting using conservative management strategies by means of acute caloric restriction and structured weight-management. In their open-label, cluster-randomised trial mean bodyweight was reduced by 10.0 kg in the intervention group versus 1.0 kg in the control group at 12 months and 24% of the intervention group achieved a weight-loss of $\geq$ 15 kg versus 0% in the control group ($p < 0.0001$). Correspondingly, 46% of patients in the intervention group achieved remission of their T2DM at 1 year versus only 4% in the control group (odds ratio 19.7, 95% CI 7.8-49.8; $p < 0.0001$). Sustained remissions at 24 months were achieved for more than a third of patients with T2DM.[151] These outcomes are very similar to those achieved by the Endobarrier group in the current study and so a comparative examination of these two interventions would be of great interest.

7.2 Endoscopic Sleeve Gastroplasty

7.2.1 Summary of main outcomes

The technique of Endoscopic Sleeve Gastroplasty (ESG) continues to evolve and we have reported on the first UK experience of using ESG as a primary weight-loss treatment for patients with obesity. The findings of this study further support ESG as a safe and effective means of achieving significant weight loss via the transoral route. In this retrospective review, patients undergoing ESG with unique longitudinal-compression sutures decreased their weight by 21.1 kg (%TBWL 19.5%, %EWL 52.6%) and their BMI decreased by 7.8 kg/m^2 after 6 months. These results are in line with the best available systematic review and meta-analysis of this device published by

Hedjoudje et al. in which they reported a mean %TBWL of 15.1%, a %EWL of 57.7% and a decrease in BMI by 5.65 kg/m^2.[312]

It is postulated that using longitudinal compression sutures results in superior 'concertina-like' compression of the greater curvature during ESG. This further reduces the volume of the gastroplasty, increases satiety, and decreases caloric intake, which is analogous with the changes that occur following laparoscopic sleeve gastrectomy (LSG). Adopting this new suture pattern resulted in no additional morbidity or mortality and can be considered as safe practice. Additionally, fashioning of these longitudinal compression sutures is technically straightforward and did not result in any significant increase in procedural time. The transient post-procedural symptoms of nausea and abdominal discomfort are in line with other reported studies and there was no demonstrable difference in symptoms between different suture patterns.

If the weight-loss outcomes of this retrospective cohort are compared to those treated with the Endobarrier, the ESG produces significantly greater weight loss after six months of therapy. Although the precise mechanisms of weight-loss following ESG are not clear, the procedure is performed with the intention of reducing the stomach volume, altering gastric motility and increasing satiety. This is in contrast to Endobarrier therapy in which there is no gastric restriction. It has been hypothesised that the ESG modifies several neurohormonal pathways that augment appetite and weight loss, as has been observed following LSG. For example, ghrelin originates from neuroendocrine cells of the stomach, duodenum and pancreas and has many complex functions relating to energy homeostasis as discussed in **section 1.2.1.4**.[29,30] Concentrations of this orexigenic hormone have been found to decrease following LSG and there are reports in support of similar changes following ESG.[311,405] As with Endobarrier, knowledge of the mechanisms underlying these bariatric and metabolic interventions is fundamental to identifying new therapeutic targets for obesity and its metabolic complications.

7.2.2 Current status of ESG

The technique of ESG is developing a robust evidence-base in the form of multiple prospective observational studies, which have reported excellent short-term clinical and safety outcomes in a high number of patients. However, high-level RCT data is lacking. This places it within Stage 2b of the IDEAL framework and is awaiting further assessment before it can be safely adopted as part of routine clinical practice. The Multicenter ESG Trial (MERIT Trial) (ClinicalTrials.gov Identifier: NCT03406975) is an open-label, multi-centre RCT that is currently underway in the United States. Two hundred patients have been randomised to receive either ESG therapy or dietary and lifestyle interventions alone with potential for crossover of the control arm to undergo ESG after

12 months. The primary outcome measure will be %EWL at 12 months and secondary outcome measures will include changes in hypertension and T2DM after 24 months. The expected study completion date is December 2020 and is eagerly awaited as the first RCT of ESG therapy.

In 2018, Apollo Endosurgery introduced the OverStitch™ Sx, which is compatible with a vast range of single-channel endoscopes. This is in contrast to the first-generation OverStitch™ device that was used in the patients reported within this study and affixes to the end of a double-channel endoscope. The broader compatibility of this new system is hoped to improve accessibility to the device and is also expected to improve visualisation and manoeuvrability for the endoscopist.

7.2.3 Limitations and future work

There are some important limitations in this research on ESG, including its retrospective nature, the short duration of follow-up data available and the small number of patients included. Additionally, all procedures were conducted by a single surgeon, which limits the generalisability of the findings, and the operator learning curve of this procedure may be considered as a potential confounding factor. Nonetheless, these outcomes support ESG as a safe and effective means of achieving significant weight loss via the transoral route and provide preliminary evidence that additional longitudinal compression plications along the greater curvature of the stomach may result in superior weight loss. Further research of this technique in the form of a prospective RCT is justified with inclusion of quantitative measurements of sleeve length and volume. Using the 6-month follow-up data and %TBWL as the primary outcome measure, a sample size of n = 29 would be required (90% power with a 0.05 significance level and accounting for 20% loss to follow-up).

In the literature to date there is a relative paucity of studies that report on metabolic improvements following ESG and even fewer studies which examine the mechanisms behind these changes. These outcomes should therefore form the focus of any future work as well as examination of pre-operative factors that may predict superior weight-loss and metabolic outcomes. Similarly, as has occurred with the Endobarrier, assessment of ESG in combination with other metabolic therapies (such as an injectable GLP-1 agonist) would also be of great interest.

7.3 Conclusions

In conclusion, 12 months of therapy with the Endobarrier DJBL in patients with obesity and T2DM resulted in superior weight loss when compared to control patients managed with intensive lifestyle and dietary interventions alone. The number of patients achieving a meaningful weight loss of > 15% was also significantly greater within the first year of Endobarrier therapy, which

could potentially have long-term effects on cardiometabolic morbidity and mortality. Patients implanted with the Endobarrier device demonstrated early, weight-loss-independent improvements in hepatic and peripheral insulin sensitivity, ß-cell function and fasting blood glucose concentrations, but these changes were not superior to improvements achieved in control patients through calorie restriction alone. Increasing weight loss related to the Endobarrier device however was associated with significantly greater improvements in insulin sensitivity and lipid profile after 12 months of therapy. A degree of weight-loss and metabolic improvement was maintained one year following removal of the Endobarrier device but this was not statistically different from control patients. Weight recidivism and loss of treatment effect should be an important consideration when referring a patient for a temporary EBT and highlights the importance of implementing medical and lifestyle interventions alongside these therapies in order to maximise their efficacy and longevity. Furthermore, absolute blood concentrations of EFAs and their long chain ω-3/-6 PUFA derivatives were significantly depleted in participants receiving Endobarrier therapy and, in view of this, dietary supplementation should be advised.

ESG is now established as a safe and efficacious minimally-invasive treatment modality in the armamentarium against obesity and obesity-related diseases. The technique of ESG is evolving and outcomes from EBTs continue to improve. We provide preliminary evidence of superior weight loss achieved through a modified gastroplasty suture pattern but further prospective data is required to validate these findings.

Appendices

Appendix A

A.1 Summary patient information sheet (PIS)

University Hospital Southampton NHS
NHS Foundation Trust

EndoBarrier TM Gastrointestinal Liner Diabetes Trial

SUMMARY OF RESEARCH STUDY

Study Project Title: EndoBarrier TM Gastrointestinal Liner Diabetes Trial

You are being invited to take part in a research study taking place University Hospital Southampton The study investigates the effect of a new non-surgical device (EndoBarrier) on type 2 diabetes mellitus and weight loss over a period of 2 years. If after reading this you would like more information about the EndoBarrier trial, please complete the attached questionnaire and return in the envelope provided.

What is the purpose of the study?

A new device called the EndoBarrier Gastrointestinal Liner helps patients with diabetes manage their blood sugar levels and lose weight without the need for surgery. This study will compare how effective the EndoBarrier device is compared to standard medical care in the treatment of type 2 diabetes mellitus.

Why have I been invited?

You are being invited because your body mass index (BMI) is between 30-50 kg/ m^2 and you have type 2 diabetes mellitus.

How does EndoBarrier work?

The EndoBarrier has been shown to help control type 2 diabetes mellitus and reduce weight. It is a 60 cm long impermeable sleeve-like device that is inserted through your stomach (endoscopically) and creates a thin plastic layer between food and the wall of the intestine. This could prevent food from coming into contact with the gut until further down the intestine and may alter natural your level of hunger and fullness.

What will happen to me if I take part?

If you are interested in this study we will invite you for a screening visit. If you decide to participate you will be asked to sign an informed consent form. Your doctor will then perform some tests and procedures to determine whether you are eligible for the study.

If you are eligible for the study you will be randomised to either receive the EndoBarrier device for 12 months and subsequently a diet for a further 12 months after the implant, or you will receive a standard medical therapy and a diet for 24 months. All patients will receive specialist support from a doctor specialising in the treatment of diabetes and a dietitian.
Randomisation means that a group of people are split into two groups at random; one group is given one intervention (the EndoBarrier device) and the other is given a different intervention (standard medical therapy/ control group). We then measure how each group is doing and see if one group has achieved its supposed outcome any better.

On your second study visit we will inform you about the treatment for which you have been randomised for the duration of the study. We will further inform you about other tests performed while being on this trial. Your dietitian will assess your diet and give you specific dietary information.

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On some of the study visits, we will also ask you to participate in specific tests which will help us to assess your metabolism, brain activity, insulin sensitivity and food preference. Participation in these tests is entirely optional. More information about these tests can be found in the patient information sheet.

On visit 4 you will either have the EndoBarrier device inserted (EndoBarrier group) or you will see the diabetic specialist doctor or nurse (Medical Therapy group). You will also see the dietitian for review.

During some study visits, you will see the diabetic specialist doctor or nurse, the gastroenterologist (EndoBarrier group only), and the dietitian for review.

If you are in the EndoBarrier group, your EndoBarrier will be removed after 12 months.

What happens if I want to withdraw from the study?

There are no foreseeable reasons why you should end your participation but you may withdraw from the study at any time.

What are the possible advantages and disadvantages of taking part?

Your direct benefit of taking part in this research will be the possible improvement in your blood glucose and HbA1c, blood pressure, weight loss and reduction in long term health risks particularly cardiovascular diseases.

The risks associated with the EndoBarrier procedure include the same risks observed with other upper gastrointestinal endoscopic procedures.

If you are randomised into the EndoBarrier group of the study, the frequent side effects are: cramps/abdominal pain, nausea/vomiting and/ or bloating. Other rare side effects are described in the Participant Information Sheet. If you are randomised into the control arm of the study, no side-effects are expected. More information of potential benefits and risks are outlined in the Participant Information Sheet.

What are the payments for this study?

On each visit, you will be reimbursed for your travel to the hospital.

Contact Information

Dr Michael Glaysher
D Level
Southampton Centre for Biomedical Research SCBR,
Southampton General Hospital
Mailpoint 218
Southampton
SO16 6YD

Email: EndoBarrierNurses@uhs.nhs.uk, Telephone: 02381208591/ 02381205339, FAX 02381204730

Thank you for taking the time to read and consider this information sheet.

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University Hospital Southampton NHS
NHS Foundation Trust

EndoBarrier TM Gastrointestinal Liner Diabetes Trial

Please complete this reply slip in the envelope provided to the research centre. This will let the research team know if you would like more information about the EndoBarrier study.

Name	
Address	
Telephone	
Mobile	
E mail	
I am interested in finding out more about the EndoBarrier research project. I am happy for a member of the research team to contact me. ☐	
I give permission for the Research Team to contact my GP or access the Hampshire Health Records to check whether there is any medical reason why I should not take part, and to confirm my medical history. ☐	
SIGN :	
DATE :	

To speak to the research team about the EndoBarrier study, please contact:

Dr Michael Glaysher/ Miranda Kean
Email: EndoBarrierNurses@uhs.nhs.uk
Telephone: 02381208591/ 02381205339
Mobile: 07717720077
FAX 02381204730

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A.2 Participant consent form for main study

Imperial College London

University Hospital Southampton NHS
NHS Foundation Trust

EndoBarrier TM Gastrointestinal Liner Diabetes Trial

Participant Consent Form (Main Study)

Title of Project: EndoBarrier TM Gastrointestinal Liner Diabetes Trial
Principal investigator: Mr James Byrne
Site number: 2
Participant I.D number:

The participant should complete the whole of this sheet him or herself.
(Please initial each statement if it applies to you)

1. I confirm that I have read and understand the Participant Information Sheet **Southampton Version 3.0 (dated 16/03/15)** for the above study. ☐

2. I have had the opportunity to ask questions and discuss this study. All my questions have been answered fully and I have received enough information about the study including the participant information sheet ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

4. I agree that my medical notes and data collected from the study may be accessed by individuals involved in the study, or by regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I understand that my personal data will be processed and stored securely in compliance with the 1998 Data Protection Act. ☐

5. I agree that all my samples (blood, urine, faeces, tissue) will be stored for research purposes and this material can only be used with ethical committee approval. All testing of my samples, will be coded such that I cannot be identified by the data. ☐

6. I give permission for the data collected in the questionnaires and computer tasks to be used for the purposes of the study. ☐

7. I give permission for my General Practitioner to be informed of my participation in this study and the results of any medical tests from my visits i.e. blood tests. ☐

8. I give permission for tissue samples to be collected during the implant and removal of the Endobarrier device. ☐

9. I agree for a DNA/RNA sample to be taken and stored to look for changes that may be involved in obesity, diabetes, brain function and the control of appetite and hormones, and understand that the results will not be fed back to me. ☐

10. I give permission for my blood or DNA/RNA samples to be sent to laboratories in the United Kingdom or abroad for analysis as long as all personal information is removed. ☐

11. I give permission for any stored samples that I give during this study, to be used in future studies which have been granted suitable ethical approval. ☐

12. The indemnity arrangements have been discussed with me. ☐

One copy for patient, 1 for site file, 1 for patient notes
Participant Consent Form [Southampton (Main Study)] Version 3.0 –16th March 2015

13. I agree to take part in the above study. ☐

14. In the event that I have private medical insurance, I agree to inform my provider of the study. ☐

15. I am happy to be contacted for possible participation in future research studies in the event that I am not eligible for this study. ☐

16. I agree to be contacted by the research team after 4 years in order to provide them with an update on my body weight and health status. ☐

_____	_____	_____
Name of Subject (block capitals)	Signature	Date
_____	_____	_____
Name of Person taking consent	Signature	Date

A.3 Participant consent form for sub-group 2: insulin clamp study

Imperial College London

University Hospital Southampton NHS
NHS Foundation Trust

EndoBarrier TM Gastrointestinal Liner Diabetes Trial

Participant Consent Form (Sub-group 2: Insulin Clamp study)

Title of Project: EndoBarrier TM Gastrointestinal Liner Diabetes Trial

Principal investigator: Mr James Byrne
Site number: 2
Participant I.D number:

The participant should complete the whole of this sheet him or herself.
(Please initial each statement if it applies to you)

1. I confirm that I have read and understand the Participant Information Sheet **Southampton Version 3.0 (dated 16/03/15)** for the above study. ☐

2. I have had the opportunity to ask questions and discuss this study. All my questions have been answered fully and I have received enough information about the study including the participant information sheet. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

4. I agree that my medical notes, data and images collected as part of this study may be accessed by individuals involved in the study, or by regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I understand that my personal data will be processed and stored securely in compliance with the 1998 Data Protection Act. ☐

5. I agree to take part in sub-group 2, the insulin clamp study, which is part of the main EndoBarrier study. ☐

6. I agree that all my samples (blood, urine, faeces, tissue) will be stored for research purposes and this material can only be used with ethical committee approval. All testing of my samples, will be coded such that I cannot be identified by the data. ☐

7. I give permission for the data collected in the questionnaires and computer tasks to be used for the purposes of the study. ☐

8. I give permission for my General Practitioner to be informed of my participation in this study and the results of any medical tests from my visits i.e. blood tests. ☐

9. I give permission for tissue samples to be collected during the implant and removal of the Endobarrier device. ☐

10. I agree for a DNA/RNA sample to be taken and stored to look for changes that may be involved in obesity, diabetes, brain function and the control of appetite and hormones, and understand that the results will not be fed back to me. ☐

11. I give permission for my blood or DNA/RNA samples to be sent to laboratories in the United Kingdom or abroad for analysis as long as all personal information is removed. ☐

One copy for patient, 1 for site file, 1 for patient notes
Participant Consent Form [Southampton (Sub-group 2: Insulin Clamp Study)] Version 3.0 –16th March 2015

12. I give permission for any stored samples that I give during this study, to be used in future studies which have been granted suitable ethical approval. ☐

13. The indemnity arrangements have been discussed with me. ☐

14. I agree to take part in the above study. ☐

15. In the event that I have private medical insurance, I agree to inform my provider of the study. ☐

16. I am happy to be contacted for possible participation in future research studies in the event that I am not eligible for this study. ☐

167. I agree to be contacted by the research team after 4 years in order to provide them with an update on my body weight and health status. ☐

_____	_____	_____
Name of Subject (block capitals)	Signature	Date
_____	_____	_____
Name of Person taking consent	Signature	Date

Appendix B

B.1 Medicines, reagents and materials

Methodology	Reagent/material	Manufacturer	Identifier
Hyperinsulinaemic euglycaemic clamp	[6, 6-$^2H_2$] glucose	Cambridge Isotope Laboratories	DLM-349-MPT
	Actrapid® Vial 100iu/ml solution for injection	Novo Nordisk, Copenhagen, Denmark	
	Fortisip compact (125ml) assorted flavours	Nutricia Ltd, UK	Product code: 53141, 79749, 79784, 79786
GC-MS	Ethyl alcohol	Thermo Fisher Scientific, UK	E/0650DF/C17
	Methylhydroxamine hydrochloride	Sigma Aldrich (Gillingham, Dorset, UK)	226904
	Pyridine	Sigma Aldrich (Gillingham, Dorset, UK)	P57506
	N,O-bis(Trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane	Sigma Aldrich (Gillingham, Dorset, UK)	T6381
	Decane	Honeywell, Loughborough, UK	D901
Total Lipid Extraction	C15 Phosphatidylcholine	Sigma Aldrich (Gillingham, Dorset, UK)	P7285
	Butylated hydroxytoluene	Sigma Aldrich (Gillingham, Dorset, UK)	W218405
	Chloroform	Sigma Aldrich (Gillingham, Dorset, UK)	C2432
	Methanol	Sigma Aldrich (Gillingham, Dorset, UK)	34860
FAME preparation	Toluene	Sigma Aldrich (Gillingham, Dorset, UK)	244511
	2% H_2SO_4	Sigma Aldrich (Gillingham, Dorset, UK)	339741
	0.25 M $KHCO_3$	Sigma Aldrich (Gillingham, Dorset, UK)	237205
	K_2CO_3	Sigma Aldrich (Gillingham, Dorset, UK)	P5833
	Hexane	Sigma Aldrich (Gillingham, Dorset, UK)	296090

Appendix C

C.1 Insulin analysis using ARCHITECT Insulin Assay (Abbott)

Serum insulin concentrations from clamp study participants were determined with an ARCHITECT Insulin assay (Abbott Laboratories, IL, USA). Insulin present in the samples was measured with a one-step chemiluminescent immunoassay that uses anti-insulin antibody coated microparticles and anti-insulin acridinium-labeled conjugate. Samples, microparticles, and conjugate are added to a tube to form a particle–insulin–conjugate sandwich. After incubation, washing removes materials not bound. After washing, pre-trigger and trigger solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). The RLUs are proportional to the insulin concentration in the sample. The standards include 6 concentrations (0, 3, 10, 30, 100, and 300 mU/L). These are measured in duplicate to establish the standard curve calculated by a 4-parameter logistic curve fit method. The intra-assay variation and inter-assay variations were less than 2.7%. The ARCHITECT Insulin assay has a sensitivity of ≤1.0µU/ml.

Appendix D

D.1 Food Preference sub-group nutritional outcomes

Clinical results

Out of the 160 patients taking part in the entire Endobarrier RCT, 47 took part in food preference mechanistic studies, the food preference arm of the Endobarrier trial.

There was a significant reduction in the absolute weight within each group at 10 days, 6, 12 and 24 months compared to baseline ($p < 0.001$). Also, there was a significant difference between groups at 24 months.

There was a significant reduction in % weight loss within each group at 10 days, 6 and 12 months compared to baseline ($p < 0.001$) but no significant differences between the groups. At 24 months the Endobarrier group lost 4 ± 5% total body weight vs 7 ± 7% in the control group.

There were no significant differences in the percentage of body fat between groups both in the men and women when assessed separately.

There was a significant difference between men and women at all-time points within each group ($p < 0.001$).

Mechanistic results

- *Total caloric intake using 24-hour recall and food diaries*

Total caloric intake per day obtained from both the 24-hour recall and 3-day food diaries was significantly reduced within both groups at all-time points except for 24 months compared to baseline ($p \leq 0.001$), but there were no significant differences between the groups.

Table: Results of total caloric intake using 24-hour recall and 3-day food diaries

									Mixed Model Analysis	
	Group									
		Endobarrier				Control			Effect	*P* value
Calories (kcal) from the 3-day diary										
	n				n					
Baseline	24	1911	±	506	17	1740	±	285		
10 Days	22	1097	±	407***	17	1194	±	203***	Group	0.5
6 months	16	1575	±	410**	14	1443	±	321*	Time	<0.001
12months	13	1423	±	647***	13	1504	±	470*	Group x Time	0.3
24months	12	1788	±	761	14	1525	±	494		
Calories (kcal) from 24-hour recall										
Baseline	23	2049	±	851	18	1939	±	758		
10 Days	21	1122	±	436***	17	1145	±	386***	Group	0.4
6 months	20	1655	±	421*	14	1463	±	641*	Time	<0.001
12months	15	1382	±	716***	15	1441	±	495**	Group x Time	0.6
24months	12	1683	±	660	14	1337	±	454**		

Results presented as mean ± SD

* P <0.05 compared to baseline within the same group

** P <0.01 compared to baseline within the same group

*** P <0.001 compared to baseline within the same group

- *Food preferences using 24-hour recall and food diaries*

There was a significant reduction in the % contribution from carbohydrates ($p \leq 0.001$) and a significant increase in the % contribution from protein to daily caloric intake within both groups ($p \leq 0.001$), the results of the pairwise comparison at the specific time points within the groups are detailed in the table below. However, there were no significant differences between the groups. There was no significant reduction in the % contribution from fat either within or between groups.

Table: The three-day food diary results of the Endobarrier and Control group overtime:

	Group							Mixed Model Analysis	
	Endobarrier				Control			Effect	*P value*
Carbohydrates (%of total calories)									
Baseline	24	40	±	7	17	40	±	8	
10 Days	22	46	±	6**	17	47	±	2**	Group	0.8
6 months	16	41	±	7	14	39	±	9	Time	<0.001
12 months	13	37	±	8	13	41	±	9	Group x Time	0.5
24 months	12	42	±	7	14	40	±	7		
Protein (%of total calories)									
Baseline	24	19	±	5	17	19	±	4		
10 Days	22	19	±	6	17	16	±	1*	Group	0.9
6 months	16	21	±	6	14	24	±	5**	Time	<0.001
12 months	13	22	±	7*	13	21	±	4	Group x Time	0.05
24 months	12	19	±	5	14	22	±	7		
Fat (%of total calories)									
Baseline	24	38	±	6	17	38	±	7		
10 Days	22	35	±	4*	17	37	±	2	Group	0.9
6 months	16	36	±	7	14	36	±	10	Time	0.5
12 months	13	38	±	7	13	36	±	9	Group x Time	0.6
24 months	12	36	±	7	14	37	±	8		

Results presented as mean±SD

* P <0.05 compared to baseline within the same group

** P <0.01 compared to baseline within the same group

*** P <0.001 compared to baseline within the same group

Table: The 24-hour recall results of the Endobarrier and Control group overtime:

	Group								Mixed Model Analysis	
	Endobarrier				Control				Effect	*P value*
Carbohydrates (%of total calories)										
Baseline	23	44	±	7	18	40	±	11		
10 Days	21	47	±	8	17	45	±	7	Group	0.3
6 months	20	40	±	10	14	33	±	9*	Time	0.001
12 months	15	37	±	10*	15	41	±	10	Group x Time	0.2
24 months	12	41	±	10	14	42	±	11		
Protein (%of total calories)										
Baseline	23	17	±	5	18	19	±	5		
10 Days	21	19	±	4	17	17	±	4	Group	0.2
6 months	20	21	±	7*	14	26	±	11**	Time	<0.001
12 months	15	20	±	7	15	21	±	6	Group x Time	0.09
24 months	12	20	±	5	14	20	±	6		
Fat (%of total calories)										
Baseline	23	37	±	8	18	38	±	8		
10 Days	21	34	±	8	17	37	±	5	Group	0.9
6 months	20	39	±	10	14	40	±	11	Time	0.4
12 months	15	40	±	8	15	36	±	13	Group x Time	0.4
24 months	12	38	±	9	14	35	±	9		

Results presented as mean ± SD

* P <0.05 compared to baseline within the same group

** P <0.01 compared to baseline within the same group

*** P <0.001 compared to baseline within the same group

- *Food preferences using food frequency questionnaires (FFQs)*

There was a significant reduction in the total calories within the Endobarrier group at all-time points compared to baseline ($p \leq 0.001$). Also, there was a significant difference between groups at baseline ($p < 0.05$).

There was a significant reduction in the consumption of carbohydrates within the Endobarrier group at all-time points compared to baseline ($p \leq 0.001$). Also there was a significant difference between groups at baseline ($p < 0.05$).

There was a significant reduction in the consumption of fat within the Endobarrier group at all-time points compared to baseline ($p \leq 0.001$), and at one year in the control group ($p < 0.05$). Also, there was a significant difference between groups at baseline ($p < 0.05$).

There was a significant reduction in the consumption of nuts and seeds within the Endobarrier group at six months only ($p = 0.02$), but not in the control group.

There was a significant reduction in the consumption of meat and meat products within the Endobarrier group at one year compared to baseline ($p < 0.05$), but not in the control group.

The following food groups had no significant differences between groups or within groups at any time point; protein, alcohol, cereal and cereal products, eggs and eggs dishes, fish and fish products, fruit, milk and milk products, non-alcoholic beverages, potatoes, soups and sauces, sugars, preserves and snacks, and vegetables.

Results of the groups that showed significant changes in the EPIC Food Frequency Questionnaire:

	Group								Mixed Model Analysis	
	Endobarrier				Control				Effect	*P value*
Calories (kcal)										
	n				n					
Baseline	22	2170	±	1046	15	1572	±	653 †	Group	0.3
6 months	22	1538	±	614***	15	1493	±	787	Time	<0.001
12 months	16	1668	±	863***	15	1361	±	683	Group x Time	0.03
24 months	14	1759	±	744**	15	1519	±	641		
Carbohydrates (g)										
Baseline	22	244	±	131	15	168	±	76 †	Group	0.3
6 months	22	167	±	75***	15	164	±	97	Time	0.002
12 months	16	175	±	102***	15	157	±	96	Group x Time	0.03
24 months	14	200	±	87*	15	169	±	84		
Fat (g)										
Baseline	23	92	±	45	15	65	±	31†	Group	0.1
6 months	20	60	±	28***	15	58	±	31	Time	<0.001
12 months	15	71	±	40***	15	51	±	25*	Group x Time	0.05
24 months	12	71	±	37**	15	59	±	27		
Nuts and seeds										
Baseline	22	9	±	12	15	8	±	11	Group	0.5
6 months	22	4	±	8*	15	13	±	13	Time	0.1
12 months	16	9	±	12	15	9	±	11	Group x Time	0.01
24 months	14	9	±	13	15	7	±	8		
Fats and oils group (g)										
Baseline	22	27	±	20	15	16	±	13 †	Group	0.07
6 months	22	15	±	12***	15	11	±	9	Time	0.001
12 months	16	21	±	26	15	8	±	7*†	Group x Time	0.06
24 months	14	13	±	10**	15	13	±	9		
Meat and meat products group (g)										
Baseline	22	147	±	126	15	137	±	72	Group	0.8
6 months	22	117	±	77	15	117	±	92	Time	0.03
12 months	16	115	±	86*	15	102	±	58	Group x Time	0.1
24 months	14	109	±	79	15	110	±	71		

Results presented as mean ± SD

* P <0.05 compared to baseline within the same group

** P <0.01 compared to baseline within the same group

*** P <0.001 compared to baseline within the same group

† P <0.05 Between groups

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