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Metabolic effects of Duodenal Mucosal Resurfacing on Insulin Resistant women with Polycystic Ovary Syndrome

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Everything is theoretically impossible,

until it is done

Robert A. Heinlein

Abstract

Background

Insulin resistant conditions such as T2DM, obesity and PCOS are significant contributors of morbidity and mortality worldwide. At present, the principal treatment modalities are lifestyle measures, pharmacotherapy and metabolic surgery.

Although metabolic surgery is a highly-effective option, there remains no ideal remedy. This has resulted in the development of endoluminal procedures such as duodenal mucosal resurfacing (DMR) to fill the treatment gap. Initial DMR results suggests efficacy in patients with T2DM.

The DOMINO trial aimed to investigate the insulin-sensitising effect of DMR in women with PCOS, as a model of insulin resistance, as it additionally allowed assessment of reproductive function.

Methods

This was a mechanistic study conducted using a multi-centre prospective double-blinded sham-controlled RCT design. Thirty women of reproductive potential with PCOS, insulin resistance and oligomenorrhoea were randomised to receive either DMR or a sham endoscopic procedure with 6 months follow-up.

All participants were investigated with OGTTs and hyperinsulinaemic-euglycaemic clamps pre- and post-procedure. Participants were also investigated with weekly reproductive blood tests and pelvic ultrasound scans from 3-months post-procedure to completion of the trial.

Results

Thirty women (mean age 31.1years, mean BMI 42.5kg/m^2, mean HOMA-IR 6.2) were recruited. The rate of glucose appearance (Ra) and disappearance (Rd)– to quantify insulin sensitivity– were not significantly different between the DMR and sham groups.

Ovulatory events from pelvic ultrasound scans and reproductive blood tests did not demonstrate a difference between the two groups.

Conclusion

DMR use did not result in significant improvement in insulin sensitivity or reproductive function in women with PCOS, insulin resistance and oligomenorrhoea.

This suggests that the improvement in glycaemia and insulin resistance seen in patients with Type 2 diabetes melitus post-DMR is likely secondary to a pathophysiological difference that is not evident in a cohort of patients without T2DM.

However, further evidence is needed to substantiate this hypothesis.

300 words

Declaration of Originality

I confirm that the presented work is my own. When referring to others' data, I have referenced it in the main text, figures and tables as well as in the bibliography. All quotations have been provided in inverted commas.

Copyright Declaration

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Abbreviations

ACTH	Adrenocorticotropin Hormone
ADA	American Diabetes Association
AE	Adverse Event
AFV	Above Fasting Value
AIR	Acute Insulin Response
AMH	Anti-Müllerian Hormone
ANCOVA	Analysis of Co-variance
AUC	Area Under the Curve
BMI	Body Mass Index
BPD	Biliopancreatic diversion
CCK	Cholecystokinin
CE	*Conformité Européenne*
CV	Coefficient of variation
DHEAS	Dehydroepiandrosterone Sulphate
DI	Disposition Index
DJB	Duodenal-Jejunal Bypass
DJBL	Duodenal-Jejunal Bypass Liner
DMR	Duodenal Mucosal Resurfacing
DPP-4	Dipeptidyl peptidase-4
EGP	Endogenous Glucose Production
ELF	Enhanced Liver Fibrosis
FAI	Free Androgen Index
FDA	Food and Drug Administration
FFA	Free Fatty Acids
FFM	Fat Free Mass
FGF	Fibroblast Growth Factor
FPG	Fasting Plasma Glucose
FSH	Follicle-Stimulating Hormone
GA	General Anaesthesia

GCMS	Gas Chromatography Mass Spectrometry
GI	Gastrointestinal
GIP	Glucose-dependent Insulinotropic Polypeptide (previously Gastric Inhibitory Polypeptide)
GLP-1	Glucagon-like Peptide-1
GLUT-2	Glucose Transporter type-2
GLUT-4	Glucose Transporter type-4
GnRH	Gonadotrophin-Releasing Hormone
HbA1c	Haemoglobin A1c (glycated haemoglobin)
HDL	High-Density Lipoprotein
HGP	Hepatic Glucose Production
HOMA-B	Homeostatic Model Assessment of Beta-cell Function
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HSP	Heat Shock Protein
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IQR	Inter-Quartile Range
ITT	Intention to Treat
IV	Intravenous
IVGTT	Intravenous Glucose Tolerance Test
LDL	Low-density lipoprotein
LFTs	Liver Function Tests
LH	Luteinising Hormone
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention to Treat
NAFLD	Non-alcoholic Fatty Liver Disease
NEFA	Non-esterified Fatty Acid
NHS	National Health Service
NICE	National Institute of Health and Care Excellence
NIH	National Institutes of Health
ODI	Oral Disposition Index

OGTT	Oral Glucose Tolerance Test
PCOS	Polycystic Ovary Syndrome
PP	Per Protocol
PPARγ	Peroxisome Proliferator-Activated Receptor gamma
PPI	Patient and Public Involvement
PT	Preferred Term
QUICKI	Quantitative Insulin Sensitivity Check Index
RCT	Randomised Controlled Trial
Ra	Rate of appearance
Rd	Rate of disappearance
RIA	Radioimmunoassay
RYGB	Roux-en-Y Gastric Bypass
SAE	Serious Adverse Event
SD	Standard Deviation
SGLT	Sodium-dependent Glucose co-transporter
SHBG	Sex Hormone-Binding Globulin
SOC	System Organ Class
T2DM	Type 2 Diabetes Mellitus
TEAE	Treatment Emergent Adverse Event
TG	Triglyceride
UK	United Kingdom
US	United States
VAT	Visceral Adipose Tissue
VSG	Vertical Sleeve Gastrectomy
WHO	World Health Organisation

Reference Range

Parameter	Reference Range
Alkaline Phosphatase (unit/L)	30 –130
ALT (unit/L)	0 – 34
Androstenedione (nmol/L)	0 – 9
AST (unit/L)	0 –40
AST/ALT	<0.8
BMI (kg/m^2)	Underweight: <18.5 Healthy weight: 18.5 – 24.9 Overweight: ≥ 25; Obese: ≥ 30
Body Fat %	Underfat: <21 Healthy: 21-33 Overweight: 33 – 39 Obese: >39%
Cholesterol (mmol/L)	< 5.0
Cholesterol: HDL ratio	<3.5
DHEAS (µmol/L)	0.7 – 11.5
Diastolic BP (mmHg)	60 – 89
Epworth Sleepiness Score	<10
ELF	<7.7
FAI	<10
Fasting Glucose (mmol/L)	3.0 – 7.8
Fasting Glucose (mg/dL): Fasting Insulin (mIU/L)	> 7.0
Fasting Insulin (mIU/L)	3.0 – 15.0
FSH (unit/L)	Follicular phase: 1.5-10 Mid-cycle: 7-20 Luteal phase: 1.5-8;
GGT (unit/L)	< 40

HbA1c (mmol/mol)	Normal range: 20 – 39 Pre-diabetes: 39 – 48 Diabetes: ≧ 48
HDL (mmol/L)	> 1.2
HOMA-IR	< 3.0
Insulinogenic Index	> 0.4
LDL (mmol/L)	< 3.0
LH (unit/L)	Follicular phase: 2-10 Mid-cycle: 20-60 Luteal phase: 4-14
LH: FSH	~1
Matsuda index	> 2.5
Neck circumference (cm)	<32
Non-HDL (mmol/L)	< 2.5
Oestradiol (pmol/L)	Follicular: 200-500 Pre-ovulatory peak: 500-1500 Luteal: 250-1000
Progesterone (nmol/L)	Follicular: <5 Luteal: >20
QUICKI	> 0.335
SHBG (nmol/L)	30 – 100
Systolic BP (mmHg)	90 – 139
Testosterone (nmol/L)	0 – 2
Triglyceride (mmol/L)	< 1.7
Waist circumference (cm)	<80
Waist: Hip ratio	<0.85

1 Chapter 1 Introduction

1.1 Type 2 diabetes mellitus

The prevalence of diabetes mellitus has escalated at an alarming rate over recent decades and it is now one of the largest epidemics the world is facing[1].

In 2006, the United Nations General Assembly passed Resolution 61/225 calling for diabetes mellitus to be recognised as an international public health concern and for every nation to target prevention and control of diabetes[1, 2]. While this call was certainly applauded, it has not stifled the burgeoning growth of diabetes mellitus.

In 1996, the number of people diagnosed with diabetes mellitus in the United Kingdom (UK) was 1.4 million[3]. This figure has more than tripled in the ensuing two decades and currently there are at least 4.7 million people in the UK living with a diagnosis of diabetes mellitus[4]. The United Kingdom is by no means singular in this. In fact, in 2019, the International Diabetes Federation projected that there were 463 million adults living with diabetes mellitus worldwide and this figure is likely to rise to 700 million by 2045[5], surpassing estimates from 2017 by 70 million[6].

These figures are particularly significant for patients with Type 2 diabetes mellitus (T2DM) which accounts for over 90% of patients with diabetes mellitus[7]. This concerning trend is attributed to a multitude of factors including population growth, urbanisation, increasing aging population size, physical inactivity, economic deprivation and the symbiotic relationship of T2DM to obesity[8].

The twin rocketing levels of T2DM and obesity is of particular concern. Public Health England report that 90% of patients with T2DM are overweight or obese and, predictably, there is a five-fold greater risk of developing T2DM in patients who are overweight or obese[9, 10].

A diagnosis of diabetes is associated with a significant life-long burden of macro- and micro-vascular complications leading to disability, morbidity and mortality. With increasing diagnosis of diabetes in younger people, complications are becoming more

prevalent in middle-aged adults and include coronary heart disease, myocardial infarction, heart failure, stroke, nephropathy, end-stage renal disease, neuropathy, lower extremity amputation, retinopathy and blindness[11] as well as a decreased life expectancy of at least a decade[12].

The economic cost of managing diabetes and its related complications is exorbitant. In 2018, NHS England reported that it costs £6 billion pounds annually to treat diabetes mellitus and its complications[13] and globally, 10% of adult health expenditure in 2019 was used for the care of patients with diabetes[5].

Collectively, these factors are a symbolic call to arms for all healthcare providers worldwide to improve prevention, detection and management of T2DM.

1.1.1 Defining hyperglycaemia

Ever since T2DM was identified as a disease, it was recognised as a progressive disorder that is on one end of a continuum of worsening glycaemia with normoglycaemia on the other end.

Subsequently, conditions of intermediate hyperglycaemia – impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) – were acknowledged as 'pre-diabetic states' in between the two extremes. Although arbitrary, defining set values globally for each glycaemic state allows clarity and standardisation for research and clinical practice.

After multiple attempts at attaining consensus, in 2000, the American Diabetes Association (ADA) published revised diagnostic criteria[14] for the diagnosis of T2DM which are now internationally used[15] and include the following:

- A fasting plasma glucose (FPG) level of ≥ 126 mg/dL (≥ 7.0 mmol/L); or
- A 2-hour plasma glucose level of ≥ 200mg/dL (≥ 11.1 mmol/L) during a 75g oral glucose tolerance test (OGTT); or

- A random plasma glucose level of ≥ 200mg/dL (≥ 11.1 mmol/L) in a patient with classic symptoms of hyperglycaemia or of hyperglycaemic crisis; or
- A glycated haemoglobin (HbA1c) level of ≥ 6.5% (≥ 48 mmol/mol) measured in patients without precluding conditions (e.g. haemoglobinopathies)

The World Health Organisation (WHO) diagnostic criteria for IGT is a plasma glucose level of between 140 - 200 mg/dL (7.8 -11.1 mmol/L) 2 hours following a 75g OGTT while IFG is defined as having a FPG of between 110 - 125 mg/dL (6.1 - 6.9 mmol/L) with a 2 hour plasma glucose level below 140 mg/dL (7.8 mmol/L)[16]. The ADA criteria for pre-diabetes include a lower threshold for the diagnosis of IFG and also includes a HbA1c criteria between 5.7- 6.5% (39 - 48 mmol/mol)[17]. The NHS Diabetes Prevention Programme uses the term *'non-diabetic hyperglycaemia'* as an umbrella terms to include pre-diabetes, IFG and IGT, with similar definitions[18].

Worsening glycaemia has been consistently identified as a predictor of poor outcome in patients with T2DM[19] as highlighted in the landmark UK Prospective Diabetes Study[20], a study which eventually followed-up more than 5,000 patients with T2DM for 20 years.

However, sanguinely, the same group also determined that improvements in glycaemia was associated with improved outcomes and that each 1% reduction in HbA1c was associated with a risk reduction of 21% for diabetes-related deaths, 14% for myocardial infarction and 37% for microvascular complications[21].

1.1.2 Pathogenesis of Type 2 diabetes mellitus

Augmented scrutiny into the pathogenesis of T2DM is imperative to revolutionise the therapeutic landscape of diabetes with novel approaches that may help improve glycaemia and direct the course of diabetes.

However, assimilating the pathogenesis of T2DM is challenging for a variety of reasons.

Firstly, T2DM is a complex condition with multiple genetic and environmental determinants[22]. T2DM is also a heterogenous condition with at least 5 distinct subtypes[23] and patients present at different stages of the disease with varying degrees of glycaemia, insulin secretion and insulin sensitivity. This obscures the sequence of events when examining the development of T2DM.

Furthermore, T2DM often co-exists with other features of the metabolic syndrome such as obesity, dyslipidaemia and hypertension[24]. These conditions have overlapping pathogenetic paths making it difficult to separately elucidate the processes involved in the development of T2DM. Additionally, the circularity of many aspects of T2DM such as hyperinsulinaemia, increased free fatty acid (FFA) levels and raised levels of inflammatory cytokines further obfuscates the cause and effect of these features in the pathogenesis of T2DM. Finally, glucose homeostasis is a highly regulated system comprising many inter-connected pathways which involve feedback loops and processes that are still elusive.

Accounting for this, the pathogenesis of T2DM can be divided into several principal physiological abnormalities, the crux of which will be explored here.

1.1.2.1 Beta-cell dysfunction

Diminished insulin secretion, resulting from abating β-cell function, is among the core defects responsible for the development of T2DM[25].

Insulin is a 51-amino acid peptide hormone synthesised and released by β-cells of the islets of Langerhans in the endocrine pancreas. Fifty percent of insulin is secreted under fasting conditions[26] at a low basal rate. This is required to preserve normal FPG levels by promoting utility of glucose by insulin-dependent tissue, maintaining hepatic glucose production (HGP) and limiting gluconeogenesis[27]. The remainder is secreted post-prandially.

In response to a glucose stimulus in an individual with normal glucose tolerance, β-cells secrete insulin in a biphasic manner. Following an OGTT, the first phase sees an acute rise in plasma insulin concentration over 30 minutes followed by an equally rapid

fall in insulin levels before the second phase of steady insulin secretion at a lower level commences. A similar pattern is seen following an intravenous glucose tolerance test (IVGTT) although the rise and fall in insulin during the first phase is much swifter – with peak levels achieved with 10 minutes of the intravenous (IV) glucose stimulus.

This first phase response in insulin secretion is characteristically lost early in patients destined to develop T2DM[28] and is an independent predictor of T2DM[29]. The resultant monophasic pattern (see Figure 1.1)[30] to insulin secretion is typically evident when FPG exceeds 110 - 120 mg/dL (6.1 - 6.7 mmol/L)[31].

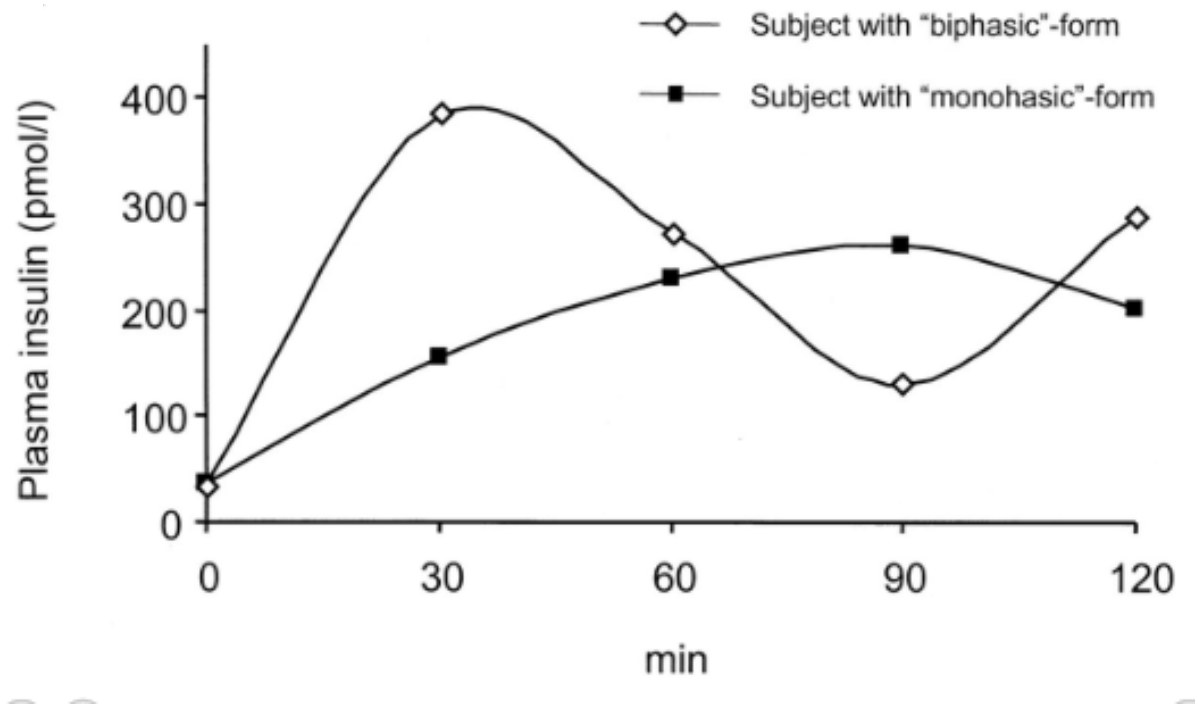

Figure 1.1 Biphasic and Monophasic patterns of insulin secretion

Plasma insulin concentration during a 75g OGTT. (Source: Tschritter O, Fritsche A, Shirkavand F, Machicao F, Häring H, Stumvoll M. Assessing the Shape of the Glucose Curve During an Oral Glucose Tolerance Test. Diabetes Care. 2003;26(4):1026-33.)[30]

This blunted response has significant pathophysiological consequences as the early burst of insulin secretion is necessary for (1) the dampening of HGP required to maintain normal plasma glucose levels in response to a glucose stimulus and (2) for priming insulin-dependent tissues for more efficient uptake of glucose.

In the early phase of disease progression – from normal glucose tolerance to T2DM – a decrease in tissue sensitivity to insulin is well-documented. However, insulin insensitivity does not result in T2DM without changes to insulin secretion.

This defect in insulin secretion is likely secondary to glucotoxicity and lipotoxicity and can be partially restored with enhanced glycaemic control[28] and following metabolic surgery[32]. Histological studies suggest that the reduction in insulin secretion is likely secondary to a combination of a progressive decrease in β-cell mass, diminished insulin content in β-cell islets and reduced islet sensitivity to glucose stimulus.

In an attempt to maintain normal glucose tolerance in the early stage of disease progression, pancreatic β-cells adapt by increasing insulin secretion to compensate for the rise in plasma glucose[28]. However, as plasma glucose concentrations continues to increase, β-cells are unable to sustain this level of escalating insulin secretion and insulin levels start to wane[28, 33]. This was also highlighted in a UK Prospective Diabetes Study which reported a contemporaneous decline in β-cell secretory capability in line with worsening glycaemia when following up 3,867 patients recently diagnosed with T2DM[34]. Further, worsening peripheral insulin resistance results in chronic augmented release of fatty acids into the circulation which has a lipotoxic effect on β-cells and result in decreased glucose-stimulated insulin secretion[35].

Thus, without treatment, hyperinsulinaemia is a harbinger for the development of IGT, IFG and the ensuing T2DM.

This transformation is apparent when mean plasma insulin response during an OGTT is plotted against FPG levels. The resultant bell-shaped curve, referred to as Starling's curve of the pancreas (see Figure 1.2)[36], has pertinent physiological significance.

Firstly, FPG exceeding 120mg/dL (6.7mmol/L) appears to be the 'turning point' at which the precipitous downward trend in insulin secretion is seen[36]. This matches the point at which HGP begins to rise[28]. Together with poor peripheral insulin sensitivity,

the rising HGP contributes to hyperglycaemia and lipaemia. The resultant gluco- and lipotoxicity, places further stress on the β-cells, leading to β-cell failure and worsening insulin resistance[37].

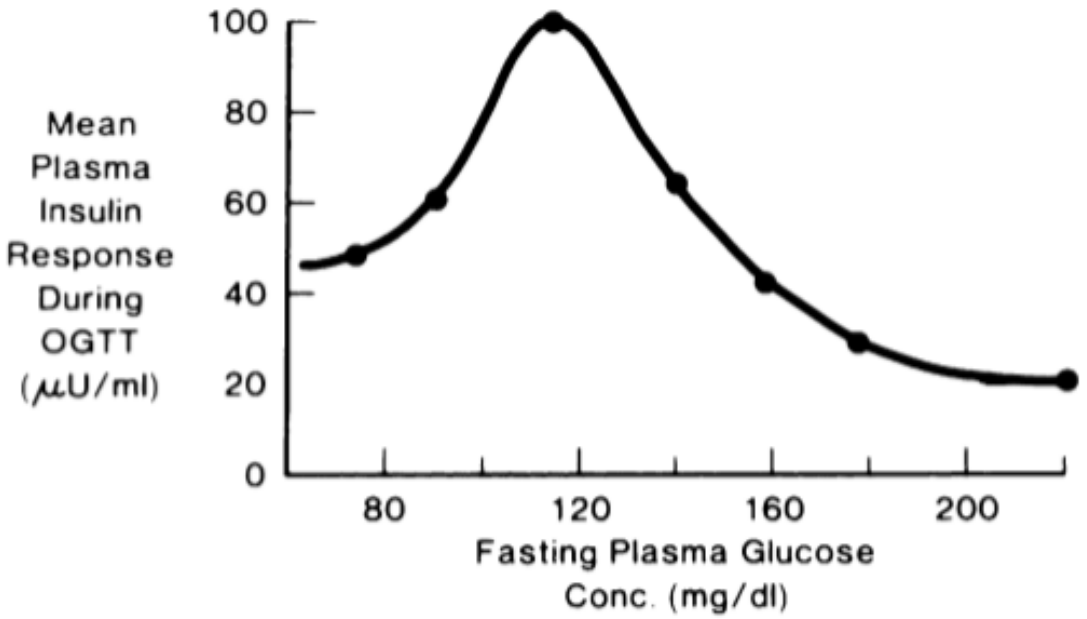

Figure 1.2 Starling's curve of the pancreas for insulin secretion.

In normal-weight patients with impaired glucose tolerance and mild diabetes mellitus, plasma insulin response to ingested glucose increases progressively until the fasting glucose concentration reaches 120 mg/dl. Thereafter, further increases in fasting glucose level are associated with progressive decline in insulin secretion. (Source: eFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 1988;37(6):667-87.)[36]

Secondly, although plasma insulin secretion remains elevated early in the development of T2DM (FPG < 140mg/dL or 7.8mmol/L), β-cell dysfunction is already well-established at this stage[28]. In fact, a staggering loss of β-cell function of 60-70% is evident in patients with IGT when Disposition Index (DI), a composite measure of β-cell function, is mapped against 2-hour plasma glucose concentration levels[33]. Further, individuals without T2DM with 2-hour plasma glucose levels between 120-140mg/dl (6.7 – 7.8 mmol/L) only have 50% residual β-cell function despite normal glucose tolerance[28, 33]. This suggests that, contrary to previous tenet, B-cell

dysfunction occurs early in the natural history of T2DM and is established by the time hyperglycaemia is evident[38].

Thirdly, it is important to appreciate that although the' typical' patient with T2DM will display fasting hyperinsulinaemia with a FPG around 150 - 160 mg/dL (8.3 - 8.9 mmol/L) and a mean post-OGTT insulin level similar to normoglycaemic individuals[36], they are effectively insulinopaenic as their mean insulin levels should be markedly higher at such raised levels of glucose. Thus further expounding the essential role of β-cell failure and decreased insulin secretion as core defects in the development of T2DM and indeed all forms of diabetes[39].

Finally, unlike other physiological systems, glucose homeostasis is solely reliant on a single hormone, insulin, acting on a single receptor to decrease glycaemia[40]. This is in sharp contrast to the multiple agents that can elevate blood glucose. This discrepancy in glucose homeostasis may be a result of human evolutionary history as an excess of insulin and the resultant hypoglycaemia results in more immediate and devastating sequelae than the reverse[40]. Nonetheless, in the current climate of plentiful high-calorie diets and sedentary lifestyle, insulin is the only glucose-lowering physiological agent available, which is why T2DM rapidly ensues as insulin secretion wanes.

1.1.2.2 Insulin resistance

Insulin resistance refers to an impaired ability of tissues in the body to respond to the actions of insulin, requiring higher concentrations of insulin to achieve the expected effect.

Insulin resistance, predominantly to hepatic, muscular and adipose tissue; is a key contributor to the underlying pathology in T2DM and glucose intolerance. In addition, it is a predominant characteristic of obesity, polycystic ovary syndrome (PCOS), non-alcoholic fatty liver disease (NAFLD), dyslipidaemia, hypertension and the eponymous metabolic syndrome. It is also a feature of aging, lack of exercise and of some diets.

The clinical heterogeneity of insulin resistance occurring to variable extents in different tissues, different individuals and different associated conditions can be partly explained by the complexity of insulin action at the molecular level[41].

Insulin, a peptide hormone, initiates a cascade of intracellular events by binding to its glycoprotein cell surface receptor which is made up of an α- and β-subunit[42]. Insulin binds to the extracellular α-subunit which activates the partially-intracellular β-subunit, mediating the effector function of the receptor[42]. The β-subunit is a tyrosine kinase and auto-phosphorylates to facilitate multiple intra-cellular events to effect insulin action[42].

Most of these actions are carried out by two signalling pathways:

(1) the phosphatidylinositol-3-kinase (PI3K)/Akt pathway – liable for the metabolic actions of insulin including transporting glucose into the intracellular compartment via the glucose transporter type-4 (GLUT-4) molecule and synthesis of glycogen, protein and fat as well as inhibiting hepatic gluconeogenesis[43, 44]; and

(2) the mitogen-activated protein kinases/Ras pathway (MAPK/Ras) which promotes gene expression as well as cell growth, proliferation and differentiation[43, 45]. Signalling within this pathway remains unimpeded in most insulin resistant conditions[46].

The latter finding suggests that insulin resistance is largely a result of targeted intra-cellular defect or defects involving the phosphatidylinositol-3-kinase (PI3K)/Akt pathway and not a global cell-wide inhibition of insulin-signalling.

Still, the aetiology of insulin resistance at a biochemical level can be divided into intrinsic causes arising from (1) rare pre-receptor causes (e.g. mutation to the insulin peptide, antibodies to insulin, accelerated insulin degradation), (2) receptor causes (e.g. decrease in the number or affinity of the receptors, failure to activate tyrosine kinase, mutation or antibodies to the receptor) and, most commonly, (3) post-receptor causes (e.g. defective signal transduction, alterations in intracellular pathways, mitochondrial and endoplasmic reticulum dysfunction, decreased production of glucose transporters (e.g. GLUT-4) or other downstream-located effector proteins)[42,

[43]. Augmented insulin desensitisation mechanisms which attenuate insulin signalling and is associated with obesity has also been found to cause systemic insulin resistance[44].

Additionally, extrinsic mechanisms involving inflammatory cytokines and alteration in adipokines also play a role in the aetiology of insulin resistance[43].

Different clinical conditions may be associated with different defects in insulin signalling or occur as a result of a combination of defects. For instance, insulin-regulated glucose transport is often selectively inhibited in many insulin-resistant conditions while other insulin-dependent processes proceed unhindered[46].

The insulin signalling defects which have been elucidated to trigger the progression to T2DM, and are often causal in obesity as well, include decreased number and activity of insulin receptors, modifications to downstream-located effector molecules, defects in GLUT-4 expression and function and mitochondrial dysfunction [43, 47]. Inflammatory cytokines also contribute to the development of insulin resistance in T2DM and obesity[42, 43].

Insulin resistance in T2DM is classically described as hepatic or peripheral (muscle and adipose tissue) in origin. Hypothalamic insulin resistance is a more recently recognised entity.

1.1.2.2.1 Hepatic Insulin Resistance

Under postabsorptive conditions, the liver is responsible for roughly 90% of endogenous glucose production (EGP) while the kidney produces the remaining glucose[48]. The liver of a healthy person produces glucose at a rate of 1.8 - 2.0 mg/kg/min[49]. Approximately half of this is produced via glycogenolysis and the remainder via gluconeogenesis[50].

This rate of glucose production is crucial to meet the needs of the brain and other neural tissue which utilises 50% of the glucose produced at a constant rate of 1.0 - 1.2 mg/kg/min, via insulin-independent glucose transporters. This rate of glucose

utilisation persists regardless of absorptive circumstance or diabetes state[28]. Splanchnic tissues utilise 25% of the glucose produced, also in an insulin-independent fashion while the remaining 25% of glucose produced is taken up by insulin-dependent tissues, primarily muscle[49]. At basal conditions, the rate of glucose utilisation is precisely matched to the glucose production rate[49].

Following nutrient ingestion, this delicate balance is disrupted. The influx of glucose, fatty acids and amino acids stimulates insulin release by pancreatic β-cells. The resultant hyperglycaemia, lipaemia and hyperinsulinemia triggers glucose uptake by splanchnic and peripheral tissues while simultaneously suppressing EGP[49].

In the liver, insulin acts by promoting glycogenesis and inhibiting gluconeogenesis, glycogenolysis and ketogenesis. If the liver is resistant to this powerful suppressive signal and continues producing glucose, there will be two simultaneous streams of glucose input, resulting in significant hyperglycaemia[28]. Most of the excess glucose produced by the liver is via accelerated gluconeogenesis[51, 52].

In patients with T2DM, fasting plasma insulin concentrations are triple or quadruple that in individuals without T2DM[36]. Despite this, HGP is not suppressed indicating a degree of hepatic insulin resistance as well as hepatic gluco-resistance[49]. The rate of HGP is strongly correlated to the severity FPG and is the major abnormality responsible for raised FPG levels[28]. Consequently, as hepatic insulin resistance worsens so does the severity of T2DM.

In the liver, insulin also acts to increase synthesis of cholesterol and triglyceride (TG). In high glucose states, when glycogen reserves are saturated, insulin acts to shunt excess glucose taken up by hepatocytes toward the synthesis of fatty acids[53]. Hence, it would be reasonable to predict that the reverse would occur under insulin resistant conditions.

However, paradoxically, in insulin resistant conditions such as T2DM, the liver increases synthesis of cholesterol and TG suggesting that the pathways involved in these processes remain selectively sensitive to the actions of insulin[54]. This deleterious selective sensitivity results in worsening fat accumulation in the liver as

insulin resistance and glucose intolerance worsens[54]. Consequently, as adipose tissue develop insulin resistance, there is increased flow of FFA from adipocytes to hepatocytes[55]. Hence, it comes as no surprise that NAFLD frequently co-exists and often precedes the onset of T2DM[53, 56].

The consequent build-up of fatty acid and its conversion to TG results in the accumulation of diacylglycerol, a compound that has been identified as a key culprit in the development of hepatic insulin resistance[57] – resulting in a vicious cycle of worsening glucose intolerance and hepatic adiposity.

Animal studies have also demonstrated other pathways involved in the development of hepatic insulin resistance including upregulation of the mammalian target of rapamycin (mTOR) complex 1[58], disruption of glucose-glycogen conversion[59], over-expression of enzymes involved in gluconeogenesis[60] and resistance mediated by inflammatory cytokines[53]. Confirmatory human studies are awaited.

1.1.2.2.2 Peripheral Insulin Resistance

Peripheral insulin resistance refers to the decreased uptake of glucose by muscle and adipose tissue despite supra-basal glucose concentrations.

Studies measuring insulin resistance using the gold-standard euglycaemic insulin clamp technique have demonstrated that peripheral insulin uptake is primarily by skeletal muscle, which accounts for 80% of glucose utilisation while adipocytes only account for 5-10% of total body glucose disposal[28].

Despite a quantitatively low acute uptake of whole-body glucose in the fed state, adipose tissue induces a potent effect on glucose homeostasis and lipid metabolism in health and in diabetes. Further, adipose tissue insulin resistance in T2DM is thought to precede that of skeletal muscle and has a bidirectional relationship with insulin resistance[46, 61].

Briefly, in healthy individuals, at basal conditions, glucagon and other hormones stimulate lipolysis in adipocytes to provide fatty acid as an additional substrate for

cellular metabolism[62]. In the post-prandial state, the circulating glucose, amino acids and fatty acids stimulate insulin release from β-cells which acts to suppress lipolysis, promote hepatic lipid synthesis for storage in adipocytes, stimulate glycogen synthesis within myocytes and stimulate rapid and efficient peripheral glucose uptake[46, 62].

The efficiency of glucose uptake by myocytes and adipocytes is dependent on the insulin-stimulated trafficking of the GLUT-4 glucose transporter to the cell surface[46, 63]. This process is thought to be the rate-limiting step in peripheral glucose utilisation and the subsequent downstream insulin-mediated events such as glycogenesis and lipogenesis in myocytes and adipocytes respectively[64].

In insulin resistant states such as T2DM, glucose uptake by peripheral tissues is both delayed and diminished[28]. Studies using the gold-standard euglycaemic insulin clamp technique have demonstrated a 20 – 30 minute delay and 50% decrease in glucose uptake when compared to subjects without T2DM, indicating resistance of skeletal muscle and adipose tissue to the action of insulin[28]. Defects in GLUT-4 expression or function have been repeatedly seen in models of peripheral insulin resistance and is likely to play a central role in insulin resistance in people with T2DM[46, 63].

Conversely, despite significant decline in insulin-mediated glucose uptake, insulin resistant adipose tissue have not been consistently found to demonstrate increased lipolysis to the rate expected[46]. Similarly, the decrease in protein synthesis in skeletal muscle is not to the same degree as the decrease in glucose uptake[65]. This suggests that insulin still retains selective sensitivity within the adipocyte and myocyte for selective functions and displays a preferential inhibition of glucose uptake.

In *vitro* and animal studies have suggested pathways for the pathogenesis[66] for this preferential insulin resistance including mitochondrial dysfunction[67], endoplasmic reticulum stress[68], impaired glucose oxidation[69] and altered lipid profile resulting in accumulation of ceramides[70].

Increased lipolysis leads to increase in circulating FFA which accumulates in adipose and lean tissue, instigating lipotoxicity and aggravating insulin resistance[71].

Further, inflammatory adipokines have an extrinsic role in the development of peripheral insulin resistance. In addition to the release of FFA into the circulation from increased lipolysis, adipose tissue also release a number of pro-inflammatory cytokines which negatively influence insulin resistance. These include mediators such as TNF-α and IL-6 both of which are raised in people with obesity and have been found to impair insulin signalling, increase lipolysis, promote leptin production and increase HGP[35, 72]. This causal association between adipose tissue and glucose homeostasis provides further insight into diabetogenesis in people with obesity.

Interestingly, the relationship between obesity and T2DM is stronger in people with high visceral fat deposition compared to those with subcutaneous or gluteal fat depots[73, 74]. This diabetogenic regional variance is attributed to insulin resistant-causing properties specific to visceral adipose tissue (VAT) – which may have more pertinence to dysmetabolism than body mass index (BMI).

Firstly, the products of visceral fat lipolysis are drained via the portal vein, exposing the liver to high concentrations of FFA and glycerol[72]. This results in increased hepatic TG production causing dyslipidaemia, increased HGP leading to hyperglycaemia and reduced hepatic insulin extraction leading to hyperinsulinaemia; all of which predispose to T2DM[72].

As VAT accumulates and adipocytes hypertrophy, macrophages accumulate within VAT leading to subclinical low-grade inflammation of the fat tissue[75]. This results in a preferential VAT production of pro-inflammatory cytokines such as TNF-α, IL-6 and leptin (as discussed below) and reduced whole-body formation of the anti-diabetic, anti-inflammatory adipocytokine, adiponectin.

Indeed, VAT production and expansion is itself a marker of ectopic fat deposition that result when subcutaneous fat tissue are unable to accommodate further fat storage. This results in fat storage in classically ‘lean’ tissues such as the liver, pancreas and the heart. Significantly, people with obesity with a comparable BMI but higher VAT are more likely to develop insulin resistance, glucose intolerance and T2DM[76, 77].

1.1.2.2.3 Central insulin resistance

The role of insulin in the brain and neural tissue has only come to light more recently following discovery by Havrankova and her colleagues[78]. Since then, with advances in the field of functional neuroimaging, significant investigation into the role of insulin as a neuroregulatory peptide in the brain has surfaced [79]. Differences in functional MRI responses in individuals with obesity and insulin resistance, at baseline and following glucose ingestion have highlighted links between central and peripheral insulin resistance[80, 81]. Reversibility in hypothalamic function towards that of lean individuals has also been documented following metabolic surgery[82].

The brain in general and the arcuate and ventromedial nuclei of the hypothalamus in particular are now acknowledged as insulin-sensitive tissue[83]; and further, insulin is recognised to play a positive role in the neuromodulation of (1) body weight, (2) eating behaviour (by increasing expression of pro-opiomelanocortin (POMC), a polypeptide associated with reduced food intake), (3) glucose metabolism (via neuronal connections that regulate HGP, myocyte glycogen synthesis and adipocyte fat metabolism), (4) neuronal development and (5) key cognitive processes including memory and learning[83, 84].

Studies using intranasal insulin sprays conclusively demonstrate the centrally mediated role of insulin[85], while an interesting study using magnetoencephalography and 2-stage hyperinsulinaemic euglycaemic clamps demonstrated a differential response to cerebrocortical insulin in lean and obese individuals[86]. This suggests that a disruption of central insulin signalling may lead to weight gain and glucose dysregulation[83, 86]. The ensuing obesity may further attenuate central insulin sensitivity, exacerbating systemic insulin resistance and progression to T2DM[83].

Additionally, leptin, a hormone predominantly made in adipocytes and enterocytes, also shares a common signalling pathway with insulin and can influence insulin resistance via its central function[87]. Leptin primarily acts on receptors within the arcuate nucleus to help regulate adipose tissue mass by inhibiting hunger, increasing insulin sensitivity and promoting lipolysis[87].

Although leptin is a satiety hormone, obese individuals have been found to have higher plasma levels of leptin than individuals with a normal body weight[87]. Despite these increased levels, the ratio of leptin concentration in cerebrospinal fluid to plasma concentrations is lower in individuals with obesity that those with normal body weight[87]. This is likely a result of a leptin resistance in these individuals resulting in increased food intake, triglyceride storage and ensuing events that lead to obesity and insulin resistance[87].

Dopamine has also been highlighted to have a centrally-acting role in insulin resistance[79]. Dopamine plays a key role in the reward circuitry in the brain and is released in response to eating and perceived pleasantness of a meal[88]. Dopamine and insulin signalling have a bi-directional regulatory function and this relationship appears to be altered by the chronicity of the signal[89]. Although research in this area is still developing, it is evident that altered dopamine signalling contributes to insulin resistant conditions[89].

1.1.2.3 The role of the small intestine

The notion that the small intestine plays a pivotal part in glucose homeostasis appears intuitive, as it is the main channel for dietary glucose input. However, its key role in regulating glucose levels was only established more tangibly at the turn of the century.

This was instigated by the publication of the sentinel paper by Pories *et al* highlighting the emergence of Roux-en-Y gastric bypass (RYGB) as a useful therapeutic option in the management of patients with obesity and diabetes[90].

The serendipitous finding of significant, rapid and enduring improvement in glucose parameters, independent of caloric restriction and weight loss were consistently noted; and the elusive mechanisms for these observations were keenly sought. This led to a paradigm shift in focus away from the, now obsolete, ‘restrictive’ and ‘malabsorptive’ modes of action for bariatric procedures towards novel metabolic concepts.

Now, more than two decades later, a number of resounding glucoregulatory mechanisms of action for the gut have emerged. These have largely been developed

around the RYGB model and include proximal intestinal exclusion, rapid distal nutrient delivery, incretin effect, the postulated anti-incretin effect, nutrient sensing, glucose utilisation and absorption mechanisms, changes in the entero-hepatic circulation of bile acids, gut microbiota and the gut-brain-liver axis. These models are particularly significant as they are being used to develop novel therapeutic strategies – effectively changing the landscape for the treatment of T2DM.

1.1.2.3.1 The incretin effect

The incretin effect refers to the greater amount of insulin secretion stimulated when glucose is consumed orally than when glucose is introduced parenterally at the same plasma glucose conditions.

Two gastrointestinal (GI) hormones have been identified to account for more than 90% of this effect; they are (1) Glucose-dependent Insulinotropic Polypeptide (GIP) - a GI hormone secreted by K cells in the proximal small intestine and (2) Glucagon-like Peptide-1 (GLP-1) – the main incretin hormone, secreted by the L cells of the distal small intestine and colon (and to a lesser extent from the duodenum and jejunum) and by the nucleus solitarius in the brainstem[28, 91-93].

Both these hormones act to increase glucose-dependent insulin secretion by binding to their specific receptor on the pancreatic β-cells, in response to intraluminal glucose[92]. The effect of this enteroinsular axis is blunted in patients with T2DM, either through a deficiency or a resistance to the incretin effect[94, 95]. This blunting has been linked to the development of β-cell dysfunction[94].

In patients with T2DM, GIP secretion to enteral glucose is similar or enhanced when compared to individuals without T2DM, indicating that β-cells are resistant to its effect[92]. Conversely, GLP-1 which is the more potent incretin hormone although released in smaller volumes, is diminished.

While continuous IV administration of GLP-1 corrects hyperglycaemia in patients with T2DM, this effect is not seen with infusion of GIP[92]. Further, the incretin effect and GLP-1 levels are also restored in patients following RYGB, prior to significant weight

loss[96]. This may be a result of accelerated nutrient transit to the distal small intestine, where GLP-1 is produced.

Both incretins are rapidly degraded by the action of dipeptidyl peptidase-4 (DPP-4), an enzyme expressed throughout the body and this property has been exploited pharmacologically with the creation of DPP-4 inhibitors (-gliptins), an oral hypoglycaemic agent.

The incretins also have other important functions. Both hormones also promote β-cell proliferation, oppose β-cell apoptosis, regulate bone metabolism and control food intake and satiety[92].

Further, GLP-1 has also been shown to suppress glucagon secretion and also induces weight loss. However, due to its short half-life of several minutes, GLP-1 is not a suitable therapeutic target. GLP-1 receptor agonists (e.g. liraglutide, semaglutide) have been developed instead to utilise the many beneficial effects of GLP-1 clinically in the management of diabetes and obesity.

Paradoxically, GIP enhances glucagon secretion and promotes fat accumulation[92], which, together with its β-cell resistance in patients with T2DM, hinders its clinical usage in diabetes and obesity[92].

The GLP-1's incretin effect is supported by the hindgut hypothesis which postulates that the early weight-independent changes seen following RYGB are secondary to early arrival of undigested nutrients to the hindgut resulting in quicker and greater increase of GLP-1.

In keeping with the hindgut hypothesis, post-prandial plasma concentrations of GLP-1 levels have been consistently found to be raised following RYGB, biliopancreatic diversion (BPD) and, to a lesser extent, vertical sleeve gastrectomy (VSG)[97]. This rise in GLP-1 levels is evident from two days following surgery and persists long-term[97].

However, the role of the raised post-op levels of GLP-1 is unclear as blockage of GLP-1 action following RYGB has very limited effect on glucose tolerance[98, 99]. Further studies using exendin-9, 39, a GLP-1 receptor antagonist, in patients post-RYGB has demonstrated minimal or no changes to glucose tolerance and insulin sensitivity[100, 101]. These findings suggest that GLP-1 and the hindgut hypothesis are not the chief contributors of early post-operative weight loss-independent glycaemic improvements as they were once thought to be[98, 100].

1.1.2.3.2 The foregut theory

While the precise molecular basis for the foregut theory and attendant anti-incretin factor mitigation remains to be eluded, the theoretical model is undoubtedly persuasive. It coherently reconciles the physiological and pathological evidence surrounding insulin sensitivity and T2DM; with observations of the effects of metabolic surgery[98]. Metabolic surgical procedures which have most consistently been found to elicit diabetes remission, characteristically include foregut bypass (e.g. RYGB, BPD) or accelerated transit (e.g. VSG)[102, 103]. Correspondingly, this principle also underlies the philosophy on which novel metabolic therapies such as duodenal mucosal resurfacing (DMR) and duodenal-jejunal bypass liner (DJBL) have been developed.

The role of proximal intestinal bypass in alleviating hyperglycaemia and insulin resistance was first highlighted by Pories and colleagues[104-106]. The team conducted studies comparing insulin and glucose excursions in female patients who had undergone RYGB to age- and weight-matched female patients who were surgically-naïve[106]. Results from these studies established that eliminating nutrient contact with the foregut facilitated weight loss-independent mechanisms for improved glucose and insulin modulation. Further, these studies concluded that the foregut, and modulators released from nutrient transit through the foregut, had a role in the pathogenesis of T2DM[105].

With that, the foregut – the duodenum and proximal jejunum – were promptly highlighted as metabolically active centres with significant pathological potential.

Rubino and Gagner then drew parallels between RYGB and BPD as operations that both resulted in improved glycaemic profiles and were similar in (1) the exclusion of the duodenum and part of the jejunum from transit of nutrients; as well as in (2) the premature arrival of partially digested food to the ileum[107]. These two occurrences led Rubino and Gagner to propose the presence of the hypothetical anti-incretin factor[107].

It was suggested that physiologically, anti-incretins have a protective negative feedback role as they are purportedly released, following nutrient stimulation in the foregut, to prevent hypoglycaemia from the glucose-lowering actions of incretin-induced insulin secretion[98, 107, 108]. Dysfunctional enhancement of this factor, from chronic exposure of the foregut to high levels of dietary fat and carbohydrates triggers the overproduction of the ant-incretins which induces glucose intolerance and insulin resistance, predisposing the onset of T2DM[107].

They proposed that surgical bypass of the foregut in such patients resulted in reduction of this factor, restored balance between the incretin and anti-incretin signals, improved insulin sensitivity and conferred a potent ant-diabetic effect[109].

They deduced that further indication for the presence of the anti-incretin effect was the rarity of β-cell proliferative tumours, despite the ubiquitous nature of incretin peptides and its proliferative effect on β-cell mass - suggesting an anti-incretin regulating factor prevented uncontrolled proliferation of β-cells[107, 110]. This concept was re-enforced with the finding of enlarged β-cell mass in an animal model following RYGB[111].

Further evidence has since amassed in support of the foregut theory and the presence of anti-incretins[110]. Notably, Salinari and her colleagues conducted a well-designed experiment comparing duodenal-jejunal bypass (DJB) procedure with jejunal resection, ileal resection, sham procedure and non-surgical controls using normal weight animals with and without diabetes[112]. She found that 2 weeks post-procedure insulin sensitivity improved in subjects with diabetes after DJB and jejunal resection but not ileal resection, without changes in circulating incretin levels[112].

This was consistent with the theory of a putative foregut anti-incretin signal which is reduced following bypass or resection of the proximal small intestine leading to improved insulin sensitivity. These experiments were conducted in normal weight animals suggesting that the inflated anti-incretin signal was evident in animals with T2DM, even in the absence of obesity – highlighting the role of weight-independent mechanisms underlying the effect. Further, the lack of glycaemic improvement seen following ileal resections, which allowed greater distal delivery of nutrients (hindgut theory) without a foregut sparing effect, fortified the foregut hypothesis.

Additionally, in keeping with the theory proposed by Rubino, foregut bypass or resection in this model did not 'over-correct' glucose homeostasis in subjects with normal glucose tolerance[112]. However, in clinical practice post-prandial hypoglycaemic events are noted, albeit infrequently, following RYGB[113]. Post-prandial hypoglycaemia often develops as a delayed post-operative feature, often in association with raised GLP-1 levels[97]. This suggests that, similar to other biological traits, anti-incretin stimulus may occur on a spectrum with raised and diminished anti-incretin effects at opposite ends. Hence, it is fathomable that in patients who develop post-RYGB hypoglycaemia the equilibrium between incretin and anti-incretin is predisposed to the former and that this post-op complication may be a consequence of increased β-cell mass, secondary to this tipped balance[97].

In another elegant experiment conducted by Mingrone and her colleagues, jejunal proteins obtained from individuals with obesity and insulin resistance were demonstrated to induce insulin resistance *ex vivo* in human skeletal muscle cell cultures[114]. This provided convincing evidence for the existence of an anti-incretin effect in the foregut responsible for the development of insulin resistance.

To concretely assess the anti-incretin effect *in vivo* in humans, Salinari *et al* conducted two landmark experiments. In the first, they carried out an experiment comparing normal weight, healthy individuals to individuals with obesity and insulin resistance before and after BPD[109]. All participants were assessed with an OGTT and an isoglycaemic IV test. As expected, insulin sensitivity was globally lower following oral

glucose stimulus compared to IV[109]. However, this difference was more profound in individuals with obesity and insulin resistance, although normalised following BPD[109].

Mathematical simulations confirmed that if the same level of insulin sensitivity present during IV isoglycaemic glucose administration was evident during oral simulation, hypoglycaemia would ensue[109]. Thus, establishing the presence of an insulin-lowering anti-incretin effect following GI nutrient stimulation and providing a compelling explanation for the improvement in insulin resistance seen following foregut-sparing surgery[109].

In the second experiment, designed to assess the acute effect of foregut sparing, nutrients were infused directly into the duodenum, proximal jejunum and mid-jejunum in obese individuals with and without diabetes[115]. Endoscopically placed feeding tubes were used for nutrient delivery. This study found that insulin sensitivity to glucose and fat, plasma GLP-1 levels and insulin clearance rates were all higher with more distal nutrient delivery[115].

A similar experiment, Zhang and his colleagues also demonstrated improved glucose modulation with distal glucose delivery in individuals with T2DM and healthy controls[116]. They also additionally found slower glucose absorption and lower blood glucose excursions with distal nutrient exposure[116].

This reinforced the value of nutrient sparing in the foregut in improving glycaemic markers either by way of (1) anatomical bypass (e.g. RYGB, BPD, DMR, DJBL), (2) expedited GI nutrient transit (e.g. VSG) or (3) reduction of nutrient stimuli (e.g. very low-calorie diet (VLCD) regimes)[98].

However, the exact nature of this anti-incretin factor has remained obscure. One potential anti-incretin is dopamine[110]. Dopamine functions both as a hormone and a neurotransmitter. In the brain it plays a major role in the motivational-reward circulatory which is deeply intertwined with eating behaviour[79]. Outside the nervous system, the bulk of dopamine is produced in the GI mesentery and dopamine has been shown to reduce insulin production, slow GI motility and affect intestinal mucosa[117, 118]. Plasma levels of dopamine has also been found to increase post-prandially[117, 118].

Another possible explanation revolves around heat shock proteins (HSP), an evolutionary-conserved protein released in response to cellular stress. An interesting study by Professor's Mingrone's team found that HSP70 is released in the foregut, particularly to calorie-rich meals high in sugar and fat[119]. Patients with obesity and insulin resistance were found to have higher levels of HSP70; and when samples from these patients were used *in vitro*, impairment of insulin-mediated glucose uptake and enhancement of lipid accumulation was evident[119]. However, following foregut-sparing surgery (BPD), HSP70 levels in these patients normalised with good correlation to improvements in insulin sensitivity and secretion[119]. Further, *in vitro* studies using samples taken following BPD revealed good glucose uptake and reduced lipid uptake and storage[119].This study suggests that limiting nutrient contact in the foregut may improve insulin resistance by reducing the release of these proteins.

Alternative enteropeptides that have been postulated to have an anti-incretin role include ghrelin, serotonin, GIP, oxyntomodulin and enterostatin[110]. Further, the vagus nerve, the major basis for gut-brain-liver interaction, has also been cited as a possible mechanism for the anti-incretin effect[110]. Several of these factors are discussed in greater detail in subsequent sections.

1.1.2.3.3 Nutrient sensing

Nutrient sensing, the ability of the GI tract to accurately recognise and react to fuel substrates, such as glucose, amino acids and lipid; is a requisite for metabolic health. Dysregulation of these nutrient sensing pathways are alleged to result in metabolic disease states[120].

Nutrient sensing starts with food preferences and taste and continues after meal ingestion. In the GI tract, pre-absorptive intra-luminal contents are closely monitored by chemosensors. These chemosensors send complex vagal and chemical negative feedback signals via the gut-brain-liver axis to regulate levels of particular substrates, by inhibiting exogenous intake and endogenous production[121, 122]. These signals usually act via peptides produced by enteroendocrine cells. Enteroendocrine cells are specialised intestinal endocrine or paracrine cells which are exposed to the intestinal

luminal contents on their apical side and are able to act as chemosensors in response to these contents[121].

Although there is significant peptide co-expression, the small intestine can be crudely divided into proximal and distal halves. The proximal small intestine contain I cells and K cells which produce cholecystokinin (CCK) and GIP respectively; while the distal small intestine is more abundant in L cells which produces GLP-1 and 2, peptide YY (PYY) and oxyntomodulin[121].

Non-nutrient stimuli of these enteroendocrine cells include bile acids and inflammatory cytokines (e.g. IL-6), which can result in increased release of GLP-1 and PYY from L-cells[123]. PYY is a peptide hormone released by L-cells that promotes satiety and reduces post-prandial insulin levels. It has been intensely studied since it was found to have an important role in weight loss and glucose remediation following metabolic surgery and DJBL implantation[123].

The duodenum is likely the main area for intestinal nutrient sensing as it is the site for the majority of nutrient absorption and the region with the most dense vagal innervation[121]. Intra-duodenal lipid stimulates CCK production which inhibits endogenous HGP[122]. However, in environments of chronic high fat exposure, this physiological negative feedback signalling mediated by nutrient sensing is likely disrupted resulting in unregulated EGP and potentially leading to dysmetabolic consequences[122].

This makes the duodenal luminal mucosa an attractive target site for novel therapeutics as theoretically, reversal of this nutrient sensing defect could potentially result in superior glucose homeostasis. This may be the *modus operandi* for procedures such as DMR, which are engineered to instigate duodenal mucosal renewal.

Studies indicate that, although the duodenum is the main site of nutrient absorption, a measure of ingested nutrients still reach the jejunum shortly after a meal[122]. Jejunal nutrient sensing mechanisms here have been shown to act to reduce plasma glucose and food intake[122]. In fact, even in the absence of decreased intake, direct

administration of glucose or lipid into the jejunum have been shown to inhibit EGP[124]. This is in keeping with Salinari's finding of direct mid-jejunal nutrient infusion resulting in higher insulin sensitivity than more proximal nutrient delivery[115, 124]. Further, the nutrient sensing pathways in the jejunum are independent of those in the duodenum as CCK is synthesised to a much lower extent in the midgut[122]. The mechanism of action for jejunal nutrient sensing is obscure, although anorexigenic vagally-acting peptides such as GLP-1 and PYY have been proposed as potential mediators.

To assess the role of nutrient sensing in patients following duodenal-sparing surgical procedures (e.g. RYGB, DMR, DJBL), Lam and his team conducted a sophisticated *in vivo* animal experiment on non-obese subjects with diabetes that had undergone DJB[124]. They found that the animals with disrupted jejunal nutrient sensing mechanisms lost the glucose-lowering effect conferred by DJB[124].

This was significant as it demonstrated that distalisation of nutrient delivery triggered a glucose-lowering signalling mechanism mediated by jejunal chemosensors.

1.1.2.3.4 Glucose absorption

Luminal glucose absorption into the small intestinal epithelial cells is predominantly via the high-affinity sodium-dependent glucose co-transporter 1 (SGLT 1) present on the apical side of the cell[125]. At the basolateral end, glucose is transported out of the enterocyte into the circulation via GLUT-2, a low-affinity high-capacity facilitative uniporter[125]. Apical accumulation of GLUT-2 has been reported in murine subjects with insulin resistance and obesity but this finding remains to be verified in *in vivo* human studies[125, 126].

SGLT1 is a symporter and transports 2 sodium ions with each molecule of glucose. SGLT1 expression is upregulated in response to a high glucose and high sodium diet, suggesting it has a function in nutrient sensing[127]. Unsurprisingly, in patients with diabetes, glucose uptake and SGLT1 expression were found to be increased[128]. However, SGLT1 upregulation was evident even in patients with T2DM on a carbohydrate-restricted diet[128]. This suggests that factors independent of the presence of luminal glucose were contributory to the increased SGLT1 expression[129].

SGLT1 expression also has a fascinating regional effect on incretin levels. Studies utilising SGLT1 inhibition have demonstrated the role of SGLT1 as a proximal small intestinal glucose-sensor, resulting in raised levels of both GLP-1 and GIP with proximal intestinal glucose uptake[129].

Nguyen and his colleagues revealed increased prominence of this regional variance in patients with obesity compared to lean controls[130]. Using a glucose absorption marker, patients with obesity were shown to exhibit enhanced proximal small intestinal SGLT1 expression and accelerated glucose absorption leading to a hyperinsulinaemia-hyperglycaemia incretin profile[130].

Conversely, as cited above, preferential delivery of glucose to the distal small intestine is associated with slower glucose absorption, lower plasma glucose excursions and raised GLP-1 levels in both patients with T2DM and healthy controls, when compared to glucose delivery to the duodenum[116].

Inhibition of SGLT1 decreases foregut glucose absorption and causes increased delivery of glucose more distally[131]. This results in a more sustained pattern of GLP-1 release from L cells in the small intestine[131, 132]. It has been proposed that bacterial fermentation of glucose to short-chain fatty acids, which are known to trigger GLP-1 secretion in animal studies, mediates this effect of SGLT1 inhibition[129].

Thus, from these studies, it can be extrapolated that procedures which isolate bile acids from proximal intraluminal glucose, such as RYGB, BPD and DJBL; may actuate some of the positive effects seen by exploiting this regional variance of glucose absorption. As bile provides the obligatory sodium ions needed for glucose co-transport in the GI tract, distalisation of nutrient contact with bile will result in slower glucose absorption, lower post-prandial glucose levels and a superior incretin effect[116, 129].

A possible explanation for the increased glucose absorption in patients with diabetes is from increased enterocyte proliferation and villi size[133]. Verdam and his team discovered evidence of increased enterocyte mass in patients with chronic hyperglycaemia compared to patients with normal HbA1c levels. To avoid the

confounding effect of weight differences, biopsy samples from both groups were taken from patients with obesity undergoing metabolic surgery[134]. Differences in bile acid composition, microbiota and nutrient-handling have been proposed as mechanism for the differences in enterocyte mass in patients with hyperglycaemia[133].

These findings suggest that therapeutic modalities that can attenuate enterocyte function in insulin resistant populations to emulate enterocyte function of lean individuals would be a very attractive strategy to correct glucose homeostasis.

1.1.2.3.5 Microbiome and bile acid in the small intestine

With more than a trillion cells per gram of faecal matter, unravelling the human gut microbiome is a colossal undertaking. The Human Microbiome Project, now in its twelfth year with microbiota from 300 subjects using metagenomic DNA sequencing, is starting to scratch the surface of this vast field[135].

The majority of gut bacteria belong to two phyla, *Bacteroidetes* and *Firmicutes*, and the number and diversity of bacteria in the gut increases distally throughout the small intestine[127]. In the small intestine, microbiota have a number of key functions including carbohydrate and lipid metabolism, bile acid circulation, regulation of epithelial permeability and immune system modulation[127].

The role of the intestinal microbiota in adiposity was first highlighted by Backed and his team in 2004 with the observation that germ-free mice were leaner than their counterparts from the same litter but became more obese when they received caecal bacteria from the latter despite no change in energy intake[136].

Since then multiple murine studies, including RYGB-related studies, have firmly established the causal role of dysbiosis and metabolic disease[123]. This link has been attributed to a number of factors including acting as a trigger of low-grade inflammation exacerbating insulin resistance, modification of bile acid and the production of active metabolites[137].

However, results from human studies have been less convincing. For example, Kootte *et al* found that transplantation of lean donor faecal microbiota to recipients with obesity and metabolic syndrome only resulted in modest, short-term improvements in insulin sensitivity[138].

Bile acid composition and cycling have been shown to be altered in patients with T2DM when compared to patients without diabetes[139]. Although the majority of data comes from animal studies, two bile acid signalling pathways in particular have been highlighted for its relevance to metabolic disease. These are the pathways involving the farnesoid X receptor (FXR) and the G-protein coupled receptor (TGR5)[140].

Activation of the FXR pathway results in increased secretion of fibroblast growth factor (FGF) 19 and 21[140]. These growth factors are associated with weight loss and improved insulin sensitivity and are now the focus of intense research for its potential pharmacotherapy role[140].

Further it is theorised that T2DM decreases gallbladder contraction, causing a diminished secretion of bile acid into the small intestine leading to a decreased activation of TGR5 in the L cells. This will result in a reduced GLP-1 induced incretin effect, lower insulin secretion and the resultant glucose dysmetabolism[141].

1.2 Polycystic Ovary Syndrome (PCOS)

Accounts of women with polycystic ovary syndrome-type symptoms date back to the 5th century B.C in the Hippocratic Corpus '*Diseases of Women*'[142]. However, it was only following Stein and Leventhal's seminal article *'Amenorrhea associated with polycystic disease'* in 1935 did the link to ovarian morphology emerge[143]. Alarmingly, more than half a century elapsed before PCOS was included by WHO in the International Classification of Diseases in 1992[144].

Today, PCOS is emphatically recognised to deleteriously contribute to significant health and socio-economic outcomes worldwide[145]. Currently it is estimated that PCOS affects up to 10% of pre-menopausal women – a similar worldwide prevalence as T2DM – making it the most prevalent endocrinopathy in women of reproductive potential worldwide[146, 147].

However, despite its substantial frequency and the documented antiquity of this disorder, the aetiology of PCOS remains elusive. Furthermore, the condition remains inadequately defined, understood and managed worldwide. This is clearly evident from (1) the multiple differing definitions associated with the condition (see Table 1.1), (2) the severe lack of licensed PCOS-specific pharmacological agents and (3) the relatively scant number of clinical trials studying women with PCOS, compared to conditions with a similar prevalence[148].

In fact, even its appellation is a misnomer as the 'polycystic' appearance alluded to is in fact caused by the accrual of follicles containing trapped oocytes, at different stages of maturation and atresia[149]. This inaccuracy focuses physicians' and patients' attention erroneously away from the pathophysiological basis of the disease and adds to the suboptimal diagnosis and management of women with PCOS[150].

1.2.1 Definition and Diagnosis

The main reason for the lack of a consistent classification of PCOS is due to its heterogeneity – in terms of pathophysiology, symptomatology and severity[148].

PCOS is a multifaceted and complex condition defined by a constellation of intertwined features associated with androgen excess, dysregulated menstrual cycles and a 'poly-follicular' ovarian morphology. In addition, PCOS has profound metabolic consequences including obesity, insulin resistance, glucose intolerance and T2DM. Recognition of the latter features only began to materialise in the 1980s[151, 152].

Criteria (year)	Definition
NIH (1990)	Hyperandrogenism & chronic anovulation
Rotterdam (2003)	Two of: hyperandrogenism, polycystic ovarian morphology or chronic anovulation
Androgen Excess Society (2006)	Hyperandrogenism and either polycystic ovarian morphology or oligo / anovulation
All criteria require the exclusion of other aetiologies of menstrual dysfunction (hyperprolactinaemia, thyroid disease, adrenal disease)	

Table 1.1 Diagnostic criteria for PCOS

(Source: Author's own)

To address this, in 1990, the National Institutes of Health (NIH) developed the first PCOS diagnostic criteria[153]. The defining criteria were selected by expert participants via a voting system at the National Institute of Child Health and Development conference in 1990[151]. The features with the highest votes were included in the definition. The eventual criteria required all three of the following features to be present for diagnosis[153].

1. Menstrual irregularity (anovulation > 35-day cycle)
2. Clinical or biochemical hyperandrogenism
3. Exclusion of other aetiologies menstrual dysfunction

However, it is worth noting that more than two thirds (69%) of the experts present voted for insulin resistance to be included as a diagnostic feature[153]. In fact, there were more votes for insulin resistance to be included as a defining feature of PCOS than even its titular polycystic ovarian morphology (54%)[153].

In 2003, following the rise of ovarian hyperstimulation syndrome in women with PCOS, the value of ovarian morphology as a diagnostic criterion surfaced and another conference on the diagnostic features of PCOS was held[151]. The outcome of this conference, held in Rotterdam, Netherlands for members of the European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine, was that ultrasonographic evidence of polycystic ovarian morphology was needed in the diagnostic criteria for PCOS[154]. Hence, the Rotterdam criteria determined that at least two of the following three criteria needed to be present for the diagnosis of PCOS, in the absence of other aetiologies of menstrual dysfunction[154].

1. Clinical or biochemical hyperandrogenism
2. Chronic anovulation
3. Polycystic ovarian morphology

In 2006, the Androgen Excess Society recommended that hyperandrogenism be considered an essential criterion for the diagnosis of PCOS alongside either chronic anovulation or a polycystic ovarian appearance[155] (see Table 1.1). This, like the NIIH criteria, highlights the central position of hyperandrogenism in the characterisation of PCOS.

Notably, none of the three definitions were based on a formal consensus process but rather on expert opinion, which is widely deigned the lowest form of evidence[155].

In 2012, in an attempt to achieve a single evidence-based consensus, the NIH sponsored an evidence-based methodology workshop for PCOS[156]. Dishearteningly, the final report, which recommended phenotyping patients with 4 subtypes according

to the Rotterdam criteria, was never published in a peer-reviewed journal, limiting its impact and clinical reach[151].

Consequently, all three definitions for PCOS are still valid and widely used – which has only added to the heterogeneity of the condition.

Of the three, the NIH criteria is the most restrictive as it only includes women with the hyperandrogenic-anovulatory phenotype while the Rotterdam criteria is the most inclusive and the most commonly used[148]. Pertinently, the NIH diagnostic criteria is also more consistently associated with metabolic dysfunction than either of the later criteria[151, 157]. In particular, women with ovulatory cycles, regardless of androgen status or ovarian morphology, were more likely to be leaner and have normal insulin sensitivity[158, 159]. Similarly, ovarian morphology *per se* did not correlate well with symptom severity, be that reproductive or metabolic[160, 161]. Equally, normo-androgenaemic women with PCOS are recognised as being metabolically disparate from patients with hyperandrogenic features[162].

Further, lean and obese women diagnosed with PCOS based on the NIH criteria were significantly more likely to have basal and post-prandial hyperinsulinaemia following an oral glucose load compared to age- and weight-matched women with non-PCOS ovulatory hyperandrogenism as well as non-androgenic women[158].

These findings suggest that the NIH criteria is the best criteria to use when identifying women affected by both the reproductive and metabolic consequence of PCOS.

1.2.2 Pathophysiology of insulin resistance in PCOS

Although dysmetabolic conditions, such as obesity, insulin resistance, NAFLD, T2DM and dyslipidaemia, do not form part of any PCOS diagnostic criteria; it is well-recognised as being an integral part of the PCOS phenotype; and the link between the reproductive and metabolic features of PCOS has been repeatedly demonstrated in multiple observational studies and clinical trials.

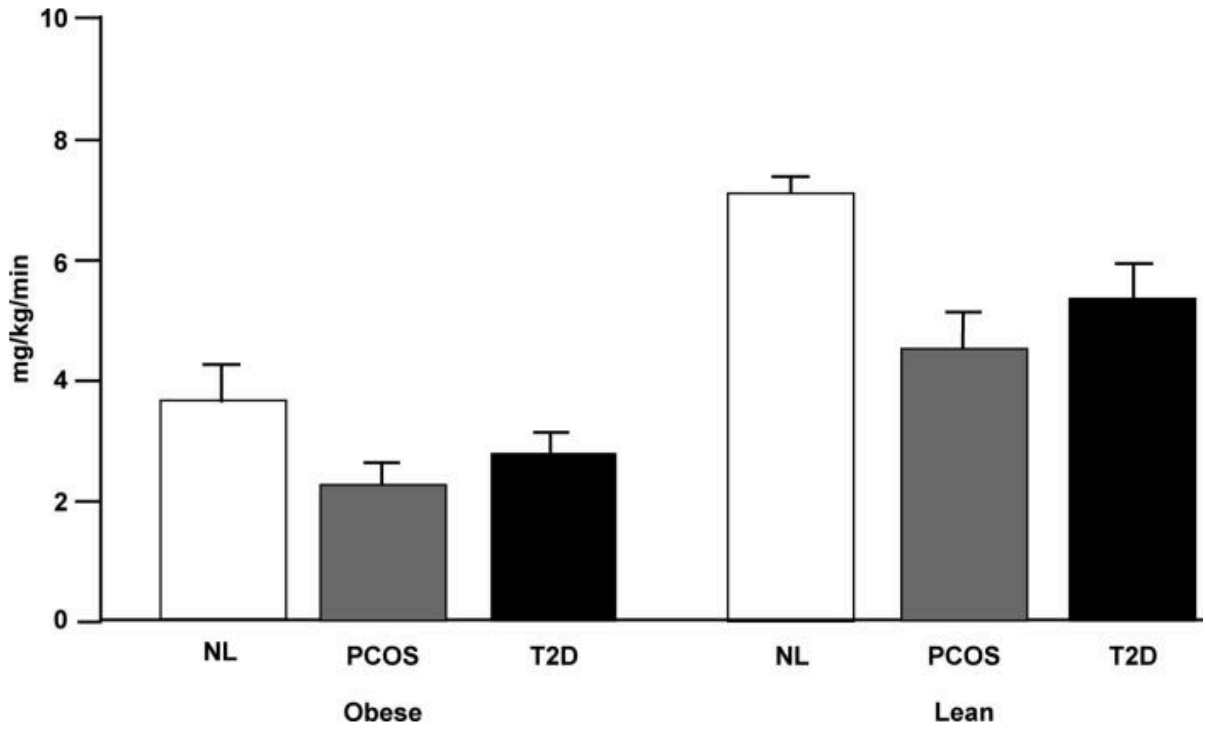

Figure 1.3 Decreased insulin-mediated glucose disposal in women with PCOS

Decreased insulin-mediated glucose disposal in women with PCOS. Insulin-mediated glucose disposal at steady-state insulin levels of 100 μU/ml is significantly decreased by 35 – 40% in women with PCOS (gray bars), independent of obesity, compared to age- and weight-matched control women (white bars) . This decrease is similar in magnitude to reported levels of insulin-mediated glucose disposal in patients with T2DM (black bars) (Source: Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovarian syndrome. Diabetes 1989; 38(9) :1165-74)[(163)]

This relationship was recently quantified in a robust systematic review and meta-analysis of clinical trials which used euglycaemic hyperinsulinaemic clamps, the gold-standard technique, for the assessment of insulin sensitivity. Cassar *et al* analysed data from 28 clinical trials comparing insulin sensitivity in women with PCOS to controls. Using mixed-effects analysis and magnitude-based inference, they found a significant (27%) reduction in insulin sensitivity in women with PCOS when compared to matched controls[(164)]. Further, they established that having a raised BMI in patients

with PCOS exacerbated insulin sensitivity by 15% and this effect was more apparent in women with PCOS than in controls[164].

One such study utilising euglycaemic hyperinsulinaemic clamp studies, carried out by Dunaif and her team, demonstrated that the decrease in insulin-mediated glucose disposal in women with PCOS was comparable in magnitude to previously reported levels in patients with T2DM (see Figure 1.3)[163]. This decrease in insulin sensitivity was present in both lean and obese women with PCOS, almost all (~95%) of whom had normal glucose tolerance[163].

Further, to isolate the effect of obesity on insulin resistance in these women, insulin-mediated glucose disposal was calculated with total weight and fat free mass (FFM)[163]. Dunaif and her team demonstrated that although obesity and PCOS worked synergistically to decrease glucose tolerance; PCOS itself conferred a significant (35%) reduction in insulin sensitivity in both lean and obese women with PCOS compared to age- and weight- matched control subjects[163]. This was also independent of body composition, glucose tolerance and insulin clearance rate[163]. Subsequent studies have confirmed these findings of profound resistance in glucose uptake in women with PCOS[151, 164, 165].

In addition, although obesity is more common in women with PCOS, the distribution of fat in these women is indistinguishable from BMI-matched insulin-resistant women without PCOS[166].This suggests that PCOS instigates a pathophysiological disorder of insulin action causing insulin resistance, independently of obesity[163, 167]. However, the adipose tissue in women with PCOS has a different adipocytokine profile from women without PCOS and the significance of this remains to be elucidated[166]. In addition, obesity exacerbates insulin resistance and hyperinsulinaemia, precipitating a more severe clinical portrait of PCOS[168]. What is clear is that the relationship between PCOS and obesity is complex and still obscure.

It is estimated that around two-thirds of all women with a diagnosis of PCOS will have a degree of insulin resistance and this prevalence increases to 80% in women with obesity and PCOS[169, 170]. Clinical features that indicate a degree of underlying insulin

resistance in women with PCOS include a raised BMI, a waist-to-hip ratio of >0.85, acanthosis nigricans, glucose intolerance, raised triglyceride level, low high-density lipoprotein (HDL) level and reduced sex-hormone binding globulin (SHBG)[167].

The mechanism by which insulin resistance develops in women with PCOS remains contentious. Many studies have shown increased levels of FFA in women with PCOS which, as detailed above, is a notorious instigator of peripheral and hepatic insulin resistance[71, 168]. Further, studies of women with PCOS have identified lower levels of GLUT4, increased lipolysis and decreased rate of glucose uptake when compared to matched controls[168]. These findings have also been evident in studies of lean subjects with normal glucose tolerance suggesting intrinsic PCOS specific defects in insulin signalling[168].

Dunaif and her colleagues carried out a euglycaemic clamp study with serial skeletal muscle biopsies in women with PCOS and age-, weight- and ethnicity-matched controls and found that although insulin receptor numbers were unaltered in the skeletal muscle biopsies, women with PCOS demonstrate evidence of a post-binding defect in insulin signalling resulting in a decrease in glucose transport[165]. This decrease in insulin receptor activity has been linked to PCOS-specific exaggerated serine phosphorylation of the β-subunit of the insulin receptor as seen in studies examining fibroblasts and skeletal muscle in women with PCOS[168, 171].

While the severity of insulin resistance in women with PCOS varies, women with PCOS who have insulin resistance and sub-optimally compensatory hyperinsulinaemia will have a degree of β-cell dysfunction, and hence will be at higher risk of developing metabolic sequelae, such as IGT and T2DM[172]. A systematic review of 35 studies suggests that the prevalence of T2DM was 4.5 times higher in women with PCOS compared to matched controls[173].

Studies in young and middle-aged women with PCOS with a prospective design revealed a prevalence of IGT in a third of the participants and 10% prevalence of T2DM[174, 175]. Similarly, a large cohort study of 101,073 women aged between 18 and 22 years who were followed up for 8 years, identified approximately a two-fold

increased incidence of T2DM in women below the age of 30 years with oligomenorrhoea compared to those with normal-length menstrual cycles, indicating an increased prevalence and earlier onset of T2DM in women with PCOS[167, 176].

Intriguingly, insulin resistance in women with PCOS has more than just a metabolic impact. For instance, improvements in insulin resistance surrogates, such as body weight, leads to improvements in both metabolic and reproductive function[177]. Conversely, weight gain and worsening insulin resistance in women predisposed to PCOS, have been shown to have the opposite effect[177]. This suggests that the metabolic and reproductive effects of PCOS are intricately linked.

This interdependent effect is secondary to the compensatory hyperinsulinaemia that is typical in women with PCOS and insulin resistance[178]. Paradoxically, although women with PCOS are frequently resistant to the metabolic actions of insulin, hyperinsulinaemia acts as a potent driver of pro-androgenic changes and reproductive dysfunction (as detailed below).

This apparent contradiction in tissue resistance to the action of insulin is related to its intracellular effects which are carried out via two distinct pathways[178] (as detailed in Section 1.1.2.2) (1) phosphatidylinositol-3-kinase (PI3K)/Akt pathway – which mediates the metabolic actions of insulin – defects in this pathway are largely liable for the development of the classical metabolic picture of insulin resistance and glucose intolerance[43, 44]; and (2) mitogen-activated protein kinases/Ras pathway (MAPK/Ras) which promotes gene expression, cell growth and steroidogenesis[43, 45, 178]. Signalling within this pathway remains unimpeded in most insulin resistant conditions[46].

The discrepancy in intra-cellular signalling leads to diminution in the metabolic effects of insulin while concurrently augmenting the effects of steroidogenesis[178]. Further, in patients with PCOS, the MAPK/Ras pathway appears autonomous of the usual feedback mechanisms; promoting the development of hyperandrogenism and reproductive dysfunction – features which are typically absent in other insulin resistant states.

Enhancement of this pathway, and the resultant hyperandrogenaemia, is believed to also lead to worsening of the dysmetabolic state secondary to accumulation of visceral and abdominal adiposity which promotes hepatic insulin resistance and glucose intolerance[148]. Further, genomic and proteomic studies have also identified masculinisation of VAT in women with PCOS, resembling VAT in men than that in women without PCOS[179, 180].

However, the assumption that visceral fat promotes insulin resistance in women with PCOS has come under scrutiny following studies of women with PCOS and controls using MRI assessment of body fat distribution in BMI-matched pairs[181, 182]. These studies demonstrate that despite a significant difference in insulin resistance, there was no significant difference in the measurement of visceral and subcutaneous fat depots in these women[181, 182]. This finding has also been seen in lean women with PCOS[183]. This suggests that while visceral adiposity may promote insulin resistance, it is not the major cause of augmented insulin resistance in PCOS.

On the opposite end of this *causal nexus*, compensatory hyperinsulinism is recognised as the principal driver of androgenic changes via its actions (1) as a co-gonadotrophin on the ovaries, (2) in facilitation of androgen release from the adrenal cortices, (3) in the modulation of luteinizing hormone (LH) pulsatility in the anterior pituitary gland and (4) diminution of SHBG production in the liver[148].

Androgens, principally testosterone and androstenedione; are steroid hormones produced from a cholesterol precursor following a few key enzymatic steps[184, 185]. In women, androgens are produced primarily by the ovaries and the adrenal cortex.

In the ovary androgens are produced, in response to stimulation by LH, in the thecal cell layer as these cells express the cytochrome P450c 17 gene which regulates dehydroepiandrosterone and androstenedione production[185, 186]. The bulk of these precursors are converted to oestrogens. The remainder are converted to androgens, while some ovarian androgens are produced directly from a progesterone precursor[185, 186]. Unlike in men, ovarian androgens do not send negative feedback signals to decrease LH stimulation, regardless of free androgen levels[168].

The adrenal gland is the other major producer of androgens in women. Adrenal androgens are produced from the inner layers of the adrenal cortex in response to adrenocorticotropin hormone (ACTH) stimulation. ACTH is also the primary stimulus for the production of the major steroid adrenal hormones such as cortisol and aldosterone, and is closely regulated by cortisol production. As such, free androgen levels, in men and women, do not have a significant feedback impact on ACTH production[187].

Thus , in women, androgen production by the ovary and adrenal gland is not regulated by levels of circulating free androgen[168].

Hyperandrogenism – often presenting as hirsutism, acne, deepening of voice and androgenic alopecia – is present in 65 - 75% of women with PCOS and is increasingly being identified as the core functional abnormality of PCOS[172, 188].

Apart from the metabolic and cutaneous effects listed above, hyperandrogenism also results in the development of increased numbers of pre-antral and small antral follicles with arrested maturation causing reduced fertility[189]. These aberrant follicles and ovarian granulosa cells in women with PCOS cause accumulation of anti-Müllerian hormone (AMH) which inhibits pituitary release of follicle-stimulating hormone (FSH) and ovarian aromatase expression[190]. This prevents the selection of a dominant follicle and the subsequent oocyte release which leads to anovulation[190].

On top of this, hyperandrogenaemia in women with PCOS also provokes upregulation of the frequency and amplitude of the pulsed release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus; and LH from the anterior pituitary gland which further augments AMH release and its effects[189, 190]. The increased LH and inhibited FSH production produces the typical raised LH to FSH ratio seen in women, particularly lean women, with PCOS[191]. Further, the hypothalamo-pituitary axis appears resistant to the suppressive effects of progesterone on GnRH pulse frequency[172].

This heralds that more than ovarian dysfunction is culpable for the pathophysiology of the reproductive changes seen in PCOS; and instead implicates the entire hypothalamic-pituitary-ovarian axis[189].

Further, in women with PCOS, the ovaries provide almost two-thirds of the androgens present while the adrenals generate the rest[168]. Androgen overproduction from both ovaries and the adrenal cortex contribute to the hyperandrogenic picture[192]. This has been established in multiple studies using suppression of androgen production form one or the other organ[193, 194]. About a third of women with PCOS have raised levels of dehydroepiandrosterone sulphate (DHEAS), a metabolite of dehydroepiandrosterone chiefly produced in the adrenal cortex.

A further source of excess active androgen in women with PCOS is from decreased levels of SHBG[195]. SHBG is a glycoprotein produced in the liver which regulates the bioavailability of sex hormones such as testosterone and oestradiol. Decreased levels of SHBG in women with PCOS contributes to raised levels of free testosterone and hyperandrogenism[196]. Interestingly, high circulating levels of FFA have also been shown to increase androgen levels in women, perhaps via serine phosphorylation of the P450c 17 gene with resultant increase in downstream enzymatic activity[168].

Insulin has a key role in ovarian androgen production. Nestler and colleagues used cultured ovarian tissue from women with PCOS and controls to demonstrate testosterone production from ovarian thecal cells of women with PCOS at physiological doses of insulin[197] In thecal cell cultures from women without PCOS, this effect is only seen at supra-physiological insulin doses which suggests increased ovarian sensitivity to insulin in women with PCOS[168]. Similarly, insulin also promotes adrenal androgenesis by increasing basal and ACTH-stimulated production of androgens from cultured human adrenal cells[168, 198].

This was also evident from a study using metformin, to reduce insulin concentration, versus placebo in women with PCOS and obesity, which found a reduction in basal and LH-stimulated cytochrome P450c 17 activity, basal LH levels, free testosterone levels and an increase in SHBG levels[199]. Similar results were also seen in a study

using diazoxide in women with PCOS and obesity, which demonstrated a reduction of testosterone and SHBG levels without change in gonadotrophin levels[200].

This effect is similarly evident in lean women with PCOS who are normo-insulinaemic and insulin sensitive. Lowering of insulin with diazoxide in these women resulted in significant reduction of free testosterone and increased levels of SHBG, without change in gonadotrophin levels[201]. The same effect was not seen in lean women without PCOS when given diazoxide[202]. This suggests that women with PCOS have an intrinsic susceptibility to the androgenic effects of insulin, irrespective to body habitus and insulin resistance. Further, it is likely that lean women with the hyperandrogenic phenotype of PCOS have augmented ovarian sensitivity to insulin, resulting in the classical androgenic symptoms even at physiological insulin levels.

Further insulin has been shown in studies using diazoxide to have a direct suppressant effect on SHBG production by the liver, resulting in increased levels of free testosterone and hyperandrogenism[195]. A analogous effect on SHBG levels is not seen in women without PCOS when given diazoxide[202].

As neither hyperandrogenaemia nor insulin resistance are universally present in women with PCOS, it is difficult to ascertain which feature is the instigator. There are proponents for each in the literature[155, 167]. Further, some experts have concluded that it is likely that a circularity exists, propagating this incessant vicious cycle[148]. The author of this thesis is of the opinion that insulin resistance and ovarian hypersensitivity to insulin is more likely the crucial element for reasons stated below, although a cyclical effect may propagate the consequences (see Figure 1.4).

Firstly, men naturally have high levels of androgens but do not physiologically have hyperinsulinaemia or insulin resistance. Further, studies using LH suppressants have demonstrated that chronic LH stimulation is not primarily responsible for ovarian hyperandrogenaemia[168]. Patients with PCOS who have undergone oophorectomies or been on long-term GnRH agonists treatment remain insulin resistant despite normo- / hypoandrogenaemia[167, 203]. Conversely, treatments aimed at improving insulin resistance, such as weight loss, metformin, inositol, peroxisome proliferator-activated

receptor gamma (PPARγ) agonists; have consistently been shown to reduce androgen levels and hyperandrogenism suggesting a causal role for insulin sensitisation in the pathogenesis of PCOS[168, 204, 205].

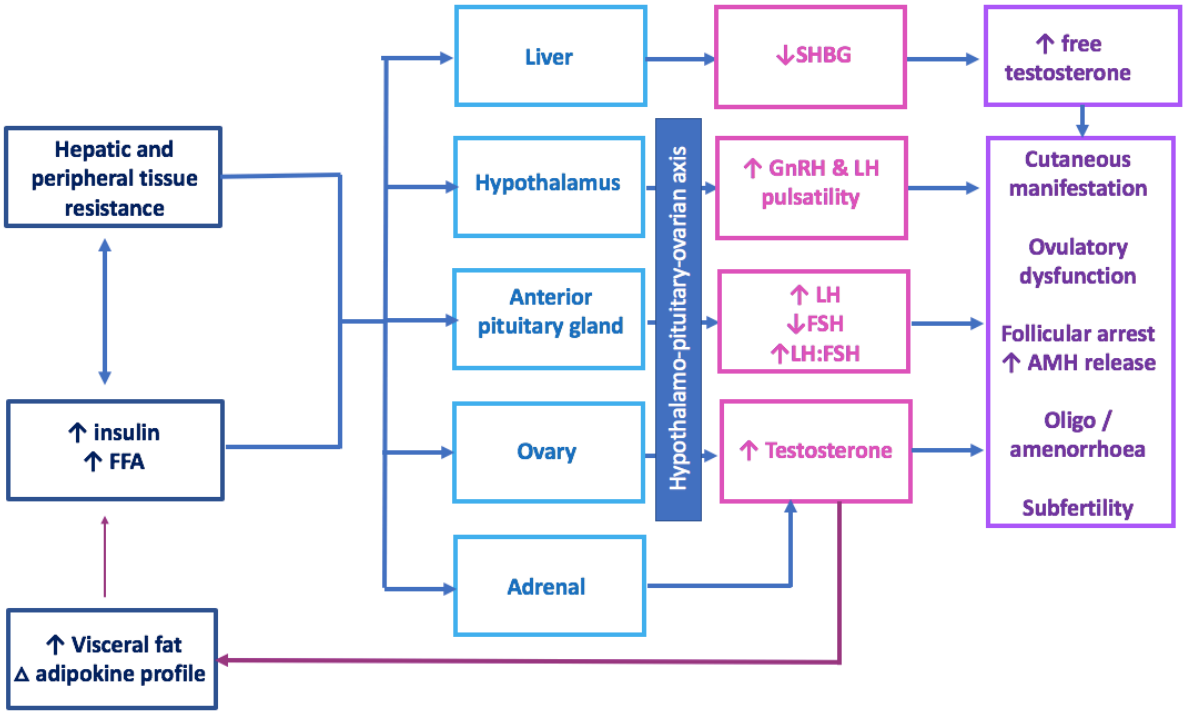

Figure 1.4 Schematic diagram of relationship between metabolic and reproductive features in PCOS

(SHBG Sex Hormone-Binding Globulin, GnRH gonadotrophin-releasing hormone, LH luteinizing hormone, FSH follicle-stimulating hormone, AMH Anti-Müllerian Hormone Source: Author's own)

Interestingly, the effect on insulin levels have not been as consistent. Studies using metformin in women with PCOS have demonstrated decreased insulin level as well as improved androgenaemia[206]. However, conflictingly, insulin sensitisers such as PPARγ agonists improve insulin sensitivity and androgen levels in women with PCOS but have not been shown to affect insulin levels[206].

Further evidence for this comes from an interesting study where hyperinsulinaemia was artificially induced in women with insulin-resistant PCOS and controls. In women with PCOS, artificial hyperinsulinaemia resulted in increased level of androstenedione

but decreased levels of pooled plasma testosterone, while there was no change in the level of either androgen in participants without PCOS[207]. Correspondingly, diazoxide studies in women with PCOS have shown decrease in testosterone levels but not androstenedione level[s(200)].

This suggests that the relationship between hyperinsulinaemia and hyperandrogenaemia is not a simple, direct one and that there is much more to discover about this liaison[207]. However, increased ovarian sensitivity to insulin seems to be crucial to the pathophysiology.

Further, as all insulin-sensitising treatments in use at present acts to improve adipocyte insulin sensitivity and decrease FFA levels as well as androgenaemia, it would be useful to explore if FFA is the common mechanism linking insulin resistance and androgen hypersecretion as increased FFA levels deleteriously affects both reproductive and metabolic manifestation of PCOS.

1.3 Obesity

Obesity is a chronic progressive multifactorial disease that results in excessive and widespread deposition of adipose tissue. The resultant pathological adiposity has the propensity to adversely affect nearly all physiological function; and people with obesity are at greater risk of developing a host of different co-morbid conditions, having a poor quality of life and dying a premature death.

The exact aetiology of obesity is complex and yet to be eluded but it is clear that it does have genetic, epigenetic, biological, environmental, behavioural and sociocultural determinants.

The global prevalence of obesity has steadily increased. Currently, at least a third of the world's population are either overweight or obese with obesity directly contributing to poor health and socioeconomic outcomes in most countries[208, 209]. There has consistently been a greater propensity of women with obesity and this gender disparity is evident globally[208].

However, promisingly, in many developed countries, including the UK, a slight deceleration in the prevalence of obesity has been evident in the past decade[208]. Unfortunately, this has not been the case in the rest of the world, where most of the global population resides; and obesity continues to attain pandemic status[208].

1.3.1 The mechanisms causing obesity

In recent years the set-point theory has received significant support by clinicians and scientists as an underlying pathophysiological basis of obesity.

The set-point theory is based on the knowledge that under constant environmental conditions, the degree of adiposity and energy balance in adults stays remarkably stable within a narrow margin and is refractory to short-term perturbations[210]. This is theorised to be secondary to innate homeostatic feedback mechanisms that autoregulate energy intake and expenditure to defend a pre-determined fat mass[210].

This has been shown to be evident regardless of weight and adiposity, including in subjects with obesity[211].

A powerful example of this was illustrated in a study using SGLT-2 inhibitors which results in insensate glycosuric loss of calories[212]. Despite ongoing caloric losses, participants did not continue to lose weight as might be expected[212]. Instead it was observed, after an initial loss of ~3kg, participants' weights quickly stabilised via an adaptive and balanced increase in energy intake[212].

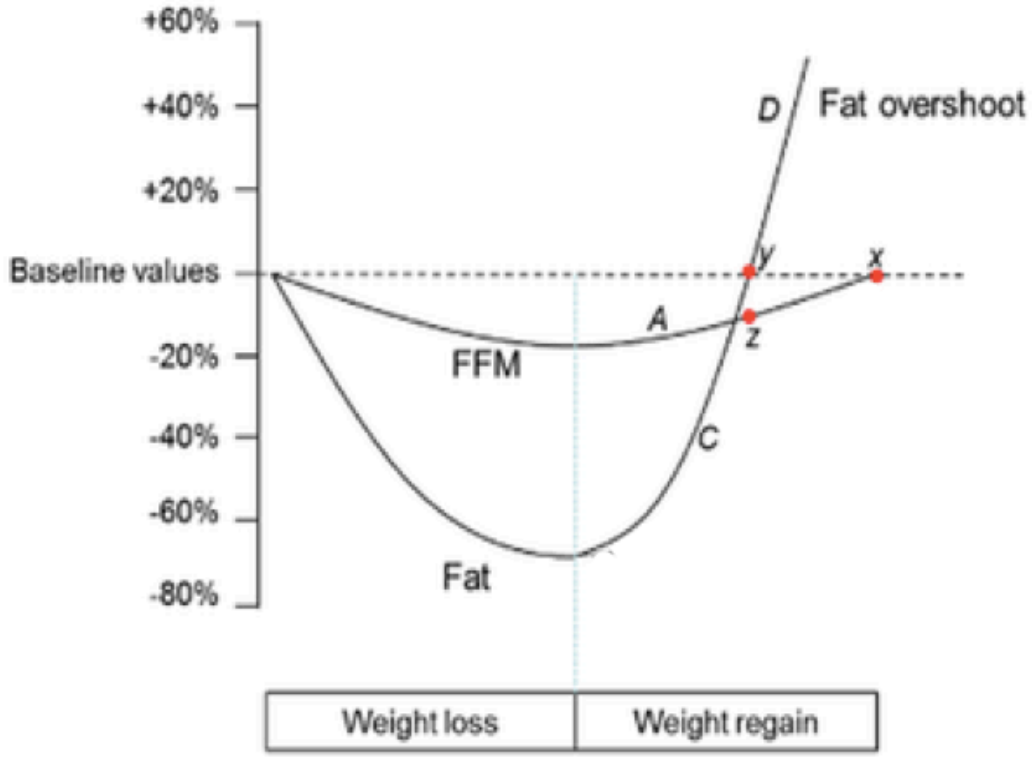

Figure 1.5 Schematic diagram showing dynamics of fat mass and FFM during one cycle of weight loss and weight regain in lean humans.

(Adapted from: Dulloo AG, Jacquet J, Montani JP, Schutz Y. How dieting makes the lean fatter: from a perspective of body composition autoregulation through adipostats and proteinstats awaiting discovery. Obes Rev. 2015;16 Suppl 1:25-35.)[213]

Further, this provides an explanation for why weight loss through short-term diets or lifestyle changes tends to be regained over time and in fact, is often seen to cause an overshoot of weight gain[213]. Dulloo *et al* examined data from multiple prospectively conducted studies and demonstrated that, following short term weight loss, adaptive processes are employed (e.g. suppression of thermogenesis, hyperphagia) to regain the loss fat and muscle mass[213]. These processes persist until both fat mass and FFM are completely regained and usually results in overshoot of fat mass and weight gain, as FFM takes longer to recover (see Figure 1.5)[213]. Even in normal weight individuals, repeated cycling of this process, as seen in with yo-yo dieting, could result in a cumulative weight gain and obesity, despite concerted attempts at weight loss[213].

This theory is critical to recognise for 2 important reasons. First, it highlights obesity as a disease of abnormal energy balance with involuntary physiological autoregulation processes involved in its persistence; and second, it effectively shifts the focus (and ideally the stigma) around culpability away from a person-centred discussion to a biological one.

However, it is also vital to recognise that these mechanisms can be overwhelmed by powerful and persistent external stimuli, including surgical manipulation, significantly altered energy intake and physical activity or lack thereof[214]. Unfortunately, the vast majority of external stimuli in our present climate promotes the deposition of adiposity, promoting in the ever-increasing prevalence of obesity worldwide.

Another interesting point this theory suggests is the pre-determined nature of fat mas and correspondingly, adult weight. This suggests that genetics or epigenetics plays a crucial role in weight attainment.

The role of genetics in obesity has long been apparent from countless familial, twin and adoption studies[215, 216]. However, the reproducible heritability values computed from these studies ranged from 25 to 90%%[215, 216]. Further, the rarity of single-gene and syndromic forms of obesity (e.g. leptin deficiency, MC4R deficiency, Prader-Willi Syndrome) and the wide spectrum of weight differences seen in many populations, point to the highly polygenic nature of heritability in obesity.

Khera *et al* recently attempted to investigate this using data from genome-wide association studies to devise a polygenic prediction score developed from 2.1 million common genetic variants[217]. They validated this score using long-term information from more than 300,000 individuals. [217] His team found that individuals with polygenic scores in the highest deciles were at greatest risk of developing obesity and metabolic disease, confirming the polygenic nature of heritability in obesity[217]. However, not all individuals with high polygenic predictive scores developed obesity advocating incomplete genetic penetrance in obesity and the influence of non-genetic factors in weight attainment[217].

Further, although the score found little association with birthweight, a gradient in weight emerged early in childhood and became more prominent in subsequent decades[217]. When comparing individuals in the top decile to the bottom decile, a difference of 3.5kg was seen at age 8 which steadily increased to 12-13kg by the time these individuals reached adulthood[217]. This provides strong evidence for the genetic predictability of adult weight and metabolic health from early childhood[217].

Although this certainly provides compelling evidence the role of genetics in individual susceptibility to developing obesity, the human genome is unlikely to have evolved significantly in the past half a century and this does not explain the pandemic that has arisen in this short time.

Epigenetics, the study of non-genetic influences on gene expression, has provided some logical solutions for this phenomenon[218]. A plethora of factors including the influence of diet, the environment and gut microbiota can influence genetic programming and susceptible gene expression[218]. Epigenetic studies suggest that insults, such as maternal metabolic hardships (e.g. malnutrition, obesity, diabetes) endured during the offspring's embryo-foetal and during perinatal period of development can have significant impact on metabolic and weight regulation in subsequent life[218, 219]. At the molecular level, epigenetic modifications include DNA methylation, histone modifications, chromatin remodelling and non-coding RNA alterations[218, 219]. Crucially, these modifications, which programme for increased risk

of obesity during the carrier's lifetime, can also be transmitted to future generations, accelerating the obesity pandemic[214].

This gene-environmental relationship is influenced by number of key environmental conditions that has changed significantly over the past few decades and has resulted in a propensity to obesity and metabolic disease. Paradoxically, these changes start with the provision of better healthcare and improved safety and hygiene practices leading to lower infection rates and longevity[220]. Alongside this, there has been increased per capita food availability and accordingly increased food consumption[221]. Food composition has also changed considerably and there are more easily accessible high-calorie, high-palatability food options often served in large portions and often available at low prices[222]. Urbanisation and industrialisation leading to widening wealth discrepancy and the rising prices of more nutritious food options has also acted as socioeconomic drivers of obesity[223].

In addition, occupational and leisure-time physical exertion has been drastically displaced by automation and sedentary behaviour, limiting energy expenditure[224]. Further, all these changes have also altered human behaviour in other ways, resulting in unprecedented reports of stress and anxiety disorders as well as sleep deprivation, all of which are obesogenic.

Chronically elevated levels of psychological stress can lead to a variety of physiological responses including decreased basal energy expenditure, increased fat storage, increased ghrelin release and increased release of cortisol which promotes visceral fat deposition and compulsive eating tendencies of high-fat and high-sugar foods (i.e. comfort eating)[225, 226]. Conversely, having adequate sleep and rest promotes healthy weight and glycaemic modulation as sleep is a vital modulator of neuroendocrine function[227]. Sleep deprivation has been consistently reported to result in dysglycaemia, insulin resistance, lower leptin levels and hyperphagia[227].

As eluded to at the start of this section, these genetic and environmental stimuli can influence long-term energy balance, weight gain and obesity. Energy balance is regulated by complex systems involving central neural circuits and peripheral signals

of satiety and energy stores together with higher cortical function[228]. The latter, which regulates emotional and hedonistic reward-based eating, is often influenced by environmental – visual, olfactory and gustatory – stimuli of food which can supersede homeostatic satiety signals and stimulate non-physiological consumption of highly palatable, frequently sweet and salty, food[229].

Physiologically, the key areas of long-term energy regulation are the adipose tissue and gut peripherally, the hypothalamic nuclei centrally as well as the integration of signals from these areas.

Over the past decade, the role of adipose tissue as a metabolically dynamic endocrine organ has been discovered and its function is no longer merely as primary site of excess energy storage[230]. It is now recognised as the key organ for release of metabolically active adipokines and has important functions in glucose and lipid metabolism, body weight homeostasis, immunity and a host of other physiological processes[230]. Further, it contains many different cell types such as adipocytes, endothelial cells and immune-mediator cells[231].

During periods of excess energy balance, adipose tissue deposits triglycerides in lipid droplets by promoting hypertrophy of adipocytes[231]. The number of adipocytes in adulthood remains fairly constant regardless of fat mass or weight loss, however, overfeeding has been shown to increase fat deposition in the lower body via hyperplasia[231]. Further, this state of constant positive energy balance together with insulin resistance also promotes fat deposition on classically lean tissue such as the liver, cardiac muscle, skeletal muscle and the pancreas.

Expansion of visceral adiposity, but not subcutaneous adiposity, is associated with significant dysmetabolic sequelae even after correcting for BMI and subcutaneous adipose tissue volume[231]. VAT has been associated with larger very low-density lipoprotein particles, smaller HDL particles, higher lipolytic rates with FFA delivered directly to the liver via the portal vein, increased pro-inflammatory profile, and higher association with T2DM, NAFLD and cardiovascular disease[231]. In fact increased abdominal adiposity, evident as increased abdominal girth, is one of the five defining

features of metabolic syndrome together with raised triglyceride levels, decreased HDL levels, hypertension and glucose intolerance[232].

Another major effect of increased adiposity is the release of multiple adipokines.

Chief among the cytokines released by adipocytes is leptin. Leptin, as discussed in a previous section, is a satiety hormone secreted by adipocytes that plays a major role in the central regulation of energy stores[87]. Leptin acts on the hypothalamus as a negative feedback regulator of adiposity, suppressing appetite, limiting energy intake and promoting energy expenditure to counteract the presence of excess fat stores[233]. The vital role that leptin has in energy regulation is clearly evident from the hyperphagia and severe childhood-onset obesity consistently seen in individuals with the rare syndrome of congenital leptin deficiency[234].

This led to the idea that obesity may be related to leptin deficiency. However, instead of low levels of plasma leptin, obese individuals have raised levels of leptin as plasma concentration of leptin parallels adipose tissue mass and triglyceride content[87]. Hence, people with more adiposity secrete more leptin, and higher levels of leptins are found in the cerebrospinal fluid of people with obesity compared to leaner individuals. However, the ratio of plasma leptins to cerebrospinal concentrations is at least five times lower in individuals with obesity compared to their leaner counterparts and its effect on appetite suppression and energy balance appears blunted in individuals with supranormal adiposity. This implies that, first, there is a saturable mechanism mediating cerebrospinal leptin transport to the brain and second, an apparent resistance to its action as suggested by the lack of a dose-response relationship[235]. This precludes the use of exogenous leptin as an anti-obesity agent.

Another key adipocytokine secreted by adipose tissue is adiponectin. The atheroprotective adiponectin also has a number of other favourable actions including regulation of food intake and energy homeostasis via its centrally-mediated action; enhancement of insulin sensitivity by suppression of hepatic gluconeogenesis and increase in skeletal muscle glucose uptake; suppression of inflammation and inhibition of cell death[236].

However, while the release of most adipokines increases with enlarging fat mass, adiponectin concentration tends to diminish with enlarging visceral fat deposition[237]. It is unclear if the decrease in adiponectin levels is a primary event or secondary to the release of VAT-derived factors that may inhibit its synthesis or suppression, such as IL-6 and TNF-α[237]. Regardless, diminished adiponectin and increased VAT leads to a dire combination of insulin resistance, β-cell dysfunction and abdominal obesity with all its attendant risks[237].

While both leptin and adiponectin have favourable, although surmountable, effects on energy balance and metabolic function, many adipokines released by adipose tissue have detrimental effects. As adipocyte mass increases in adipose tissue, so do the number of immune-mediator cells such as macrophages[231]. This enhances the release of various pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-8 which promotes obesity-induced inflammation, augments insulin resistance, increases lipolysis, promotes low-grade inflammation and increase HGP[72]. These inflammatory processes may also act to increase the deposition of visceral adiposity and propagating this vicious cycle[238].

Centrally, energy balance is regulated by neural or hormonal signals derived from the gut or adipose tissue[229, 239]. These signals either act directly on the hypothalamus or exert their effect via the vagus nerve[229, 239]. In the hypothalamus, the arcuate nucleus plays a crucial role in energy homeostasis. It contains two groups of neurons with opposing functions. First, laterally located anorexigenic neurones express POMC and cocaine-and-amphetamine-regulated transcript, which inhibits eating[229, 239]. Conversely, orexigenic neurons, which express appetite-stimulating chemicals such as neuropeptide Y and agouti-related peptide, are located medially in the hypothalamus[229, 239]. Further, the activity of the arcuate nucleus can be influenced directly by circulating hormones that cross the blood-brain barrier such as insulin and leptin[229, 239].

Vagal afferents from the GI tract, activated by mechano- and chemosensors, converge in the nucleus of the solitary tract which then transmits these signals to the hypothalamus[229, 239]. Gut hormones, such as CCK, GLP-1, oxyntomodulin and PYY

can also regulate food intake by inhibiting hypothalamic orexigenic signalling and inhibiting feedback mechanisms involved in intestinal transit[229, 239]. Conversely, ghrelin, a peptide hormone released in the stomach, acts centrally in the medial hypothalamus to promote feelings of hunger and induce eating[229]. Further, as previously discussed, the gut microbiome also has a key role to play in obesity although the exact mechanisms underlying this has to be elucidated[240]. Evidence does suggest that a lack of microbial diversity is adversely associated with obesity[240].

In contemporary society, obesity is often portrayed as a disease of choice and that people who are obese choose to eat more than they should and exercise less than required. However, current evidence strongly suggests that increased energy intake and sedentary behaviour may not simply be a consequence of individual choice; but is in fact regulated by strong hormonal and hedonic pathways[241]. Mitochondrial dysfunction, oxidative stress and lower ATP production have been reported in the muscle, liver and brain of individuals with obesity suggesting increased fatiguability, lower oxygen consumption and decreased exercise capacity than leaner subjects[241, 242]. Lower hepatic ATP concentration is also associated with stimulation of hunger, further increasing energy intake[241].

Additionally, neural imaging in subjects with obesity have highlighted a downregulation of dopamine receptors suggesting an addictive response to food in some individuals[243].

Finally, certain medical conditions and pharmacological agents can also predispose individuals to obesity. Examples of the former include PCOS, Cushing's syndrome and hypothyroidism while culprit drugs include steroid hormones, anti-depressants, insulin, antipsychotics.

All this strongly suggests that, while culture and advertising certainly have a role, the core mechanism underlying obesity is likely rooted in biology.

1.3.2 Evaluation of obesity

Recognising the significant mortality and morbidity associated with obesity as well as the widespread prevalence of the disease, it is vital that all healthcare professionals are proficient in the recognition, assessment and management of obesity and its associated conditions. This is particularly in light of results from the ACTION (Awareness, Care and Treatment In Obesity maNagement) study which highlighted that of 3008 people with obesity surveyed, only 55% had ever received a formal diagnosis of obesity by their healthcare provider and less than a quarter had a follow-up management plan by their clinician[244]. Without a diagnosis and a proper assessment, people with obesity are unlikely to receive accurate and appropriate management and care for their health. Common assessment tools used in the evaluation of people with or at risk of obesity include the following.

(1) Body Mass Index

The most common means of assessing for obesity is by calculating an individual's BMI. While not a true measure of adiposity, BMI provides a practical, reproducible and inexpensive first step in enumerating adiposity. In addition, it provides a better correlation with fat mass than body weight alone, although this is age- and gender-dependent[245].

Further, in the Global BMI Mortality Collaboration Study, a mammoth meta-analysis of 10 million participants from 4 different continents, the relationship of a BMI above 25kg/m^2 or higher was consistently associated with increasing all-cause mortality in a log-linear relationship[246]. The hazard ratio per 5 units of BMI was between 1.3 – 1.4 in all the different continents[246].

However, BMI measures are not unflawed and it may underestimate the risk in individuals who are not overweight that may have high body fat content (e.g. South and East Asians ethnicity, elderly with loss of muscle mass) and conversely, overestimate the risk for people with high muscle mass (e.g. athletes, bodybuilders)[247, 248]. Also, a BMI measurement does not provide

quantification of the different types of fat (i.e. visceral *vs* subcutaneous) which is a better marker for metabolic disease and cardiovascular risk. For this reason, BMI measures should be used in conjunction with other methods of assessing obesity.

(2) Anthropometric Measures

Commonly used and validated anthropometric measures in the assessment of obesity include waist circumference and waist-to-hip ratio. Akin to BMI, these measures are again simple, reproducible and inexpensive to perform. However, in contrast, these measure provide a better gauge of abdominal adiposity than BMI and are a particularly useful adjunct in patients with a BMI of 25 to 35 kg/m^2[249].

Regardless of BMI, patients with evidence of significant abdominal adiposity are at increased risk of developing multiple cardiometabolic diseases including cardiac disease, T2DM and dyslipidaemia, and also have a higher overall mortality rate[250, 251]. Hence, anthropometric measures such as waist circumference are now included in all major obesity assessment guidelines[249, 252, 253].

A waist circumference exceeding 102cm in men and 88cm in women, even in an individual with a normal BMI, is considered raised and is an indicator of cardiometabolic risk[251]. A meta-analysis of more than 250,000 individuals illustrated the superiority of waist circumference as a predictor of T2DM compared to BMI[251].

Similarly, the WHO state that a waist-to-hip ratio exceeding 1.0 in men or 0.8 in women are at increased risk because of their fat distribution[254]. However, recent studies suggest that waist-to-hip ratios do not provide any added advantage over waist circumference alone and is no longer recommended in the American Heart Association / American College of Cardiology / The Obesity Society guideline for obesity assessment[252].

Interestingly, a study from the SOON (Severe Obesity Outcome Network) cohort suggested that in women with obesity, neck circumference was the most appropriate measure to assess for fat distribution and cardiometabolic risk. However, these findings have yet to be validated elsewhere[255].

(3) Body Composition Studies

Body composition studies are the most accurate method of quantifying fat mass and assessing fat deposition. These studies are increasingly carried out using a dual-energy x-ray absorptiometry (DEXA) scans or using the quantitative MRI method[256]. Other methods to assess body composition include air-displacement plethysmography (BodPod) and bioelectrical impedance[257]. These are all highly accurate but also considerably more expensive techniques and are often reserved for research studies rather than routine clinical assessment[256]. However, the latter is also often available in commercial health facilities such as gymnasiums. Fat mass exceeding 25% in men and 33% in women are often cited as indicative of increased cardiometabolic risk of disease.

(4) Assessment of obesity-related health risk

In patients found to be overweight or obese, a thorough history, full physical examination and routine blood tests should be undertaken to rule out treatable underlying causes (e.g. hypothyroidism, insulinomas) and to assess for presence and severity of co-morbid disease (e.g. T2DM, dyslipidaemia, sleep apnoea, NAFLD)[249]. Also, a complete medication history should be obtained and current medications should be rationalised for potential causes of weight gain[249]. Further assessment and management should only be made once these tests have been undertaken[249].

Further, the American Association of Clinical Endocrinologist Practice Guidelines promotes a co-morbidity focused management plan for patients with

obesity, rather than a BMI-centric approach; and tools such as the Edmonton Obesity Staging System and the King's Obesity Staging Criteria may help clinicians to effectively risk stratify patients[258-260]. Although this is not formally indicated in current guidelines in the UK, the merits for such practice are clear. Evidence suggests that patients who are likely to benefit the most from medical and surgical interventions are those with co-morbid conditions in two general categories (1) those with cardiometabolic disease and (2) patients who suffer with biomechanical consequences of a high body weight and it would be beneficial to identify and manage such patients expediently[258, 261].

1.4 Current Treatment Options of Metabolic Disease

This section will focus on the current therapeutic options available for patients with metabolic disease in general, but specifically those relating to the management of T2DM, obesity and the metabolic components of PCOS. As presented in earlier sections of this chapter and widely cited in the literature, there is a significant degree of overlap in terms of aetiology, pathophysiology as well as clinical progression of these conditions and they often co-exist in the same patient.

Hence, it is unsurprising that many of the therapeutic strategies currently available are used in the management of more than one of these conditions. Further, encouraging results have also been seen with novel treatment modalities focused on the presumed pathophysiological principal linking these conditions – insulin resistance.

Contrarily, it is also important to appreciate that each of these conditions occur with a myriad of disease-specific associations and pathophysiological distinctiveness. Indeed, even within the same condition there exists subtypes and categories of severity. There remains no universal treatment for each of these conditions, let alone metabolic disease in general. Therefore, it should be anticipated that response to treatment is likely to vary considerably from patient to patient and a tailored individual approach is more likely to be efficacious[262].

Further, these conditions are lifelong disorders and treatment should be aimed at (1) achieving a healthy baseline metabolic status, (2) avoidance or delay of complications, (3) reducing mortality risk and (4) maintaining a good quality of life.

With that in mind, a few therapeutic strategies for metabolic conditions are discussed here.

1.4.1 Lifestyle Modification

Lifestyle changes for metabolic disease, essentially diet modification and increasing physical activity levels, but also promoting good sleep hygiene, emotional well-being and avoidance of toxic substances (alcohol, tobacco, illicit drugs); are the first line

treatment strategies for T2DM[263], obesity[264] and the metabolic component of PCOS[265].

The primary driver of this tactic is weight loss, and the subsequent improvements in metabolic health in terms of insulin resistance, cardiometabolic disease and life expectancy[266, 267]. Alongside this, lifestyle modifications have also been reported to improve body composition[268], muscle strength[269], bone health[270], immune mediation[271], depression[272], quality of life[273] cancer incidence and prognosis[274].

This was highlighted in a double-blinded randomised controlled trial of 343 overweight women with PCOS and infertility comparing a lifestyle modification program (low-calorie diet and 30 minutes of exercise a day) to clomiphene citrate alone, metformin alone or a combination of metformin and clomiphene citrate[275]. After 6 months, women in the lifestyle modification group did better than their medicated counterparts in terms of waist circumference, serum levels of insulin, low-density lipoprotein (LDL), testosterone and SHBG[275]. Further, although not statistically significant, women in the lifestyle medication group had a higher pregnancy rate (20%) than women in the combination group (14.4%) or metformin alone (14.4%) or clomiphene alone (12.2%) groups[275]. A 2019 Cochrane Database systematic review of 500 women with PCOS also identified improvements in weight, BMI and free androgen index following lifestyle treatment in women with PCOS[276].

Although the longevity of the benefit gained from lifestyle interventions is still unconvincing, a short-term benefit may still be useful in women with PCOS who are seeking gestation. An oft-cited study found that women with PCOS who lost 5% of their initial body weight within a 7-month diet plan, had improvements in their fasting serum insulin and SHBG levels with a corresponding reduction in their free testosterone levels[277]. These changes were accompanied by improvements in reproductive function as well with most women having more regular cycles or successfully conceiving (n=5). However, only 50% of the women recruited to the study managed to lose 5% of their body weight.

A systematic review of various low-calorie diets in women with PCOS with different macronutrient composition found, although there were subtle differences with each diet, there were no significant differences in terms of weight loss between different diet plans[278].

The positive effect of diet modification, in particular caloric restriction, in promoting remission of T2DM was highlighted on a national platform in 2018 following publication of the results from the Diabetes Remission Clinical Trial (DiRECT) in the Lancet[279]. The contentious results from this trial led to discussions on numerous platforms including a parliamentary debate[279, 280]. The open-label randomised clinical trial recruited 298 patients with recent onset T2DM (less than 6 years) to receive either standard medical therapy or complete discontinuation of medical therapy and total diet replacement with a very low calorie liquid diet (approximately 825 kcal/day) for a minimum of 3 months, followed by structured food reintroduction over 2- 8 weeks with intense follow up for the duration of the trial[279]. Patients in the intervention group were also encouraged to increase physical activity at the start of the food reintroduction phase for weight loss maintenance[279].

At 12 months, despite a drop-out rate in excess of 20% and a liberal definition of diabetes remission, 24% of participants in the intervention group had weight loss of 15kg or more and 46% of patients achieved remission of T2DM[279]. This was in stark difference to the participants in the control group where no patients achieved comparable weight loss and only 4% demonstrated an equivalent reduction in HbA1c[279]. Similarly, the mean weight loss was 10 ± 8 kg for the intervention group and 1± 3·7 kg in the control group[279]. Quality of life measures were also greater in the intervention group than in the control group[279].

While these initial results were certainly impressive, enthusiasm for this treatment modality waned somewhat following publication of the group's results at 24 months post-intervention. In the follow-up report, only 11% of participants in the intervention group maintained a weight loss of at least 15kg while T2DM remission rates had fallen to 36%[281]. This is despite (1) the more motivated cohort trial participants usually

represent and (2) participants being followed up in an intensely structured and supported setting.

When these findings are considered together with the considerable drop-out rate and the poor tolerance many patients are likely to have to a very low-calorie liquid diet replacement, it is unlikely that this treatment strategy can be effectively rolled out at a population level for the management of T2DM. However, with careful patient selection, very low calorie and low-calorie diets may be a useful adjunct in the management of T2DM.

Similarly, the Action for Health for Diabetes (Look AHEAD), the largest RCT of an intensive lifestyle modification trial in patients with T2DM, also showed poor long-term durability of lifestyle interventions in achieving and maintaining weight loss and diabetes remission[282]. Of the 5,145 patients recruited, participants in the intensive lifestyle intervention arm loss more weight at year 1 (8.6% vs 0.7%) and were more likely to attain partial or complete diabetes remission (11.5% vs 2%) than patients in the control group[282].

However, once the weekly or fortnightly health practitioner input became less frequent, the difference between the two groups narrowed and was halved by the year 4 follow-up[282]. By year 8, there was only a 2.6% difference in terms of weight loss between the intervention and the control groups[283]. Unsurprisingly, at 9 years, the study group concluded that intensive lifestyle intervention did not reduce the rate of cardiovascular outcomes in overweight and obese patients with T2DM[284]. These findings again highlights the difficulty with utilising lifestyle interventions to achieve sustained health benefits in patients with metabolic disease.

Interestingly, data from the Look AHEAD study did suggest that early weight loss patterns may help predict durability of a patient's response to intensive lifestyle interventions[285]. Patients who achieved >6% weight loss at 2 months were 3.85 times more likely to have >5% weight loss at 4 years and 2.28 times more likely to have the same 8 years later, when compared to patients who lost less than 3% of their weight in the first 2 months of lifestyle intervention[285]. This was in spite of adherence being

reported as excellent in the early weeks of the trial[285]. This suggests that lifestyle interventions may be physiologically well-suited to a minority of patients with metabolic disease.

Likewise, this has also been evident from studies assessing the outcome of lifestyle interventions in patients with obesity. In the UK, three tiers of the national 4-tiered obesity service commissioned by NHS England are focused on lifestyle interventions. A recent systematic review of Tier 3 specialist weight management services in 11,735 patients highlighted a significant and consistent drop-out rate seen in almost (90%) of the studies included. However, of the patients who completed 24 months, 44.4% achieved >5% weight loss[286].

Biologically, evidence suggest a major reason for the ineffectiveness of lifestyle measures is a result of the many powerful signals which act counter-intuitively in response to weight loss. These signals act in concert to increase perception of hunger, heighten seeking of energy-dense food, decrease energy expenditure by reducing lean muscle mass and sympathetic activity, enhance neural response to food cues and diminishing vagal transmission, alteration to BA cycling and gut microbiome, increase ghrelin release and by reducing secretion of leptin, GLP-1 and PYY[287].

Hence, it is strongly suggested that lifestyle management is best used to complement other therapeutic modalities in metabolic disease as it is unlikely to result in significant benefit on its own.

1.4.2 Pharmacotherapy

The following sections will focus on the key drugs used for glucose mediation in T2DM, insulin sensitisation in PCOS and weight management in obesity. Additionally, patients with these conditions will also often need drugs for the many accompanying co-morbid conditions that co-exist with T2DM, PCOS and obesity.

1.4.2.1 Pharmacotherapy for T2DM

The primary goal of pharmacotherapy in T2DM is to safely achieve normoglycaemia and, for most patients, the HbA1c target of < 6.5% (48 mmol/mol). This is with the aim of avoiding or reducing macro- and microvascular complications of T2DM[288]. In the past decade there has been a progressive change in the landscape of T2DM pharmacotherapy and currently there are 7 major classes of glucose-lowering medication available for use, either as a single agent or in combination.

The order in which the drugs are discussed below represents the hierarchy of recommended use by the American Association of Clinical Endocrinologists and the American College of Endocrinology in their 2019 Consensus Statement[288].

(1) Metformin

Metformin, a biguanide, is the most widely used glucose-lowering agent worldwide. It primarily acts on the liver but also affects glucose metabolism in many other tissues including skeletal muscle, adipose tissue and the ovaries[289]. It acts by decreasing hepatic glucose production and improving peripheral glucose uptake[290]. A meta-analysis of 35 trials estimated a HbA1c reduction of 1.12% when metformin was used as monotherapy[291]. It has a low risk of hypoglycaemia, can promote modest weight loss, has a good safety profile and durable effects[290]. It should be used with caution in patients at risk of lactic acidosis[291].

(2) GLP-1 Receptor Agonists

GLP-1 receptor agonists, such as liraglutide and semaglutide, are synthetically produced agonists of the GLP-1 receptor[292]. It has a largely homologous amino acid sequence to the incretin, GLP-1 and has a similar therapeutic profile[292]. However, it is characteristically resistant to DPP-4 degradation and newer agents like semaglutide has a half-life of 5-7 day[292]. Most GLP-1 receptor agonists are administered in an injectable form, although an oral form of semaglutide has recently been released[293].

GLP-1 receptor agonists have a robust HbA1c lowering capacity of 1-2% depending on the agent and dose[292]. This is augmented when used in combination with other classes of glucose-lowering agents[292]. Further, GLP-1 receptor agonists also induces clinically meaningful weight loss (>10% reported with semaglutide) and reductions in BP and lipid profiles[292, 294]. Results from the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial and the Sustain -6 (Trial to Evaluate Cardiovascular and Other Long-Term Outcomes with Semaglutide in Subjects with Type 2 Diabetes) trial have demonstrated reduction in the risk of cardiovascular death as well as non-fatal myocardial infarction and strokes in patients with T2DM and cardiovascular disease[295, 296].

As it promotes glucose-dependent insulin secretion, it has a low risk of hypoglycaemia[288]. In addition, this class of drug also decreases glucagon secretion, reduces hepatic glucose production, slows gastric emptying and has an anorexigenic effect [288]. It, theoretically, may have a proliferative effect on β-cell mass and hence should be used with caution in patients with a history of pancreatitis[288]. GLP-1 receptor agonists are contraindicated in patients with a personal or family history of medullary thyroid cancer or multiple endocrine neoplasia syndrome Type 2[288].

(3) Sodium-dependent Glucose Co-Transporter 2 Inhibitor

SGLT2 inhibitors, the '-gliflozins', are a class of drug which act by inhibiting the SGLT2 receptors in the kidney tubules, obstructing glucose reabsorption and lowering plasma glucose level[297].

SGLT2 Inhibitors effect a 1.1% reduction of HbA1c as a monotherapy and can be used in combination with any other class of glucose-lowering agents as well[297]. It has the added advantage of reducing major adverse cardiac events and improving renal outcomes as illustrated in the EMPA-REG OUTCOME (Empagliflozin, Cardiovascular Outcome Event) Trial and CANVAS (Canagliflozin Cardiovascular Assessment Study)[298, 299]. SGLT2 inhibitors also has a modest weight loss and BP-lowering effect[297]. However, there was a

higher incidence of amputation seen in patients receiving Canagliflozin in the CANVAS Trial[299].

Due to its mode of action, SGLT2 inhibitors cause glycosuria which increases the risks of urinary tract infections and genitourinary fungal infections[297]. For similar reasons, it will also have limited efficacy in patients with an eGFR < 45 mL/ min/ 1.73 m^2 and can cause dehydration and related complications (including renal dysfunction) [297]. There is a small risk of diabetic ketoacidosis with SGLT2 inhibitor use and it is recommended to be stopped prior to scheduled surgery and metabolically stressful activities[288].

(4) Dipeptidyl peptidase-4 Inhibitors

DPP-4 inhibitors, the '-gliptins' exert an anti-hyperglycaemic effect by inhibiting DPP4 and thereby reducing incretin degradation[288]. This results in an increased incretin (GLP-1 and GIP) effect including glucose-dependent insulin synthesis and HbA1c reduction (of <1%) with minimal risk of hypoglycaemia[300]. In contrast to GLP-1 receptor agonists and SGLT2 inhibitors, DPP-4 inhibitors are weight neutral and do not exert a cardioprotective effect[301].

(5) Thiazolidinedione

Thiazolidinedione, the '-glitazones', are the only insulin sensitizing drugs available for use in patients with T2DM[25]. The act by increasing expression of the nuclear receptor, PPARγ, which increases storage of fatty acids and adipocytes resulting in a lower concentration of circulating fatty acids and increased utilization of glucose oxidation for cellular processes[25]. This results in an increase in peripheral insulin sensitivity, durable glycaemic effects and HbA1c lowering effect of 1-1.5%[288]. It has also been shown to suppress hepatic gluconeogenesis and lipogenesis[25].

However, its use has been limited as it causes weight gain and has an increased risk of fractures, heart failure and bladder cancer[288]. Further,

although pioglitazone is still available for use with caution, rosiglitazone has been withdrawn from use in the UK due to the increased risk of myocardial infarction[302].

(6) Sulfonylureas

Sulfonylureas, insulin secretagogues, act by increasing insulin release by β-cells[288]. They have a potent HbA1c lowering capacity (1-2%) but lack durability of action and is associated with weight gain and hypoglycaemia[288, 303]. Further, concerns have been raised with regard to its cardiovascular safety[288].

(7) Insulin

Insulin is the most potent anti-hyperglycaemic agent available[288]. It is usually reserved for use when endogenous insulin secretion is depleted, as often the case with patients with poor glycaemic control despite optimal therapy with non-insulin agents[288]. There are multiple preparations of insulin available with varying duration of actions and patients can use a combination of different insulin to optimize glycaemic control at different times of day[288]. The main concern with insulin use is hypoglycaemia and patients are encouraged to monitor their blood glucose with capillary glucose monitors[288]. Further, insulin can also cause modest weight gain. [288]

1.4.2.2 Pharmacotherapy for obesity

All current guidelines in the management of obesity suggest that obesity pharmacotherapy should be used as an adjunct to lifestyle interventions. Current indications for use of such agents are (1) history of falure to achieve clinically meaningful weight loss or to maintain weight loss in (2) patients with a BMI >30kg/m^2 (or BMI >27kg/m^2 in the presence of an obesity-related co-morbidity)[264]. Further, if less than 5% weight loss is achieved within 3 months of commencing the drug, the drug should be stopped and another anti-obesity treatment attempted[264]. However, if the drug does result in the desired weight loss, it should be continued long-term[264].

At present there are 3 major classes of drugs in use for weight loss in the UK, however, orlistat is the only drug approved by the National Institute of Health and Care Excellence (NICE) and available on the NHS for weight loss[249, 304].

(1) Orlistat

Orlistat is a selective inhibitor of pancreatic and gastric lipase and is able to decrease dietary fat absorption by 30%[305]. As a result, orlistat therapy induces weight loss (2-3% of body weight) and, unsurprisingly, also causes a decrease in total cholesterol, LDL, HDL and triglyceride levels[306]. These effects were recently illustrated in a large meta-analysis of 33 RCTS including almost 10,000 patients[306].

Further, the XENDOS trial, a large RCT of 3305 patients with obesity followed up for 4 years, found that orlistat use also significantly reduced the risk of T2DM onset when compared to a placebo (6.2% vs 9.0%)[307]. Orlistat has also been shown to decrease glucose levels, insulin sensitivity and blood pressure[307].

However, its use is limited by unpleasant side-effects including faecal urgency, liquid flatulence and steatorrhoea which, when coupled with the fairly modest weight loss, often leads to cessation of treatment[305].

(2) Naltrexone-bupropion

This is a combination drug which acts synergistically on the central nervous system pathways to decrease appetite and increase energy use. It results in weight loss of 5-6% as well as improvement in HbA1c (0.4% reduction) and lipid profile[305].

Naltrexone-bupropion was previously approved for use in the UK until an update by NICE in 2017 which stated its use was no longer advised due to a paucity of evidence for its long-term effect and difficulty justifying its cost/benefit ratio[304]. However, patients who were already on Naltrexone-bupropion with good effect have been allowed to continue with their treatment[304].

(3) Liraglutide

Liraglutide, a GLP-1 receptor agonist (discussed above), is also used as an anti-obesity agent when given at the higher dose of 3.0 mg daily (titrated up from 0.6mg daily) via subcutaneous injection. It plays a role in weight and appetite regulation through its anorexigenic effect as well as its recognised incretin-mimetic actions.

Evidence for its use in obesity come from three large randomised placebo-controlled clinical trials which examined the effect of 3.0 mg liraglutide daily on weight loss in 3,755 people with a BMI > 30kg/m^2 and either T2DM, pre-diabetes or normal glucose tolerance who were followed up for 56 weeks[308-310]. All three trials demonstrated clinically-relevant weight loss (6-8%) and increased efficacy of liraglutide over placebo (mean difference in weight loss ranging from 4-6%). In addition, improvements were also seen in other metabolic parameters including in the levels of fasting glucose, HbA1c, lipids and blood pressure[308-310].

More recently, semaglutide use has also exhibited clinically-meaningful weight loss in obese patients without diabetes[311]. When combined with lifestyle intervention, a phase II, 52-week RCT demonstrated a mean weight loss of 13.8% with 0.4mg injection of semaglutide (vs 7.8% with liraglutide injections and 2.3% with placebo injections)[311].

1.4.2.3 Pharmacotherapy for PCOS

Pharmacotherapeutics aimed at improving insulin resistance in women with PCOS has the dual target of ameliorating metabolic and reproductive parameters.

(1) Metformin

In women with PCOS and obesity, metformin use has been associated with improvements in insulin sensitivity, insulin secretion, lipid profile, androgenaemia and ovulatory derangements[172, 312, 313]. These effects,

particularly in insulin secretion, insulin sensitivity and body composition, are also seen in women with PCOS who are lean and insulin sensitive[172, 312, 313], However, these effects are more consistently seen in women with PCOS who have a raised BMI (>32 kg/m^2), suggesting that fat distribution may be pertinent to its action[172, 312, 313]. Conversely, metformin use does not have a significant effect on the dermocutaneous manifestations of PCOS[148].

Despite documented evidence of its benefit, in the UK, metformin is still not licensed for use in women with PCOS[314]. However, the Royal College of Obstetricians and Gynaecologists and the Androgen Excess and PCOS Society, both internationally recognised authorities on PCOS management, advocate the use of metformin, together with lifestyle intervention, in women with PCOS who have risk factors for cardiometabolic disease including glucose intolerance and dyslipidemia[314, 315]. Hence, metformin is commonly used off-label in the UK in the management of women with PCOS and insulin resistance[314].

(2) Thiazolidinediones

Thiazolidinediones have been shown, in a systematic review of 278 women with PCOS, to be more effective than metformin at improving insulin sensitivity and decreasing insulin secretion in women in PCOS[316]. However, similar to T2DM, its use in women with PCOS has fallen out of favour due to potentially serious side-effects and weight gain.

(3) Inositol

Myo-inositol, and its stereoisomer, D-chiro-inositol, are physiological carbohydrate compounds which have intra-cellular functions in insulin signaling. Further, inositol is also intimately involved in glucose uptake and FSH expression. In women with PCOS, the metabolism of inositol may be dysregulated.

In a systematic review of 12 RCTs, inositol use in women with PCOS was shown to decrease insulin secretion and HOMA-IR levels[317]. However, there was significant heterogeneity among the studies, hampering the reliability of its conclusions. Further, while other studies have also shown similar improvements as well as improved menstrual regularity, there has been considerable inconsistency in the degree of benefit conferred in women with PCOS[172]. Hence, its use is not currently clinically indicated although it is available as over-the-counter preparations.

(4) Anti-obesity agents

In women with PCOS and obesity, anti-obesity agents such as orlistat, liraglutide and even metformin, when compared to lifestyle management, have been shown to induce significant weight loss when used singly or in combination[318]. The indication for use of these agents are similar to patients without PCOS.

(5) Combined oral contraceptives and anti-androgenic agents

These two classes of drugs are typically used to improve the dermocutaneous manifestations of PCOS and provide endometrial protection in women not seeking fertility[319]. Generally, combined oral contraceptive agents are recognised to worsen glucose metabolism. However, in women with PCOS and hyperandrogenaemia, the testosterone-lowering improvement in dysglycaemia offsets this[319]. Similarly, antiandrogenic agents also improve glucose metabolism as well as body composition[319].

Further, the effect of both these classes of drugs is potentiated when combined with metformin[320].

1.4.3 Metabolic surgery

Metabolic surgery is the most effective treatment available to-date for the treatment of patients with obesity and metabolic dysfunction, particularly T2DM[321]. Recognition of

its potent metabolic effects via '*weight-loss dependent'* and *'weight-loss independent'* pathways, aptly led to a change in nomenclature from the traditional *'bariatric surgery'* to the term currently preferred – *'metabolic surgery'*.

The most commonly performed metabolic surgical procedures in the UK are the Roux-en-Y gastric bypass (RYGB) procedure and the vertical sleeve gastrectomy (VSG)[322]. This echoes the worldwide frequency of operative procedures as depicted in the figure below (see Figure 1.6)[323].

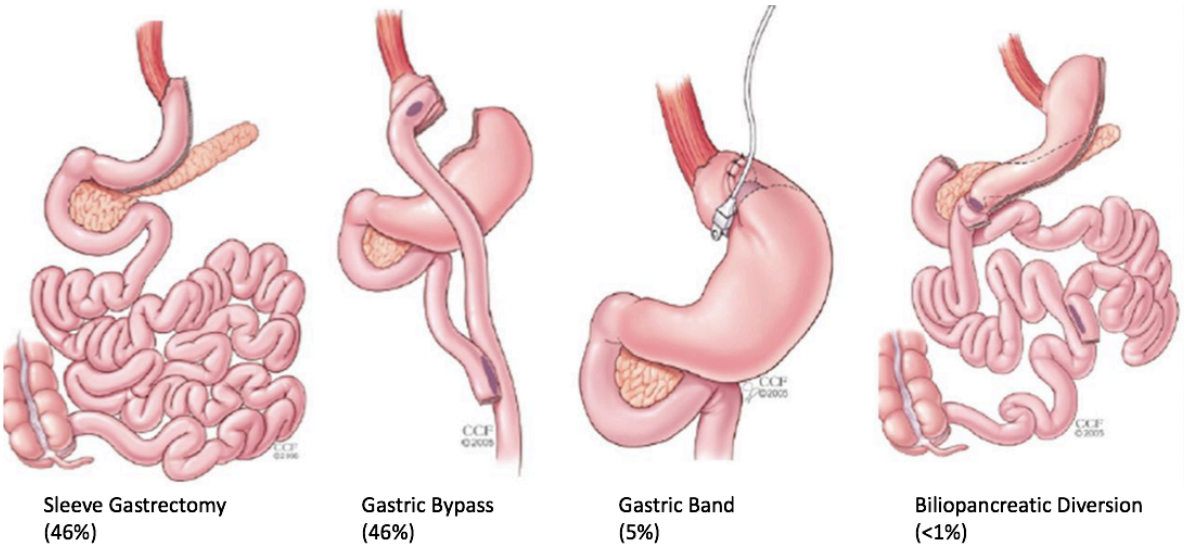

Figure 1.6 Current metabolic procedures and their global frequency of use from the IFSO Global Registry Report 2018

[Source: Adapted from Welbourn R, Hollyman M, Kinsman R, Dixon J, Liem R, Ottosson J, et al. Bariatric Surgery Worldwide: Baseline Demographic Description and One-Year Outcomes from the Fourth IFSO Global Registry Report 2018. Obes Surg. 2019;29(3):782-95 and *Schauer PR, Mingrone G, Ikramuddin S, Wolfe B. Clinical Outcomes of Metabolic Surgery: Efficacy of Glycemic Control, Weight Loss, and Remission of Diabetes. Diabetes Care. 2016;39(6):902-11.][323, 324]*

Laparoscopic adjustable gastric banding (LAGB) was pervasive in the last decade but its popularity has declined steadily due to poor long-term outcomes and high complication rates[325].

The value of metabolic surgery in eliciting T2DM remission has been effectively demonstrated in a number of well-designed studies. The prospectively matched SOS (Swedish Obese Subjects) cohort study demonstrated T2DM remission rates of 72.3% at 2 years, 38.1% at 10 years and 30.4% at 15 years post-metabolic surgery[326]. Although in contrast to the UK, two-thirds of patients in this cohort were treated with a vertical banded gastroplasty[326]. Conversely, the STAMPEDE (Surgical Treatment and Medications Potentially Eradicate Diabetes Efficiently) RCT had a more reflective population and assessed the effect of intensive medical therapy alone vs intensive medical therapy plus RYGB or VSG[327]. They reported T2DM remission (Hba1c ≦ 6%) in 29% of patients in the RYGB group, 23% in the VSG group and 5% of patients treated with intensive medical therapy alone[327]. This study further established the superiority of metabolic surgery, particularly RYGB, in the management of patients with obesity and T2DM.

This was in keeping with evidence that metabolic surgical procedures which bypass the upper GI tract result in better T2DM remission rates than those that maintain GI continuity[328, 329]. Furthermore, the BPD procedure has consistently been found to stimulate greater rates of T2DM remission than RYGB, even when matched for weight loss[329, 330]. This suggests weight-independent mechanisms contribute to its therapeutic efficiency. Using mechanistic studies including hyperinsulinaemic euglycaemic clamps, BPD has been demonstrated to provoke greater improvement in peripheral insulin sensitivity and slower rate of intestinal glucose absorption, which may contribute to the superior rates of T2DM remission[330]. Although BPD procedures are infrequently carried out due to its complexity and adverse event profile (protein-calorie malnutrition, excess flatulence), understanding the mechanism underling its effectiveness is valuable in illuminating the pathophysiology of metabolic disease and developing novel therapeutic modalities.

In contrast to pharmacotherapy, metabolic surgery acts via multiple different pathways. Although many of these remain incompletely elucidated, it is clear that there are weight loss-dependent and independent mechanisms in action.

1.4.3.1 Weight-loss dependent mechanisms of metabolic surgery

Weight loss is undoubtedly the primary outcome for patients undergoing metabolic surgery. Twenty year follow-up data from the SOS study suggests that weight loss from metabolic surgery is more profound and sustained for longer post-operatively than other weight loss methods[331]. The 5-year outcomes from the STAMPEDE RCT reported 23% weight loss in the RYGB group and 19% in the VSG group compared to only 5% in the intensive medical therapy group[327]. Weight loss is also associated with significant improvements in obesity-related co-morbidities such as T2DM, PCOS, hypertension and obstructive sleep apnoea syndrome. However, clinical response at the individual level is variable and evidence suggests that the degree of improvement is related to the amount of weight lost[287].

The mechanisms underlying sustained weight loss is likely multifactorial, and still only partly understood, but it is clear that the effect is primarily biological; and not merely mechanical as previously perceived with the obsolete restrictive-malabsorptive model. Physiological changes that have been identified that may be involved in stimulating weight loss include altered hypothalamic signalling leading to blunted food-reward response and changes in food preference to less energy-dense options, diminished hunger and neural responsiveness to food cues leading to reduced food intake, increased vagal stimulation, increased circulating bile acid levels, enriched microbiota and altered gut hormone profile[332]. Malabsorption of nutrients is not believed to play a key role in the regulation of weight following the most commonly used metabolic surgical procedures[332].

In terms of gut hormones, ghrelin secretion is often found to be reduced and GLP-1 and PYY levels raised post-operatively, in contrast to diet-induced weight loss[287]. The latter is most likely due to the reconfiguration of the intestinal anatomy leading to increased nutrient stimulation of the distal intestinal L-cells. Further, it is believed that

all these changes in body weight regulation act in concert to re-establish the weight set-point to a new, lower level thereby eliciting sustained weight loss[333].

The substantial weight loss seen is likely to be a major pathway in provoking glycaemic improvements in patients with obesity. Studies show improvement in peripheral insulin sensitivity in parallel with weight loss and this is likely resultant from contracted visceral fat depots[332]. In contrast, improvements in hepatic insulin sensitivity precedes substantial weight loss suggesting weight loss-independent mechanisms are responsible for its initial change[332]. However, its sustained improvement is probably related to weight loss and diminished glucotoxicity and lipotoxicity. Further, β-cell function and early insulin secretion have also been demonstrably improved post-operatively as a result of weight loss, redistribution of fat mass and less potent glucolipotoxicity[97].

1.4.3.2 Weight loss-independent mechanisms of metabolic surgery

The concept of weight-loss independent mechanisms triggering the benefits seen after metabolic surgery was borne following the observation of improved glycaemic markers and insulin sensitivity early in the post-operative course before the onset of any significant weight loss.

Many of the physiological changes responsible to this effect has been scrutinised earlier in this chapter and hence will be approached briefly in this section.

Firstly, an enhanced incretin effect leading to improved post-prandial glucose tolerance and post-prandial insulin secretion has been consistently identified in patients following metabolic surgery[96]. Using GLP-1 receptor antagonists in patients with obesity and T2DM in the early post-RYGB period, Shah *et al* demonstrated the small but significant contribution GLP-1 has in improving glucose tolerance, insulin secretion and β-cell sensitivity in this early period[101]. This effect is attributed to more rapid delivery of nutrients to the distal intestinal tract but it is clearly not the chief contributor to the metabolic improvements seen.

A larger contributor of the early improvements seen in the metabolic profile of patients undergoing foregut-sparing surgery is related to the profound improvement in hepatic insulin sensitivity, most likely through reduced hepatic gluconeogenesis[334]. In an elegantly-designed experiment, Mingrone and her colleagues demonstrated this by conducting hyperinsulinaemic euglycaemic clamps, HOMA-IR measurements and muscle biopsies in 10 patients with obesity 4 weeks following RYGB[335]. They found normalisation of hepatic insulin clearance and improvement in total insulin sensitivity. However, the latter was incompletely normalised indicating a lag in peripheral insulin sensitivity[335]. Further, hyperinsulinaemic euglycaemic clamps in patients with obesity 2 weeks after BPD also demonstrated significant improvements in insulin sensitivity.

Correspondingly, a comprehensive meta-analysis assessing the change in insulin resistance following different metabolic surgical procedures (RYGB, BPD, VSG and LAGB) only identified significant decrease in insulin resistance at 2 weeks in patients who had undergone RYGB and BPD[336]. This is, again, in keeping with the foregut-sparing effect detailed earlier.

1.4.3.3 RYGB vs VSG

Much of the discussion around the improvements seen following metabolic surgery are focused on operations that include a foregut-bypass component (e.g. RYGB, BPD). This is juxtaposed to procedures such as VSG which also prompt significant weight loss and glycaemic improvements but does not involve an anatomical bypass[102]. The reasoning behind the improvements seen are believed to be secondary to more rapid nutrient transit through the foregut – essentially establishing a functional foregut bypass.

However, as nutrients still pass through the foregut, the increase in GLP-1 and PYY levels are less pronounced[287]. This may also explain the less marked decrease in visceral fat seen following VSG and the less significant improvements in glycaemia[287]. Conversely, a more striking decrease in ghrelin level is seen following VSG when compared to RYGB and this is likely secondary to the obligate fundal excision of VSG[287]. This will contribute to some of the weight loss seen with VSG.

Further, although studies such as SLEEVEPASS and SM-BOSS have demonstrated weight loss in a similar region to RYGB at 5 years post-op, the difference between the two procedures increases with time and this disparity is clearly discernible in studies with longer follow-up times, suggesting a dulling of some of the functional changes seen with VSG[102, 337].

1.4.4 Endoluminal and less-invasive metabolic devices

The management of metabolic diseases has improved significantly over the past two decades. Yet, the twin epidemic of diabesity persists and the majority (>60%) of people with T2DM living in England and Wales have failed to achieve their metabolic targets as set by NICE[338].

This suggests that despite novel, glucose-lowering agents, such as GLP-1 receptor agonists and SGLT-2 inhibitors – which not only significantly decrease HbA1c levels but also effect substantial weight loss and improvement of cardiometabolic status – pharmacotherapeutic strategies are not sufficiently meeting the needs of patients with metabolic disease. The proportion of patients with HbA1c levels above the recommended 48mmol/L has remained concerningly high at around 70%, despite the introduction of these newer agents[339]. This reflects real-world problems affecting clinical response of medication in chronic disease including issues with compliance and side-effects.

In contrast, surgical management of metabolic conditions, RYGB in particular, has consistently demonstrated superiority in achieving significant weight loss and effecting metabolic improvements. A review of RCT data by international diabetes organisations suggested that between 30-63% of people achieved diabetes remission for 1 to 5 years after surgery[340].

Despite this, far fewer than 1%[1] of the target population are being treated with metabolic surgery worldwide, reflecting serious patient and provider concerns around the acceptability and scalability of metabolic surgery[341, 342]. This clearly highlights a wide treatment void in metabolic disease.

Endoluminal interventions, such as DMR, are perhaps the most promising candidates available currently to fill this void as they mimic the anatomical components of metabolic surgical procedures but are delivered in a less invasive manner and often carried out in a day-case setting.

Additionally, laparoscopically-placed electrical pacing devices have also been seen to effect metabolic improvement in patients with obesity.

All devices that result in metabolic improvements, and not only in weight loss, are discussed below, except for DMR which is detailed in the following section.

(1) Duodenal-Jejunal Bypass Liner (DJBL)

This device, commercially branded as Endobarrier (gi-Dynamics, Lexington Massachusetts), is designed based on the concept of foregut exclusion used in RYGB and BPD. It is a sterile, single-use endoscopic implant which is deployed under radioscopic control and can remain *in situ* for a maximum of 12 months.

The device is comprised of (1) a 62-cm open-ended conduit, made of an impermeable fluoropolymer, (2) a proximal fixating anchor which secures the liner in the duodenal bulb, proximal to the ampulla of Vater and (3) drawstrings attached to the anchor to facilitate subsequent removal[343].

[1] Percentage calculated for the year 2016 from data published in the World Health Organization Factsheet: Obesity and Overweight and the 4th IFSO Global Registry Report.

Once in place, it directs chyme from the stomach through it and into the proximal jejunum. Concurrently, it precludes mixing of chyme with bilio-pancreatic juices, which flows on the outside of the impermeable liner for the length of the device[343]. This effectively circumvents nutrient digestion and absorption in the post-papillary duodenum and proximal jejunum.

The device has demonstrated promising metabolic improvements with early studies demonstrating improvements in glycaemic indices and BMI[343]. More recently, Ruban *et al* reported findings in a clinical trial on 45 patients with T2DM (mean baseline HbA1c 8.5%) and obesity (mean baseline BMI 39.9 kg/m^2) with planned explantation at 1 year[344]. Despite almost a third of patients needing early explantation, there was a mean reduction of HbA1c of 0.8% and BMI of 4.9 kg/m^2 at 1 year, and these changes seemed to be maintained for at least 6-months after[344].

However, the use of the device has been limited following the publication of a systematic review which highlighted a significant adverse event profile[345]. Of the 38 studies included, there were 891 reported events in 1056 patients, with 20.5% of events graded as moderate and 3.7% severe[345]. Complications in the latter category included 11 hepatic abscesses, 8 patients with GI haemorrhage and 4 cases of oesophageal perforation[345].

There were also other concerning adverse events reported (including oesophageal mucosal laceration resulting from trauma associated with the anchor barbs, ulceration, pancreatitis, cholangitis, device migration and obstruction) and further, nearly a quarter of all devices had to be removed prematurely due to adverse events or intolerability[345].

Troublingly, the systematic review also identified inadequate reporting of adverse events in the studies included, suggesting an underestimation of the true incidence of adverse events[345]. Subsequently, the European CE

(Conformité Européenne) mark for implantation was withdrawn and has not been re-instated. The device has not been approved by the FDA (Food and Drug Administration).

However, gi-Dynamics is currently re-designing the device to allow a more favourable safety profile and further information is expected in 2020.

(2) Gastroduodenojejunal Bypass Sleeve

Based on a similar concept of foregut exclusion, the 120cm fluoropolymer gastroduodenojejunal bypass sleeve was developed for use in humans in 2011[346]. This device is anchored at the level of the gastro-oesophageal junction and requires endoscopic and laparoscopic visualisation for fixation[346]. In a small initial study, the device was explanted after 12 weeks and demonstrated favourable findings of weight loss and diabetes resolution[346]. Similar to DJBL, a quarter of patients needed the device explanted (for dysphagia)[346]. A further study was published in 2015 with similar findings but the device *in situ* for 12 months[347]. However, since then, there remains very limited information on the device in the literature and it has not been approved for use.

(3) Satisphere

Another device focused on the duodenum is the Satisphere duodenal implant. This is an endoluminal mechanical device with multiple small plastic spheres, designed to be *in situ* for 3 months, during which it acts by delaying duodenal transit time and promoting duodenal fullness[348].

An early feasibility study demonstrated, at 12 months, a mean BMI loss of 2.4 kg/m^2 (mean weight loss 6.7kg) as well as delayed glucose absorption and insulin secretion (from 30 minutes to 60 minutes) [348]. Interestingly, patients with the device also displayed a constant GLP-1 level resembling exogenous

GLP-1 administration, which may, in part, account for some of the weight loss observed[348].

However, the study had to be terminated prematurely as the device migrated in almost 50% of trial participants, with one patient requiring a hemicolectomy and intensive care management[348]. Hence, the device has not been approved for use and it is currently being re-designed.

However, conceptually, it does raise questions on the key role the duodenum plays in metabolic health and disease.

(4) Magnetic Anastomosis Devices

An incisionless jejuno-ileal anastomosis – created using self-assembling magnets introduced endoscopically, to simulate an RYGB – has been used safely with encouraging results in terms of total weight loss (14.6%) and HbA1c levels (1.9% in T2DM, 1% in pre-diabetic) at 12 months[349]. The procedure is based on the premise that the magnetic force will cause transmural ischaemia and subsequent necrosis focally to create a wide-bore, side-to-side, full-thickness anastomosis, before the magnets disengage and get expelled faecally[349]. The magnets are concurrently introduced endoscopically 50 - 100cm from the proximal and distal ends of the small intestine[349].

Although early studies have delivered promising results, application of this system in a real-world setting remains to be seen.

(5) Aspire Assist

This FDA approved device is essentially a gastrostomy which requires users to aspirate their gastric contents 20 minutes after ingestion of all meals exceeding 200kcal[350]. Results of a 4-year multicenter RCT has published favourable weight loss (18.7%) and modest glycaemic improvement (mean HbA1c difference 0.33%). However, this device has had issues with acceptability by

both patients and health practitioners, as is perhaps evident from the fact that only 15 of the 111 randomised patients maintained the device in place for the duration of the 4-year trial[350].

(6) Endoluminal Gastric Plication

Endoluminal gastric restrictive procedures for metabolic disease was first introduced 12 years ago but, despite many different application systems being manufactured since, its utility remains controversial and oft debated[351].

Currently the two primary FDA-approved systems in use are POSE (Primary Obesity Surgery Endoluminal) and the Apollo Overstitch. In the first, plication is carried out using expandable tissue anchors while the latter involves forming an endoscopic sleeve gastroplasty (ESG) using full-thickness sutures. A recent meta-analysis comparing the two techniques illustrated greater weight loss with ESG at 6 months and 12 months post-procedure[352]. However, there remains no convincing data on metabolic improvements with either of these devices.

TOGa (transoral gastroplasty), a gastric stapling system, has thus far been the only plication system which has been shown to have metabolic effects as well as significant weight loss [353]. A study by Mingrone and her colleagues on 9 normoglycaemic subjects with obesity demonstrated an increase in insulin sensitivity and a decrease in insulin secretion even as early as 3 months following the procedure[354].

Unfortunately, despite such promising results, the parent company declared insolvency due to insufficient funding and the future development of TOGa remains to be seen[355].

(7) Neuro-electrical Modulation

The mechanism of action of these devices is based on vagal modulation. Electrical leads are usually placed laparoscopically within the stomach wall and the pace generator is usually affixed in the subcutaneous tissue.

The Diamond TANTALUS system is activated by intraluminal contents in the stomach and delivers intermittent antral contraction for 75 minutes[355]. Initial results illustrate improvements in weight, glycaemic markers and lipid profile[356]. A more recent cross-over study in patients with well-controlled T2DM also demonstrates this with a 1% reduction in HbA1c[357]. However, the company is currently unable to procure the components needed for its manufacture, and production of the device has been discontinued.

Conversely, the FDA-approved vagal blocking therapy (VBLOC) uses high-frequency electrical currents to intermittently block the intrabdominal vagus nerve at the level of the gastro-esophageal junction. At 18 months, total weight loss (8.8%) appears sustained[358]. Additionally, it has also been shown to result in a sustained decrease in HbA1c and BP in patients with T2DM and arterial hypertension[359]. Despite this, VBLOC has yet to penetrate mainstream clinical practice in the management of metabolic disease.

Despite encouraging initial results with many of the therapeutic modalities listed here, there remains no panacea for metabolic disease.

Many treatment options have been hampered by adverse events; while those that have been deemed safe and feasible, have had problems with device manufacture (e.g. insufficient funding, challenges in the procurement of core material)

Further, even FDA-approved treatment options with good efficacy and safety profiles (e.g. VBLOC) have yet to be incorporated into conventional practice and continues to be viewed as experimental by many.

1.5 Duodenal Mucosal Resurfacing

Duodenal mucosal resurfacing is a novel metabolic procedure aimed at patients with insulin resistant conditions.

1.5.1 The role of the duodenum

The principle of the duodenum as a key metabolic signalling hub is central to the development and philosophy of the DMR technology. As the role of the duodenum and the foregut has been described in detail earlier in this chapter, it will only be re-visited briefly in this section.

Firstly, from a physiological perspective, the duodenum has a crucial role in nutrient metabolism. As the first site of fuel recognition, it is intimately involved in rapid nutrient-sensing mechanisms and glucose absorption. In addition, the putative insulin-resisting anti-incretin signal is assumed to emanate from the foregut as well. Putting this together, the duodenum functions to facilitate efficient fuel assimilation and to prevent the occurrence of hypoglycaemia.

From an evolutionary perspective, it is supposed that these features of duodenal function are selectively favoured over time, in keeping with the natural selection theory[241]. A highly absorptive duodenal mucosa would confer a survival advantage in times of food scarcity. According to the thrifty genotype theory, carriers are able to efficiently accumulate and process food, as fat, in times of food abundance[241]. This will then act as a reservoir in times of food shortage; improving their chances of survival, procreation and continued transmission of the genotype to future generations[241].

While historically advantageous for the hunter-gatherer, this genotype is no longer favourable in today's environment of copious calorific food as it deleteriously prepares the individual for a famine that never comes; while predisposing them to dysmetabolic conditions.

This notion also explains the high prevalence and the familial aggregation of metabolic conditions such T2DM, PCOS and obesity, which would have been expected to be selected out due to its detrimental effects.

To comparatively highlight the physiological role of the duodenum in nutrient absorption, Zhang *et al* and colleagues assessed the glycaemic and incretin response of nutrient administration directly into the duodenum or ileum in patients with T2DM and those without[116]. In both groups, direct duodenal nutrient introduction, resulted in higher blood glucose concentration, faster glucose absorption, lower GLP-1 concentrations and reduced incretin effect compared to ileal nutrient administration[116].

These findings are also similar to those of Nguyen *et al* who investigated glucose absorption in patients with obesity using duodenal biopsies before and following duodenal nutrient infusion[130]. The group found accelerated glucose absorption in the proximal small intestine with increased SGLT-1 expression, leading to an incretin profile predisposing to hyperinsulinaemia and hyperglycaemia[130].

These findings further support the role of the foregut in augmenting post-prandial glucose levels and insulin resistance while its bypass results in a glucose-reducing, insulin-sensitising effect.

In addition to its glycaemic-promoting effects detailed above, histological examination of the duodenal mucosa has also been seen to demonstrate maladaptive morphological and functional changes, particularly in response to chronic high sugar and high fat exposure.

Multiple studies have demonstrated that individuals with obesity, irrespective of glycaemic status, have longer small intestinal length and higher enterocyte mass, both of which contribute to a greater absorptive surface leading to the assimilation of nutritional excess[133, 134, 360]. In addition, patients with dysmetabolic disease, such as T2DM and obesity, also exhibit augmented intestinal permeability, increased intestinal inflammatory cells and altered microbiota compared to individuals without metabolic disease[361-363]. Further it has been demonstrated that patients with T2DM have

greater duodenal enterocyte turnover and proliferation of enteroendocrine cells compared to those with normoglycaemia[134, 364]. Much of these changes are focused on the hub of absorption – the foregut.

In studies conducted in animals induced with different obesity models, intestinal changes were seen to occur secondary to hyperphagia and increased nutrient presence in the intestinal lumen, regardless of the aetiology of obesity[365]. The intestine in these animals were seen to exhibit similar adaptive changes including greater villi lengths, increased cell hyperplasia, augmented cell permeability and amplified nutrient absorption[365]. Other studies using increased luminal nutrient content identified increased villi length, crypt depth, cell quantity and proliferation rate – all of which increased the absorptive surface of the intestine[133]. Similarly, Gniuli *et al* demonstrated mucosal hyperplasia of enteroendocrine cells with a propensity of GIP-secreting K cells in a high-fat fed animal experiment[366].

Conversely, the exclusion of luminal nutrients has the opposite effect, as seen in patients following surgical intestinal bypass and patients receiving total parenteral nutrition[367, 368]. Further, the adaptive changes of intestinal morphology following increased nutrient exposure appear unrelated to BMI or body composition, although subjects with obesity were seen to absorb more nutrients than their leaner counterparts[133].

These studies suggest that chronic overexposure to nutrients result in maladaptive changes in intestinal morphology and that luminal nutrient exposure may drive the changes seen in metabolic disease.

1.5.2 Manipulation of the foregut

As discussed in the preceding sections, metabolic procedures which incorporate an element of foregut bypass (e.g. RYGB, BPD, DJBL) have been shown to elicit profound glycaemic improvements and reversal of many of the changes seen in metabolic disease[334]. This has been demonstrated in multiple studies such as a recent study by Salinari *et al* in patients with obesity and insulin resistance who

underwent BPD and were found to have weight-independent normalisation of the anti-incretin effect and improvement in insulin sensitivity measures post-operatively[109].

A number of potential candidates have been postulated as possible anti-incretin factors, and several have been detailed in earlier sections, but thus far none have been identified with certainty. Alternatively, it is conceivable that the anti-incretin effect is driven by a combination of different factors.

Nevertheless, evidence for the presence of the effect is mounting[110]. In addition to evidence from foregut bypass procedures, studies investigating the effects of re-introduction of nutrients to previously bypassed foregut segments also point to the presence of an ant-incretin effect as illustrated by Rubino *et al* and more recently, Shimizu *et al*[108, 369]. Further, investigation in patients with the rare complication of gastrogastric fistula post-RYGB and its subsequent repair, also suggest a strong association between foregut nutrient re-exposure and the re-appearance of dysmetabolic state[370, 371]. These studies demonstrated T2DM relapse and decreased incretin factors in patients with a fistula which resolved following repair[370, 371].

Taken altogether, it is clear that the duodenum is a key metabolic signalling centre and critical regulator of glucose homeostasis. It emanates an insulin-resisting, glycaemia-enhancing effect which is upregulated with exposure to high-fat, high-sugar luminal contents. Further, evidence suggest that these potentially reversible pathological signals are likely associated with duodenal mucosal hyperplasia.

The underlying hypothesis for DMR is based on the theory that metabolic disease develops when there is either hyperplasia of the insulin-resisting proximal small intestine and / or hypoplasia of the satiety-inducing distal small intestine; and that effective metabolic therapies should aim to correct this balance by altering at least one side of this equilibrium[116]. This unifying hypothesis is coherent and congruent with current understanding of the pathophysiology of metabolic disease and the effect of metabolic surgery.

Hydrothermal ablation and the subsequent healing is thought to reverse the duodenal mucosal maladaptation, restore a normal mucosal interface, diminish the anti-incretin

effect and correct abnormal metabolic signalling. The desired effect of the DMR procedure is a compliance-independent mechanism for improvement in insulin resistance without needing to undergo permanent anatomical modifications and while eluding the risks associated with invasive surgery and/or polypharmacy.

1.5.3 The DMR technology

The Fractyl® DMR procedure is a safe and reliable, minimally-invasive, endoscopic procedure. This novel technique utilises a single-use polyethylene terephthalate balloon catheter attached to an electro-mechanical console to execute the 2-step process (see Figure 2.3 and Figure 2.4).

First, it induces a circumferential mucosal lift with sub-mucosal saline (in solution with methylene blue) injection to protect the deeper tissue layers from thermal injury. Subsequently, the 2cm balloon attached to the catheter is inflated with water heated to 90°C and used to thermally ablate each section of lifted mucosa for approximately 10 seconds each time.

The steps are repeated sequentially for 10-14cm and the treated area will encompass almost the entire post-papillary duodenum. The catheter is then removed fully and the patients is not left with any indwelling device.

The whole procedure is carried out under direct endoscopic visualisation with fluoroscopic control, in a day-case setting and usually takes between 45 – 60 minutes. Similar to other therapeutic endoscopic procedures, it can be carried out under GA or sedation with the patient in the left lateral decubitus or supine position. As a safety precaution, the device manufacturer also requires that all patients demonstrate evidence of a negative *Helicobacter pylori* test prior to the procedure.

As submucosal lifts and ablations are fairly commonplace therapeutic endoscopic techniques, the DMR procedure can be easily implemented in high-volume centres. Qualified therapeutic endoscopists usually only require a 1- or 2-day training programme to gain competence with the DMR procedure. The procedure does not necessitate any complicated steps or surgical incisions.

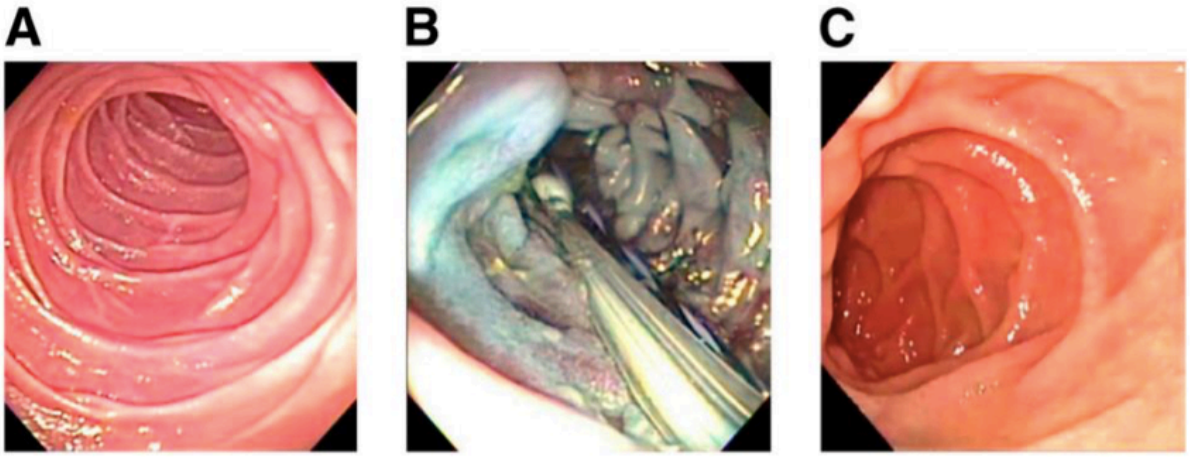

Figure 1.7 Duodenal mucosa prior to DMR (A), immediately after hydrothermal ablation (B) and healthy mucosa 1 month post-procedure at follow-up endoscopy (C)

(Source: Fractyl, Lexington. Used with permission)

Pre-procedure, all patients need to comply with standard pre-endoscopy regimes including a period of fasting. Prior to the procedure a thorough endoscopic examination of the upper GI tract is performed to assess for feasibility and to evaluate for potential precluding conditions such previous GI surgery or duodenal inflammatory conditions. Once suitability is confirmed, a guidewire is advanced past the ligament of Treitz and the DMR catheter is then advanced over the guidewire and the guidewire is removed. Post-procedure, all patients will need to adhere to a liquid and pureed diet regime for 10 days. By 12 weeks post procedure, rejuvenation of the new duodenal mucosa is complete (see Figure 1.7).

The Revita System received CE marking in June 2016 for its use in improving glycaemic control in patients with T2DM who have preserved pancreatic β-cell function and whose T2DM is poorly controlled with oral glucose lowering medications. In the UK it is currently being used in the research setting and is being introduced for use in the commercial sector imminently. It is also presently being appraised by NICE as a modality in metabolic disease.

1.5.4 Clinical Trial Data

To date, DMR has only been used in a research setting and over 200 patients have been treated with DMR worldwide. Aside from the DOMINO study, only patients with medically-treated T2DM have received the DMR procedure thus far.

The first-in-human, proof-of-concept study was published 3 years ago and reported favourable results in terms of improved glycaemia and HbA1c levels as well as improved insulin sensitivity in 44 patients with T2DM and a BMI of 25-40kg/m^2[372]. Patients were treated with both short-segment (mean 3.4cm) and long-segment (mean 9.3cm) ablation as the device was still in a procedure development stage; and safety and tolerability were also being assessed[372]. Patients who had a longer length of treatment experienced similar tolerability to the procedure but displayed superior metabolic results (2.5% reduction in HbA1c vs 1.2% at 3 months post-procedure) indicating an ablation dose-response[372].

Further, significant improvements in HOMA-IR and hepatic transaminases were reported as well[373]. Of note, only modest changes in body weight was evident early in the post-procedure course and this returned to pre-procedure levels by 6 months[373]. This indicates that the positive metabolic effects seen were a result of weight-independent mechanisms[373].

Interestingly, unpublished data presented at an international meeting (awaiting publication) illustrated changes in the metabolomic profiles in the subset of 14 patients who were analysed[374]. There was a pattern of diminished gluconeogenic dive, reduced lipotoxic stress, decreased Warburg effect (pro-oncogenic metabolic profile) and improved mitochondrial function[374].

More recently results from the REVITA-1 trial – an open-label, prospective, multicentre study in 46 patients with sub-optimally controlled T2DM (mean BMI 31.6 kg/m^2, mean HbA1c 8.6%) – was published and demonstrated sustained and durable improvements in glycaemic indices, insulin resistance and hepatic transaminase

levels at 12 months (see Figure 1.8)[375]. Again, there was only minimal weight loss seen, indicating a weight-independent mechanism[375].

Since publication, 24-month results have emerged which show continued sustained reduction in HbA1c at 2 years following DMR with a mean (±SEM) HbA1c of 8.5 (±0.1)% at baseline, 7.6 (±0.2)% at 12 months and 7.5 (±0.2)% at 24 months][374].

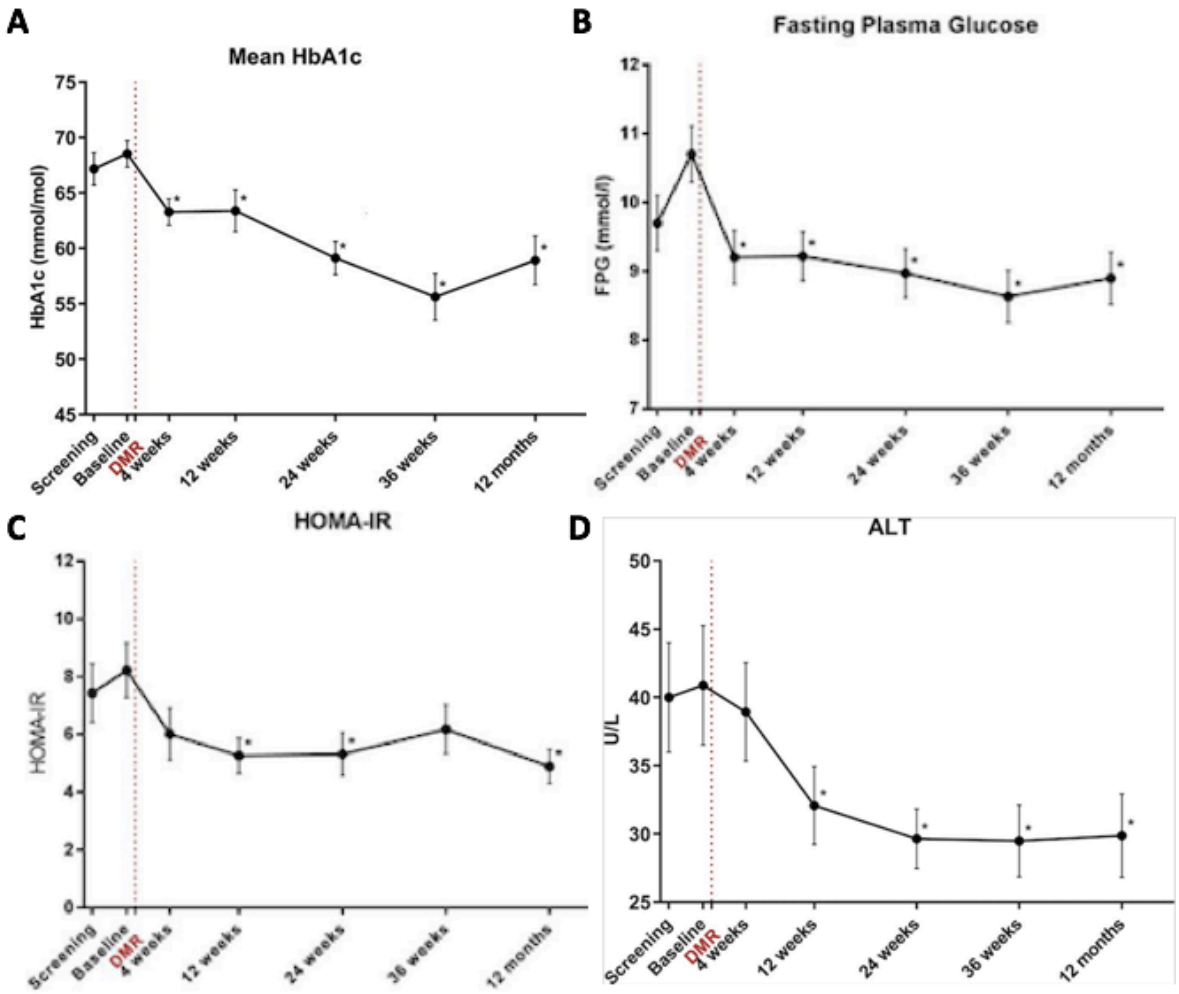

Figure 1.8 Changes in HbA1c (A), Fasting Plasma Glucose (B), HOMA-IR (C) and ALT (D) at 12 months following DMR.

(Presented as Mean ±SE. Source: Adapted from van Baar ACG, Holleman F, Crenier L, Haidry R, Magee C, Hopkins D, et al. Endoscopic duodenal mucosal resurfacing for the treatment of type 2 diabetes mellitus: one year results from the first international, open-label, prospective, multicentre study. Gut. 2019.)

In the recently completed REVITA-2 study, the first randomised, sham-controlled DMR trial, participants who were on the treatment arm demonstrated significant reduction in HbA1c at 24 weeks, significant decrease in liver fat content on MRI at 12 weeks and significant reduction in the level of transaminases at 12 weeks[374].

These initial results are very favourable to the metabolic benefits of the DMR procedure in T2DM and other insulin resistant conditions such as NAFLD, and further results are eagerly awaited[374].

Pertinently, there were no serious adverse events or unanticipated adverse device events reported in either REVITA-1 or REVITA-2 for the duration of both trials.

1.5.5 Safety and acceptability

DMR provides a very attractive model in the treatment of metabolic disease.

It proposes the use of a minimally invasive technique with good metabolic efficacy and is carried out as a day-case procedure under deep sedation or general anaesthetic. In addition, patients who have undergone the DMR procedure would have no indwelling device, surgical insult or suturing and no requirement for daily medication and its associated compliance. No other therapeutic option in chronic metabolic disease has been able to manage a similar endeavour.

With more than 200 patients worldwide treated so far, DMR has displayed an acceptable safety profile. Thus far, there have been no unanticipated adverse device effects and no device or procedure related deaths. In contrast to DJBL, there have also been no procedure-related infections including occurrence of abscess or sepsis. Further there have been no incidence of procedure-related pancreatitis, GI bleeding or injury to surrounding organs.

In the first iteration of the device, used in the proof-of-concept study, a more rigid dual-catheter system was utilised. This was associated with longer procedure time (~90 minutes) and higher risk of duodenal stenosis. Since then the device has been

significantly modified and all subsequent procedures have been carried out using the less rigid second generation single-catheter system.

With the previous system, three patients developed duodenal stenosis within a few weeks of the procedure. All three patients were managed with endoscopic balloon dilatation with full symptom resolution and no long-term sequelae. No episodes of stenosis have been reported with the use of the modified catheter system.

The most commonly described adverse events have been transient and mild or moderate in severity and related to abdominal discomfort, flatulence or sore throat in the early post-procedure period, most often related to air insufflation or the endotracheal tube. Uncommonly, patients who are on concomitant glucose-lowering medication have had mild, short-lived episodes of hypoglycaemia in the early post-procedure period.

1.5.6 Future Direction

Currently, measures are underway to implement DMR utilisation in the clinical setting for patients with T2DM in both the private and public sectors.

However, in view of the promising findings from DMR studies indicating improvement in insulin sensitivity, it is reasonable to postulate that the procedure has potential application in other insulin resistant conditions such as PCOS and NAFLD.

PCOS is a particularly interesting target due to its high prevalence, dearth of PCOS-specific therapies and the potential to impact fertility via the complex interaction between its metabolic and reproductive features.

Therefore, the DOMINO Trial was conceived – the first randomised, double-blinded sham-controlled clinical trial assessing the efficacy of DMR in insulin-resistant women with PCOS and a BMI ≧30kg/m^2. This was also the first DMR trial being conducted in patients without T2DM and the first trial utilising hyperinsulinaemic, euglycaemic clamps – the gold-standard method – to assess insulin sensitivity.

1.6 Trial Hypothesis

The hypotheses of the DOMINO randomised controlled trial (RCT) are:

1. DMR will improve insulin sensitivity in women with PCOS, insulin resistance and oligomenorrhoea.

2. Improved insulin sensitivity in this population will lead to improved ovulation and menstrual regularity.

Women with PCOS but without T2DM were selected as the model of insulin resistance for the DOMINO RCT to help circumvent the pharmacological effect of concurrent glucose-lowering medication on insulin sensitivity and insulin secretion.

In addition, this cohort permits further evaluation of the impact of metabolic changes on reproductive function in terms of ovulation and menstrual regularity.

1.7 Trial Objectives

The main objectives of the DOMINO RCT are as follows:

1. Investigate the efficacy of the Fractyl® DMR procedure using the Revita System compared to a sham procedure for the treatment of women with PCOS, insulin resistance and oligomenorrhoea.

2. To study the effect of DMR on mechanistic and clinical endpoints 24 weeks post-procedure, in terms of

    a. Insulin sensitivity

    b. Ovulation and Menstruation

3. To gain further understanding of the mechanism of action of the DMR procedure in this population.

2 Chapter 2 Methodology

2.1 Study Design

The DOMINO Trial was a mechanistic study conducted using a multi-centre prospective double-blinded sham-controlled RCT design at two academic clinical centres in the UK – Imperial College London and University Hospitals Coventry and Warwickshire.

Thirty women of reproductive potential with PCOS, insulin resistance and oligomenorrhoea were recruited. All participants were randomised, using a computer software algorithm at a 1:1 ratio to receive lifestyle modification with DMR or a sham endoscopic procedure.

Following randomisation, all participants were followed up for 6 months (see Figure 2.1).

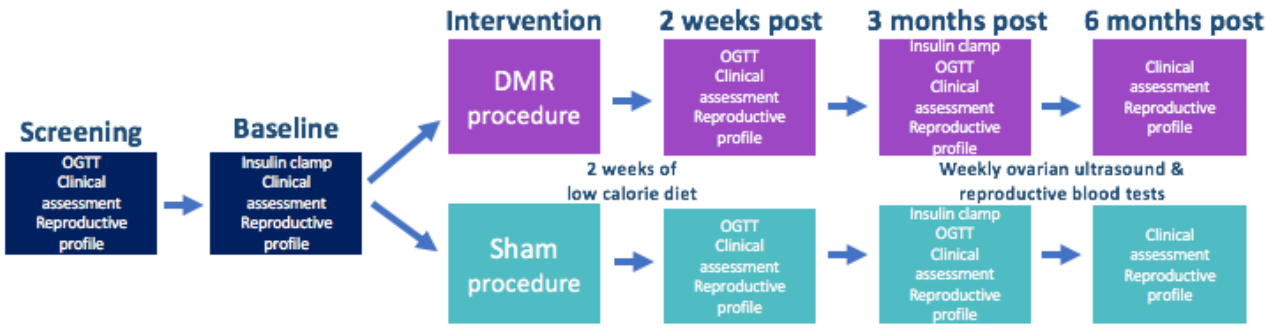

Figure 2.1 DOMINO Trial Flow Diagram

(Source: Author's own)

2.1.1 Trial Registration and Regulatory Aspects

2.1.1.1 Ethical Approval

The study protocol (see Appendix 1) and design was approved by the London-Dulwich Research Ethics Committee (REC reference number: 17/LO/1095). The study was

conducted in accordance with the guidelines for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions[376].

2.1.1.2 Trial Registration

The trial is registered with the International Standard Randomized Controlled Trial Registry (ISRCTN Number: 76278694) and with the Health Research Authority via the Integrated Research Application System (IRAS ID: 225278).

2.1.1.3 Trial Funding and Sponsorship

The DOMINO Trial was funded through the Fractyl® investigator-initiated study programme, and was sponsored by Imperial College London. The funder and sponsor had no role in any part of the trial handling including recruitment of participants, management of the trial, the accrual of data or the analysis of results.

2.1.1.4 Trial Management

The study was conducted in adherence to the applicable regulatory requirements of the International Conference on Harmonization Good Clinical Practice[377] with all applicable subject privacy requirements including observance of the European Union General Data Protection Regulation[378] and was conducted in accordance with the recommendations of the Declaration of Helsinki[376].

Consent to participate in the study was sought from each participant only after a full explanation has been given, an information leaflet provided and time allowed for consideration. Informed written consent was then obtained from all participants (see Appendix 2).

The Chief Investigator of the Trial is Dr Alexander Miras, Senior Clinical Lecturer in Endocrinology at the Department of Investigative Medicine, Imperial College London.

I conducted the day-to-day management of the trial at the NIHR Imperial Clinical Research Facility, Imperial College London. Co-investigators and collaborators on the

trial are Dr Channa Jayasena Dr Dev Bansi, Dr Belen Perez-Pevida, Professor Harpal S Randeva, Dr Georgios K. Dimitriades, Dr Barbara Fielding and Dr Bu'Hussain Hayee.

2.1.2 Blinding Procedure

This was a double-blinded randomised controlled trial. Participants, investigators and all members of the research team at both study sites were blinded to treatment allocation until the trial was completed.

Only the team carrying out the endoscopy and an external researcher responsible for randomisation were aware of the treatment allocation, neither of whom were involved in trial management, data collection or analysis.

2.1.3 Patient and Public Involvement

Advice on the trial concept, protocol and information sheet were sought from Patient and Public Involvement (PPI) panels in accordance with the NIHR Imperial Clinical Research Facility PPI strategy. This advice was utilised to aid in participant recruitment and retention.

In addition, the trial was supported by Verity, the largest national UK PCOS Charity Group. Regular consultations were carried out with the charity at multiple points throughout the duration of the trial. This consisted of face-to-face meetings with Verity Trustees as well as a podium presentation to Verity members about the trial at the Verity National Conference (Leeds, Nov 2018).

2.2 Inclusion and Exclusion Criteria

2.2.1 Inclusion Criteria

Participation in the trial was limited to female patients aged between 18 and 50 years with:

(1) a diagnosis of PCOS based on the National Institutes of Health (NIH) criteria which required all of the following
   - Menstrual irregularity (anovulation or > 35-day cycle)
   - Clinical or biochemical hyperandrogenism
   - Exclusion of other aetiologies of menstrual dysfunction (e.g. thyroid dysfunction, hyperprolactinaemia)

(2) Insulin resistance as defined by a 2-hour 75g OGTT concentration of >7.8 mmol/l and/or a Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) ≥ 3.0.

(3) A body mass index (BMI) ≥30 kg/m and

(4) willing to comply with study requirements and able to give informed consent

2.2.2 Exclusion Criteria

The exclusion criteria for the trial included the following

- Type 1 or Type 2 diabetes mellitus
- Medications affecting insulin sensitivity at screening or 2 months previously.
- More than 6 menstrual bleeds in the previous 12 months
- Unable to maintain abstinence or barrier contraception for the duration of the study
- Pregnancy, breastfeeding or smoking at screening or in the 6 months prior
- Blood donation in the 3 months prior to recruitment or intended blood donation during the trial
- Without access to a telephone / other factor that interferes with ability to participate reliably in the trial
- History of any condition or medication use that, in the opinion of the investigator, may interfere with study participation including:
   - Active *Helicobacter pylori* infection
   - Previous GI surgery that could affect ability to access or treat the duodenum
   - Active systemic infection, liver disease, severe kidney disease (eGFR <30 ml/min/1.73m^2), symptomatic gallstones or kidney stones

- History of pancreatitis, duodenal inflammatory disease including Crohn's disease and coeliac disease,
- History of coagulopathy or upper GI bleeding conditions
- Use of anticoagulation / P2Y12 inhibitors / non-steroidal anti-inflammatory drugs / corticosteroids / drugs affecting GI motility and unable to stop for the required period pre- and post-procedure
- Persistent anaemia (haemoglobin <10 g/dl)
- Active malignancy within the last 5 years
- Poor candidate for surgery or general anaesthesia
- Active illicit substance abuse or alcoholism

2.3 Sample Size Calculation

Assumptions of effect size for the primary efficacy endpoints in the treatment arm were derived from previous publications in which insulin sensitising medications were administered in similar groups of women[204, 379] and also takes into account information from previous studies on DMR.

It is assumed that:

1. a difference in mean change in total insulin sensitivity (as assessed by insulin clamp) between treatment and control of 2.79 µmol/kg.min at 12 weeks with equal variance in both groups (standard deviation (SD) of 4.50), which gives a standardised effect size of 0.62 (= 2.79/4.50). Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity.
2. a standardised effect size of 0.91 for the change from baseline in insulin sensitivity at 24 weeks as assessed by HOMA-IR.
3. a difference in the number of menses between treatment and control of 1.0 over 24 weeks with equal variance in both groups (SD of 1.0), which gives a standardised effect size of 1.0.

The Hochberg procedure to adjust for multiple endpoints and decrease the risk of Type 1 error was used and described in the DOMINO Trial Statistical Analysis Plan (see

Appendix 4). Under this procedure 24 randomised subjects (12 per group) provides approximately 82.8% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.050 significance level, and provides approximately 72.9% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.025 significance level.

Thirty participants will be randomised to account for potential participants lost to follow up prior to the primary endpoint assessment. Participants who for technical reasons cannot have the DMR will be replaced.

2.4 Participant Recruitment

Participant recruitment for this study was conducted in 3 ways:

(1) Referral from Medical Clinics

Participants who were reviewed in participating clinics at the 2 research centres and who fit the trial criteria and were willing to take part in the trial were contacted by the research team with further information about the trial.

(2) Review of Clinic Letters

I reviewed clinic letters for all patients who had attended the Reproductive Endocrinology clinic at Imperial College Healthcare NHS Trust from to December 2016 to April 2018. I then contacted any patients who fit the study criteria with further information about the trial.

(3) Online recruitment using Social Media

I worked with Clariness GmbH, Hamburg, an international participant recruitment company for clinical trials, to build and develop a recruitment website (www.clinlife.com/pcos).

I designed the website with an online pre-screener questionnaire based on the trial inclusion and exclusion criteria. I then created online awareness of the trial via targeted social media banner advertising (see Figure 2.2) and search engine marketing with links to the recruitment website.

All participants who completed the online pre-screener questionnaire (see Appendix 3) and fit the trial criteria were invited to leave their contact details, if they wished to be in the study. I then contacted these women with further information about the trial.

Figure 2.2 Examples of banner advertisements used on social media

(Source: Design by author and Clariness GmbH, Hamburg, Used with permission)

2.5 Trial Structure

2.5.1 Study visits

The trial structure comprised of 6 main study visits. These consisted of 2 pre-procedure visits (Screening Visit and Baseline Visit), intervention visit and 3 follow-up visits at 2 weeks, 12 weeks and 6 months post-intervention (see Figure 2.1)

Thirteen weekly reproductive visits were undertaken from week 12 to week 24 following the intervention.

The visit components are as detailed following.

Participants also had open telephone and email access to me throughout the trial duration, and if required were reviewed more frequently.

2.5.1.1 Screening visit

Following recruitment, participants who were willing to enrol in the study were invited to attend a screening visit at the NIHR Imperial Clinical Research Facility, Imperial College London. Participants were instructed to attend following a 12-hour overnight fast.

The trial design, including recruitment criteria, format and expectations for each clinical and mechanistic visit, risks and benefits of trial participation, randomisation process and the intervention were all re-discussed with the participant.

Formal, written consent was then obtained (see Appendix 2).

The screening visit was used to assess each participant for eligibility based on the trial inclusion and exclusion criteria (see Section 2.2).

This visit comprised of the following detailed assessments

1. A full medical and reproductive history
2. Routine physical examination

3. Height, weight, anthropometric measures and body composition analysis using a bioelectrical impedance analyser (Tanita MC-780MA P, Tanita, Amsterdam)
4. Electrocardiogram
5. Urine pregnancy test
6. Blood tests – full blood count, urea and electrolytes, liver function tests (LFTs), thyroid function tests, HbA1c, lipid profile, vitamin, mineral and metabolite levels and a coagulation screen.
7. Biochemical reproductive profile for luteinising hormone (LH), follicle-stimulating hormone (FSH), progesterone, oestrogen, testosterone, sex hormone-binding globulin (SHBG), androstenedione and dehydroepiandrosterone sulphate (DHEAS).
8. Assessment of insulin secretion with an OGTT performed as per standard protocol used for human studies at the Department of Investigative Medicine, Imperial College London (see Section 2.5.2.1)
9. Assessment of insulin sensitivity with HOMA-IR
10. Stool collection for *Helicobacter pylori* antigen (unless recent negative *Helicobacter pylori* stool test or rapid urease test result available). If found positive, the participant would undergo standard *Helicobacter pylori* eradication as per local hospital guidelines and would be re-tested following completion of eradication. Participation would only proceed once negative test result available.

If any incidental clinical abnormalities were identified at the screening visit, participants were managed as per standard NHS management practice.

All participants who fit the trial criteria were given a course of Medroxyprogesterone (10mg a day for 10 days) to induce a menstrual period before they attended the Baseline Visit. The induction of the menstrual period was to allow study of all participants at the same phase of their menstrual cycle.

All participants were provided a link to an online menstrual diary tool (SurveyGizmo, Boulder, CO) and asked to record their periods throughout the duration of the trial

following confirmation of eligibility. Participants were also provided a food diary and asked to fill it in for 3 days in the week prior to their next visit.

2.5.1.2 Baseline Visit

All participants who were eligible following their Screening Visit, were invited join the trial and attend a Baseline Visit. Baseline visits were arranged at the NIHR Imperial Clinical Research Facility, Imperial College London approximately 4 weeks prior to the planned date of intervention.

Participants were asked to refrain from alcohol consumption and strenuous physical activity for 48 hours prior to their visit attendance and to come in after a 12-hour overnight fast.

On arrival for their visit participants were assessed with the following tests

1. Height, weight, anthropometric measures and body composition analysis using a bioelectrical impedance analyser (Tanita MC-780MA P, Tanita, Amsterdam)
2. Vital signs
3. Urine pregnancy test
4. Blood tests – Full blood count, urea and electrolytes, liver function tests (LFTs), HbA1c, lipid profile, vitamin, mineral and metabolite levels and a biochemical reproductive profile (LH, FSH, progesterone, oestrogen, SHBG, DHEAS, testosterone and androstenedione).
5. The 2-phase euglycaemic hyperinsulinaemic clamp performed as per standard protocol used for human studies at the Department of Investigative Medicine, Imperial College London (see Section 2.5.2.2).

Participants were asked to provide their completed food diary. Participants were also provided a food diary and asked to fill it in for 3 days in the week prior to their next mechanistic visit.

2.5.1.3 Randomisation

All eligible participants were randomised using a computer software in a 1 : 1 manner (DMR : sham). The elected randomiser, Prof Tricia Tan , Professor of Endocrinology used sealed envelopes to convey the randomisation allocations to the Endoscopy Team on the day of the intervention for each participant.

2.5.1.4 Intervention

All procedures were performed endoscopically under General Anaesthesia by a single endoscopist (Dr Devinder Bansi, Consultant Gastroenterologist) at Imperial College Healthcare NHS Trust.

Prior to the day of the intervention, participants were instructed to fast overnight and attend the Endoscopy Unit in the morning. Dr Bansi discussed the procedure with each participant and they were then asked to provide procedural consent for the intervention.

All participants were then anaesthetised in a standard manner. Once anaesthetised, participants were placed in a lateral decubitus position and a standard screening endoscopy would be carried out to assess for procedural feasibility before proceeding. If appropriate to proceed, a guidewire would then be delivered past the ligament of Treitz to assist in delivering the catheter. Anti-peristaltic agents may be used during the procedure.

Then the randomisation envelope would be opened and the allocated procedure would be carried out. Participants in both arms of the trial would have the Revita Catheter (see following section and Figure 2.3) inserted and advanced under fluoroscopic guidance. The use of fluoroscopy is limited to use during catheter placement and verification of location during treatment. A lead apron drape would be placed on the participant's abdomen and pelvis during the procedure to protect the reproductive organs.

The entire procedure is completed in the endoscopy suite under general anaesthesia.

Unforeseen events (findings or procedures) may occur during either the DMR or sham procedure. These unforeseen events are those that are not planned as part of this procedure (e.g. a drop in oxygen saturation or evidence of intestinal bleeding, etc.). Unforeseen events that are emergent in nature should be recorded as adverse events and the investigator would reassess the subject's suitability for continued participation in this study.

2.5.1.4.1 Duodenal Mucosal Resurfacing (DMR)

The DMR procedure is carried out using the Fractyl® Revita System (Fractyl, Lexington) which consists of two main components – the Revita catheter and a console.

Figure 2.3 Revita Catheter

(Source: Fractyl, Lexington. Used with permission)

The Revita Catheter (see Figure 2.3) is a sterile, single use device that performs two functions:

1. it injects saline (in solution with methylene blue) into the submucosa of the duodenum to create a thermal barrier while also lifting the mucosa with saline to create a more uniform surface for ablation; and

2. ablates the mucosal surface using heated water recirculating inside a balloon.

To achieve its function, the Revita Catheter is constructed of a multi-lumen shaft with a 24mm (outer diameter) balloon affixed to its distal end. Affixed to the outside of the balloon are three narrow shafts with a port that are used to draw a vacuum when placing the saline during the mucosal lifting portion of the procedure. Within each shaft is a fluid lumen with a miniaturized needle affixed to the distal end. Each needle is wholly constrained within the port ensuring its safe use.

During the mucosal lift, the tissue is drawn into the needle port, and saline (in solution with methylene blue) is injected into the submucosal space through the needles. The proximal end of the shaft is fitted with a handle and saline and vacuum lines that are affixed to a console unit to control its function.

Console: The console is a reusable electro-mechanical piece of equipment and provides functionality to the submucosal lift and hot fluid ablation steps of the procedure. It is controlled through the use of a software user interface monitor. Prior to use, it is fitted with a sterile single use line set that serves as the pathway for the saline to be placed into the duodenal submucosa during the procedure.

The Revita catheter is placed in the proximal duodenum distal to the papilla. Using the console interface, the balloon is inflated and vacuum delivered to draw the intestinal mucosal tissue onto the ports located on the balloon. The actuator on the handle is moved to advance the needle into the submucosal space within each of the ports.

The console delivers saline into the submucosal space through the needles within the lumens of the catheter resulting in complete circumferential lift of the mucosa. Once complete, the ablation cycle is started and hot water is circulated into the balloon to complete an ablation of the previously expanded tissue (see Figure 2.4). The balloon is deflated and the catheter repositioned distally to the next segment to be treated. Following completion of all submucosal lifts and ablation, the Revita catheter and endoscope are then removed entirely.

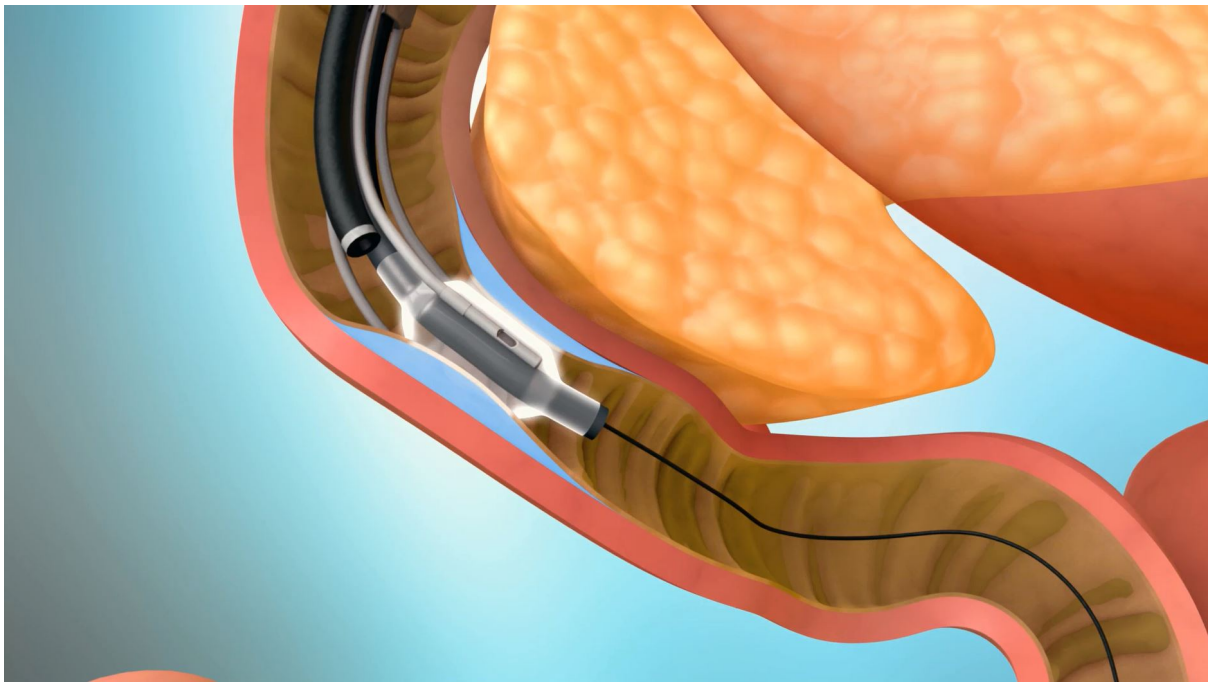

Figure 2.4 Schematic image of endoscopic submucosal lift and mucosal ablation using the Fractyl® Revita System

(Source: Fractyl, Lexington. Used with permission)

The number of ablations completed in the duodenum was determined by the distance between the papilla and the ligament of Treitz. This distance is variable based on individual anatomy.

2.5.1.4.2 Sham

The sham procedure consisted of placing the DMR catheter into the participant's duodenum under general anaesthesia and leaving it in place for a minimum of 30 - 45 minutes and then removing it from the participant.

2.5.1.4.3 Post-procedure care and diet

Immediately following the procedure, participants were transported to the recovery area and monitored according to the hospital standard care protocol for endoscopic procedures and post-anaesthesia recovery.

Prior to discharge, all subjects were examined and evaluated for the presence of any adverse events that may have occurred between the procedure and discharge. A

participant's hospital stay can be extended based on need as determined by the Investigator. Subjects were eligible to be discharged when they met the hospital's criteria for discharge as per the local recovery from general anaesthesia protocol.

Following intervention participants in both groups were asked to consume the same low-calorie diet (1200 kcal/d consisting of 16% protein, 49% carbohydrate and 35% fat) for 14 days using 125 ml Fortisip Compact Protein (Nutricia, Wiltshire). No recommendation for diet or calorie restriction were made beyond the first 14 days.

2.5.1.5 2-week post-procedure visit

These visits were scheduled 14 days after the intervention. Participants were asked to attend the NIHR Imperial Clinical Research Facility following a 12-hour overnight fast. The following assessments and procedures were performed:

1. Clinical assessment
2. Height, weight, anthropometric measures and body composition analysis using a bioelectrical impedance analyser (Tanita MC-780MA P, Tanita, Amsterdam)
3. Vital signs
4. Urine pregnancy test
5. Blood tests – full blood count, urea and electrolytes, liver function tests (LFTs), thyroid function tests, glucose, insulin, HbA1c, lipid profile, vitamin, mineral and metabolite levels, coagulation screen and a biochemical reproductive profile (LH, FSH, progesterone, oestrogen, SHBG, DHEAS, testosterone and androstenedione).
6. Assessment of insulin sensitivity with HOMA-IR
7. Assessment of insulin secretion with an OGTT performed as per standard protocol used for human studies at the Department of Investigative Medicine, Imperial College London (see Section 2.5.2.1)

Information were also collected with regard to the participant's current medications and any adverse events.

Participants would also be asked to provide their completed food diary and were also provided a food diary and asked to fill it in for 3 days in the week prior to their next visit.

2.5.1.6 12-week post-procedure visit

Twelve weeks following the intervention all participants were asked to come in for their 12-week post procedure assessment. Participants were asked to attend the NIHR Imperial Clinical Research Facility following a 12-hour overnight fast. This visit was usually carried out over two consecutive days. The visit consisted of:

1. Height, weight, anthropometric measures and body composition analysis using a bioelectrical impedance analyser (Tanita MC-780MA P, Tanita, Amsterdam)
2. Vital signs
3. Urine pregnancy test
4. Blood tests – full blood count, urea and electrolytes, liver function tests (LFTs), thyroid function tests, glucose, insulin, HbA1c, lipid profile, vitamin, mineral and metabolite levels, coagulation screen and a biochemical reproductive profile (LH, FSH, progesterone, oestrogen, SHBG, DHEAS, testosterone and androstenedione).
5. Assessment of insulin sensitivity with HOMA-IR
6. Day 1: Assessment of insulin secretion with an OGTT performed as per standard protocol used for human studies at the Department of Investigative Medicine, Imperial College London (see Section 2.5.2.1)
7. Day 2: 2-phase euglycaemic hyperinsulinaemic clamp performed as per standard protocol used for human studies at the Department of Investigative Medicine, Imperial College London (see Section 2.5.2.2).

Information would also be collected with regard to the participant's current medications and any adverse events.

Participants would also be asked to provide their completed food diary.

2.5.1.7 Weekly reproductive assessments

From 12 weeks following the intervention to week 24 post-intervention, participants were invited to attend weekly visits for reproductive assessments. These visits were held at the both Imperial College Healthcare NHS Trust as well as at University Hospital Coventry and Warwickshire NHS Trust and participants attended at the site that they were recruited from.

At the weekly reproductive visits participants were assessed with

1. Pelvic ultrasound scan measuring
    - endometrial thickness (in millimetres)
    - mean ovarian volume (in cubic centimetres)
    - mean follicle number
    - maximum diameter of largest follicle in each ovary (in millimetres).
2. Biochemical reproductive profile including blood samples for the measurement of LH, FSH, progesterone, oestrogen, SHBG, DHEAS, testosterone and androstenedione

Ovulation will be defined as a rise in serum progesterone >10 nmol/L together with suggestive radiological features (visualization of a dominant follicle with subsequent appearance of a preovulatory follicle and/or corpus luteum).

The ultrasounds will be either transabdominal or transvaginal depending on views obtained and participant preference.

The ultrasonographers at both sites were well-experienced at gynaecological scans. The ultrasonographers were blinded to the treatment allocation.

2.5.1.8 6-month clinical visit

The 6-month post-procedure follow-up visit is the final trial visit for participants. This visit is held at the NIHR Imperial Clinical Research Facility and participants were asked to fast for 12 hours prior to attending. It comprised of the following assessments.

1. Height, weight, anthropometric measures and body composition analysis using a bioelectrical impedance analyser (Tanita MC-780MA P, Tanita, Amsterdam)
2. Vital signs
3. Urine pregnancy test
4. Blood tests – full blood count, urea and electrolytes, liver function tests (LFTs), thyroid function tests, glucose, insulin, HbA1c, lipid profile, vitamin, mineral and metabolite levels, coagulation screen and a biochemical reproductive profile (LH, FSH, progesterone, oestrogen, SHBG, DHEAS, testosterone and androstenedione).
5. Assessment of insulin sensitivity with HOMA-IR

Information would also be collected with regard to the participant's current medications and any adverse events.

Participants would also have their re-imbursement for travel, time and inconvenience processed at this visit.

2.5.2 Mechanistic visits

Mechanistic visits for the DOMINO trial consisted of

1. OGTT, and / or
2. Euglycaemic hyperinsulinaemic clamps

All mechanistic visits were carried out the NIHR Imperial Clinical Research Facility, Imperial College London.

2.5.2.1 Oral Glucose Tolerance Tests

Participants were assessed with a 75g 180-minute OGTT at

- Screening Visit
- 2 weeks post-intervention
- 12 weeks post-intervention

All participants were instructed to fast overnight for 12 hours prior to attending the research facility. An arm warmer was applied to promote vasodilation to facilitate sequential sampling. An intravenous catheter was placed in a vein in the antecubital fossa.

A solution of 75g of glucose powder (Oxford Pharmaceuticals, Alabama) dissolved in 300ml of water was made up.

Blood samples were obtained 30 minutes prior to the start of the OGTT (-30 min) and at time point 0. Participants were instructed to consume the solution entirely at time point 0 minute.

Blood samples were then taken at a further 5 time points following consumption of the 75g glucose solution at +15 minutes, +30 minutes, +60 minutes, +120 minutes and + 180 minutes. Vital signs were taken at each time point and participants were asked to complete visual analogue scales to indicate their levels of hunger, sickness, fullness, sleepiness and stress.

Following venesection, all blood samples were immediately placed on ice except for the samples for glucose, insulin and c-peptide. Glucose samples were handled as detailed below while the tubes containing samples for insulin and c-peptide were left out at room temperature to allow clot formation.

Blood glucose measurements were immediately taken at each time point using a bed-side glucose analyser (YSI 2300 STAT Plus Glucose and Lactate Analyser, YSI Inc, Yellow Springs, OH). Glucose samples were also taken at each time point to be sent for analysis at the NHS Laboratories of Imperial College Healthcare NHS Trust (North West London Pathology).

Blood samples were additionally taken at each of these time points for storage for the analysis of insulin, c-peptide, gut hormones, ghrelin, bile acids, FGFs, FFA and plasma metabolites. Samples were centrifuged for 10 minutes at 4°C at 4000 rpm. The separated plasma was initially stored in a -20°C freezer until the end of the visit and then transferred to -80°C freezer for long-term storage.

Following completion of the OGTT assessment, participants were provided a lunch option of their choice. They would have their blood glucose checked again with the bed-side glucose analyser after the meal. They were discharged from the NIGH Clinical Research Facility once they had a blood glucose measurement above 4.0 mmol/L (72 mg/dL).

2.5.2.2 Euglycaemic Hyperinsulinaemic Clamp

Participants were assessed with a 2-phase euglycaemic hyperinsulinaemic clamp using an isotope dilution technique when they attended for the

- Baseline visit
- 3-month post-intervention visit

On the evening before the visit, participants were instructed to consume a standardised meal which consisted of two bottles of Ensure Plus 200mls (Abbott, Maidenhead) at 5pm. Following this, participants were permitted to consume a liquid diet (including soup) until 9 pm. Participants were only allowed to plain, clear water overnight.

On the morning of the visit, a blood glucose measurement was first taken to check that blood glucose measurements were between 4.0 - 6.0 mmol/L (72 – 108 mg/dL). Two intravenous cannulae were then placed, one in each antecubital fossa. One cannula was used for blood sampling and the other for administration of the infusates. An arm warmer was applied on the side used for blood sampling to promote vasodilation to facilitate sequential sampling.

Before the clamp is started, the participant's vital signs were checked and the participant was asked to complete a visual analogue scale to indicate their levels of hunger, sickness, fullness, sleepiness and stress.

At time point -120 minute, 1.7mls of the [6, 6-$^{2}H_{2}$] stable glucose isotope was injected as a priming bolus. A pre-prepared infusion of [6, 6-$^{2}H_{2}$] glucose isotope was then

started at a fixed rate of 5.9 ml/hr and continued for the duration of the clamp. It is estimated that the isotope will achieve equilibrium within 120 minutes.

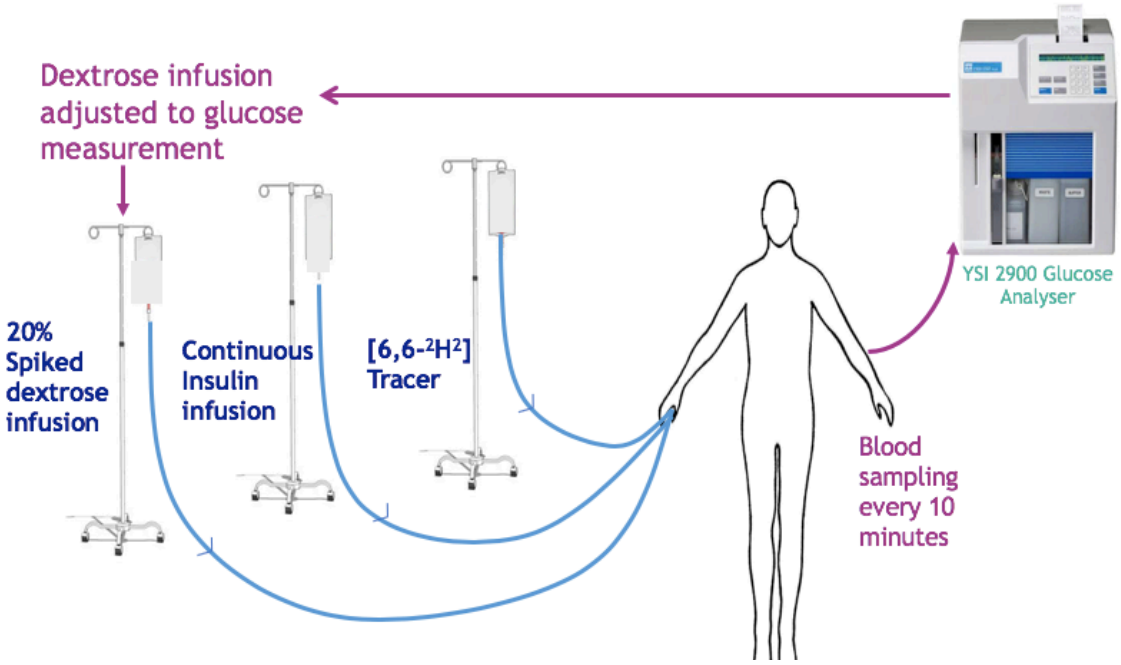

Figure 2.5 Schematic image of the set-up used for the 2-phase euglycaemic hyperinsulinaemic clamp studies

(Source: Adapted from Kamocka, A, Imperial College London. Used with permission)

The tracer used for euglycaemic hyperinsulinaemic clamps is a naturally occurring metabolite which is labelled with a stable, non-radioactive label. It was supplied for use in the DOMINO Trial by Cambridge Isotopes Ltd through their UK suppliers CK Gases Ltd and prepared as a quality-assured sterile solution suitable for intravenous infusion by the Pharmacy Production Unit at Guys & St Thomas' NHS Trust.

In the final 20 minutes of the equilibrium phase, blood glucose samples were taken every 5 minutes (-20, -15, -10, -5 minutes) using the bed-side glucose analyser (YSI 2300 STAT Plus Glucose and Lactate Analyser, YSI Inc, Yellow Springs, OH). These levels were used as a reference basal glucose level for the euglycaemic hyperinsulinaemic clamp. The participants vital signs are also checked.

At time point 0, the first stage of the 2-phase euglycaemic hyperinsulinaemic clamp was started. An insulin infusion was commenced at a rate of 0.5 mU/kg/min (low-dose) for 2 hours. A second infusion made up of 500mls of 20% dextrose admixed with 8ml of the [6, 6-$^{2}H_{2}$] glucose isotope is infused at a variable rate to maintain blood glucose concentrations of ±0.5 mmol/L of the basal level. Hepatic insulin resistance is assessed at this stage.

After 2 hours (time point +120), the 2nd stage of the euglycaemic hyperinsulinaemic clamp was commenced. For this stage, the insulin infusion rate is tripled to 1.5 mU/kg/min (high-dose) to effectively clamp endogenous glucose production by the liver. This allows assessment of peripheral (muscle and fat) insulin resistance. A further 2 ml bolus of the stable [6, 6-$^{2}H_{2}$] glucose isotope is admixed into the 20% dextrose infusate to maintain the plasma tracer enrichment for accurate assessment of endogenous glucose production. This stage lasts a further 2 hours (from +120 to +240 minutes).

To maintain euglycaemia throughout the euglycaemic hyperinsulinaemic clamp, frequent blood glucose analysis was required. Blood glucose measurements were taken every 10 minutes using the bed-side glucose analyser (YSI 2300 STAT Plus Glucose and Lactate Analyser, YSI Inc, Yellow Springs, OH). The dextrose infusion rate was then adjusted accordingly to maintain blood glucose concentrations of ±0.5 mmol/L of the basal level.

Blood samples were also taken for storage for the analysis of glucose, insulin, c-peptide, NEFA and glucagon. These samples were taken at -120, 0, +30, +60, +90, +100, +110, +120, +150, +180, +210, +220, +230, and +240 minute. Insulin and c-peptide samples were left at room temperature to clot for 10 minutes and the other samples were put on ice. All samples were then centrifuged for 10 minutes at 4°C at 4000 rpm. The separated plasma was initially stored in a -20°C freezer until the end of the visit and then transferred to -80°C freezer for long-term storage.

At the end of the 2nd phase of the euglycaemic hyperinsulinaemic clamp, the insulin and isotope infusion were stopped. The dextrose infusion was continued for a further

30 minutes to prevent hypoglycaemia. Participants were then provided a lunch of their choice and discharged from the NIHR Imperial Clinical Research.

Diagrammatic representation of the 2-phase euglycaemic hyperinsulinaemic clamp is as presented (see Figure 2.5).

2.5.3 Special Considerations

2.5.3.1 Contraception

Trial participants were asked to maintain effective non-hormonal contraception for the duration of the study. Participants on hormonal contraception of any form were not eligible to participate in the trial as this could interfere with the trial outcomes.

Effective non-hormonal contraception methods that were permitted included

1. Barrier method
2. Non-hormonal intra-uterine device
3. Vasectomised solo sexual partner
4. Complete sexual abstinence

2.5.3.2 Menstrual History

Menstrual history information from menarche to the start of the trial was collected from participants at the time of the screening visit.

Following screening, participants who were successfully enrolled into the trial were asked to complete a brief questionnaire relating to their periods using an online data collection platform, SurveyGizmo (Boulder, Co). Participants were asked to complete this questionnaire for each period they had throughout the duration of the trial to provide measures of frequency and duration.

The menstrual history data from this platform was further corroborated during the reproductive visits as well as at the time of US scan at by the presence of a corpus luteum and by endometrial thickness.

2.5.3.3 Food Diaries

Total caloric intake and macronutrient composition was assessed through the use of food diaries. All participants were asked to complete food diaries over 3 consecutive days in the weeks preceding their Baseline, 2-week and 12-week post-procedure visits.

2.5.3.4 Body composition studies

Al participants were assessed with body composition studies using a bioelectrical impedance analyser (Tanita MC-780MA P, Tanita, Amsterdam) at screening, at every mechanistic visit and at the 6-month clinical visit. The bioelectrical impedance analyser provided measures of weight, BMI, fat-mass and percentage, fat-free mass and percentage and an estimate of the resting metabolic rate (RMR).

2.5.3.5 Cross over

Following the completion of the last visit for the last participant of the DOMINO Trial, investigators and participants were unblinded. All participants were informed of preliminary results for the trial and participants in the sham arm of the trial were offered the DMR procedure.

2.5.3.6 Unblinding Procedure

The randomisation list will be created and held by Prof Tan in a secure area within the NIHR Imperial Clinical Research Facility. This copy is to be kept as a code-break envelope in case emergency unblinding was indicated.

Only in an emergency or a serious medical condition and when knowledge of treatment allocation is essential for clinical management or participant welfare should the decision to unblind be taken. This should be done by obtaining the code-break envelope.

The opened envelope must be signed and dated by the investigator with the reason for the unblinding documented on the envelope as soon as possible after the opening

and within 24 hours of the code break. This information should also be recorded in the participant's research and medical notes.

2.5.3.7 Withdrawals and Exclusions

All participants are free to withdraw at any point in the study.

Participants may be excluded in the event of a major protocol violation (e.g. non-compliant with study visits, pregnancy, accidental unblinding) or if the procedure is not feasible for the participant (e.g. anatomical or technical reasons).

Withdrawals and exclusions that occurred up to and including the time of the procedure were replaced. Dropouts and exclusions that occur following the intervention were not replaced.

2.5.3.8 Participant Re-imbursement

All participants received £300 upon completion of the study as reimbursement for their time and inconvenience. Participants based outside the M25 were additionally reimbursed for their travel expenses.

Reimbursement was not cited in advertising for the trial to avoid trial participation with a financial motivation.

2.6 Trial Endpoints

The trial outcomes are as listed following:

2.6.1 Primary Efficacy Endpoint

The primary trial outcomes were

1. The change from baseline in total insulin sensitivity at 12 weeks.
   Total insulin sensitivity was defined as the sum of the hepatic and peripheral insulin sensitivity as assessed by the 2-phase euglycaemic hyperinsulinaemic clamp.

2. The change from baseline in insulin sensitivity at 24 weeks as assessed by HOMA-IR.

3. The number of menses during the 24-week duration of the trial

2.6.2 Secondary Efficacy Endpoints

Secondary trial outcomes are listed below.

1. Change from baseline in the following at 2 weeks following intervention
    a. Matsuda Index and Disposition Index at OGTT
    b. Area under the curve (AUC) 0-180 minutes for the concentrations of glucose and insulin at OGTT
        i. Full
        ii. Area above fasting value (AFV) for the specified variable

2. Changes from baseline in the following at 12 weeks following intervention
    a. Hepatic insulin sensitivity
    b. Peripheral insulin sensitivity
    c. Insulin sensitivity as assessed by HOMA-IR
    d. Matsuda Index and Disposition Index at OGTT
    e. Area under the curve (AUC) 0-180 minutes for the concentrations of glucose and insulin at OGTT
        i. Full
        ii. Area above fasting value (AFV) for the specified variable
    f. Concentration on enhanced liver fibrosis (ELF) test

3. Change from baseline in the following at 24 weeks following intervention
    a. HbA1c
    b. Free Androgen Index (FAI)
    c. Concentrations of LFTs

d. Percentage body weight loss

4. Number of ovulatory cycles defined by an increase in serum progesterone and/or ultrasound (US) evidence of ovulation followed by menstrual bleeding between weeks 12-24.

2.6.3 Exploratory Endpoints

As this was a trial investigating the mechanism behind a novel investigation, several exploratory endpoints were proposed, as listed below.

1. Change from baseline in the following at 12 weeks following intervention
    a. Energy expenditure
    b. Body composition
    c. Plasma lipid concentration
    d. Arterial blood pressure

2. Change from Week 12 to Week 24, and change from Week 12 to each visit, for the following:
    a. Endometrial thickness
    b. Ovarian volume
    c. Follicle number
    d. Diameter of largest follicle in each ovary
    e. Serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), Oestradiol, sex hormone-binding globulin (SHBG), testosterone, androstenedione, dehydroepiandrosterone (DHEAS)

2.7 Trial Risks

As with any interventional procedure, there are inherent risks associated. Below is a list in alphabetical order of the risks associated with the Fractyl® Revita System (Fractyl, Lexington) and procedure and means by which these may be mitigated.

2.7.1 Procedural Risks

Risks related to endoscopic procedure as well as the DMR ablation procedure

- Abdominal pain and cramping
- Diarrhoea
- Difficulty swallowing
- Hypoglycaemia
- Infection
- Mucosal injury to the GI tract
- Pancreatitis
- Perforation
- Sore throat
- Stricture
- Transient bleeding

2.7.2 Device-specific risks

Below is a list of risks to the participant associated with the materials selected, design and construction of the catheter and console of the Fractyl® Revita System (Fractyl, Lexington).

- Allergic reaction to the device materials or endoscopic labelling dye or injectate
- Component degradation
- Control module delivers incorrect ablation time and temperature profile
- Device breakage
- Disarticulation of components from the device
- Device/Component lost in GI tract or wall
- Hole in hot fluid catheter balloon resulting in leakage of hot fluid
- Lost catheter component in the GI tract or wall
- Thermal damage to the duodenum wall or surrounding structures
- Unforeseen adverse events

2.7.3 General anaesthesia risks

Risks associated with general anaesthesia are rare (fewer than 1 in 10, 000 cases). These include

- a serious allergic reaction to the anaesthetic (anaphylaxis)
- an inherited reaction to the anaesthetic that causes breathing difficulties
- waking up during the intervention – this is rare, and the amount of anaesthetic given will be continuously monitored to help ensure this does not happen
- death – this is very rare, occurring in 1 in every 100,000 to 1 in every 200,000 cases

2.7.4 Minimising study risks

The following steps have been taken to minimise the risks associated with the Fractyl® Revita System (Fractyl, Lexington).

- The tissue or fluid contacting materials used in the construction of the Revita Catheter are known medical grade materials that are well characterized and have a long history of use. In addition, bio-compatibility testing has proven that the materials are safe.
- The device design uses known technologies including sub-mucosal injection and hot fluid balloon to complete the procedure. Similar technologies are currently in use for such accepted procedures as endoscopic mucosal resection and treatment of menorrhagia.
- The device design has been rigorously tested in the laboratory, animal models and clinical trials to characterize its performance and confirm the safety and performance of the procedure.
- All endoscopists receive detailed training in the use of the Fractyl® Revita System (Fractyl, Lexington) and the DMR procedure. The training includes hands on use of the system in a lab setting.
- The anaesthetist will review the participant's medical history and adapt the anaesthesia so that its risks are minimised.

2.8 Adverse Events

Safety outcomes of the DOMINO Trial were evaluated by adverse event (AE) occurrence. All AEs are coded using the standardised central coding dictionary Medical Dictionary for Regulatory Activities (MedDRA) version 19.1 or greater. Adverse event analyses was performed on the safety analysis population.

A list of all AEs including the participant identification number, AE number, days since index procedure, the investigator description of the AE, the AE System Organ Class (SOC) and Preferred Term (PT) as per MedDRA, severity of AE, whether or not the AE is classified as a Serious Adverse Event (SAE), the relationship of the AE to the investigational device or procedure, the action taken, the outcome and the adjudication status was collated.

A treatment emergent adverse events (TEAEs) is an event starting or worsening in severity at or after initiation of the index procedure for the randomised treatment. For subject counts, subjects experiencing a given event more than once was counted only once for that event. Any TEAEs leading to withdrawal was listed.

Adverse Events of Special Interest (AESIs) are AEs that may be related to the mechanism of action of the DMR procedure, a consequence of the procedure or rare events that may not be related to the device or procedure but are of special interest. These include

- Hypoglycemia
- Diarrhea
- Abdominal pain, nausea, vomiting
- Gastrointestinal bleeding
- Unexplained fever
- Stenosis

AEs were recorded as SAEs if they met any of the following criteria:

- Led to death or were life-threatening

- Led to hospitalisation or prolongation of existing hospitalisation
- Caused persistent of significant disability
- Linked to congenital anomaly

All SAEs were reported to the Chief Investigator using an SAE form within 24 hours of the event. Related or unexpected SAEs were reported to the Joint Research Compliance Office and the Research Ethics Committee within 15 days. .

All SAE and AE were actively followed up by me (or the clinician responsible for the participant's care) until resolution or stabilisation of the event.

2.9 Blood Samples Processing

2.9.1 Standard Clinical and Specialised Blood Tests

All routine biochemical and haematological blood tests were carried out by the NHS Laboratories of Imperial College Healthcare NHS Trust (North West London Pathology). These included full blood count, urea and electrolytes, liver function tests (LFTs), glucose, insulin, thyroid function tests, HbA1c, lipid profile, vitamin, mineral and metabolite levels, a coagulation screen as well as reproductive blood tests (LH, FSH, progesterone, oestrogen, testosterone, SHBG, androstenedione and DHEAS).

Glucose was analysed using the hexokinase / G-6-PDH method. Insulin levels were measured using an immunoassay and HbA1c measurements were carried out using ion exchange chromatography.

Testosterone values refer to total testosterone levels. Testosterone and androstenedione were measured using liquid chromatography / tandem mass spectrometry. The values for DHEAS, oestradiol, FSH, LH, progesterone and SHBG were measured using the immunoassay principle.

Samples obtained during the OGTT and hyperinsulinaemic euglycaemic clamps were processed as detailed above.

Insulin radioimmunoassay, glucose quantification with gas chromatography mass spectrometry and enhanced liver fibrosis score calculation is as detailed below.

2.9.2 Insulin radioimmunoassay

Insulin measurements were quantified with the radioimmunoassay (RIA) technique using the Millipore Human Insulin Specific RIA HI-14K kit (Millipore Corporation, Billerica, USA) and conducted as per the manufacturer's specifications and protocol. All RIA measurements were performed at the Department of Medicine, Hammersmith Campus, Imperial College London.

RIA is a highly sensitive *in vitro* assay methodology used to determine the concentrations of specific substances. The principle underlying RIA is based on the ability of the technique to measure the level of radioactivity of a specific antibody to an antigen labelled with a radioisotope.

To measure the concentration of a substance using RIA, a fixed concentration of a radio-labelled tracer is mixed with a specified amount of antibody for that particular antigen. The tracer utilised was the widely used ^{125}I, a gamma-radioisotope of iodine, labelled with insulin (^{125}I-Insulin).

The serum containing the concentration of insulin to be measured is then added to the tracer and the antibody mixture. This will result in competitive binding of the unlabelled insulin (from the serum sample) and the radiolabelled tracer at the antibody sites, resulting in displaced radiolabelled tracer.

The higher the concentration of insulin in the serum sample analysed, the higher the binding-ability of the unlabelled insulin and the lower the amount of bound tracer to the antibody. All the bound antigen is then separated from the unbound antigen and the radioactivity of the antibody-antigen complex is measured using a gamma counter.

The Millipore Human Insulin Specific RIA kit was selected for use as it does not cross-react with pro-insulin and hence provides a more reliable and accurate measure of

true insulin levels. All RIAs assays were conducted in duplicate and all samples from a single mechanistic visit for each participant were analysed at the same time.

Serum samples from time points 0, +15, +30, +60, +120, and +180 during the 3 OGTT visits and from time points -120, 0, +60, +100, +110, +120, +180, +220, +230, and +240 during the 2 hyperinsulinaemic euglycaemic clamps clamp visits as well as 2 quality control samples were analysed in duplicate for insulin levels using the RIA technique, resulting in a total of 2,520 samples for 30 participants.

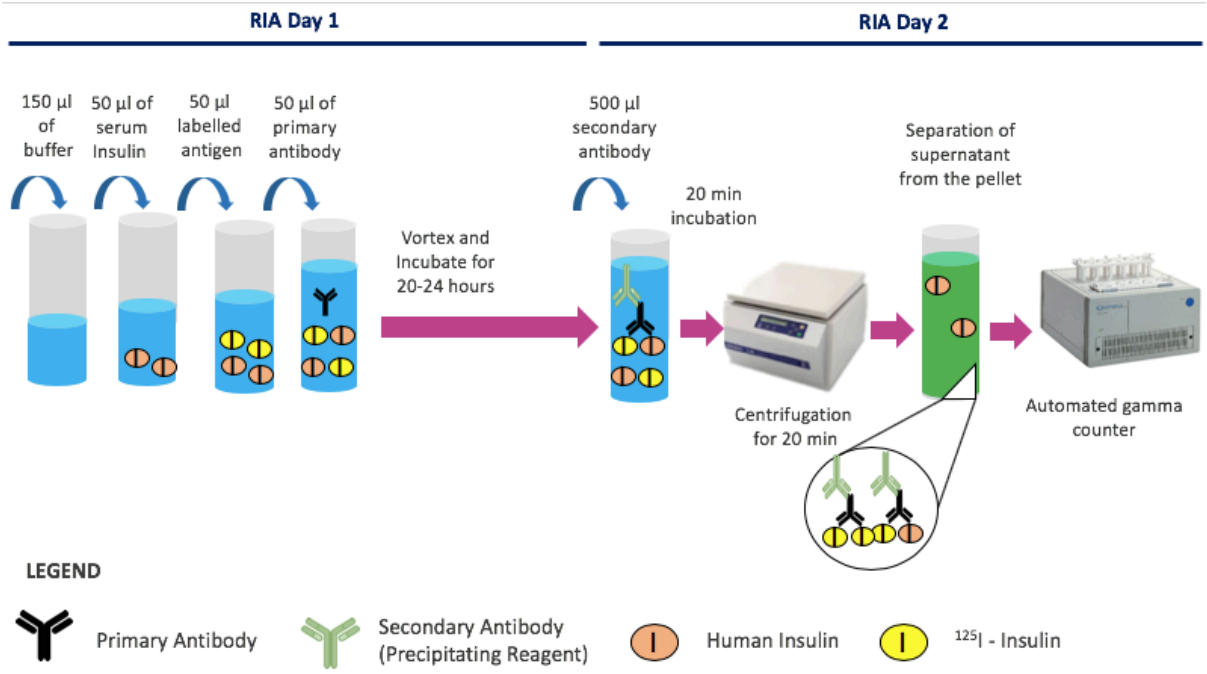

Figure 2.6 Schematic image of insulin radioimmunoassay (RIA) methodology

(Source: Adapted from Petropoulou, K, Imperial College London. Used with permission)

Each RIA measurement is conducted over two days (see Figure 2.6) . Before the first day, serum samples to be analysed were removed from the -80 ^{o} C freezer and allowed to defrost in a -20 ^{o} C freezer. The following day, the samples were placed at room temperature and allowed to defrost completely.

A volume of 50 µl serum insulin was pipetted out of each serum cryotube and added to the 150 µl 0.05M phosphosaline buffer containing 0.025M

Ethylenediaminetetraacetic acid (EDTA), 0.08% sodium azide and 1% RIA Grade Bovine Serum Albumin (BSA) to optimize the pH. A volume of 50 µl hydrated ^{125}I-Insulin and 50 µl antibody were then added to all the tubes.

A standard curve or a calibration curved was then created using serial dilution of the insulin standard provided. Six vials containing differing volumes (100 µl, 50 µl, 25 µl, 12.5 µl, 6.25 µl and 3.125 µl) of standard insulin was prepared the same way as the samples above by adding buffer, tracer and antibody. The readings provided from these tubes will be used to create a standard curve to allow reliable and consistent determination of the insulin levels from the serum samples being analysed.

All the tubes were then vortexed, covered and left to incubate at room temperature for 20-24 hours.

On the second day, 500 µl of cold precipitating reagent (secondary antibody) was added to all tubes. The tubes were then vortexed and incubated for a further 20 minutes. All the tubes were then centrifuged for 20 minutes at 4^{o} C at 1500g. Immediately after centrifugation the supernatant was carefully decanted, leaving only the pellet.

Radiation levels in the pellets was counted using an automated gamma counter (Multi Crystal LB2111 Gamma Counter, Berthhold Technologies, Bad Wildbad, Baden-Wurttenberg). Using the standard curve, calculations were performed using, GraphPad Prism Version 8 and the insulin measurements in the serum samples was quantified. According to manufacturer's specification, the minimum limit of detection is 2.715 µU/ml.

Prior to unblinding, the coefficient of variation (CV) was calculated. The within-assay CV cut-off was 15%; values with CV over 15% were withdrawn. If there was a significant difference between duplicates, caution was used in selecting the appropriate value. All measured values and standard curves were also reviewed by an independent blinded researcher for consistency and quality of measurements.

2.9.3 Gas Chromatography Mass Spectrometry

Isotope-labelled plasma glucose ([6, 6-$^{2}H_2$] glucose) levels were derivatised using gas chromatography mass spectrometry (GCMS) by the Nutrition and Dietetics laboratory team, Faculty of Health and Medical Science, University of Surrey. This was conducted using a modified technique of a well-described methodology as described here[380].

GCMS is a highly specific analytical technique which combines the features of gas chromatography and mass spectrometry (see Figure 2.7) to measure the abundance of ionised particles at specific masses.

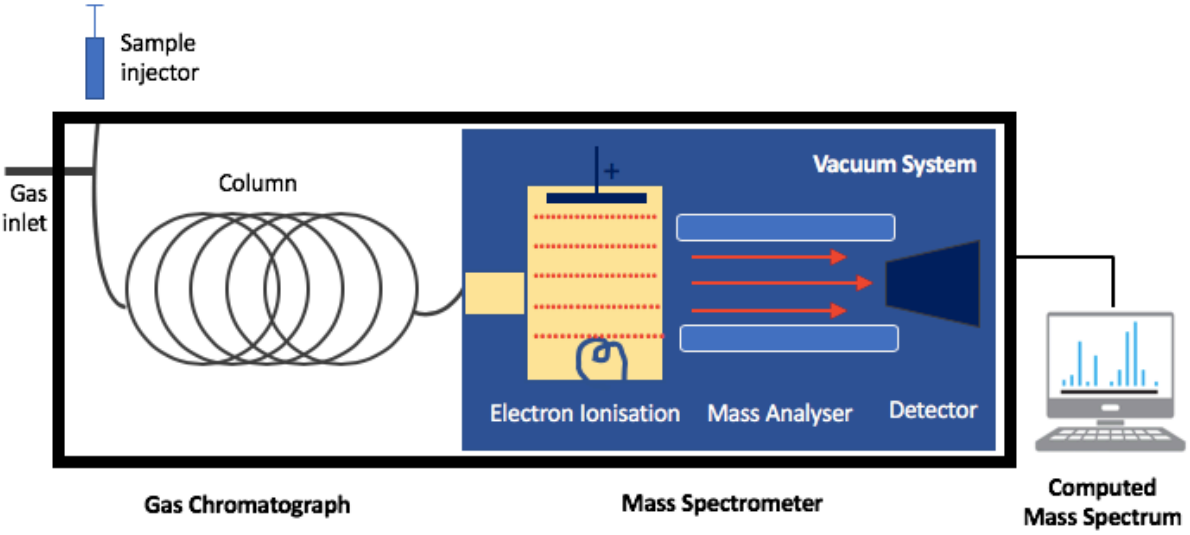

Figure 2.7 Schematic Image of Gas Chromatograph Mass Spectrometer

(Source: Author's Own)

Gas chromatograph vapourises a liquid sample to allow separation and analysis of its compounds. The sample to be analysed is heated to 250^{O}C to produce a vapour without particle decomposition. Gas chromatography has 2 phases the – the mobile phase, uses an inert carrier gas (e.g. Helium) and the stationary phase which allows separation on a capillary column coated with an inert non-volatile liquid. The different molecules in the vapour of the compound being analysed will display different affinity to the stationary phase of the column. This will promote separation of the different

molecules as it travels through the column. The molecules are then retained for analysis in the mass spectrometer.

Mass spectrometer measures ionic mass-to-charge ratio and computes this result into a mass spectrum detailing the abundance of each type of ion. When a sample is introduced into the vacuum pumps of the mass spectrometer, the first step is electron ionisation to produce gas phase ions. All the resultant ions are then separated according to their mass-to-charge ratio by accelerating them and subjecting them to an electric (i.e. quadropole mass filter) or magnetic field. The ion are then detected by an electron multiplier and the results are computed and displayed as a mass spectrum. This is a graphical plot of the ion signal as a function of its mass-to-charge ratio.

Isotopes are variants of an element that have the same number of protons as the element but different number of neutrons and consequently have a different atomic mass. This difference in atomic mass allows it to be separately detected. Deuterium is a naturally occurring isotope of hydrogen and contains and extra neutron in its nucleus. The glucose isotope used in the hyperinsulinaemic euglycaemic clamps were labelled with deuterium in 2 places giving it a different mass than standard glucose and allowing it to be quantified in a mass spectrometer.

Plasma samples from 18 time points during the hyperinsulinaemic euglycaemic clamp and 8 quality control samples were analysed for isotope-labelled glucose levels using the GCMS technique, resulting in a total of 1,560 samples for 30 participants.

Frozen samples and quality controls were transferred from Imperial College London to the University of Surrey and stored in a -80 ^{O}C freezer. Samples and quality controls were allowed to fully thaw prior to commencing the GCMS process.

All the test samples were then mixed using a vortex and centrifuged for 10 minutes at 4 ^{O}C at 2500rpm to spin down ay proteinaceous material. Then, using a pipette, 50μL of sample (or quality control) was transferred into a glass test tube and 0.5 mL of ethyl alcohol was then added to each test tube. All the test tubes were then centrifuged for 10 minutes at 4 ^{O}C at 2500rpm, as previous.

The resulting supernatant was transferred to 1.75 mL vials using a glass Pasteur pipette and dried under oxygen free nitrogen (OFN) at 50 ^{O}C in the SpeedVac sample concentrator for 1 hour. Methoxamine hydrochloride (100μL in pyridine 2% w/v) was added to the dry residue, vortexed and heated for a further 2 hours at 90 ^{O}C. The tubes were then allowed to cool to room temperature.

Once cooled, 50 μL of N,O-bis(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane (BSTFA) was added. All tubes were subsequently heated again at 120°C for 15 minutes. Samples were then cooled to room temperature before drying under OFN at room temperature. Once dried, samples were reconstituted with 500 μL of decane and a final dilution of 50 μL reconstituted sample to 500 μL of decane was performed before transfer for GCMS analysis.

Gas chromatography was performed on the derivatised samples using the Agilent® 7890A model gas chromatograph with an Agilent® 7683B injector series. Separation was completed on a Rxi® 1ms crossband dimethyl polysiloxane capillary column with dimensions of 30m x 0.25 mm i.d. 0.25 μm. For the gas chromatography, helium was used at 11.7 psi. Samples of 1 μL were injected into the autosampler in the splitless mode with the injection port a temperature of 250°C. The temperature programme for the column oven was 120 °C for 2 minutes with a rise of 20°C min^{-1} to the temperature of 300°C for 1 minute. The gas chromatograph had a run time of 12 minutes per sample.

Mass spectrometry was performed using an Agilent® 5975C inert XL EI/CI MSD model using the software MSD Chemstation version E 02.00.493. The ion source of the mass spectrometer was directly heated to 230°C with the transfer line of the GCMS at a set temperature of 280°C. The mass spectrometer was operated in electron impact (EI) mode, with an electron ionisation of 70 eV. In addition the mass spectrometer was operated in Selected Ion Monitoring (SIM) mode, with ions measured including: *m/z* 319.2 (m+0), 321.2 (m+2), 322.20 (m+3) and 323.2 (m+4) with a dwell time of 25 ms between 7.0 and 9.0 minutes of the GCMS run.

These results were then used to calculate the rates of endogenous glucose production and glucose uptake as detailed below.

2.9.4 Enhanced Liver Fibrosis

The Enhanced Liver Fibrosis (ELF) score is a validated, sensitive combination biomarker used to assess the severity of chronic liver disease such as NAFLD. It is calculated from measures of extracellular matrix metabolism, principally hyaluronic acid (HA), pro-collagen III amino terminal peptide (P3NP) and Tissue Inhibitor of Metalloproteinases-1 (TIMP-1), using a linear algorithm (as detailed in the next section).

For the DOMINO study, all ELF analysis was performed using a Siemens Advia Centaur XP analyser at the Specialist Biochemistry Laboratory, Southampton General Hospital, Southampton University Hospital NHS Trust, using the principles of ELISA.

For analysis, the levels of HA, P3NP and TIMP-1 would first be calibrated using the Centaur ELF Siemens calibrators. Prior to commencing analysis, a quality control evaluation would be run using the Centaur ELF Siemens quality control and further analysis only carried out if these values were within the acceptable range. A quality control analysis was carried out for every batch of sample analysis.

Calculations were conducted automatically by the analyser but values were double-checked manually in results were unusually high.

2.10 Derived Variables and Surrogate Indices

Derived variable and surrogate indices are defined as listed below

- **BMI** = Weight (kg) / [height x height (cm^2)]
  BMI $\geq$ 30 kg/m^2 indicates obesity

- **Waist-to-Hip ratio** = Waist circumference (cm) / Hip circumference (cm)
  Waist-to-Hip ratio $\geq$ 0.85 in women indicates abdominal obesity

- **HOMA** = Homeostatic Model Assessment is a validated method for the assessment of insulin resistance (IR) and β-cell function (β)

- **HOMA-IR** = [Fasting insulin (μIU/L) x Fasting glucose (mmol/L)] / 22.5
  HOMA-IR > 2.5 indicates insulin resistance

- **HOMA-B** = [20 x Fasting insulin (μIU/L)] / [Fasting glucose (mmol/L) – 3.5]
  HOMA-β provides a measure of β-cell function

- **QUICKI** = Quantitative Insulin Sensitivity Check Index = 1/ [log Fasting glucose (mg/dL) + fasting insulin (μIU/L)]
  QUICKI < 0.335 indicates insulin resistance

- **Cholesterol: HDL ratio** = Cholesterol (mmol/L) / HDL (mmol/L)
  Cholesterol: HDL ratio > 3.5 indicates increase cardiovascular risk

- **LH:FSH ratio** = LH (U/L) / FSH (U/L)
  LH: FSH ratio > 2 indicates raised LH levels relative to FSH

- **Free Androgen Index (FAI)** = [Total testosterone (nmol/L) / SHBG (nmol/L)] x 100, FAI levels > 10 in women indicate raised androgen levels.

- **Ovulatory Event** = Each dominant follicle (>14mm) separated by >14 days + corpus luteum sighting (in the absence of a dominant follicle 7 days prior) + progesterone rise >10nm/L separated by >14 days (in the absence of a dominant follicle or corpus luteum)

- **Enhanced Liver Fibrosis (ELF) Score** = 2.278 + 0.85 (Hyaluronic acid) + 0.751 (Pro-collagen III Amino terminal peptide) + 0.394 (Tissue Inhibitor of Metalloproteinases-1)
  ELF Score $\geq$ 7.7 – moderate liver fibrosis, ELF Score $\geq$ 9.8 – severe liver fibrosis

- **Area under the Curve (AUC)** = calculated using the trapezoid rule on GraphPad Prism Version 8.0

- **Area above Fasting Value (AFV)** = AUC for the area above the fasting value for the specified variable

- **Insulinogenic Index (II)** = [Insulin concentration 30 minutes after the start of the OGTT(μIU/L) – FPI]/ [Glucose concentration 30 minutes after the start of the OGTT (mg/dL) – FPG), where FPI: Fasting plasma insulin (μIU/L) and FPG: Fasting plasma glucose (mg/dL)
  Insulinogenic Index <0.4 indicates inadequate insulin secretion

- **Matsuda Index or Insulin Sensitivity Index (ISI)** = 10000 / √**(**FPG x FPI x MPG $_{(0\text{-}120)}$ x MPI $_{(0\text{-}120)}$), where √: square root, FPG: fasting plasma glucose (mmo/L), FPI: Fasting plasma insulin (μIU/L), MPG $_{(0\text{-}120)}$: mean plasma glucose calculated from glucose measurements at time points +0, +15, +30, +60 and +120 during the OGTT, MPI $_{(0\text{-}120)}$: mean plasma insulin calculated from insulin measurements at time points +0, +15, +30, +60 and +120 during the OGTT.
  Matsuda Index or ISI </= 2.5 indicates insulin resistance

- **Oral Disposition Index (ODI)** = [AUC Insulin $_{(0\text{-}120)}$ / AUC glucose $_{(0\text{-}120)}$] x Matsuda Index, where AUC Insulin $_{(0\text{-}120)}$: Area under curve calculated using the trapezoid rule from time point +0 to +120 during the OGTT, AUC glucose $_{(0\text{-}120)}$: the corresponding value for glucose.
  ODI has also been calculated for 0-30 minutes to give an indication of the 1st phase insulin secretion

- **Change from baseline** = Baseline value of variable – New value for variable at outcome timepoint

- **Percentage (%) change from baseline** = (Change from baseline / Baseline value of the variable) x 100

- **Total Insulin Sensitivity** = Hepatic insulin Sensitivity + Peripheral Insulin Sensitivity

- **Hepatic Insulin Sensitivity** = the rate of glucose infusion required to maintain a state of euglycaemia during the first phase (low dose insulin infusion) of the hyperinsulinaemic euglycaemic clamp = mean of glucose infusion from +90 to +120 of the clamp

- **Peripheral Insulin Sensitivity** = the rate of glucose infusion required to maintain a state of euglycaemia during the second phase (high dose insulin infusion) of the hyperinsulinaemic euglycaemic clamp = mean of glucose infusion from +210 to +240 of the clamp

- **Ra / Rd** = Glucose rate of appearance (Ra) and rate of disappearance (Rd) were calculated using the Steele's non-steady state equations and modified for stable isotope use and the inclusion of the glucose isotope in the dextrose infusion[381, 382]. Volume was distribution was assumed to be 22% of body weight based on previous calculations. Before commencing calculation of Ra and Rd, plasma glucose concentration and glucose enrichment time courses were smoothed out using the Optimal Segments Technique Analysis[383].

  Ra, the endogenous glucose production rate, was calculated at basal conditions and following the administration of low dose insulin.

  Rd, the rate of glucose uptake, was calculated at basal conditions and following administration of high dose insulin.

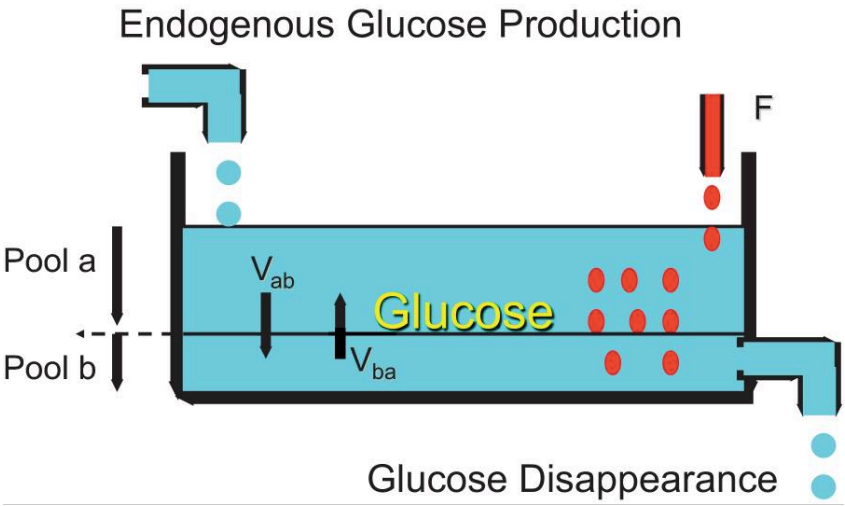

Figure 2.8 Schematic diagram illustrating the multiple-pool concept in tracer dilution studies
Pool a represents the readily accessible pool in which the tracer distributes rapidly, in contrast to the less readily accessible pool (Pool b). The relative size of each pool can be altered as can flow of tracer and tracee from Pool a to b (Vab) or vice versa (Vba). F: tracer infusion rate. (Source: Vella A, Rizza RA. Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes. 2009;58(10):2168-74.)

Steele equation is used to quantify glucose metabolism using the tracer dilution technique. A simplified explanation for this was proposed by Vella and Rizza using the presumption that glucose metabolism in the fasting state can be equated to volume in a single compartment with a faucet at one end (endogenous glucose production, Ra) and a drain at the other (glucose uptake or disappearance, Rd) (see Figure 2.8)[384]. During a clamp, when peripheral glucose concentrations are kept constant, the rate of glucose appearance will equal the rate of glucose disappearance, (Ra=Rd). If an isotope is added to the compartment and it instantly and uniformly distributes within the compartment, glucose disappearance will not change its concentration[384]. Conversely, endogenous glucose production will not contain the isotope and will dilute the concentration of the isotope[384]. However, in human beings, glucose (and thus isotope) is not confined to a single compartment. Hence, it was postulated that there is at least one rapidly equilibrating compartment (pool a) and a slower equilibrating pool (pool b)[384]. This was the basis for the non-steady state equation used in the mathematical modelling of Ra and Rd.

- **Glucose Metabolic Clearance Rate (MCR)** = Glucose Rd / glucose concentration. Similar to Rd, this was calculated at basal conditions and following administration of high dose insulin.

  (Calculation of Ra, Rd and MCR were carried out by Prof Margot Umpleby, Department of Diabetes & Metabolic Medicine Research, University of Surrey)

2.11 Statistical Analysis

2.11.1 Study Population

Intention to Treat (ITT) Population – this includes all randomised subjects

Modified Intention to Treat (mITT) Population – this includes all randomised subjects where the DMR procedure has been attempted (i.e. at least one ablation performed). This is the primary analysis population for the primary and secondary efficacy endpoints.

Per Protocol (PP) Population – this is a subset of the mITT population who received the treatment as randomised and excludes any participants with major protocol deviations (see Appendix 4 for list of major protocol deviations).

Safety Population – this includes all treated participants. Safety analysis was based on actual treatment received.

2.11.2 Method of Analysis

All analysis and outputs were generated using statistical software packages GraphPad Prism version 8.0, IBM SPSS Software version 26.0 and SAS version 9.3 or later.

Variables are presented according to the treatment group using descriptive statistics. Analysis was performed for the mITT population. Normality was assessed for all continuous variables by analysing the Q-Q plots and the histograms of residuals from an analysis of co-variance (ANCOVA) model. For normally distributed variables, data was presented as mean ± SD, with sample size stated. Data with a skewed distribution was presented with median and inter-quartile range (IQR) with sample size stated. Nominal and ordinal variables were presented using frequencies and percentage of participants in each category with sample size stated.

Baseline characteristics including demographic data, baseline medical history, clinical and laboratory measurements will be presented using descriptive statistics. Formal

statistical comparison between groups was not carried out as the groups were selected at random.

Variables collected at multiple follow-up time periods were reported using appropriate descriptive statistics of change from baseline and presented by treatment group at each follow-up interval using ANCOVA or mixed-effects analysis. Statistical evidence of significance was assumed for p-value of $p<0.05$ for 2-tailed significance and $p<0.25$ for 1-sided significance.

All efforts were made to prevent the occurrence of missing data. If missing data was encountered to be missing at random, analysis was carried out using the mixed-effects analysis and adjusted with a Bonferroni correction. This model allowed for fixed and random effect in its analysis. Variables with only one post-baseline visit were excluded if missing data was encountered.

Single comparison between groups and within groups were carried out using unpaired or paired t-tests for normally distributed data. Single comparison for skewed data and ordinal or nominal variables was carried out using Mann-Whitney test or Chi-square test (Fisher's test if the number of data points were low) for between group comparison; and Wilcoxon test or McNemar's test for within group comparison.

For some of the analyses below, statistical support was also provided by an external firm (Boston Bio-Medical, Cambridge, Massachusetts).

The primary analysis for the change from baseline to Week 12 in total insulin sensitivity comparing treatment groups was performed with an ANCOVA model. The primary analysis for change from baseline to Week 24 in insulin sensitivity as assessed by HOMA-IR was carried out using mixed-effects analysis. The primary analysis for the number of menses during the first 24 weeks after randomization was performed comparing treatment groups with an ANCOVA model on rank of measured number of menses over 24 weeks with terms for rank of number of reported number of menses in the 12 months before randomisation and treatment.

For each of the analyses of the primary endpoints, least square means is presented together with their SEs and two-sided confidence intervals.

For each analysis, tables of descriptive statistics of the primary endpoint includes sample size, mean, SD, least squares mean, standard error of least squares mean, median, quartiles, and minimum and maximum for each treatment group. Two-sided 90% and 95% confidence intervals of the difference between treatment least square means is presented. Tables are complimented by graphs as necessary.

Secondary efficacy endpoints were compared in a similar manner to primary efficacy endpoints. Analyses for the number of ovulatory cycles in weeks 12-24 was carried out in a similar manner to the analyses of menses above. Analyses for continuous secondary efficacy endpoints that are assessed at more than one post-baseline visit was carried out in a similar manner to the analyses of HOMA-IR change from baseline to Week 24 as described above. Any continuous secondary efficacy endpoints that are assessed at only one post-baseline visit will be carried out in a similar manner to the analyses of total insulin sensitivity change from baseline to Week 12 based on insulin clamp. Tables will be complimented by graphs as necessary.

Results for all exploratory endpoints were presented by treatment group with summary statistics of actual values and change from baseline.

The analyses of safety was carried out on the safety population and summarised by treatment group.

3 Results

3.1 Study Enrolment and Progress

Between May 2018 to February 2019, 32 participants were randomised to receive either the DMR procedure using the Fractyl® Revita System or a sham endoscopic procedure. Two participants were excluded from the ITT analysis due to technical difficulty intubating the duodenum and accidental unblinding of the research team at the time of endoscopy. Both participants were replaced.

Two further participants were excluded from the per protocol analysis due to major protocol deviations (incomplete laboratory samples due to inadequate intravenous access and non-adherence to dietary protocol). Both participants were included in the ITT analysis. Only one participant was lost to follow-up (due to personal commitments as she had an ill relative), but as she had attended all her mechanistic visits by this point, her data was included in the per protocol analysis and the ITT analysis.

Hence, data from 30 participants were included in the final ITT analysis, with 15 participants in each group. The DOMINO trial CONSORT diagram is as depicted below (see Figure 3.1).

3.2 Baseline Characteristics of Study Participants

At baseline, the characteristics of the two groups are as detailed in Tables 3.1 – 3.4.

Across the entire cohort, the majority of participants were of White European descent with a mean (±SD) age of 31.1 (± 6.1) years and a mean (±SD) BMI of 42.5 (± 5.7) kg/m^2. In keeping with the study requirements this was an insulin resistant cohort with a mean HOMA-IR of 6.2 (± 3.1) but without T2DM [mean (±SD) HbA1c of 38.7 (±4.6) and FPG of 5.3 (±0.67) mmol/L. The insulinogenic index was in the normal range across the whole cohort (see Table 3.2).

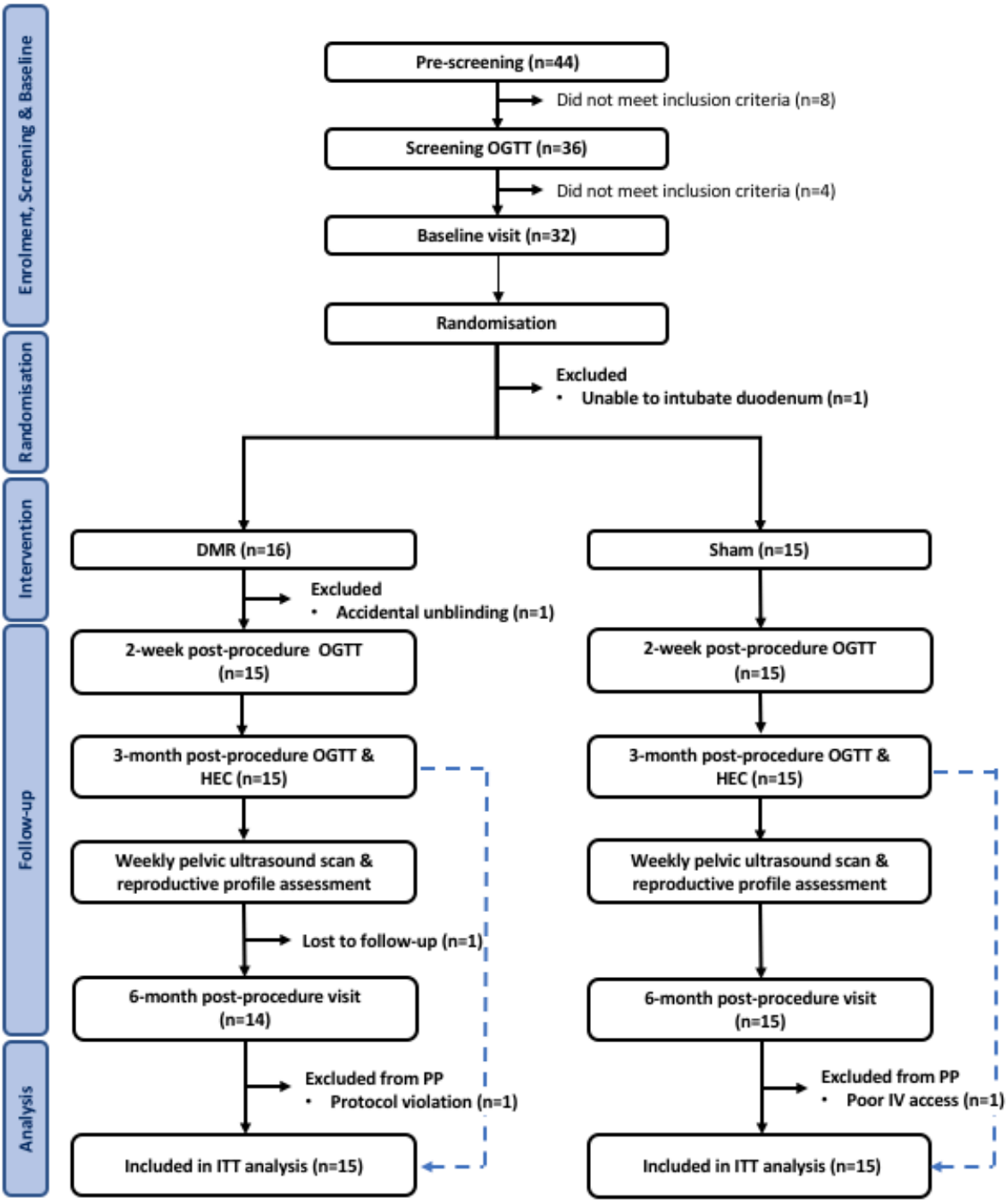

Figure 3.1 DOMINO Trial CONSORT Diagram

(DMR: Duodenal mucosal resurfacing, HEC: Hyperinsulinaemic euglycaemic clamp, ITT: Intention to Treat, OGTT: Oral Glucose Tolerance Test, PP: Per Protocol)

	DMR group (n=15)	Sham group (n=15)	P value
Demographic data			
Age (years)	30.6 (5.2)	31.6 (6.9)	ns
Ethnicity % (n)			
White	60 (9/15)	60 (9/15)	
Black	0 (0/15)	6.7 (1/15)	
Asian	26.7 (4/15)	13.3 (2/15)	
Mixed / Other	13.3 (2/15)	20 (3/15)	
Anthropometric Measures			
Weight (kg)	107.8 (19.0)	121.2 (12.6)	0.03^{+}
BMI (kg/m^2)	40.2 (6.6)	44.7 (3.3)	0.03^{+}
% Body fat	43.9 (3.5)	46.1 (2.1)	0.05^{+}
% FFM	53.2 (3.3)	49.5 (4.6)	0.25
Neck circumference (cm)	41 (4.1)	42.2 (3.0)	0.38
Waist circumference (cm)	112.8 (11.9)	120.5 (11.1)	0.07
Waist: Hip ratio	0.9 (0.1)	0.9 (0.1)	0.98
Energy Intake			
Daily Calorie Intake (kcal)	1603.3 (482.4)	1895.6 (404.4)	0.14
Fat %	40.3 (11.4)	39.8 (5.9)	0.91
Carbohydrate %	40.7 (14.3)	42.2 (6.9)	0.75
Protein %	17.9 (4.5)	17.0 (4.9)	0.63

Table 3.1 Baseline demographic, anthropometric and nutritional data

Data presented as mean ± SD, except for ethnicity which is described with % (n), $^{+}$denotes significant p values

The baseline metabolic profiles of participants are as detailed in Table 3.2. Participants in both groups exhibited a metabolic phenotype with 43% (13/30) previously diagnosed with one or more of sleep apnoea, hypertension, hypercholesterolaemia or non-diabetic hyperglycaemia. Of these, 62% (8/13) were allocated to the DMR arm. Both groups had normal fasting glycaemia levels and significant fasting hyperinsulinaemia (normal range 3-15 mIU/L). Additionally, the groups displayed similarly elevated HOMA-IR levels although the relative contribution of fasting glucose and fasting insulin to this value differed in both groups.

	DMR group (n=15)	Sham group (n=15)	P value
Glycaemic Profile			
Fasting plasma glucose (mmol/L)	5.6 (0.7)	5.1 (0.6)	0.04+
Fasting serum insulin (μIU/L)	22.0 (8.4)	27.7 (13.9)	0.18
HbA1c	39.7 (3.7)	37.7 (5.3)	0.24
HOMA-IR	6.2 (3.4)	6.1 (2.9)	0.96
QUICKI	0.3 (0.0)	0.3 (0.0)	0.17
HOMA-B	259.1 (196.7)	454.8 (288.4)	0.08
Liver Profile			
ALT (U/L)	32.9 (20.8)	35.2 (22.0)	0.77
AST (U/L)	26.6 (8.2)	30.5 (10.9)	0.27
AST: ALT	1.1 (0.6)	1.0 (0.5)	0.41
ALP (U/L)	73.7 (17.6)	82.4 (17.7)	0.19
GGT (U/L)	39.1 (48.2)	27.6 (9.7)	0.37
ELF Score	8.4 (0.8)	8.2 (0.7)	0.43
Lipid Profile			
Total Cholesterol (mmol/L)	5.3 (0.9)	4.9 (0.8)	0.21
HDL (mmol/L)	1.1 (0.2)	1.1 (0.2)	0.76
LDL (mmol/L)	3.4 (0.7)	3.2 (0.6)	0.43
Non-HDL (mmol/L)	4.2 (0.9)	3.7 (0.7)	0.17
Triglyceride (mmol/L)	1.7 (0.8)	1.2 (0.4)	0.04+
Cholesterol : HDL ratio	4.9 (1.1)	4.4 (0.8)	0.21
Clinical Features			
Systolic BP	126.6 (16.0)	131.4 (12.9)	ns
Diastolic BP	78.3 (11.0)	79.9 (12.4)	0.70
Epworth Sleepiness Scale	8.8 (3.8)	4.5 (3.5)	0.003+
Pre-existing Medical Conditions % (n)			
Sleep apnoea	6.7 (1/15)	6.7 (1/15)	
Hypertension	20.0 (3/15)	33.3 (5/15)	
Hypercholesterolaemia	33.3 (5/15)	6.7 (1/15)	

Table 3.2 Baseline Metabolic Profile

Data presented as mean ± SD (or % where indicated), +denotes significant p values

Standard liver profile tests did not indicate evidence of liver function derangement in either group. However, the Enhanced Liver Fibrosis (ELF), indicated a moderate degree of fibrosis in both groups.

The lipid profile in both groups demonstrated raised LDL and non-HDL levels but reduced HDL concentration. The DMR group had elevated mean cholesterol and triglyceride concentrations while the mean value of these parameters were in the normal range for participants in the sham group.

	DMR group (n=15)	Sham group (n=15)	P value
Markers of fecundity			
No. of menses in preceding 12 months*	2 [0, 6]	3[2,4]	0.86
Participants with parity % (n)	20.0 (3/15)	33.3 (5/15)	
Participants with prior miscarriage % (n)	26.7 (4/15)	20.0 (3/15)	
Reproductive Profile			
LH (unit/L)	5.8 (3.7)	6.7 (3.0)	0.47
FSH (unit/L)	4.0 (1.3)	5.3 (3.0)	0.14
LH:FSH ratio	1.4 (0.6)	1.4 (0.6)	0.92
SHBG (nmol/L)	21.9 (8.1)	28.1 (7.0)	0.03^{+}
Testosterone (nmol/L)	1.9 (0.9)	2.5 (1.5)	0.18
Free Androgen Index	10.1 (5.2)	9.7 (5.9)	0.85
Oestradiol (pmol/L)	259.1 (155.6)	252.5 (144.1)	0.91
Progesterone* (nmol/L)	1 [1, 1]	1[1, 1]	0.51
Androstenedione (nmol/L)	6.4 (2.4)	6.0 (2.2)	0.64
DHEAS (µmol/L)	6.3 (3.6)	5.2 (4.1)	0.41
PCOS cutaneous features %, (n)			
Hirsutism / Male pattern balding	100 (15/15)	100 (15/15)	
Acne	40.0 (6/15)	40.0 (6/15)	
Acanthosis nigricans	46.7 (7/15)	66.7 (10/15)	

Table 3.3 Baseline clinical and biochemical reproductive profile

*Data presented as mean ± SD (or % (n) or Median [Interquartile range], where indicated with *, $^{+}$denotes significant p values*

The participants' clinical and biochemical reproductive profile are as presented in Table 3.3. In keeping with the trial inclusion criteria, women in both groups were oligomenorrheic and displayed clinical and biochemical hyperandrogenism. In keeping

with an insulin resistant-PCOS cohort, women in both groups displayed low SHBG concentrations and raised testosterone concentrations. However, women in the DMR group had a significantly lower SHBG level when compared to the sham group.

Data from the baseline mechanistic studies for both groups is presented in Table 3.4.

	DMR group (n=15)	Sham group (n=15)	P value
Oral Glucose Tolerance Test			
Matsuda Index*	2.1 [1.5, 2.6]	1.8 [1.5, 2.0]	0.17
Oral Disposition Index	27.5 (10.3)	28.9 (9.1)	0.71
Insulinogenic Index	1.6 (0.9)	1.8 (1.0)	0.49
AUC 120 minutes			
Glucose	9.8 (2.0)	9.6 (1.2)	0.83
Insulin	119.3 (42.3)	149.3 (49.6)	0.09
AUC 180 minutes			
Glucose	22.5 (4.6)	22.1 (3.1)	0.73
Insulin	178.0(63.1)	203.4 (56.0)	0.25
Hyperinsulinaemic Euglycaemic Clamp			
Insulin Sensitivity			
Total	5.2 (1.7)	5.3 (2.1)	0.91
Hepatic	1.4 (0.4)	1.4 (0.5)	0.80
Peripheral	3.9 (1.3)	3.9 (1.8)	0.94
Rate of Glucose Appearance			
Basal Stage	8.8 (1.1)	8.5 (0.7)	0.32
Low dose insulin infusion stage	2.5 (0.6)	2.3 (1.0)	0.39
High dose insulin infusion stage	0.6 (1.2)	0.3 (1.3)	0.49
Rate of Glucose Disappearance			
Basal Stage	8.9 (1.1)	8.5 (0.7)	0.30
Low dose insulin infusion stage	10.7 (2.4)	10.2 (1.9)	0.47
High dose insulin infusion stage	22.3 (7.5)	22.6 (8.1)	0.93

Table 3.4 Baseline mechanistic data from Oral Glucose Tolerance Test and Hyperinsulinaemic Euglycaemic Clamp

*Data presented as mean ± SD or Median [Interquartile range], where indicated with **

3.3 Post-procedure Clinical Outcomes

The clinical outcomes post-procedure are as detailed in Tables 3.5 – 3.7.

Participants in the sham group had significantly higher body weight, BMI and percentage body fat than their counterparts who received the DMR procedure, and this difference persisted in the follow-up period (see Figs 3.2 – 3.3 below). However, participants in both groups had similar anthropometric measures at baseline.

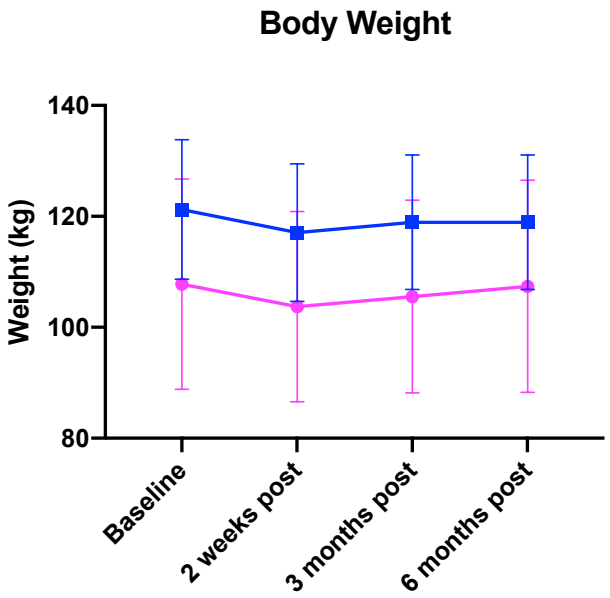

Figure 3.2 Body weight in the DMR and sham groups from baseline to 6 months post-procedure.

Data plotted as means ± SD, n=15 per group

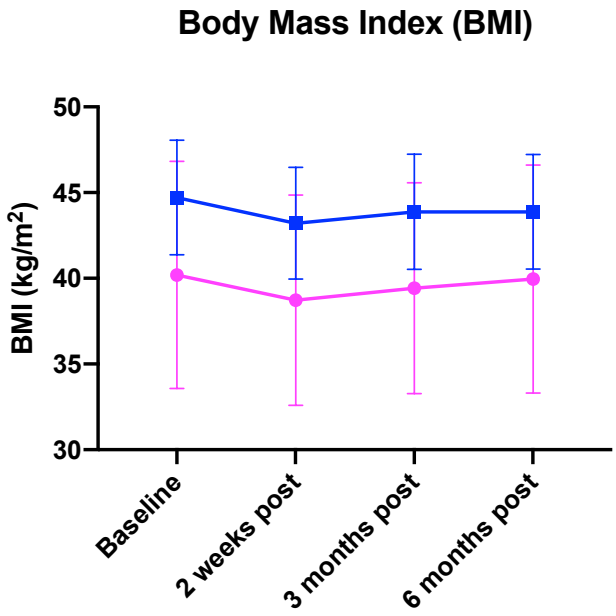

Figure 3.3 BMI in the DMR and sham groups from baseline to 6 months post procedure

Data plotted as means ± SD, n=15 per group

Timepoint	Group		Mixed-effects analyses		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
			Weight (kg)		
Baseline	107.8 (19.0)	121.2 (12.6)			
2 weeks post	103.7 (17.2)	117.1 (12.4)	**Group**	5.53 (1, 28)	0.03^{+}
3 months post	105.6 (17.4)	119.0 (12.1)	**Time**	16.64 (2, 54)	<0.0001^{+}
6 months post	107.4 (19.1)	119.0 (12.1)	**Group x Time**	0.31 (3, 83)	**0.82**
			BMI (kg/m^2)		
Baseline	40.2 (6.6)	44.7 (3.3)			
2 weeks post	38.7 (6.1)	43.2 (3.3)	**Group**	5.78 (1, 28)	0.02^{+}
3 months post	39.4 (6.1)	43.9 (3.4)	**Time**	16.57 (1.9, 53.8)	<0.0001
6 months post	39.9 (6.65)	43.9 (3.3)	**Group x Time**	0.25 (3, 83)	**0.86**
			% Fat Mass		
Baseline	43.9 (3.5)	46.1 (2.1)			
2 weeks post	43.9 (3.9)	46.1 (2.4)	**Group**	5.25 (1, 28)	0.03^{+}
3 months post	42.7 (3.2)	45.7 (2.7)	**Time**	4.77 (2.6, 71.9)	0.006^{+}
6 months post	43.4 (3.7)	45.7 (2.6)	**Group x Time**	0.9 (3, 83)	**0.44**
			% Fat Free Mass		
Baseline	53.2 (3.3)	49.5 (4.6)			
2 weeks post	53.3 (3.7)	49.5 (4.7)	**Group**	8.63 (1, 28)	0.007^{+}
3 months post	53.0 (4.5)	49.9 (5.4)	**Time**	0.39 (2.2, 61.3)	0.70
6 months post	53.5 (3.2)	50.7 (3.9)	**Group x Time**	0.13 (3, 83)	**0.94**

Table 3.5 Baseline and post-procedure weight, BMI and fat distribution

Data presented as mean ± SD, ns: not significant. Analyses conducted with mixed-effects model with Geisser-Greenhouse correction. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. $^{+}$denotes significant p values

Participants in both groups did not display clinically relevant weight loss (mean 2.2 kg in each group at 3 months) and no significant difference between or within groups in caloric intake was evident, throughout the trial (see Fig 3.5). At 3 months post-procedure participants in the DMR group had a more significant change in percentage body fat than those in the sham group (see Fig 3.4). However, this improvement was not sustained to 6 months.

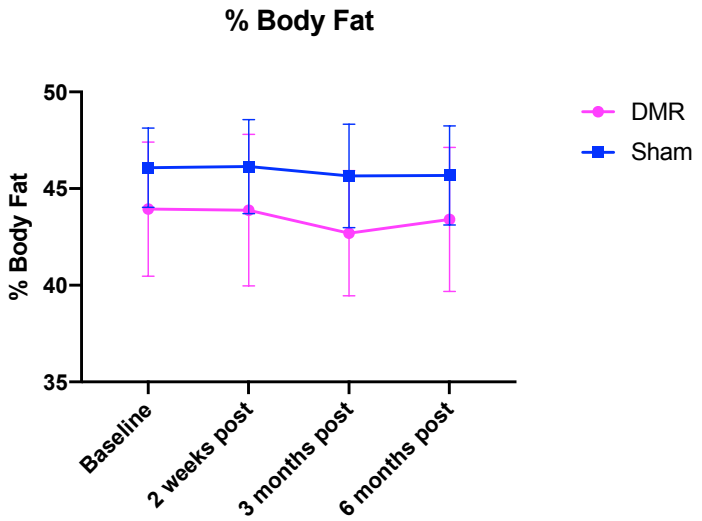

Figure 3.4 Percentage body fat in the DMR and sham groups from baseline to 6 months post-procedure

Data plotted as means ± SD, n=15 per group

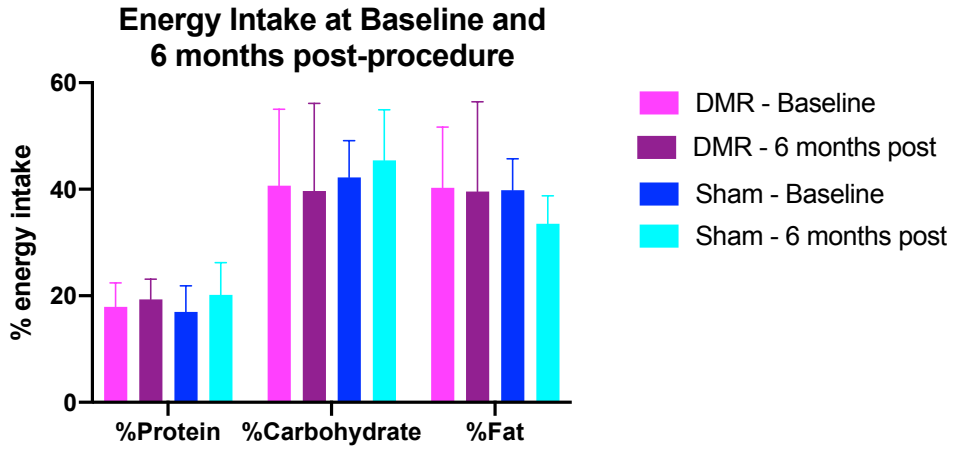

Figure 3.5 Nutritional composition for the DMR and sham groups at baseline and at 6 months post-procedure.

Data plotted as means ± SD, n=15 per group

Timepoint	Group		Mixed-effects analyses		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Fasting plasma glucose (mmol/L)					
Baseline	5.6 (0.7)	5.1 (0.6)			
2 weeks post	5.2 (0.5)	5.0 (0.8)	**Group**	3.18 (1, 28)	0.09
3 months post	5.3 (0.4)	5.2 (0.7)	**Time**	1.722 (2.42, 66.97)	0.18
6 months post	5.3 (0.7)	4.8 (0.6)	**Group x Time**	1.81 (3, 83)	**0.15**
Fasting plasma insulin (µIU/L)					
Baseline	22.0 (8.4)	27.7 (13.9)			
2 weeks post	17.32 (5.3)	17.63 (7.9)	**Group**	0.14 (1, 28)	0.71
3 months post	21.3 (8.1)	20.2 (10.4)	**Time**	4.96 (2.29, 63.36)	0.007^{+}
6 months post	23.0 (9.3)	22.3 (11.4)	**Group x Time**	1.3 (3, 83)	**0.28**
HbA1c levels					
Baseline	39.7 (3.7)	37.7 (5.3)			
2 weeks post	37.2 (3.8)	34.5 (5.24)	**Group**	2.2 (1, 28)	0.15
3 months post	40.9 (3.8)	37.7 (5.0)	**Time**	22.0 (2.91, 79.61)	<0.0001^{+}
6 months post	40.9 (4.8)	38.3 (6.1)	**Group x Time**	0.67 (3, 82)	**0.57**

Table 3.6 Baseline and post-procedure glycaemic profile

Data presented as mean ± SD, ns: not significant. Analyses conducted with mixed-effects model with Geisser-Greenhouse correction. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. $^{+}$denotes significant p values

Timepoint	Group		Mixed-effects analyses		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
			HOMA-IR		
Baseline	6.2 (3.4)	6.1 (2.9)			
2 weeks post	4.02 (1.27)	3.89 (1.63)	**Group**	0.19 (1, 28)	0.66
3 months post	5.1 (2.2)	4.7 (2.5)	**Time**	6.04 (2.69, 74.58)	0.001+
6 months post	5.1 (2.7)	5.0 (3.1)	**Group x Time**	0.1 (3, 83)	**0.96**
			HOMA-B		
Baseline	295.1 (196.7)	454.8 (288.4)			
2 weeks post	313.3 (173.7)	462.6 (371.8)	**Group**	2.39 (1, 28)	0.13
3 months post	331.6 (139.8)	372.1 (239.7)	**Time**	1.07 (2.32, 64.08)	0.36
6 months post	287.2 (160.3)	363.9 (160.2)	**Group x Time**	1.12 (3, 83)	**0.34**
			QUICKI		
Baseline	0.3 (0.0)	0.3 (0.0)			
2 weeks post	0.3 (0.0)	0.3 (0.0)	**Group**	0.00 (1, 28)	0.94
3 months post	0.3 (0.0)	0.3 (0.0)	**Time**	2.43 (2.42, 68.84)	0.09
6 months post	0.3 (0.0)	0.3 (0.0)	**Group x Time**	2.23 (3, 83)	**0.09**

Table 3.7 Baseline and post-procedure clinical markers of insulin sensitivity and insulin secretion

Data presented as mean ± SD, ns: not significant. Analyses conducted with mixed-effects model with Geisser-Greenhouse correction. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. +denotes significant p values

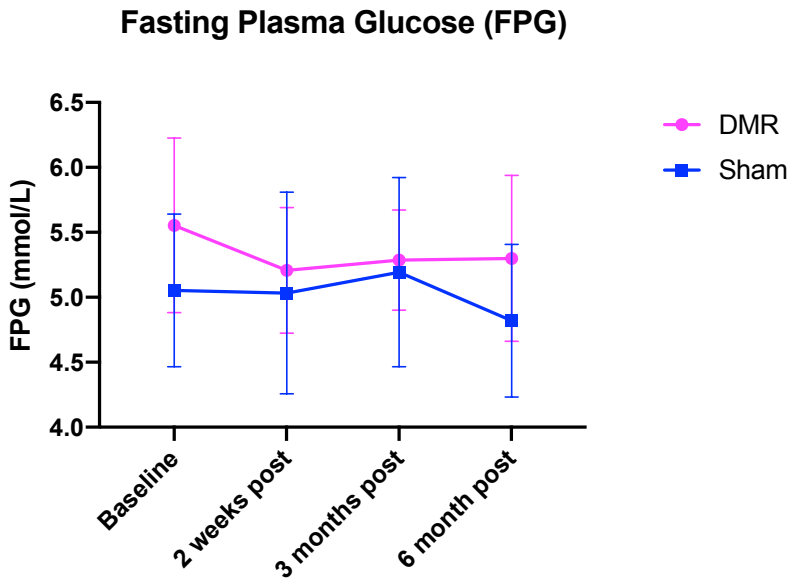

Figure 3.6 Fasting plasma glucose in the DMR and sham groups from baseline to 6 months post-procedure.

Data plotted as means ± SD, n=15 per group

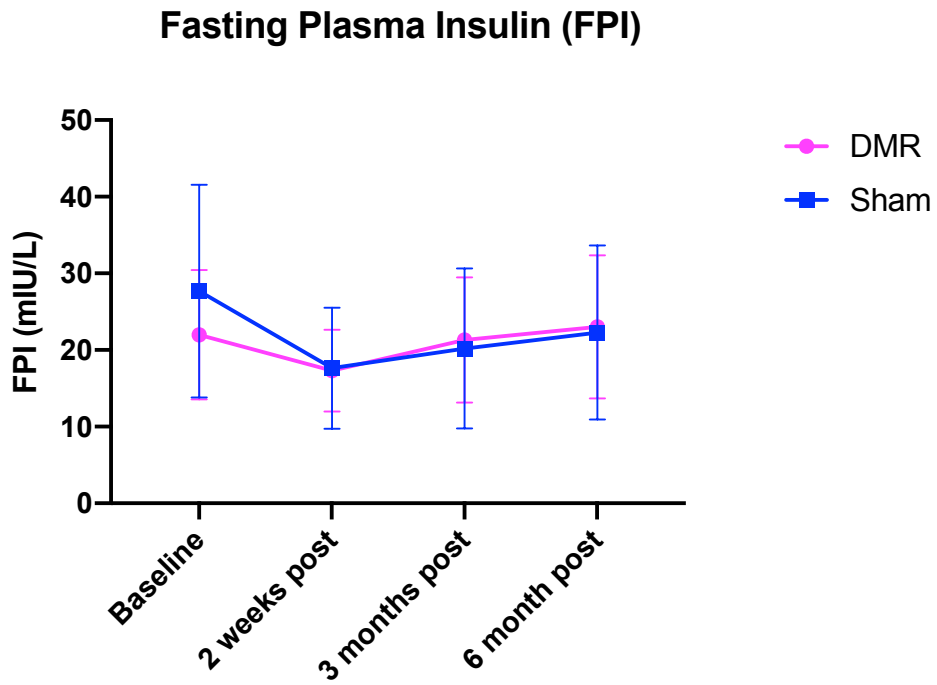

Figure 3.7 Fasting Plasma Insulin in the DMR and sham groups from baseline to 6 months post-procedure

Data plotted as means ± SD, n=15 per group

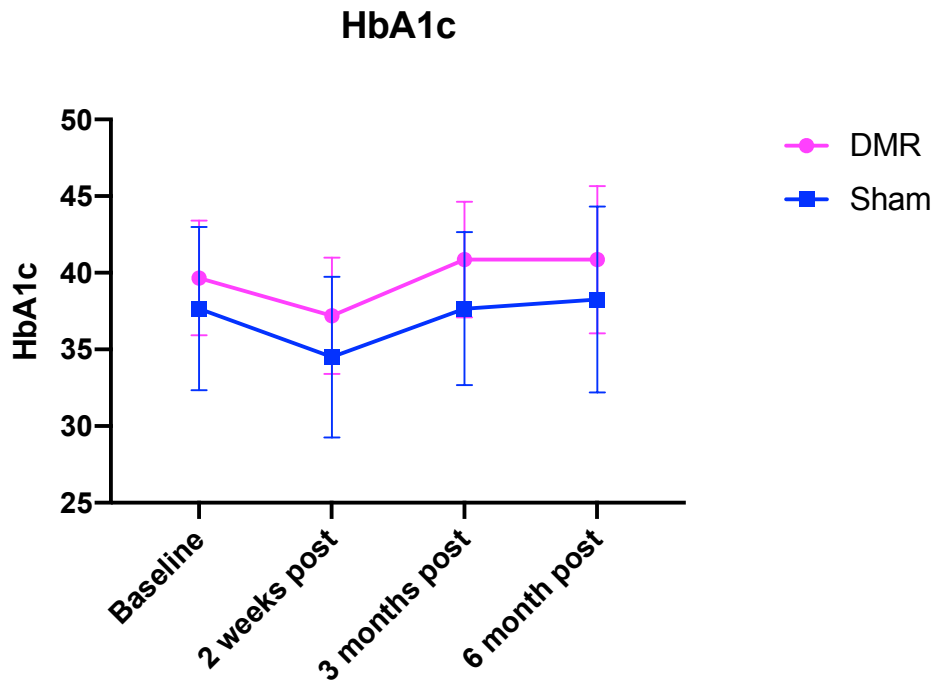

Figure 3.8 HbA1c in the DMR and sham groups from baseline to 6 months post-procedure

Data plotted as means ± SD, n=15 per group

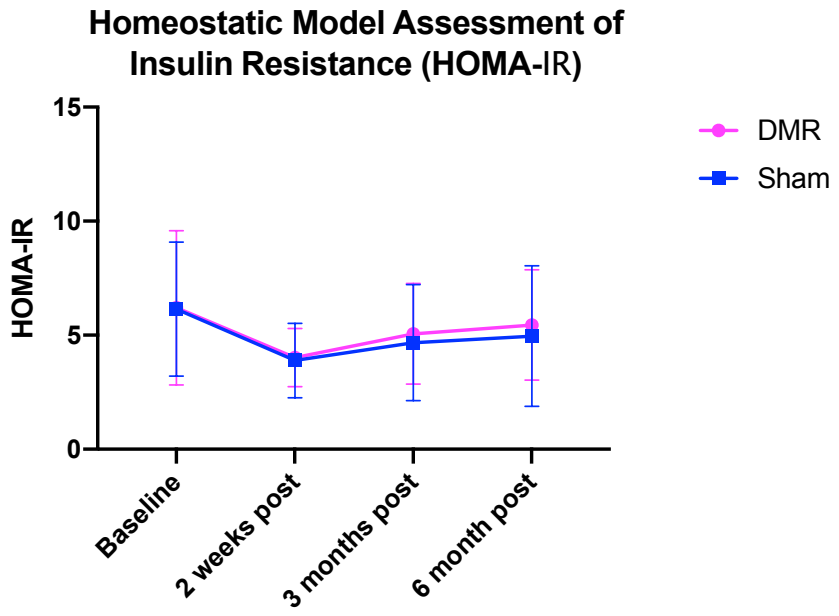

Figure 3.9 HOMA-IR in the DMR and sham groups from baseline to 6 months post-procedure

Data plotted as means ± SD, n=15 per group

Table 3.6 details the glycaemic profile of participants in both groups throughout the 6-month follow-up period.

Despite a significantly higher fasting glycaemia at baseline in the DMR group, this difference narrowed in the first 3 months following treatment mainly due to reduction in glucose concentrations in the DMR group (see Figure 3.6). However, HbA1c concentrations, although in the normal range in both groups, remained higher in the group treated with DMR reflecting a higher background glycaemia in the DMR group in the preceding 3 months (see Figure 3.8).

Conversely, the sham group displayed more profound hyperinsulinaemia, however there was no significant difference in insulin concentrations seen at baseline, or at any point post-procedure either between or within the two groups (see Figure 3.7).

This combination of changes in glucose and insulin concentrations in the two groups was reflected in the almost indistinguishable HOMA-IR profiles seen in both groups throughout the trial period. The HOMA-IR levels revealed a similar improvement in insulin sensitivity in both groups, although this was not statistically significant (see Figure 3.9).

Comparison of the participants' liver profile using mixed-effects analysis did not reveal any significant differences between or within the DMR and sham groups at baseline or during follow-up. However, the mean ELF score of the participants in the DMR group demonstrated a downward trend while that of the sham group was static (see Figure 3.10).

Similarly, mixed-effects analysis of the lipid profile of participants did not show any statistically relevant change either within each group over the follow-up period or between the two groups. However, although participants in the DMR group displayed a worse lipid profile than the participants in the sham group at baseline, this difference disappeared at 6 months for several lipid markers (see Figure 3.11 and Figure 3.12).

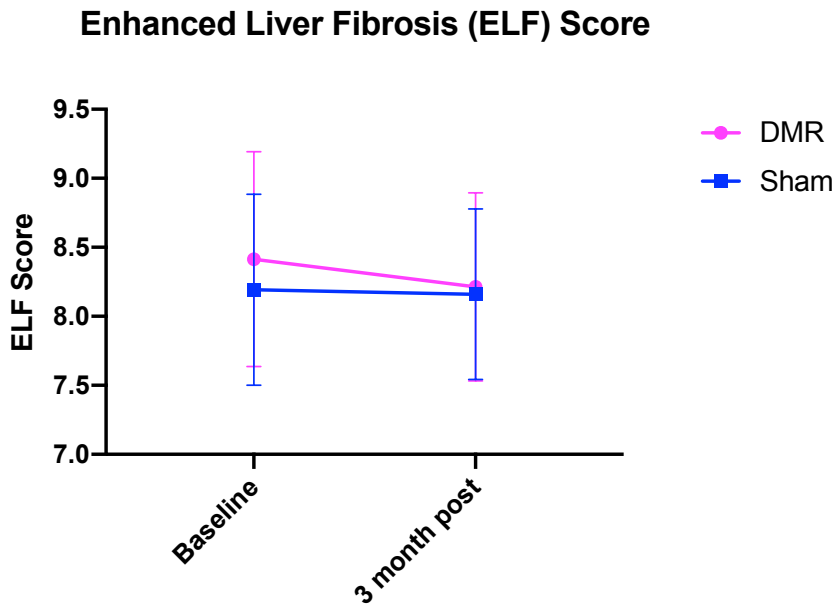

Figure 3.10 Enhanced liver fibrosis score in the DMR and sham groups at baseline and at 3 months post-procedure

Data plotted as means ± SD, n=15 per group

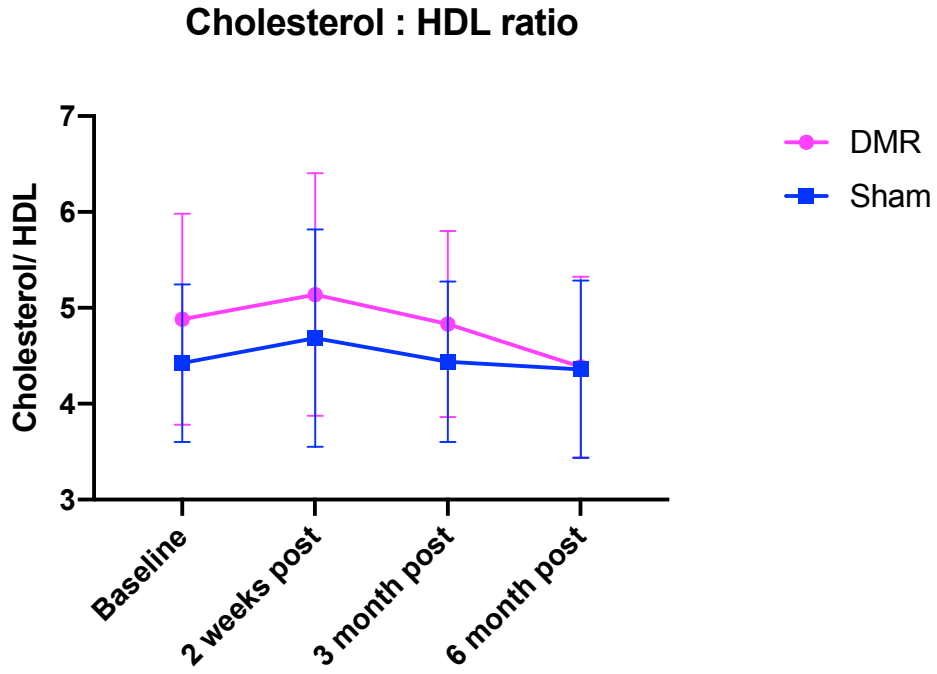

Figure 3.11 Cholesterol: HDL ratio in the DMR and sham groups at baseline and at 3 months post-procedure.

Data plotted as means ± SD, n=15 per group

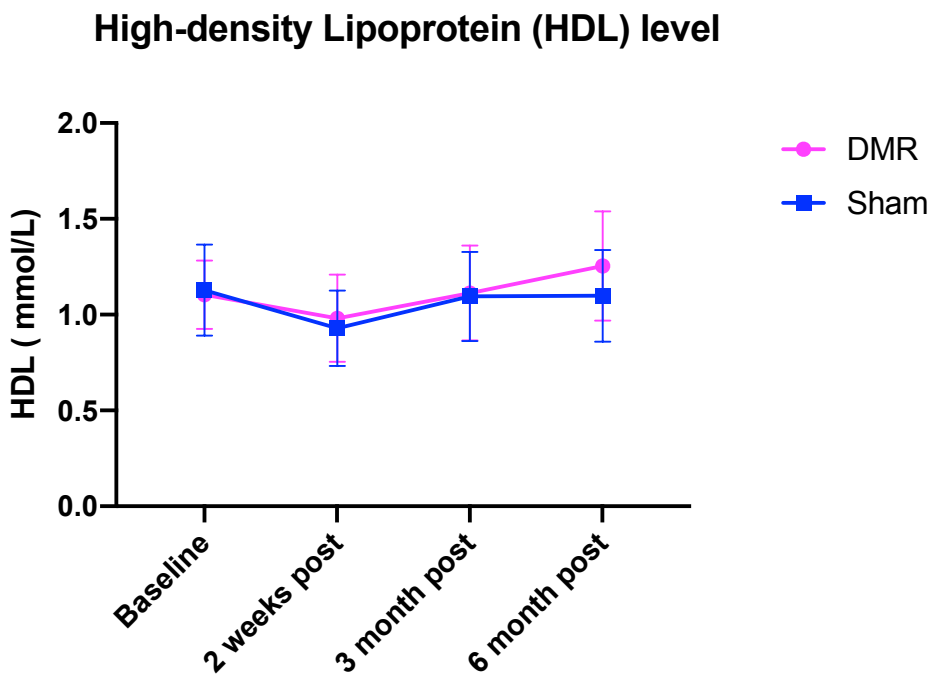

Figure 3.12 High-density lipoprotein (HDL) concentrations in the DMR and sham groups from baseline to 6 months post-procedure.

Data plotted as means ± SD, n=15 per group

3.4 Post-procedure reproductive outcomes

The reproductive changes for participants in both groups post-procedure are as detailed in Table 3.8 – Table 3. 11. There were no statistically significant differences seen between the two groups in any of the reproductive parameters in the post-operative period.

There were more menstrual periods and ovulatory events noted in the DMR group following the procedure (see Figure 3.13 and Figure 3.14). In addition, there was one pregnancy in the DMR group. Two patients in the DMR group were *virgo intacta* and were not assessed with weekly pelvic scans. Abdominal scans were attempted for both patients but did not yield adequate images.

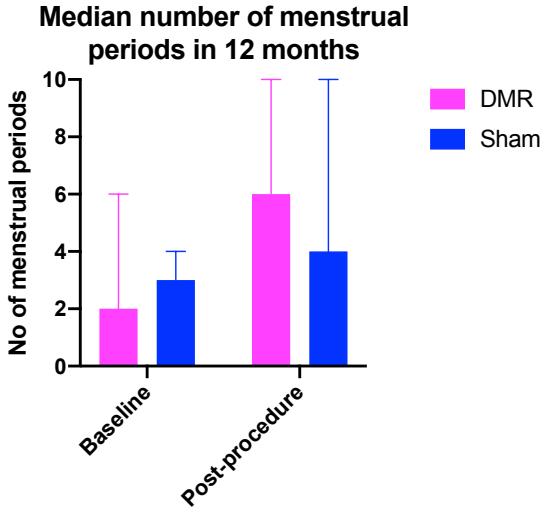

Figure 3.13 Number of menstrual periods in 12 months pre- and post-procedure

Data plotted as means ± SD, n=15 per group

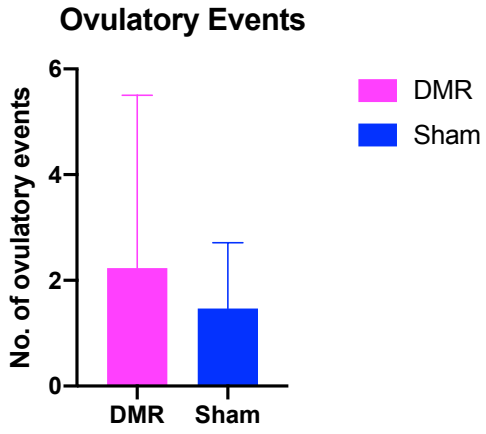

Figure 3.14 Number of ovulatory events in the DMR and sham groups at 6 months post-procedure

Data plotted as means ± SD, n=15 per group

Timepoint	Group		Mixed-effects analyses		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Luteinising Hormone (LH) (unit/L)					
Baseline	5.8 (3.7)	6.7 (3.0)			
2 weeks post	6.4 (3.2)	9.0 (6.5)	**Group**	1.20 (1, 28)	0.28
3 months post	6.7 (2.4)	6.6 (2.8)	**Time**	1.48 (1.64, 45.34)	0.24
6 months post	5.6 (3.4)	6.7 (3.4)	**Group x Time**	1.03 (3, 83)	**0.39**
Follicle Stimulating Hormone (FSH) (unit/L)					
Baseline	4.0 (1.3)	5.3 (3.0)			
2 weeks post	5.1 (1.3)	5.1 (1.62)	**Group**	2.48 (1, 28)	0.13
3 months post	4.4 (1.4)	5.5 (2.6)	**Time**	0.64 (2.10, 58.22)	0.54
6 months post	4.2 (1.3)	5.1 (1.9)	**Group x Time**	1.08 (3, 83)	**0.36**
LH: FSH ratio					
Baseline	1.4 (0.6)	1.4 (0.6)			
2 weeks post	1.25 (0.57)	1.66 (0.71)	**Group**	0.08 (1, 28)	0.78
3 months post	1.5 (0.3)	1.3 (0.4)	**Time**	0.5 (2.34, 66.35)	0.64
6 months post	1.3 (0.7)	1.3 (0.5)	**Group x Time**	3.28 (3, 83)	**0.02**$^{+}$

Table 3.8 Baseline and post-procedure levels of LH, FSH and LH: FSH ratio

Data presented as mean ± SD, ns: not significant. Analyses conducted with mixed-effects model with Geisser-Greenhouse correction. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. $^{+}$denotes significant p values

Timepoint	Group		Mixed-effects analyses		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Testosterone (nmol/L)					
Baseline	1.9 (0.9)	2.5 (1.5)			
2 weeks post	1.8 (0.5)	1.8 (1.1)	**Group**	0.84 (1, 28)	0.37
3 months post	1.9 (0.8)	2.2 (1.5)	**Time**	3.80 (2.14, 57.78)	0.026+
6 months post	1.7 (0.7)	1.9 (0.8)	**Group x Time**	1.0 (3, 81)	**0.40**
Sex Hormone-Binding Globulin (SHBG) (nmol/L)					
Baseline	21.9 (8.9)	28.1 (7.0)			
2 weeks post	22.1 (8.2)	25.0 (5.5)	**Group**	3.59 (1, 28)	0.07
3 months post	21.3 (7.4)	26.0 (7.2)	**Time**	1.15 (2.51, 69.46)	0.33
6 months post	21.1 (8.4)	26.9 (7.5)	**Group x Time**	1.27 (3, 83)	**0.29**
Free Androgen Index					
Baseline	10.1 (5.2)	9.7 (5.9)			
2 weeks post	8.9 (3.9)	7.7 (5.0)	**Group**	0.43 (1, 28)	0.52
3 months post	9.7 (5.0)	8.9 (5.8)	**Time**	1.94 (2.42, 65.38)	0.14
6 months post	9.7 (6.4)	7.6 (4.3)	**Group x Time**	0.35 (3, 81)	**0.79**

Table 3.9 Baseline and post-procedure levels of testosterone, SHBG and Free Androgen Index
Data presented as mean ± SD, ns: not significant. Analyses conducted with mixed-effects model with Geisser-Greenhouse correction. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. +denotes significant p values

Timepoint	Group		Mixed-effects analyses		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Dehydroepiandrosterone Sulphate (DHEAS) (µmol/L)					
Baseline	6.3 (3.6)	5.2 (4.1)			
2 weeks post	6.4 (3.3)	5.8 (3.4)	**Group**	0.10 (1, 28)	0.75
3 months post	5.9 (3.0)	6.0 (4.2)	**Time**	3.80 (2.51, 69.55)	0.74
6 months post	6.2 (3.0)	6.2 (4.2)	**Group x Time**	1.81 (3, 83)	**0.15**
Androstenedione (nmol/L)					
Baseline	6.4 (2.4)	6.0 (2.2)			
2 weeks post	6.6 (2.5)	5.6 (2.0)	**Group**	2.34 (1, 28)	0.14
3 months post	7.6 (3.3)	5.3 (1.9)	**Time**	1.15 (2.78, 76.92)	0.61
6 months post	6.7 (3.7)	5.3 (1.6)	**Group x Time**	2.68 (3, 83)	**0.05**

Table 3.10 Baseline and post-procedure concentrations of DHEAS and androstenedione

Data presented as mean ± SD, ns: not significant. Analyses conducted with mixed-effects model with Geisser-Greenhouse correction. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator.

	DMR	Sham	P value	
Menstrual periods*			Time	0.005^{+}
Pre-procedure	2 [0, 6]	3 [2,4]	Group	0.94
Post-procedure	6 [0, 10]	4 [2, 10]	Group x Time	**0.80**
Ovulatory Events	2.2 (3.3)	1.5 (1.2)		0.41
No of pregnancies (n)	1/15	0/15		N/A

Table 3.11 Clinical indicators of fecundity in the DMR and sham groups

*Data presented as mean ± SD, * Median [Interquartile range], ns: not significant, N/A: not applicable. Analyses conducted with repeated-measures 2-way ANOVA for Menstrual periods and unpaired t-test for Ovulatory events, $^{+}$denotes significant p values*

3.5 Post-procedure OGTT outcomes

Post-prandial glucose and insulin profiles at baseline, 2 weeks post- and 3 months post-intervention are as detailed in Table 3.12 and Table 3.13.

Timepoint	Group		Repeated Measures 2-way ANOVA		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Matsuda Index					
Baseline	2.5 (1.4)	2.0 (1.8)	**Group**	0.12 (1, 27)	0.74
2 weeks post	2.36 (1.01)	2.30 (0.72)	**Time**	1.72 (1.79, 48.36)	0.69
3 months post	2.1 (0.9)	2.3 (1.3)	**Group x Time**	2.45 (2, 54)	**0.10**
Oral Disposition Index					
Baseline	27.5 (10.3)	28.9 (9.1)	**Group**	0.41 (1, 27)	0.52
2 weeks post	29.5 (12.2)	31.7 (10.0)	**Time**	0.85 (1.69, 45.56)	0.42
3 months post	28.7 (9.2)	31.1 (9.1)	**Group x Time**	0.05 (2, 54)	**0.95**
Insulinogenic Index					
Baseline	1.6 (0.9)	1.8 (1.0)	**Group**	0.34 (1, 26)	0.57
2 weeks post	1.5 (0.8)	1.6 (0.5)	**Time**	1.83 (1.77, 46.01)	0.18
3 months post	1.8 (0.8)	2.0 (0.8)	**Group x Time**	0.17 (2, 52)	**0.85**

Table 3.12 Baseline and post-procedure levels of Matsuda Index, Oral Disposition Index and Insulinogenic Index

Data presented as mean ± SD, ns: not significant. AUC: Area under the curve. Analysis conducted with repeated-measures 2-way ANOVA. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator.

Timepoint	Group		Repeated Measures 2-way ANOVA		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
AUC Glucose (0-120 minutes)					
Baseline	9.8 (2.0)	9.7 (1.2)	**Group**	0.11 (1, 28)	0.85
2 weeks post	10.3 (1.6)	10.0 (1.7)	**Time**	0.95 (1.67, 46.72)	0.23
3 months post	9.6 (1.9)	9.7 (1.2)	**Group x Time**	0.35 (2, 56)	**0.82**
AUC Insulin (0-120 minutes)					
Baseline	119.3 (42.3)	149.2 (49.6)	**Group**	0.26 (1, 28)	0.61
2 weeks post	133.8 (43.9)	132.0 (49.6)	**Time**	0.82 (1.94, 52.26)	0.44
3 months post	141.1 (49.1)	146.6 (60.1)	**Group x Time**	1.643 (2, 54)	**0.20**
AUC Glucose (0-180 minutes)					
Baseline	14.6 (2.9)	14.3 (1.8)	**Group**	0.12 (1, 28)	0.74
2 weeks post	15.6 (2.3)	15.1 (2.6)	**Time**	3.50 (1.80, 50.38)	0.04^{+}
3 months post	14.4 (2.6)	14.4 (1.6)	**Group x Time**	0.21 (2, 56)	**0.81**
AUC Insulin (0-180 minutes)*					
Baseline	178.0 (63.1)	203.4 (56.0)	**Group**	0.27 (1, 26)	0.61
2 weeks post	187.5 (55.9)	196.3 (57.6)	**Time**	1.10 (1.93, 45.34)	0.34
3 months post	205.5 (67.5)	200.4 (76.8)	**Group x Time**	0.71 (2, 47)	**0.50**

Table 3.13 Baseline and post-procedure levels of the area under the curve (AUC) at 120 minutes and 180 minutes following glucose ingestion at OGTT

*Data presented as mean ± SD, ns: not significant. AUC: Area under the curve. Analysis conducted with repeated-measures 2-way ANOVA. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. *Analyses for AUC Insulin (0-180) conducted with mixed-effects model with Geisser-Greenhouse correction. $^{+}$denotes significant p values*

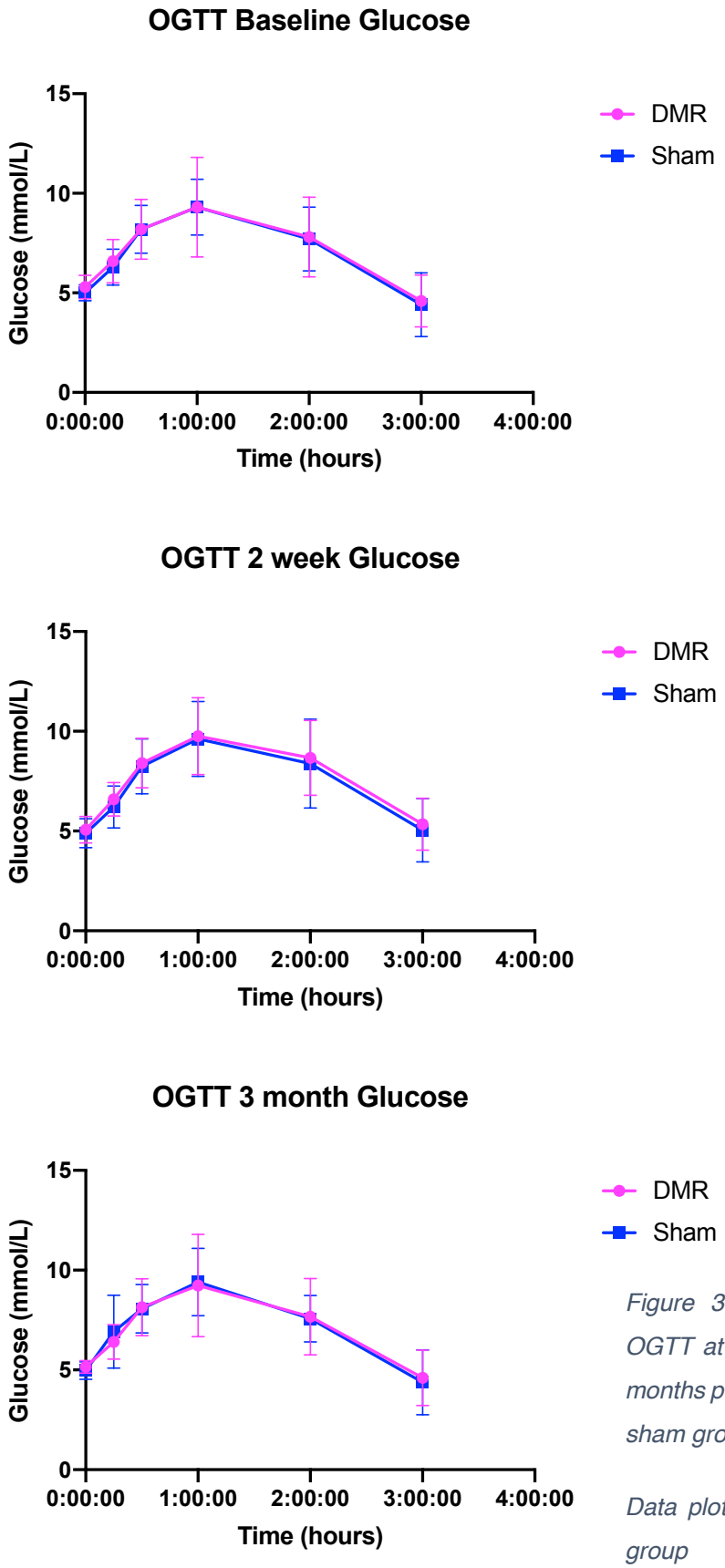

Figure 3.15 Glucose excursion during OGTT at baseline, 2 weeks post- and 3 months post-intervention for the DMR and sham groups.

Data plotted as means ± SD, n=15 per group

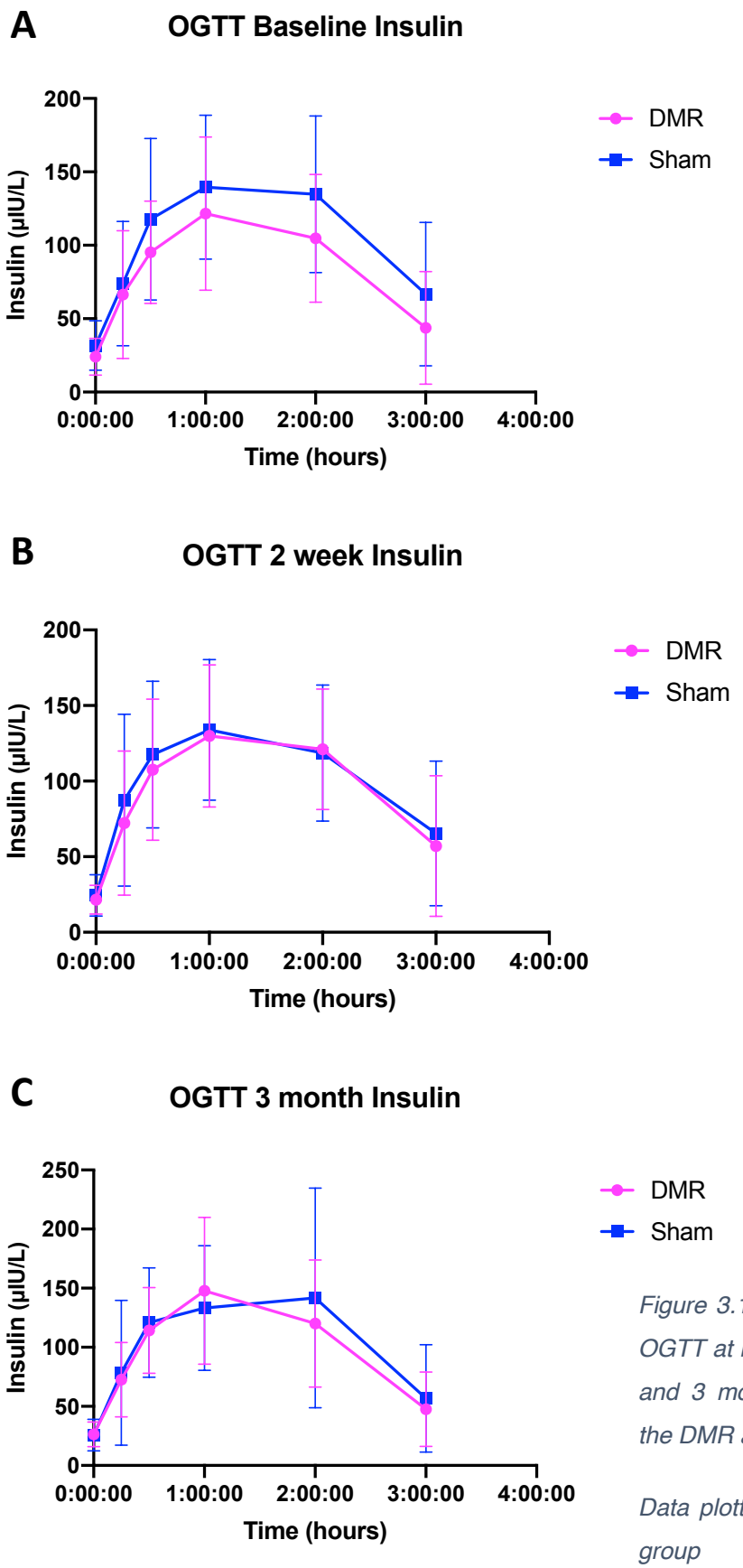

Figure 3.16 Insulin concentration during OGTT at baseline (A), 2 weeks post- (B) and 3 months post-intervention (C) for the DMR and sham groups.

Data plotted as means ± SD, n=15 per group

There was no significant difference between or within the groups in terms of glucose excursions and insulin secretion at baseline, 2 weeks after and 3 months after the intervention.

Similarly, analysis of the first phase response of insulin secretion, measured with the insulinogenic index using a mixed-effects model did not reveal any statistically significant difference either between or within groups at baseline, 2 weeks or 3 months after the intervention. On the insulin OGTT graphs, there was an increase in insulin concentration at 30- and 60-minutes post-ingestion at 2 weeks and 3-months post-intervention in the DMR group which is absent in the sham group. However, this difference did not reach statistical significance (see Figure 3.17).

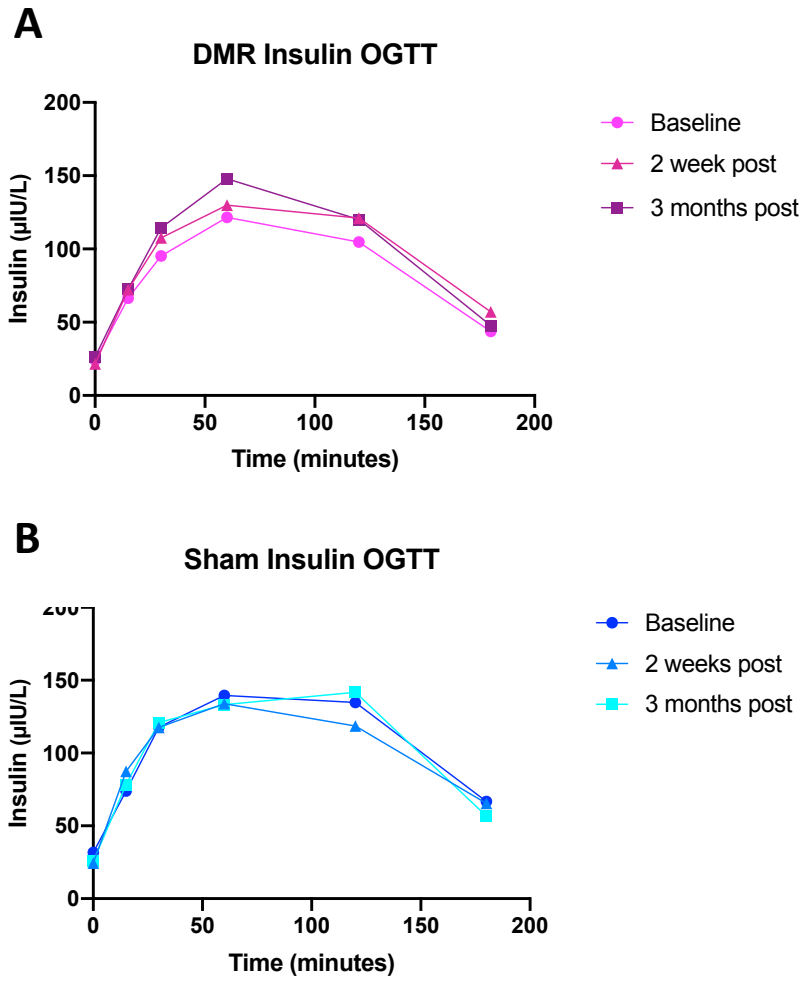

Figure 3.17 Insulin concentrations during OGTT for the DMR (A) and sham (B) groups at baseline, 2 weeks post- and 3 months post intervention.

Data plotted as means ± SD, n=15 per group

3.6 Post procedure hyperinsulinaemic euglycaemic clamp results

There was no difference in terms of insulin sensitivity (total, hepatic or peripheral) between the two groups or within the groups when comparing basal levels to levels of insulin sensitivity 3 months following intervention. Further the rate of endogenous glucose production and glucose uptake at basal conditions and during low dose and high dose insulin infusions were not significantly different either between or within the groups at baseline and 3 months post-intervention (see Table 3.14 – Table 3.16).

Timepoint	Group		Repeated Measures 2-way ANOVA		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Hepatic Insulin Sensitivity					
Baseline	1.4 (0.4)	1.4 (0.5)	**Group**	0.15 (1, 28)	0.70
3 months post	1.4 (0.4)	1.5 (0.4)	**Time**	0.14 (1, 28)	0.71
			Group x Time	0.06 (1, 28)	**0.81**
Peripheral Insulin Sensitivity					
Baseline	3.9 (1.3)	3.9 (1.8)	**Group**	0.03 (1, 28)	0.86
3 months post	4.0 (1.4)	4.1 (1.0)	**Time**	0.52 (1, 28)	0.47
			Group x Time	0.02 (1, 28)	**0.88**
Total Insulin Sensitivity					
Baseline	5.2 (1.7)	5.3 (2.1)	**Group**	0.06 (1, 28)	0.82
3 months post	5.4 (.16)	5.6 (1.4)	**Time**	0.56 (1, 28)	0.46
			Group x Time	0.04 (1, 28)	**0.85**

Table 3.14 Baseline and post-procedure levels of insulin sensitivity (hepatic, peripheral and total) for the DMR and sham groups

Data presented as mean ± SD, ns: not significant. Analysis conducted with repeated-measures 2-way ANOVA. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator.

Timepoint	Group		Repeated Measures 2-way ANOVA		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
Ra Basal					
Baseline	8.8 (1.1)	8.5 (0.7)	**Group**	1.96 (1, 28)	0.17
3 months post	9.0 (1.1)	8.4 (0.9)	**Time**	0.17 (1, 28)	0.68
			Group x Time	0.54 (1, 28)	**0.47**
Ra Low dose Insulin Infusion					
Baseline	2.5 (0.6)	2.3 (1.0)	**Group**	1.35 (1, 28)	0.26
3 months post	2.6 (1.0)	2.2 (0.9)	**Time**	0.01 (1, 28)	0.94
			Group x Time	0.16 (1, 28)	**0.70**
Ra High Dose Insulin Infusion*					
Baseline	0.6 (1.2)	0.3 (1.3)	**Group**	0.35 (1, 28)	0.56
3 months post	0.5 (0.85)	0.5 (0.9)	**Time**	0.00 (1, 27)	0.98
			Group x Time	0.25 (1, 27)	**0.62**

Table 3.15 Baseline and post-procedure levels of endogenous glucose production (Ra) under basal conditions, low-dose insulin infusion and high dose insulin infusion.

*Data presented as mean ± SD, ns: not significant, Ra: rate of appearance. Analysis conducted with repeated-measures 2-way ANOVA. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. *Analysis conducted with mixed-effects model.*

Timepoint	Group		Repeated Measures 2-way ANOVA		
	DMR	Sham	Effect	F value (DFn, DFd)	P value
			Rd Basal		
Baseline	8.9 (1.1)	8.5 (0.7)	**Group**	2.03 (1, 28)	0.17
3 months post	9.1 (1.1)	8.5 (0.9)	**Time**	0.14 (1, 28)	0.68
			Group x Time	0.54 (1, 28)	**0.47**
			Rd Low dose Insulin Infusion		
Baseline	10.7 (2.4)	10.2 (1.9)	**Group**	0.29 (1, 28)	0.60
3 months post	10.7 (1.9)	10.5 (1.7)	**Time**	0.22 (1, 28)	0.64
			Group x Time	0.77 (1, 28)	**0.39**
			Rd High Dose Insulin Infusion*		
Baseline	22.3 (7.5)	22.6 (8.1)	**Group**	0.04 (1, 28)	0.85
3 months post	22.0 (6.1)	22.2 (5.0)	**Time**	0.33 (1, 27)	0.57
			Group x Time	0.04 (1, 27)	**0.85**

Table 3.16 Baseline and post-procedure levels of glucose uptake (Rd) under basal conditions, low-dose insulin infusion and high dose insulin infusion.

*Data presented as mean ± SD, ns: not significant, Rd: rate of disappearance. Analysis conducted with repeated-measures 2-way ANOVA. DFn: Degree of freedom numerator, DFd: Degree of freedom denominator. *Analysis conducted with mixed-effects model.*

3.7 Procedural details

Sixteen participants underwent the DMR procedure during the RCT. One participant was replaced due to accidental unblinding of the investigation team peri-procedure. Another participant was replaced due to technical difficulty intubating the duodenum which precluded the procedure. Otherwise, DMR was feasible in all other participants and the required length of ablation was consistently achieved (see Table 3.17).

DMR Procedure	
Number of ablations	5.6 (0.9)
Treatment length (cm)	11.3 (1.8)
Total Procedure Time (minutes)	82.4 (49.2)

Table 3.17 Details of DMR procedure

3.8 Adverse Events

All adverse events experienced by participants following intervention, whether related to the procedure or otherwise, is reported in the table below using the MedDRA preferred terms (see Table 3.18).

The most common adverse event observed in both the DMR and sham groups were GI-related and were self-limiting in the majority of cases. Several events included in the list are likely unrelated to the DMR procedure, such as urinary tract infection.

There were two serious adverse events noted during the trial. One participant in the DMR group was admitted to hospital with cholecystitis 4 months following the procedure. One participant in the sham group developed breathlessness due to a mucous plug and was admitted for overnight observation following the procedure.

There were no serious adverse events directly related to the DMR procedure.

	DMR		Sham	
	Number of Events	% (n/N)	Number of Events	% (n/N)
GI disorders	**15**	**73.3% (11/15)**	**15**	**53.3% (8/15)**
Abdominal pain	9	53.3% (8/15)	4	26.7% (4/15)
Abdominal distension	3	20.0% (3/15)	1	6.7% (1/15)
Anal fissure & proctalgia	0	0.0% (0/15)	1	6.7% (1/15)
Constipation	0	0.0% (0/15)	1	6.7% (1/15)
Diarrhoea	0	0.0% (0/15)	2	13.3% (2/15)
Nausea	3	20.0% (3/15)	3	20.0% (3/15)
Oral candidiasis	0	0.0% (0/15)	1	6.7% (1/15)
Vomiting	0	0.0% (0/15)	2	13.3% (2/15)
Other sites of pain	**3**	**20.0% (3/15)**	**3**	**20.0% (3/15)**
Non-cardiac chest pain	1	6.7% (1/15)	0	0.0% (0/15)
Oropharyngeal pain	0	0.0% (0/15)	1	6.7% (1/15)
Pelvic pain	1	6.7% (1/15)	0	0.0% (0/15)
Muscle spasms	0	0.0% (0/15)	1	6.7% (1/15)
Migraine	1	6.7% (1/15)	1	6.7% (1/15)

Other AEs	**6**	**40.0% (6/15)**	**3**	**20.0% (3/15)**
Bronchial secretion retention*	0	0.0% (0/15)	1	6.7% (1/15)
Cholecystitis*	1	6.7% (1/15)	0	0.0% (0/15)
Urinary tract infection	0	0.0% (0/15)	1	6.7% (1/15)
Acute tonsillitis	1	6.7% (1/15)	0	0.0% (0/15)
Oral contusion	0	0.0% (0/15)	1	6.7% (1/15)
Dehydration	1	6.7% (1/15)	0	0.0% (0/15)
Pregnancy	1	6.7% (1/15)	0	0.0% (0/15)
Depressed mood	1	6.7% (1/15)	0	0.0% (0/15)
Alopecia	1	6.7% (1/15)	0	0.0% (0/15)
All TEAEs	**24**	**86.7% (13/15)**	**21**	**53.3% (8/15)**

Table 3.18 List of all Treatment Emergent Adverse Events (TEAEs) in both the DMR and sham groups during the follow-up period.
*Serious Adverse Events (SAEs) indicated with *.*

4 Discussion

4.1 General overview

4.1.1 Key Findings

The key findings of the DOMINO study are as follows.

- The results demonstrate that the addition of DMR to lifestyle modification did not yield additional improvements in insulin sensitivity or in the rate of ovulation in insulin resistant women with PCOS and obesity, during the 6-month follow-up period.
- Nonetheless, there was a trend to improvement in several metabolic and reproductive outcomes in the DMR group, which, although not statistically significant, does point toward a possible beneficial metabolic effect.
- Equally, it is important to consider that the blunted response seen may reflect key differences in the pathophysiological processes underlying insulin sensitivity in PCOS to those seen in overt T2DM.

The following discussion will be structured in terms of clinical outcomes, mechanistic outcomes and reproductive outcomes.

4.1.2 Appraisal of clinical outcomes

Despite adequate randomisation, the two groups were unbalanced for some clinical parameters. However, the groups were well-matched for primary and secondary outcomes.

The most obvious difference is that participants in the sham group weighed more and accordingly had a higher BMI and percentage body fat than the women who received the DMR procedure. However, there was no significant difference in terms of anthropometric measurements such as neck circumference, waist circumference and waist-to-hip ratios between the two groups. This indicates that the women in the two groups had different body composition; with women in the DMR group displaying a

greater propensity for visceral fat deposition. This is particularly relevant as visceral fat is well-recognised to be a greater predictor of dysmetabolism, than BMI, in women with PCOS[385]. In keeping with this, women in the DMR group also had a significantly higher Epworth Sleepiness Score at baseline[386].

Similarly, women in the DMR group had a significantly higher fasting plasma glucose concentration at baseline but this difference disappeared in the follow-up period, largely due to a decrease in fasting glucose concentrations in the DMR group. This was evident from 2 weeks post-procedure although women in both groups were on the same post-procedure liquid diet. However, this difference was small and did not reach statistical significance in the mixed-effects analysis.

This is unsurprising as DMR has been demonstrated to exert its most potent effect on patients with hyperglycaemia[374]. In fact, even in early DMR animal experiments on subjects with and without T2DM, the predilection of DMR to only exert an effect on hyperglycaemic animals was reported[372]. A similar trend has been reported in more recent human studies where the most significant improvements in HbA1 was seen in patients with the highest fasting hyperglycaemia[375]. This suggests that the insulin-sensitising effect of DMR may be specific to pathologically altered mucosa of patients with T2DM. As all the participants in our cohort were normoglycaemic and insulin resistant, the glycaemia-lowering effect of DMR, if present, would be small and a much larger cohort would be needed for a significant effect to be seen.

Further, women in the DMR group, despite weighing less than their counterparts, were more metabolically deranged as evident from the significantly higher concentrations of triglycerides seen at baseline in this group. Indeed, these women demonstrated more severe dyslipidaemia globally for all the lipid parameters measured – at baseline and for the duration of the study – although this difference was not statistically significant.

Raised triglyceride concentrations are of particular relevance, not only because it is a defining feature of metabolic syndrome, but also because it contributes to lipotoxicity in the pathogenesis of T2DM[386]. Unfortunately, no evidence of a meaningful decrease

in triglyceride concentrations was seen following the DMR procedure in our cohort although this has been demonstrated in other DMR studies[387]. There was, however, evidence of improvement in other lipid parameters such as for HDL concentrations and in the cholesterol: HDL ratio but again this did not reach statistical significance when compared to the sham group.

Similarly, ELF results indicate an improvement in liver fibrosis at 3 months post-procedure in the DMR group but this was not statistically significant. It is conceivable that a more prominent improvement in these parameters may have been evident if longer-term results were available. Recent DMR studies suggest more significant improvements in glycaemic and hepatic markers after 6 months follow-up which were sustained a year post-procedure[375]. It is possible that key analysis in this trial was conducted too prematurely and that longer-term follow-up may have yielded different results.

4.1.3 Appraisal of insulin sensitivity

The primary metabolic outcomes of the DOMINO trial was change in insulin sensitivity as assessed by the hyperinsulinaemic euglycaemic clamp and HOMA-IR.

While hyperinsulinaemic euglycaemic clamps with isotope tracers are regarded as the gold-standard for measurement of insulin sensitivity, they are also expensive, time-consuming, labour-intensive and complex to conduct. This has led to the development of simpler, quicker and cheaper methods to assess insulin sensitivity and its reciprocal, insulin resistance[388]. Assessment of insulin sensitivity can be performed from glucose and insulin concentrations in the fasted state and from dynamic investigations, as detailed below.

4.1.3.1 In the fasted state

Two of the most commonly used indices in the fasted state are HOMA-IR and QUICKI.

Both these surrogate indices use the presumption that following an overnight fast, a basal steady-state exists where glucose is homeostatically maintained in a particular

range as HGP and insulin levels are relatively stable[388]. Under these conditions, HGP will match glucose utilisation and the level of insulinaemia will reflect the degree of insulin sensitivity. This is primarily a measure of hepatic insulin sensitivity, however, under most conditions, insulin resistance is not tissue specific and the severity of hepatic and skeletal insulin sensitivity would be proportional to each other[388].

The HOMA model was developed based on the dynamic interaction between glucose and insulin and was used to predict steady-state glucose and insulin concentrations in a wide range of combinations of insulin sensitivity and β-cell function, in the fasted state[389]. The model presumes that a hepato-pancreatic feedback loop exists which acts to constantly regulate glucose concentrations by altering insulin-dependent HGP; which in turn affects insulin secretion by β-cells[389].

The HOMA model quantifies this relationship using a set of empirically derived non-linear equations to predict fasting steady-state concentrations of glucose and insulin for a wide variety of permutations of β-cells function and insulin sensitivity[388]. Computer simulations and mathematical transformation of glucose and insulin concentrations from different subjects in steady-state conditions were then used to determine insulin sensitivity (HOMA %S) and β-cell function (HOMA %B) [388]. Approximation of this model led to the development of a set of linear equations to calculate β-cell function (HOMA-B) and insulin resistance (HOMA-IR, the reciprocal of HOMA %S) [388]. This allowed a simple, quick, cheap method to assess insulin sensitivity. The equations were originally designed to be used in epidemiological studies and not individual clinical settings due to the risk of individual errors with the assays used[388]. Further, subsequent studies indicate variable correlation of the HOMA model with results derived from the hyperinsulinaemic euglycaemic clamps, particularly in hyperinsulinaemic states, such as PCOS[388, 390].

More than a decade later, an updated computer model of insulin resistance/ sensitivity and β-cell function was developed, HOMA2. This model accounted for some of the

variations in hepatic and peripheral glucose resistance, variation is hyperglycaemic states and for the contribution of proinsulin[388]. It has since been validated and an online calculator for HOMA2 is also now available to increase user convenience. Studies comparing the two models generally demonstrate superiority of HOMA2[388]. Even then, due to its simplicity, the HOMA1 models, particularly HOMA-IR, remains widely used. Its prevalent use allows ease of comparing and contrasting different studies, further fuelling its use.

HOMA-IR is calculated using the product of insulin and glucose under fasted steady-state conditions divided by a constant such that under ideal health conditions the result will be 1. People with a HOMA-IR level exceeding 2.5 are deemed insulin resistant. In this study a higher cut-off value of 3 was used for participant recruitment to ensure an insulin resistant cohort was selected. At baseline, the mean HOMA-IR of both groups was in excess of 6.0, suggesting that participants fitted the specified metabolic criteria.

QUICKI is another empirically-derived surrogate index of insulin secretion that uses mathematical transformation of fasting concentrations of glucose and insulin to provide a simple, reliable and accurate measure of insulin sensitivity with high positive predictive power[391]. To correct for the non-normal distribution of fasting values across a range of insulin and glucose concentrations, logarithmic transformation of glucose and insulin is used[388]. The QUICKI index is the reciprocal of the sum of the log transformed values and values below 0.334 are deemed insulin resistant[388].

The QUICKI index has been repeatedly demonstrated to have a high correlation with insulin sensitivity measures derived from hyperinsulinemic euglycaemic clamps[388]. Further, it has also been shown to be a more sensitive and specific marker of insulin sensitivity than HOMA-IR in women with PCOS, although it has a comparable profile to logarithmically transformed HOMA-IR values[392]. In view of this, QUICKI indices was calculated for participants in the DOMINO trial to confirm that they were appropriately insulin resistant. The mean QUICKI index for both groups at baseline was 0.3 (±0.0) with individual values ranging from 0.26 to 0.34 across both groups, indicating that insulin resistant participants were appropriately recruited. However,

participants did not display meaningful improvements in either parameter at 2 weeks, 3 month or 6 months post-procedure.

Another fasting index of insulin sensitivity is glucose: insulin (G/I) ratios which has a comparable profile to the QUICKI index in patients with PCOS[392]. A G/I ratio < 7 indicates insulin resistance. However, its general use is limited as it correlates poorly with hyperinsulinaemic euglycaemic clamp values in patients with T2DM who have fasting hyperglycaemia and impaired β-cell function. Contrastingly, the McAuley Index utilises fasting triglyceride values to calculate insulin resistance.

4.1.3.2 From dynamic tests

Surrogate indices of insulin sensitivity can also be derived from dynamic tests such as OGTT, mixed meal tolerance tests and IVGTT. In dynamic tests of insulin homeostasis, a standardised glucose load is delivered and the consequent hyperglycaemia, hyperinsulinaemia and return to basal concentrations can be measured. This reflects both hepatic and peripheral insulin sensitivity. Dynamic testing, although more expensive, more labour-intensive and more time-consuming; also has the advantage of providing information regarding insulin secretion, which tests using fasting samples lack.

For this trial, OGTT was selected for use as it is more physiological and the anatomical area being interrogated in the trial is the small intestine. The tests were carried out as described in Chapter 2. Insulin sensitivity was then derived using a specific formula which have been validated against the hyperinsulinaemic euglycaemic clamps in a relatively large number of subjects. A number of formulae exists for the calculation of insulin sensitivity from dynamic tests. These include Stumvoll Index, Avignon Index, oral glucose sensitivity index, Gutt Index and the Belfiore Index.

However, the Matsuda Index is arguably the most widely used of these and has also been validated for use in women with PCOS[392]. For this reason, it was selected for use in the DOMINO trial. The Matsuda Index was developed to provide an OGTT derived equivalent of Rd (rate of glucose disappearance measured during an insulin

clamp using a glucose tracer)[388]. A Matsuda index of 2.5 or less indicates insulin resistance. At baseline, the median Matsuda Index of the two groups was 1.8 [interquartile range 1.5 – 2.5], in keeping with the HOMA-IR and QUICKI indices. Further, similar to the fasting indices, no significant changes in the Matsuda Index was seen in either group following the procedure.

However, a caveat of using OGTT to assess insulin sensitivity, is that the rate of glucose appearance cannot be directly measured and empirical assumptions have to be used for its assessment. However, predictions here may lack precision as glucose concentrations will be affected by individual variability of glucose absorption, splanchnic glucose uptake and the incretin effect – hampering identification of insulin action in isolation. Hence, investigations which allow more reliable measurements of glucose appearance and disappearance, such as the hyperinsulinaemic euglycaemic clamp with glucose tracers, are very useful.

4.1.3.3 From hyperinsulinaemic euglycaemic clamp studies

The gold standard for measuring insulin sensitivity *in vivo* is with a hyperinsulinaemic, euglycaemic clamp[393]. As described in earlier sections, this technique allows accurate evaluation of whole-body insulin action by maintaining ('clamping') plasma glucose concentrations within the euglycaemic range (4-6 mmol/L), avoiding hypo- or hyperglycaemia and the accompanying counter-regulatory mechanisms. This is accomplished by infusing a pre-determined fixed dose of insulin and continuously adapting the glucose infusion rate to maintain euglycaemia based on the plasma glucose concentrations obtained from frequent blood sampling (every 5-10 minutes). At steady-state (usually in the final 30 minutes), the rate of glucose infusion is equal to the rate of glucose uptake by peripheral tissues.

During the first low-dose insulin infusion phase, the rate of glucose infusion during the steady-state phase is sufficient to suppress HGP and represents hepatic insulin sensitivity. The amount of HGP in response to insulin is quantified using a glucose isotope as detailed in Chapter 2. In the subsequent high-dose insulin infusion phase, the rate of glucose infusion during the steady-state phase is equal to the rate of

peripheral insulin-mediated glucose uptake (referred to as the 'M' value) and represents peripheral insulin sensitivity. Whole body insulin sensitivity is a combination of these two parameters.

As there exists significant inter-individual variability, there is no cut-off value for the definition of insulin sensitivity, making the inclusion of age- and weight matched controls a necessity when designing studies which utilise hyperinsulinaemic euglycaemic clamps[393]. M values corrected for body weight or insulin concentration is often reported in the literature but this practice is not recommended as it underestimates insulin sensitivity in individuals with obesity and increases variability. Correction of M value to FFM is useful to account for gender-related difference but was not relevant in the design of the DOMINO study.

In the DOMINO cohort, no significant change in insulin sensitivity was seen in total, hepatic or peripheral insulin sensitivity either between or within the two groups at baseline or at 3 months following intervention. Similarly, there was no difference seen in the rate of glucose appearance (Ra) and disappearance (Rd) when comparing results between or within groups. As detailed previously, Ra and Rd provide the gold-standard measure of insulin sensitivity from hyperinsulinaemic euglycaemic clamps with glucose isotope tracers.

As this is the first DMR study using hyperinsulinaemic euglycaemic clamps to investigate its effect, there is no comparator to measure against. However, evidence from mixed meal tolerance tests suggests that DMR exerts its main effect on fasting glucose levels (rather than in the post-prandial period)[394]. This indicates an effect on basal hepatic glucose production. In an effort to unravel it's still enigmatic mechanism of action, it is perhaps reasonable to postulate that DMR may result in improvement in hepatic insulin sensitivity in patients either with hyperglycaemia and/ or a pathogenic duodenal mucosa. This would be in keeping with recent data demonstrating hepatic fat-reduction and further studies in this area would be useful to glean a better understanding of its effect.

4.1.4 Assessment of β-cell function

In stark contrast to insulin sensitivity, there is no widely-regarded 'gold-standard' method to assess β-cell function *in vivo*[395]. In addition, all bar one surrogate index, requires a dynamic state to assess β-cell function, adding to the complexity of its assessment. This is because β-cell response is complicated and a degree of provocation, either intravenously or orally, is often required to assess its function[395]. Further, our current understanding of β-cell function is incomplete, making it unlikely that a single measure assessing one facet of its function would be able to provide sufficient information to be representative[395]. Thus, in practice, multiple indices are often used together, although correlation between theses indices is variable and the resultant assessment likely approximate and incomplete[395].

Of the available options, IVGTT is the typical test used and the acute insulin response (AIR) the typical index used[395]. IVGTT can also be used to provide AUC information and second phase insulin response[395]. Hyperglycaemic glucose clamps are also often used to provide information about first and second phase insulin secretion, although these are more cumbersome to perform than IVGTT[395]. Intravenous tests of β-cell function were not employed in the DOMINO trial as it was felt that it would likely be too onerous on the participant in combination with the other mechanistic and imaging studies already included into the study design, particularly as there are other measures β-cell function available.

At the most basic level, insulin concentration may be used to reflect β-cell function, as functioning β-cells are required for its release[395]. However, insulin concentrations are also affected by first pass hepatic removal and hepatic insulin clearance, the degree of which varies in different metabolic conditions[395]. This makes plasma insulin concentrations, used in isolation, a rather crude measure of β-cell function. However,

for measures assessing rapid insulin release such as first phase insulin secretion, plasma insulin concentrations is still a useful parameter[395].

In the DOMINO study, OGTT was used as an oral β-cell function test based on the presumption that glucose ingestion stimulates the entero-insular axis and provides a comprehensive assessment of insulin response. However, the caveat is that there are no specific means to distinguish insulin response from other contributors of the entero-insular axis such as incretin response, which are also perturbed by metabolic conditions[395].

Further, plasma insulin concentration measured has to be normalised to plasma glucose concentration, using modelling or empirical indices, to provide a measure of standardisation[395]. The most widely used OGTT-derived empirical index of β-cell function is the insulinogenic Index[395]. This is a measure of change in insulin concentration per unit change in glucose concentration over the first 30 minutes following ingestion of a standardised glucose load. It has a high degree of correlation with AIR from IVGTT and is widely denotated as a reasonable surrogate of early phase insulin secretion[396].

In the DOMINO study, the mean insulinogenic index of the entire cohort was 1.7 (±0.9) at baseline, in keeping with non-diabetic hyperglycaemia and preserved β-cell function. Following analysis with a mixed-effects model, there was no difference evident both between and within the groups at baseline, 2 weeks and 3 months post-intervention. However, there was an indication of increased insulin concentration in the first hour of the OGTT when compared to pre-procedure levels, although this difference did not reach significance. However, as mentioned earlier, using insulin concentrations *per se* to assess β-cell function is somewhat rudimentary and the implication of this increase in insulin secretion is unclear.

The oral disposition index (ODI) is another useful surrogate measure of β-cell function. It provides a more sophisticated measure of β-cell function as it incorporates (1) the

ratio of insulin to glucose following oral provocation and (2) the Matsuda Index, as an indication of insulin sensitivity[397]. Measurements incorporating values from the first 30 minutes as well as from 120 minutes have both been shown to be equally valuable by DeFronzo and colleagues; and indeed, have both been used as predictors of T2DM in population-based studies[397].

For the DOMINO trial, ODI was derived from the product of the Matsuda Index with the ratio of the OGTT AUC for the first 120 minutes of insulin to that or glucose. This was to prevent duplication of assessments for the early phase insulin secretion. However, similar to the mixed-effects model results for other markers of insulin sensitivity and secretion, no significant change was seen either between or within groups at baseline, 2 weeks post- and 3 months post-intervention

The only surrogate index of β-cell function derived from measurements of insulin and glucose in the fasted state is HOMA-B. The rationale of the HOMA model is as detailed in the previous section. However, as expected from a simplified test of a complex measure, HOMA-B has limited power and reliability[395]. It's use is suggested as an aid in the interpretation of data that has already been collected rather than an outcome measure[395]. Unsurprisingly, assessment of HOMA-B levels in the DOMINO cohort did not provide any additional information.

Finally, C-peptide, a polypeptide which is co-secreted with insulin in equimolar amounts and undergoes irrelevant hepatic extraction, can be measured to reflect insulin secretion[395]. However, its time-course is somewhat blunted and delayed when compared to insulin, and hence deconvolution, a mathematical operation, is required to reconstruct insulin secretion levels form C-peptide concentrations[395]. This requires specialised software and technical expertise, making its use rather limited due to its complexity and cost[395]. For these reasons, C-peptide deconvolution was not used in the DOMINO trial. Other tests of β-cell function to consider include the graded glucose infusion test, mixed meal tolerance test and tests using arginine or glucagon[395].

4.1.5 Assessment of reproductive outcomes

The assessment of reproductive function in women with PCOS is challenging for a number of reasons.

Firstly, by definition, women with PCOS have irregular menstrual periods which makes standardisation of timing of investigations complicated. This was evident in our cohort as the median number of menstrual periods reported by participants in the 12 months prior to trial enrolment was 3 with an interquartile range between 1 and 6.

Due to the multiple definitions in use for PCOS, an upper limit of 6 menstrual periods in the preceding 12 months was used to ensure that the most appropriate participants were recruited. This further compounded the problem, as apart from having infrequent periods most patients also reported highly variable cycle lengths .

To overcome this, all patients were prescribed a course of Medroxyprogesterone to induce a menstrual period before they attended the Baseline Visit. The induction of the menstrual period was in an attempt to carry out the baseline hyperinsulinaemic euglycaemic clamp in the follicular phase of the menstrual cycle for all participants. However, it is impossible to be certain this was consistently achieved due to variation in response to Medroxyprogesterone and variable cycle lengths. Further, even if participants underwent the mechanistic visit at similar times in their menstrual cycles, this had no relevance to the timing of the intervention or subsequent mechanistic and clinical visits due to the unknown cycle length of individual participants.

In view of this, to gather meaningful information with regard to ovulation, all participants who were sexually active were assessed with weekly pelvic ultrasound scans and weekly reproductive blood tests from week 12 to week 24 post-procedure. Transabdominal scans were attempted on two participants from the DMR group who were *virgo intacta* but the views obtained were inadequate. For all other participants, transvaginal ultrasound and biochemical assessment were used to collect information to facilitate quantification of ovulatory events.

This information was collated using a composite score which accounted for the presence of a dominant follicle or a corpus luteum and isolated progesterone rises. In addition, all patients were provided with an online tool to record details of each day of their menstrual period. Together this provided information with regard to ovulation and menstruation post-intervention.

However, apart from participant-reported baseline menstrual data, there were no pre-intervention ovulation data available for comparison. Although this would have been useful for comparison purposes, it that would have also necessitated a similar structure of ultrasound scans and biochemical assessments which would have substantially increased the cost and duration of the study.

Comparison of means between the two groups did not highlight a statistically different number of ovulatory events at 24 weeks post-intervention, although women in the DMR group had more ovulatory events during the assessment period. Similarly, the number of menstrual periods were not significantly different between or within the two groups, although women in the DMR group reported having more menstrual periods than their counterparts in the sham group. Further one woman in the DMR group was found to be pregnant when she attended her final ultrasound scan, despite reported adequate barrier contraception use.

These markers of increased ovulation, although none statistically significant, provide an interesting picture of the reproductive effect in the cohort who received the DMR procedure. As there was no difference seen in insulin sensitivity, it does appear unlikely that the reproductive improvements seen, if any, is related to a DMR-metabolic effect. However, it must be recalled that the mechanism of action for DMR is yet to be elucidated and it is possible that these participants have benefited from metabolic improvements in a marker that has not been measured.

4.1.6 Other considerations

There are a few other areas to consider when appraising the results of this study.

First, it is likely that the differences in pathophysiology between T2DM and PCOS may have led to the differences in response to DMR seen. The initial assumption is that patients with established T2DM have more diseased duodenal mucosa and hence a larger effect was evident following resurfacing. Conversely, the non-T2DM trial participants had less severely diseased duodenal mucosa and only experienced minimal improvements following mucosal resurfacing, which did not translate into clinically meaningful outcomes. This view suggests that a difference may have been evident if a larger sample size was used or a cohort with potentially more diseased duodenal mucosa such as patients with non-diabetic hyperglycaemia or T2DM.

Further, in keeping with the dose-dependent effect reported in the first-in-human DMR study, a longer length of treatment may result in a more profound effect. However, this concept is unlikely to be verified until a catheter than can consistently navigate beyond the ligament of Treitz is designed. This view is further corroborated by the more profound effect seen with longer intestinal bypass length such as with RYGB or DLBL and from experiments highlighted in Chapter 1 which demonstrate the anti-incretin role of the jejunum.

A second, alternative explanation for the results seen is based on the fact that not all insulin resistant states are the same and hence a difference in response to DMR is to be expected. Although insulin sensitivity has not been formally sub-typed, it is clear that it is can be rudimentarily be classified by organ-system (hepatic, peripheral, central) or predominant contributor (hyperinsulinaemia, hyperglycaemia or both) and two people with the same HOMA-IR levels may differ in both aspects.

This may underlie the lack of response seen to DMR in this study. Patients with T2DM, by definition, will have a tendency to hyperglycaemia and DMR has been shown to have a glycaemia-lowering effect. Conflictingly, the majority of patients in the DOMINO study were normoglycaemic suggesting preserved gluco-regulating ability which would not benefit from further enhancement. On the other hand, like many patients with PCOS, participants in both groups of the DOMINO trial had a significant fasting hyperinsulinaemia. This basal hyperinsulinaemic state would have been a greater contributor to the diagnosis of insulin sensitivity when using HOMA-IR for diagnosis

(or for study recruitment as in this case). However, recent DMR studies suggest that DMR does not exert a fasting plasma insulin-lowering effect which provides further explanation for the lack of response evident.

In view of this, it may be prudent for future DMR studies to limit recruitment to participants with a degree of dysglycaemia (T2DM, non-diabetic hyperglycaemia) rather than using HOMA-IR cut-off points. Although, OGTT was included as an optional test for recruitment in this study, the more convenient and quicker HOMA-IR was elected for use in the majority of cases as OGTT was not mandatory.

Likewise, it may also be worth considering using glycaemic outcome measures as primary end-points rather than insulin sensitivity, to avoid diluting the effect seen.

4.2 Safety and feasibility

This trial demonstrates that the DMR procedure is a safe and feasible procedure in this cohort. Only in one instance was the procedure not viable to be carried out due to anatomical reasons and there were no SAEs directly-related to the DMR procedure.

Notably, following DMR, no participants developed fasting hypoglycaemia at 2 weeks, 3 months or 6 months, reflecting preserved regulation of glucose homeostasis after DMR in this cohort. This is reassuring as this was the first time the procedure had been attempted in a population without T2DM.

Further, in keeping with worldwide trends, there were no GI luminal injuries noted during the trial. Again, this was the first time the procedure had been attempted in this population and it was useful to learn that even in participants with non-diabetic duodenal mucosa, the procedure can be carried out safely.

There were two SAEs during the course of the trial. In the DMR group, one participant was admitted with cholecystitis 4 months following her DMR procedure. The clinical team managing her care (at a different hospital) did not feel this was related to the DMR procedure. Further, the details of the admission were independently reviewed by the Joint Research Compliance Office (JRCO) who also did not feel it was related

to the DMR procedure. The second participant developed a mucus plug causing breathlessness shortly after her procedure. This participant was in the sham group but as this is a GA-related event, it is conceivable that it could equally occur in a participant receiving the DMR procedure.

As expected, abdominal pain was the most commonly cited AE in the DMR group and this resolved with no or mild analgesia in all cases. Abdominal distension, presumably secondary to air insufflation used for endoscopic visualisation, was also self-limiting within a few days. Several participants developed AEs relating to the post-procedure diet, most commonly diarrhoea (ascribed to possible high-dose dairy intolerance) and nausea and vomiting (relating to the taste of some flavours). Participants are directed to consume 2-3L of water a day in the first fortnight but despite this one participant developed dehydration and a second participant developed severe constipation and an acute anal fissure. Both of these participants were managed conservatively with good effect.

As stated previously, several events listed above are likely unrelated to the DMR procedure. However, as this is still a new device, it is prudent to be exhaustive in this regard.

4.3 Strengths and Limitations

The strengths and limitations of the DOMINO study are detailed in this section.

4.3.1 Strengths of the study

The DOMINO trial was a mechanistic study conducted using a multi-centre prospective double-blinded sham-controlled RCT design.

Well-conducted double-blinded RCTs are widely regarded as trial designs which provide the highest level of evidence[398]. Randomisation minimises selection bias which reduces the risk of systematic error; while double-blinding curtails behavioural modification and interpretation bias by both participants and investigators[398]. Additionally, as opposed to many procedural RCTs, this trial also had the advantage

of including an equivalent sham procedure in its design, which precluded perception of procedure allocation, further strengthening the trial design.

Further, in the interest of standardisation, all procedures – DMR and sham – were performed by a single endoscopist with the same endoscopy team each time. Similarly, participants received standardised caloric intake and lifestyle modification advice from a single obesity dietitian, who was also blinded to allocation, for the duration of the trial. All mechanistic studies were also conducted by a single researcher who was blinded to the procedure allocation. Only one participant lost to follow-up due to unavoidable personal reasons.

The main strength of this trial is in its use of gold-standard mechanistic methodologies in the assessment of insulin sensitivity. Hyperinsulinaemic euglycaemic clamps, although expensive, time-consuming and technically-demanding to perform; provide the most accurate measure of insulin sensitivity which further adds to the credibility of the trial results[399]. In addition, the use of a stable isotope tracer methodology during the hyperinsulinaemic euglycaemic clamps provides a highly reliable technique for the quantification of the rate of endogenous glucose production and glucose uptake. Further, in this trial, the quality of all glucose samples for every clamp performed were verified by an independent laboratory which specialises in insulin clamp techniques. This was to ensure that all the hyperinsulinaemic euglycaemic clamps conducted were consistently executed at a high standard.

Apart from being the first DMR trial to utilise hyperinsulinaemic euglycaemic clamps, the DOMINO trial is also the first trial to investigate the effects of DMR on a cohort of participants with insulin resistance but without T2DM. Further, this is also the only trial worldwide to evaluate the effects of DMR using PCOS as a model of insulin resistance. Improvements in insulin sensitivity in this cohort of patients would have the additional benefit of allowing assessment of change in other PCOS features including reproductive function and ovulation. Further, using a cohort of participants without T2DM helps circumvent the confounding pharmacological effect of concurrent glucose-lowering medication on glycaemia and insulinaemia.

To effectively appraise reproductive function and ovulation, all participants (excluding participants who were *virgo intacta*) were assessed weekly from week 12 to week 24 with a pelvic ultrasound scans and reproductive bloods. This provided a precise means of tracking physiological changes within the uterus and ovaries during each menstrual cycle and affords detailed information with regard to the presence of a corpus luteum or a dominant follicle.

Finally, DMR is a new device using novel technology that is only starting to become established in the management of T2DM. The rationale of investigating the effects of this state-of the-art tool with gold-standard methodology in patients at risk of diabetes, with a view to decrease metabolic risk and influence ovulation, is commendable and clearly reflects the pioneering thinking that led to its development.

4.3.2 Limitations of study

Notwithstanding the use of robust trial methodology and meticulous trial conduct, there are several inadvertent limitations in this study.

First, despite randomisation, the populations were inhomogeneous at baseline for certain parameters as discussed above. However, in term of its primary and secondary outcomes, the two populations were well-matched.

Second, the trial was designed more than 3 years previously based on the limited data available on DMR at that time. Since then, evidence around DMR has evolved. In hindsight, a larger sample size with a longer follow-up time and the use of hyperinsulinaemic euglycaemic clamps to assess insulin sensitivity at 6 months rather than 3 months may be preferable. Further, as early DMR studies suggest no effect on normoglycaemic subjects, it may have been advantageous to recruit participants with non-diabetic hyperglycaemia, rather than those with normal fasting glucose concentrations[372].

Finally, as participants were selected from the general population rather than clinical settings, it would have been beneficial to design a run-in period following recruitment

to allow clinical stabilisation and to minimise the Hawthorne effect, which describes alterations in the behaviour of trial subjects due to awareness of being observed.

4.4 Future direction

Recent studies on DMR has suggested that in addition to an insulin sensitising effect, it also exerts a hepatic fat-reducing effect[374, 394]. Further to T2DM, future studies focused on patients with NAFLD would be beneficial particularly as there remains no NAFLD-specific treatment as yet.

In terms of PCOS, it would be interesting to assess the effect of DMR in patients with PCOS and T2DM. As the current study did not find a difference in insulin sensitivity in participants who received the DMR procedure, it is difficult in this study to attribute improvements seen in reproductive function to DMR. However, as DMR has been reliably demonstrated to improve glycaemic markers in T2DM, it is feasible that in this population, DMR may also exert an effect on improving metabolically-determined PCOS features, including ovulation.

In addition, further studies establishing the mechanism of action of DMR would be valued as this is still obscure. Specifically, scrutinising markers involved in the pathogenesis of T2DM, such as gut hormones including the incretins, bile acids and the gut microbiome would be beneficial.

Additionally, although not statistically significant, this study indicated an improvement in lipid profile in the participants who underwent DMR. Focus on this area may also provide further illumination in terms of mechanism of action. It may be useful to extend this in future studies with evaluation of body fat topography with tools such as DEXA or MRI to assess for change in visceral fat. Changes in visceral fat would be in keeping with the previously demonstrated finding of hepatic fat reduction. Similarly, studies which include duodenal biopsies may also expose hitherto unknown changes at the cellular level.

As the role of DMR in the management of T2DM continues to be established, evidence surrounding the optimal treatment length and duration of effect would be valuable.

Unpublished evidence form the parent company suggests that the insulin-sensitising effect is strongest with treatment that commence in the most proximal post-papillary duodenum. While the proximal limit is clear, the distal extent is still to be determined. The first-in-human study established that a longer treatment length provided superior results compared to the shorter treatment length. However, the optimum length is unknown and even within this study, treatment lengths varied from 10 – 16cm.

Further, recent studies suggest that DMR has a durable effect with benefit still evident at 2 years. Future studies with longer follow-up times are awaited and subsequently, if required, studies investigating the feasibility, safety and efficacy of repeated DMR procedures in the same patient is anticipated. Ablative techniques are widely used on many different tissue types, from cardiac to uterine tissue, and repeated treatments have been used safely with good effect. Although, this is encouraging, the effect of repeated hydrothermal ablations on duodenal mucosa remains to be seen.

Finally, as DMR does not cause weight-loss, studies aimed at individuals with T2DM but without obesity would be of potential clinical value as these individuals are currently precluded from metabolic surgical procedures on the NHS based on current guidelines.

4.5 Conclusions

In conclusion, this is the first attempt of interrogating the metabolic effects of DMR, a novel endoscopic procedure, on insulin-resistant participants without T2DM. The DOMINO trial, a mechanistic study using gold-standard methodology was conducted with a multi-centre prospective double-blinded sham-controlled RCT design in 30 patients with PCOS. Following a 6-month follow-up period, DMR did not has not exert an insulin-sensitising effect in patients with PCOS and insulin resistance. Correspondingly, there was also no significant change in ovulation or reproductive outcome seen.

Potential explanations for these findings include the normoglycaemic nature of the participants, the small sample size and the early timing of mechanistic studies. Further,

pathophysiological differences in insulin resistant states may also contribute, as highly effective T2DM therapies may have disparate effects in PCOS or NAFLD.

This study demonstrates that although DMR is safe and feasible in the population studied, physiological evidence from mechanistic investigations suggest that it may not have a place in the management of normoglycaemic patients with PCOS.

However, as DMR continues to gain a foothold in the management of T2DM and further evidence on its mechanism of action is amassed, it's utility in patients with other metabolic conditions such as NAFLD may emerge.

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6 Appendices

Appendix 1 DOMINO Trial Protocol

Appendix 2 DOMINO Trial Information Sheet and Consent Form for Research Participants

Appendix 3 DOMINO Trial On-line Recruitment Website

Appendix 4 DOMINO Trial Statistical Analysis Plan

6.1 DOMINO Trial Protocol

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Study Protocol

Investigation of the metabolic effects of DuOdenal resurfacing on insulin resistant woMen with polycystic ovariaN syndrOme

The DOMINO Trial

Background and rationale

Polycystic ovarian syndrome and insulin resistance.

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting women, which causes the dysregulation of the menstrual cycle, excessive actions of the male hormone, testosterone (hyperandrogenism), and polycystic ovaries (1). The prevalence of PCOS is approximately 15%-20% of women (2). Clinical manifestations include reduced or absent menstrual cycles, excess hair growth, and infertility. Moreover, women with PCOS have an increased rate of obesity, with a propensity toward abdominal deposition of body fat (3) and insulin resistance which affects 50%-70% of women with PCOS (4). This insulin resistance occurs mostly in muscle and fat, and results in increased pancreatic insulin secretion

to maintain normal glucose levels (4). Hyperandrogenism, obesity and insulin resistance lead to a number of comorbidities including metabolic syndrome, hypertension, dyslipidaemia, glucose intolerance, and type 2 diabetes mellitus (T2DM) (5).

Weight loss is the most effective therapy for restoring fertility in women with PCOS (1). Reduction in weight of as little as 5% can restore regular menses and improve response to ovulation- inducing and fertility medications. However, lifestyle interventions only work in the short term and is very difficult to maintain. Other pharmacological and invasive treatments have high rates of side effects and limited efficacy (1, 6, 7). As a consequence, a large number of these women remain infertile and this has a devastating impact on their psychological health (8-10).

Duodenal mucosa resurfacing.

The Fractyl Revita System™ has achieved CE-marking and a number of studies support its safety and effectiveness in T2DM patients (11, 12). So far 102 patients have been treated with this duodenal mucosal resurfacing (DMR) technology around the world with very acceptable rates of complications (11). The procedure is performed endoscopically, without surgery, and under general anaesthesia and in most patients as a day-case. A balloon attached to a catheter is inserted endoscopically and through the use of hot water thermally (by using heat) ablates ~10cm of the duodenal mucosa (11).

The procedure is very effective for the treatment of patients with T2DM with rapid reductions in blood glucose (11). It is thought that it works mainly by increasing insulin sensitivity which the predominant mechanism in both T2DM and PCOS (1).

In this trial we will investigate both whether this non-invasive device indeed increases insulin sensitivity using gold-standard methodologies but also whether it can help women of reproductive age start menstruating. Both the intervention and control group will be given intensive NHS Tier 3 lifestyle advice for weight loss; therefore, the control group is expected to benefit from the trial.

If successful, this trial can have a substantial impact on millions of women around the world. The technology could be used as a one-off treatment to enable women with PCOS to get pregnant without exposing them to the side effects of long-term medications or the complications of invasive pelvic interventions.

Objectives

Our main objectives are to assess the effect of the Fractyl Revita System™ in women with PCOS in terms of:

Insulin sensitivity

Ovulation and menstruation

Trial team

The Chief Investigator is Dr Alexander Miras, Senior Clinical Lecturer in Endocrinology at the Department of Investigative Medicine, Imperial College London. Co-investigators and collaborators are Dr Channa Jayasena Dr Dev Bansi, Dr Belen Perez-Pevida, Miss Vasha Kaur, Professor Harpal S Randeva, Dr Georgios K. Dimitriadis, Dr Barbara Fielding and Dr Bu'Hussain Hayee.

Trial design

This will be a prospective double-blinded randomised controlled clinical trial. The setting will be a multi-entre with tertiary obesity, metabolic medicine and reproductive endocrinology expertise. Thirty female patients will be recruited and randomised to either DMR or the sham procedure. All patients will be registered at Imperial College Healthcare NHS Trust. Patients who have their procedure performed at King's College Hospital NHS Trust will also be registered there.

Both groups will receive standard NHS Tier 3 lifestyle advice and support for the duration of the trial. Lifestyle modification aimed at weight loss will be delivered by a dietician (and psychologist as necessary) in monthly group or individual sessions for a period of 6 months.

All patients will also be followed up for 6 months.

All patients will receive £300 upon completion of the study as a reimbursement for their time and inconvenience. Patients based outside the M25 will also have their travel expenses reimbursed (in addition to the £300).

Inclusion criteria

Female participants

Age 18-50

Body mass index (BMI) ≥ 30 kg/m^2

Diagnosis of PCOS based on the NIH Criteria. Require ALL of the following:

a) Menstrual irregularity (anovulation or >35 day cycle)

b) Clinical or biochemical hyperandrogenism

c) Exclusion of other causes other aetiologies of menstrual dysfunction (e.g. thyroid dysfunction, hyperprolactinaemia)

Insulin resistance as defined by a 2-hour oral glucose tolerance test glucose concentration of 7.8 mmol/l and/or HOMA-IR ≥ 3.0.

Willing to comply with study requirements and able to give informed consent

Exclusion criteria

Type 1 or Type 2 diabetes mellitus

History of any medical, psychological or other condition, or use of any medications, including over-the-counter products, which, in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer. These includes:

Active H. pylori infection (Participants with active H. pylori may continue with the screening process if they are treated via medication and re-testing verifies the condition has resolved.)

Previous gastrointestinal surgery that could affect the ability to treat the duodenum such as subjects who have had a Billroth 2, Roux-en-Y gastric bypass, or other similar procedures or conditions

History of chronic or acute pancreatitis

Known active hepatitis or active liver disease

Symptomatic gallstones or kidney stones, acute cholecystitis or history of duodenal inflammatory diseases including Crohn's Disease and Celiac Disease

History of coagulopathy, upper gastro-intestinal bleeding conditions such as ulcers, gastric varices, strictures, congenital or acquired intestinal telangiectasia

Use of anticoagulation therapy (such as warfarin) which cannot be discontinued for 7 days before and 14 days after the procedure

Use of P2Y12 inhibitors (clopidogrel, pasugrel, ticagrelor) which cannot be discontinued for 14 days before and 14 days after the procedure. Use of aspirin is allowed.

Unable to discontinue NSAIDs (non-steroidal anti-inflammatory drugs) during treatment through 4-weeks post procedure phase

Taking corticosteroids or drugs known to affect GI motility (e.g. Metoclopramide)

Persistent anaemia, defined as haemoglobin<10 g/dl

eGFR <30 ml/min/1.73m^2

Active systemic infection

Active malignancy within the last 5 years

Poor candidates for surgery or general anaesthesia

Active illicit substance abuse or alcoholism

Medications affecting insulin sensitivity (oral steroids, metformin, thiazolidinediones, atypical antipsychotics, hormonal contraceptives, weight loss medication) at screening or 2 months previously.

Other causes of anovulation (e.g. hypothyroidism, adrenal or pituitary disorders)

More than 6 menstrual bleeds within the previous 12 months

Current pregnancy or breastfeeding at screening or 6 months previously

Smoking at screening or 6 months previously

Without access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study.

Donated blood during the preceding 3 months or intention to do so before the end of the study

Any other mental or physical condition which, in the opinion of the Investigator, makes the subject a poor candidate for clinical trial participation

Patient identification

The routes of identifying potential participants will be:

Contacting the patient after review of their medical history by the direct clinical care team. This will take place at Imperial College Healthcare NHS Trust by the direct clinical care team members that are also research team members and at other research and PIC sites (Chelsea and Westminster Hospital NHS Foundation Trust, West Middlesex University Hospital and University Hospitals Coventry and Warwickshire NHS Trust). Patients who are willing to take part will be asked for their permission for their clinical information to be passed on to the Imperial research team.

Clariness which is an international patient recruitment provider for clinical trials. Clariness will run a campaign to advertise this clinical trial using online outreach strategies, such as search engine marketing, banner advertising on relevant websites, and social media advertising. Data is stationed following the privacy policies and security measures as stipulated by the (EU) General Data Protection Regulation (GDPR) and the German Data Protection Act (BDSG).

Posters: These will be placed in areas where potential participants are routinely cared for and they will contain the clinical research team contact details

Screening visit

This will be performed after the research team have made first contact with the participant. All participants will be screened to assess whether they meet inclusion criteria and this process will comprise a medical history, routine physical examination, and the following investigations:

Full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, lipid profile, vitamins, minerals and metabolites, electrocardiogram and a pregnancy test. To confirm anovulation, a serum reproductive profile will be performed: serum progesterone, LH, FSH and oestradiol (E2).

Assessment of insulin secretion – oral glucose tolerance test: An oral glucose load of 75 grams of glucose will be consumed by the patients. This will be followed by measurements of glucose, insulin, c-peptide and metabolites for 3 hours, where time zero is the time of administration of the glucose.

Urine pregnancy test

Patients will be asked to take Medroxyprogesterone to induce a menstrual bleed before the baseline visit. Medroxyprogesterone is used routinely in clinical practice for the same indication. The induction of the bleed will enable all women to be studied at the same phase of their menstrual cycle.

Baseline visit

This visit will take place approximately 4 weeks before participants undergo the intervention.

Assessment of insulin sensitivity - euglycaemic hyperinsulinaemic clamp

Patients will be asked to refrain from alcohol and strenuous physical activity for 48 hours before the study. They will also be asked to consume a standardised meal the evening before the study and only consume fluids from 10pm onwards. The visit will be performed in early follicular phase in any patients who have resumed menses. Patients will attend the Imperial NIHR clinical research facility on the day of the clamp procedure. Two venous catheters will be inserted. The first cannula will be used for infusions and the other for blood sampling. An insulin infusion may be commenced to keep their blood glucose stable between 4.0-6.0 mmol/l. A primed continuous infusion of [6, 6-$^{2}H_{2}$] glucose, a stable isotope tracer, will be started and maintained for ~7 hours. Two hours later a two-stage hyperinsulinaemic-euglycaemic clamp procedure will be started and continued for ~5 hours. During stage 1 of the clamp procedure, in which hepatic insulin resistance is assessed, insulin will be infused at a low dose (depending on patient's weight/body surface area) for 2 hours. During stage 2 of the clamp procedure, in which peripheral insulin resistance is assessed, insulin will be increased to a higher dose (depending on patient's weight/body surface area) for 2 hours. Euglycaemia will be maintained by infusing 20% dextrose at a variable rate. Blood samples will be taken every 5-10 minutes to measure blood glucose concentration and the dextrose infusion will be adjusted accordingly. The exogenous glucose infusion will be enriched with 6, 6 $^{2}H_{2}$ glucose to prevent a fall in plasma

tracer enrichment and underestimation of endogenous glucose production rate. Regular glucose monitoring is necessary to ensure safety and avoid the small risk of hypoglycaemia.

Blood samples will be obtained before the start of the tracer infusions, every 10 min during the final 30 min of the basal period and stages 1 and 2 of the clamp procedure and every 30 minutes between these periods to determine glucose enrichment and concentration and insulin. The same time points participants will be asked to complete appetite visual analogue scales.

At the end of the study, participants will undergo an oral glucose tolerance test as described above. The maximum amount of venesected blood will be 180 mls. Blood samples will be centrifuged and the separated plasma kept in a -20°C or -80°C freezer. The isotopic enrichment of plasma glucose will be determined by gas chromatography mass spectrometry (GCMS) at the Wolfson Centre for Translational Research, Postgraduate Medical School, University of Surrey.

The stable labelled isotope tracer [6, 6 $^{2}H_{2}$] glucose is not a drug, but a naturally occurring metabolite which has been labelled with a stable and non-radioactive label. Stable isotope tracers are widely and safely used in metabolic research by groups throughout the UK and worldwide. All labelled isotope tracers are ordered from Cambridge Isotopes Ltd through their UK suppliers CK Gases Ltd. They are prepared as sterile solutions suitable for intravenous use by the Pharmacy Production Unit at Guys & St. Thomas' NHS Trust to ensure they are safe for the participants. The products are supplied with the appropriate certificate of analysis and MSDS. We have used the same manufacturer to ensure the quality of the products and the supporting documentation. They will be stored at the Imperial NIHR clinical research facility.

Additional assessments

The following assessments will also take place on or around the baseline visit:

Body weight and body composition using bioelectrical impedance

Full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, lipid profile, iron indices, vitamins, minerals and metabolites.

Reproductive profile: serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, E2, sex hormone binding globulin (SHBG), testosterone, dehydroepiandrosterone sulphate (DHEAS), androstenedione.

Urine pregnancy test

Blood pressure and pulse

Total caloric intake and macronutrient composition will be assessed through the use of food diaries. These will be given to the participants at the visit prior to this one and returned to the investigators on the day of the visit.

Number of medications

Energy expenditure: All patients, including those who crossover at the end of the trial, will be offered an optional metabolic study at the Human Metabolic Research Unit (HMRU) for body composition, energy expenditure and sleep study. This metabolic study will take place at baseline and 6 months post-intervention follow-up.

Each HMRU study will commence at 8am with measurement of body composition in the BodPod, which provides an accurate measurement of fat and lean mass through air displacement (on principle of Boyles Law). Participants will have attended fasting since 22:00 pm previous night and a fasting blood sample will then be taken and spun for serum and plasma, to be stored at -20°C and then transferred to -80°C freezers. Each participant will then enter the metabolic chamber at 9:30am for a 24-hour metabolic study to measure energy expenditure profile in real time. Prior to entry into the chamber, each participant will be shown around the chamber by the HMRU nurse, for orientation purposes and will be trained on the use of a portable sleep machine . Participants will be also provided with theatre scrubs to ensure clothing standardisation whilst in the calorimeters, and will have two urine pregnancy tests. Throughout their 24-hour stay within the calorimeters, subjects will be asked to collect their urine in specific containers provided by the research team. These will be collected 8-hourly and used for the assessment of substrate oxidation.

Following chamber entry, the experiment will start at 09:50 am to allow participants to settle in the calorimeter. This period allows equilibration of and participant familiarity with the chamber environment. At 12.00 hrs standard lunch will be served and subjects will have sequential blood tests every 30 minutes at 12:30, 13:00, 13:30 and 14:00pm to assess glucose metabolism. Serum samples will be analysed for insulin, adiponectin and other adipokines, glucose and lipid

profiles. These samples taken during HMRU will be anonymised. They will be stored for 10 years and analysed by the clinical research team.

Following the post-prandial period from the standard lunch, there will be a standard activity protocol at 15.30 hrs involving stepping for 30 minutes at a rate of 90 beats per minute with one full-step up and down per second. A standard online metronome application will be used and this will enable assessment of standard activity-related energy expenditure. Following this at 18.00 hrs, a standard evening meal will be provided. Subjects following this will be asked to remain sited for 3 hours avoiding any form of activity for the assessment of post-prandial thermogenesis.

There will be a standard snack provided at 21.00 hrs, and participants will be requested to sleep from 22.30 hrs with wake-up at 07.00 hrs the next morning. Prior to going to sleep, participants will be requested to fix their portable sleep machine in place (as explained to them by the HMRU nurse prior to entry into the chamber). The next morning, subjects will be woken at 07:00am and will be asked to remain on the bed without sleeping or moving during which time, resting metabolic rate is going to be assessed after at least 8 hours fast. At 08:00am a blood test will be carried out; sleep machines will be removed and standard breakfast will be provided at 08.30 hrs. Participants will be asked to exit the chamber at 10.00 hrs. Assessment of sleep data using complete polysomnography will be carried out by our Lead Respiratory Physician at UHCW NHS Trust (Dr Asad Ali).

Throughout each of the HMRU visits, all subjects will be monitored continuously (including during the whole 24-hours HMRU study). Subjects will be encouraged to report any unusual or unpleasant sensation to the investigator immediately. Any significant adverse effects will lead to discontinuation of the visit after assessment by an investigator . Subjects will be supervised throughout their HMRU visits by a member of the research team. Throughout the study there will be at least one member of the research team available on 24-hour call via a direct line, with a second member on back up, and a secondary direct line to one of the senior investigators. Although we do not anticipate any serious adverse effects based on screening procedures and

previous experience with similar studies, participants will be able to contact a member of the research team via phone after clear instructions that, if they feel unwell, they should call us.

Randomization

Subjects who meet all criteria after screening will be randomized via computer software 1:1 (DMR to sham). This will take place at the baseline visit. The patient, the research and clinical team, except for the endoscopist, will be blinded to the type of intervention that has been performed, unless clinical need and procedure dictates the un-blinding of the clinical team (e.g. development of a complication). Unblinding will take place at the end of the trial.

Intervention

Duodenal mucosal resurfacing: The Fractyl Revita System consists of two main components: the Revita Catheter and a console.

Revita Catheter: The Revita Catheter is a sterile, single use device that performs two functions: 1) it injects saline into the submucosa of the duodenum to create a thermal barrier while also lifting the mucosa with saline to create a more uniform surface for ablation; and 2) ablates the mucosal surface using heated water recirculating inside a balloon. To achieve its function, the Revita Catheter is constructed of a multi-lumen shaft with a balloon affixed to its distal end. Affixed to the outside of the balloon are three narrow shafts with a port that are used to draw a vacuum when placing the saline during the mucosal lifting portion of the procedure. Within each shaft is a fluid lumen with a miniaturized needle affixed to the distal end. Each needle is wholly constrained within the port ensuring its safe use. During the mucosal lift, the tissue is drawn into the needle port, and saline is injected into the submucosal space through the needles. The proximal end of the shaft is fitted with a handle and saline and vacuum lines that are affixed to a console unit to control its function. The catheter will be available with a 24 mm outer diameter balloon.

Console: The console is a reusable electro-mechanical piece of equipment and provides functionality to the submucosal lift and hot fluid ablation steps of the procedure. It is controlled through the use of a software user interface monitor. Prior to use, it is fitted with a sterile single use line set that serves as the pathway for the saline to be placed into the duodenal submucosa during the procedure.

The DMR procedure using the Revita System is completed in the endoscopy suite using general anaesthesia. The patient is positioned in the left lateral decubitus position used for endoscopic procedures or preferred position as dictated by the site's requirements for endoscopic procedures. A standard endoscope is used to complete an initial endoscopic evaluation and a guidewire is delivered past the ligament of Treitz to assist in delivering the catheter. Anti-peristaltic agents may be used during the procedure. Catheter delivery and device location for treatment is verified using fluoroscopic guidance. The use of fluoroscopy is limited to use during catheter placement and verification of location during treatment. Based on data collected during earlier clinical investigations, the duration of radiation exposure is approximately equivalent to that delivered during an endoscopic retrograde cholangiopancreatography procedure, which is a common endoscopic procedure with an acceptable safety profile. A lead apron drape will be placed on the abdomen and pelvis during the procedure to protect the reproductive organs. The total procedure time is less than 70 minutes.

The Revita catheter is placed in the proximal duodenum distal to the papilla. Using the console interface, the balloon is inflated and vacuum delivered to draw the intestinal mucosal tissue onto the ports located on the balloon. The actuator on the handle is moved to advance the needle into the submucosal space within each of the ports. The console delivers saline into the submucosa through the needles within the lumens of the catheter resulting in complete circumferential lift of the mucosa. Once complete, the ablation cycle is started and hot water is circulated into the balloon to complete an ablation of the previously expanded tissue. The balloon is deflated and the catheter repositioned distally to the next segment to be treated. The Revita catheter and endoscope are then removed. The number of ablations completed in the duodenum is determined by the distance between the papilla and the Ligament of Trietz and may be variable based on the individual anatomy.

Sham

The sham procedure will consist of placing the DMR Catheter into the duodenum under general anaesthesia and leaving it in place for a minimum of 30-45 minutes and then removing it from the patient.

Unforeseen events (findings or procedures) may occur during either the DMR or sham procedure. These unforeseen events are those that are not planned as part of this procedure (e.g. a drop in oxygen saturation or evidence of intestinal bleeding, etc.). Unforeseen events that are emergent in nature should be recorded as adverse events and the investigator should reassess the subject's suitability for continued participation in this study.

Post-Procedure Care & Discharge

Immediately following the procedure, the subject is transported to the recovery area and monitored according to the hospital/physician protocol for endoscopic procedures. The subject may be released from the recovery room to the nursing unit when they have met the hospital's criteria for discharge from the recovery area. Immediate postoperative care is dictated by the hospital or physician's standard care protocol regarding post-anaesthesia recovery.

Prior to discharge, all subjects are examined and evaluated for the presence of any adverse events that may have occurred between the procedure and discharge. A subject's hospital stay can be extended based on need as determined by the Investigator. Subjects are eligible to be discharged when they meet the criteria following the local sedation protocol and discharge requirements.

Following intervention patients in both groups will be asked to consume the same low-calorie diet for approximately 14 days. Participants will be informed as to how this liquid diet will be consumed by the research team.

Early post-procedure mechanistic visit

This will take place within 14 days after the intervention. The following assessments and procedures will be performed:

Clinical assessment

Full blood count, urea and electrolytes, liver function tests, HbA1c, lipid profile

serum LH, FSH, progesterone, E2, SHBG, testosterone, DHEAS, androstenedione

Urine pregnancy test

Oral glucose tolerance test

Body weight and body composition using bioelectrical impedance

Blood pressure and pulse

Total caloric intake and macronutrient composition will be assessed through the use of food diaries. These will be given to the participants at the visit prior to this one and returned to the investigators on the day of the visit

Adverse events

12-week mechanistic visit

The following assessments and procedures will be performed:

Clinical assessment

Full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, lipid profile, vitamins, minerals and metabolites.

Reproductive profile: serum LH, FSH, progesterone, E2, SHBG, testosterone, DHEAS, androstenedione

Urine pregnancy test

Euglycaemic hyperinsulinaemic clamp as described above

Oral glucose tolerance test as described above

Body weight and body composition using bioelectrical impedance

Blood pressure and pulse

Total caloric intake and macronutrient composition will be assessed through the use of food diaries. These will be given to the participants at the visit prior to this one and returned to the investigators on the day of the visit.

Number of medications

Adverse events

Reproductive assessments weeks 12-24

During the duration of the study, information about self-reported menstrual bleeding will be collected.

The reproductive assessments that will be performed from weeks 12 to 24 will include:

Weekly pelvic ultrasound scans: The ultrasonographer will be blinded to treatment for all subjects. During each scan, the following parameters will be measured: endometrial thickness (in millimetres), mean ovarian volume (in cubic centimetres), mean follicle number, and maximum diameter of largest follicle in each ovary (in millimetres). Ovulation will be defined as a rise in serum progesterone >10 nmol/L together with suggestive radiological features (visualization of a dominant follicle with subsequent appearance of a preovulatory follicle and/or corpus luteum). The ultrasounds will be either transabdominal or transvaginal depending on views obtained and patient preference.

Measure serum progesterone 7-10 days later (i.e. mid-luteal phase).

Once-weekly progesterone/E2 ratio, LH, FSH, E2 measurement.

Patients recruited at University Hospitals Coventry and Warwickshire NHS Trust will be offered to have this follow-up done locally.

6-month clinical visit

The clinical assessments will include:

Body weight and body composition using bioelectrical impedance

Blood pressure and pulse

Blood tests: full blood count, urea and electrolytes, liver function tests, thyroid function tests, glucose, insulin, c-peptide, HbA1c, lipid profile, vitamins, minerals and metabolites

Reproductive profile: serum LH, FSH, progesterone, E2, SHBG, testosterone, DHEAS, androstenedione

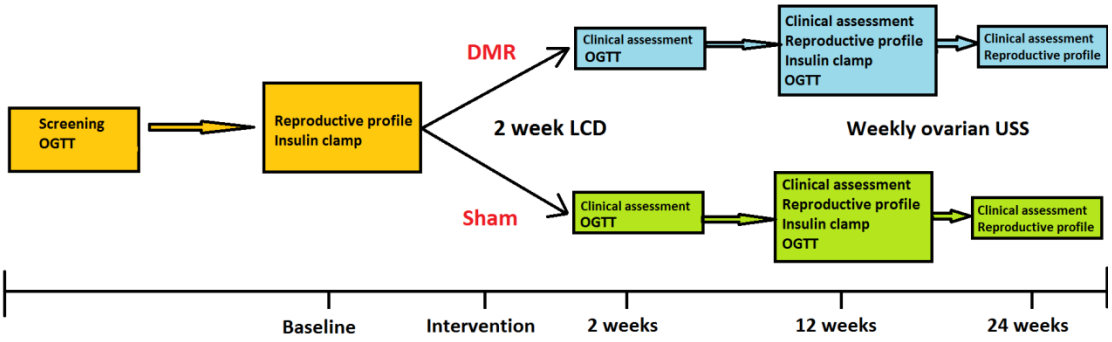
Urine pregnancy test

Number of medications

Adverse events

The energy expenditure study follow-up will be conducted for those patients who have opted to have it done.

Trial flow diagram



Primary Efficacy Endpoint

Primary Efficacy:

1. The change from baseline in total insulin sensitivity at 12 weeks. Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity as assessed by insulin clamp.

2. The change from baseline in insulin sensitivity at 24 weeks as assessed by HOMA-IR.

3. The number of menses during 24 weeks

Secondary Efficacy Endpoints

Change in hepatic insulin sensitivity from baseline at 12 weeks

Change in peripheral insulin sensitivity from baseline at 12 weeks

Number of ovulatory cycles defined by an increase in serum progesterone and / or ultrasound evidence of ovulation followed by menstrual bleeding between weeks 12-24

Change in the area under the curve (time 0-180 minutes, full and area above fasting value of the variable) of the concentrations of glucose, insulin and c-peptide at the OGTT from baseline at 12 weeks

Change in the Matsuda and Disposition indices at the OGTT from baseline at 12 weeks

Change from baseline in insulin sensitivity at 12 weeks as assessed by HOMA-IR

Change in the area under the curve (time 0-180 minutes, full and area above fasting value of the variable) of the concentration of glucose, insulin and c-peptide at the OGTT from baseline at 2 weeks

Change in the Matsuda and Disposition indices at the OGTT from baseline at 2 weeks

Change in glycated haemoglobin from baseline at 24 weeks

Change in free androgen index from baseline at 24 weeks

Change in concentrations of liver function tests from baseline at 24 weeks

Change in concentrations of the enhanced liver fibrosis test from baseline at 24 weeks

% body weight loss from baseline at 24 weeks

Exploratory Endpoints

Change from baseline in the following at 12 weeks

Energy expenditure

Body composition

Plasma lipid concentration

Arterial blood pressure

Change from Week 12 to Week 24, and change from Week 12 to each visit, for the following:

Endometrial thickness

Ovarian volume

Follicle number

Diameter of largest follicle in each ovary

Serum LH

Serum FSH

Serum Oestradiol

Serum SHBG

Serum Testosterone

Serum DHEAS

Serum Androstenedione

Risk Analysis

There are certain residual risks associated with the use of the Fractyl Revita System™ and the DMR procedure. As with any endoscopic procedure, there are risks that are associated with interventional procedures in the duodenum. Below is a listing of these risks and the means by which they may be minimized.

Procedure Risks

There are risks related to the endoscopic procedure in general, as well as, risks specific to the Fractyl Revita System™ procedural treatment (in alphabetical order):

abdominal tightness, cramping, pain

diarrhoea

difficulty swallowing

infection

mucosal injury to GI tract

pancreatitis

perforation

sore throat

stricture

transient bleeding

worsening diabetic symptoms including hypoglycaemia

Many of these risks and complications associated with the procedure would be similar to those associated with other commonly performed endoscopic procedures such as duodenal biopsies and endoscopic mucosal resection.

Device Risks

In addition to the risks listed above, the Fractyl Revita System may have unique risks associated with its catheter and console used to complete the procedure. This includes risks associated with the materials selected, its design and construction. These risks include:

Allergic reaction to the device materials or endoscopic labelling dye or injectate

Component degradation

Control module delivers incorrect ablation time and temperature profile

Device breakage

Disarticulation of components from the device

Device/Component lost in GI tract or wall

Hole in hot fluid catheter balloon resulting in leakage of hot fluid

Lost catheter component in the GI tract or wall

Thermal damage to the duodenum wall or surrounding structures

Unforeseen adverse events

General anaesthesia risks

These are rare, occurring in less than 1 in every 10,000 cases. They include:

a serious allergic reaction to the anaesthetic (anaphylaxis)

an inherited reaction to the anaesthetic that causes breathing difficulties

waking up during the intervention – this is rare, and the amount of anaesthetic given will be continuously monitored to help ensure this does not happen

death – this is very rare, occurring in 1 in every 100,000 to 1 in every 200,000 cases

Minimizing Study Risks

The following steps have been taken to minimize risks associated with the procedure and the use of the Fractyl Revita System™:

The tissue or fluid contacting materials used in the construction of the Revita Catheter are known medical grade materials that are well characterized and have a long history of use. In addition, biocompatibility testing has proven that the materials are safe.

The device design uses known technologies including sub-mucosal injection and hot fluid balloon to complete the procedure. Similar technologies are currently in use for such accepted procedures as endoscopic mucosal resection and treatment of menorrhagia.

The device design has been rigorously tested in the laboratory, animal models and clinical trials to characterize its performance and confirm the safety and performance of the procedure.

All investigators receive detailed training in the use of the Fractyl Revita System and the DMR procedure. The training includes hands on use of the system in a lab setting.

The anaesthetist will review the patient's medical history and adjust the anesthesia so that its risks are minimised

Contraception

Participants will be asked to maintain effective contraception for the duration of the study. Effective contraception methods include:

Barrier methods

intrauterine device (non-hormone releasing)

vasectomised partner: this is a highly effective birth control method provided that partner is the sole sexual partner of the trial participant and that the vasectomised partner has received medical assessment of the surgical success.

sexual abstinence: sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments and when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.

Drop-outs

Subjects will be free to withdraw at any point. Drop-outs taking place up to and including the intervention will be replaced. Drop-outs following the intervention will not be replaced.

Trial Closure

The end of the clinical trial is defined as the last visit of the last patient.

Sample size calculations

Assumptions of effect size for the primary efficacy endpoints in the treatment arm were derived from previous publications in which insulin sensitising medications were administered in similar groups of women (13, 14) and also takes into account information from previous studies on DMR.

It is assumed that:

a difference in mean change in total insulin sensitivity (as assessed by insulin clamp) between treatment and control of 2.79 μmol/kg.min at 12 weeks with equal variance in both groups (standard deviation of 4.50), which gives a standardized effect size of 0.62 (= 2.79/4.50). Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity.

a standardized effect size of 0.91 for the change from baseline in insulin sensitivity at 24 weeks as assessed by HOMA-IR.

a difference in the number of menses between treatment and control of 1.0 over 24 weeks with equal variance in both groups (standard deviation of 1.0), which gives a standardized effect size of 1.0.

The Hochberg procedure to adjust for multiple endpoints is described in Section 6.5. Under this procedure 24 randomised subjects (12 per group) provides approximately 82.8% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.050 significance level, and provides approximately 72.9% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.025 significance level.

Thirty patients will be randomised to account for potential patients lost to follow up prior to the primary endpoint assessment. Patients who for technical reasons cannot have the DMR will be replaced.

Statistical analysis plan

This is detailed in the Statistical Analysis Plan 2.0).

Procedure for emergency un-blinding

The randomisation lists will be created and held by Professor Tricia Tan, Professor in Endocrinology, Imperial College London, in a secure area within the centre (this copy to be held as code-break envelopes).

In the case of a medical emergency or in the event of a serious medical condition, when knowledge of treatment allocation is essential for the clinical management or welfare of the subject, an investigator or other physician managing the subject may decide to un-blind that subject's treatment code. They should therefore request and obtain the relevant code-break envelope.

The investigator must sign and date the open un-blinding envelope, as soon as is reasonably possible, and at the very least within 24 hours of the code break. The reason for the code break must be documented on the envelope. The Investigator will also record the date and reason for revealing the blinded treatment assignment for that subject in the clinical research facility and in the subject's medical notes.

Adverse Events

All adverse events (AEs) will be coded using the standardised MedDRA central coding dictionary, version 19.1 or greater. Adverse event analyses will be performed on the Safety analysis population.

All Adverse Events

The number of treatment emergent adverse events (TEAEs) and the number and percent of subjects with at least one TEAE will be presented overall by SOC and PT. A TEAE is an event starting or worsening in severity at or after initiation of the index procedure for the randomised treatment. For subject counts, subjects experiencing a given event more than once will be

counted only once for that event. For TEAEs occurring in the randomised phase, results will be presented by treatment group.

The proportion of patients with at least one TEAE of special interest (TEAESI) with at least one procedure-related TEAE, and with at least one device related TEAE in the double-blind phase will be plotted by time point (peri-procedure, 0-1 week, 1-4 weeks, 4-8 weeks, …, 20-24 weeks) for each treatment group.

A listing of all adverse events will include the subject number, AE number, days since index procedure, the investigator description of the AE, the AE SOC and PT, the severity of AE, whether or not the AE is classified as serious (SAE), the relationship of the AE to the investigational device or procedure, the action taken, the outcome, and the adjudication status.

Adverse Events Leading to Withdrawal

A summary of number of TEAEs and of the incidence rates (number and percentage of subjects) of TEAEs leading to study withdrawal, by SOC and PT will be presented in a similar manner as discussed above (with the exception of the plot). A data listing of TEAEs leading to withdrawal will also be provided, displaying details of the event(s) captured on the CRF.

Serious Adverse Events

Summaries of serious TEAEs will be conducted in the same manner as for all TEAEs discussed above.

Device and Procedure Related Adverse Events

Summaries of device or procedure related TEAEs will be conducted in the same manner as for all TEAEs discussed above; patients with the occurrence of more than one TEAE within a given SOC or PT will be counted only under the maximum severity/relationship experienced for that SOC or PT, respectively.

Unanticipated Adverse Device Effects

Summaries of treatment emergent unanticipated device TEAES will be conducted in the same manner as for all TEAEs discussed above.

Adverse Events of Special Interest (AESIs)

Specific events that may be related to the mechanism of action of the DMR procedure (eg, hypoglycemia)

Potential adverse consequences of the procedure (e.g., gastrointestinal adverse events)

Rare events that may or may not be related to the DMR procedure/device, but are of interest to the Sponsor (e.g., unexplained fever)

Events of Special Interest are:

Hypoglycemia

Diarrhea

Abdominal pain, nausea, vomiting

Gastrointestinal bleeding

Unexplained fever

Stenosis (GI)

The number of events and incidence of AESIs will be presented.

Deaths

If a death occurs during the course of the trial, relevant information (including study day of death relative to index initiation, cause of death, and adverse event leading to death) will be supplied in a data listing.

Reporting procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non serious AEs

All such events, whether expected or not, should be recorded.

Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to non-obesity or diabetes related causes, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All SAEs should be reported to the REC where in the opinion of the Chief Investigator, the event was:

'related', i.e. resulted from the administration of any of the research procedures; and

'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs

SAEs must be reported to the Chief investigator and the Sponsor within 24hrs of becoming aware of the event:

CI details: Fax: 0208 383 8320, attention of: Dr Alexander Miras

Sponsor details: Tel: 0207 594 9459 or jrco@imperial.ac.uk

Please send SAE forms to: Section of Investigative Medicine, Division of Diabetes, Endocrinology & Metabolism, Imperial College London

Tel: 0208 383 3242 (Mon to Fri 09.00 – 17.00) or 07551266480 (24 hours, 7 days a week).

Follow-up of AEs and SAEs

After the initial AE report, the Chief Investigator or appropriately qualified designee will proactively follow the subject at subsequent visits and contacts. Follow up information about a previously reported SAE must be reported to the Trial Management Group and Sponsor within 24 hours of receiving it. AEs and SAEs will be followed until they resolve, stabilise to a level acceptable to the Investigator or delegates even after the reporting period or the subject is lost to follow-up. Additional measures may be carried out by the Investigator to elucidate as fully as possible the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations or consultation with other health care professionals. In the event that a

subject becomes pregnant, the follow-up period will be deemed to have ended when the health status of the child has been determined on its birth.

Monitoring

The principal investigator is responsible for monitoring arrangements. The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy for Health and Social Care Research.

Regulatory issues

Ethics and regulatory approvals: The Chief Investigator has obtained approval from the London-Dulwich Research Ethics Committee and the HRA. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Consent: The study will be conducted in accordance with applicable regulatory requirements, with International Conference on Harmonization "Good Clinical Practice" (GCP), with all applicable subject privacy requirements, and with the guiding principles of the Declaration of Helsinki. Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. If a participant, who has given informed consent, loses capacity to consent during the study they would be withdrawn. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

Confidentiality: The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. All patients will be registered with Imperial College NHS Trust or King's College Hospital NHS Trust and their personal data

will be kept on password protected NHS computers. Personal addresses, postcodes, faxes, emails or telephone numbers will be used to enable the research team to contact participants during the trial. A paper copy of these data will be placed in the individual patient folders and the study Master File. These folders will be kept in locked storage at the Imperial NIHR clinical research facility or Dr Dr Bu'Hussain Hayee's NHS office at King's College Hospital NHS Trust for the duration of the trial.

All other study documentation and data stored on other non-NHS computers will use only the study code, without any personal data, and hence will be anonymised. Subjects will be given a personal study code number which will be used throughout the study and in the analysis of data. Anonymised samples will be stored for 10 years at the Department of Investigative medicine laboratories and analysed by the clinical research team. Anonymised samples of isotopic enrichment quantification will be transferred to the University of Surrey for analysis. Anonymised samples may be sent for analysis outside Imperial College London, the United Kingdom, to the European Union, USA or commercial companies.

Transfer of electronic personal or clinical non-anonymised data between the research sites will only take place via the secure nhs.net email system.

Indemnity: Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Sponsor: Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

Funding: Fractyl® is funding this study through its investigator-initiated study programme.

Audits: The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, and other also by other regulatory bodies to ensure adherence to GCP and the UK Policy for Health and Social Care Research

Study management, data monitoring and ethics

A Trial Steering Committee (TSC) and a Data Monitoring & Ethics Committee will be established.

Quality Control and Quality Assurance

The trial will be adopted by the NIHR Clinical Research Facility at Imperial and will fall under their QC/QA regime.

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6.2 DOMINO Trial Participant Information Sheet and Consent Form

DOMINO Trial Information Sheet and Consent Form

Imperial College London

Department of Investigative Medicine

Imperial College London

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Imperial College London at Hammersmith Campus

Du Cane Road, London W12 ONN, UK

Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320

INFORMATION SHEET FOR RESEARCH PARTICIPANTS

You will be given a copy of this information sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

Investigation of the metabolic effects of DuOdenal resurfacing on insulin resistant woMen with polycystic ovariaN syndrOme

The DOMINO Trial

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether

or not you wish to take part. If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Please be assured that if you decide not to take part this will not affect your clinical care in any way.

WHAT IS THE PURPOSE OF THE STUDY?

You have been invited to participate in this research study because you have polycystic ovarian syndrome and insulin resistance. This means that you do not have regular periods and the reason is that your body is not sensitive to its own insulin. Treatments that improve this sensitivity have been shown to help women with PCOS start having regular periods and eventually get pregnant.

The innermost layer (mucosa) of the duodenum (small intestine immediately after your stomach) releases hormones that control your insulin resistance. Evidence from a recent study showed that a procedure to heat the duodenum (duodenal mucosa resurfacing (DMR) procedure) was a safe and potentially effective procedure to improve Type 2 Diabetes and possibly insulin resistance. The DMR procedure uses a device manufactured by Fractyl Laboratories and has the CE mark for the treatment of metabolic diseases.

The purpose of this study is to evaluate how effective this procedure is in people like you in the treatment of PCOS, i.e. can it make you start having periods. We want to assess this effectiveness by comparing the procedure to a "sham procedure" where you receive the same follow up and care but the DMR procedure will not be performed. This means that if you take part there is a 50% chance you will not receive the DMR procedure, although you will be given intensive NHS lifestyle advice for weight loss and it may be possible that you could receive the DMR when the study finishes if the DMR is shown to be effective.

WHY HAVE I BEEN INVITED?

You have been invited because you have polycystic ovarian syndrome with few and irregular or no periods, a body mass index equal or greater than 30 kg/m^2 and insulin resistance.

You should not take part in this study if you:

have Type 1 or Type 2 diabetes mellitus.

have significant medical or surgical conditions, which in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer. These include:

Previous gastrointestinal surgery that could affect the ability to treat the duodenum such as subjects who have had a Billroth 2, Roux-en-Y gastric bypass, or other similar procedures or conditions.

History of chronic or acute pancreas inflammation, active hepatitis or active liver disease or chronic kidney disease.

Symptomatic gallstones or kidney stones, acute cholecystitis or history of duodenal inflammatory diseases including Crohn's and Coeliac Disease.

History of bleeding tendency, upper gastro-intestinal bleeding conditions such as ulcers, gastric varices, strictures, congenital or acquired intestinal telangiectasia.

Use of anticoagulation therapy (such as warfarin) which cannot be discontinued for 7 days before and 14 days after the procedure.

Use of clopidogrel, pasugrel, ticagrelor which cannot be discontinued for 14 days before and 14 days after the procedure. Use of aspirin is allowed.

Persistent anaemia, defined as haemoglobin less than 10 g/dl.

take any medicine that may affect the trial (such us oral steroids, metformin, thiazolidinediones, atypical antipsychotics, hormonal contraceptives, weight loss medication) or harm you.

have other causes of anovulation (e.g. hypothyroidism, adrenal or pituitary disorders).

more than 6 periods within the previous 12 months.

current pregnancy or breastfeeding at screening or 6 months previously.

smoking at screening or 6 months previously, active illicit substance abuse or alcohol excess.

do not have access to a telephone.

have donated blood in the last 3 months or intend to do so by the end of the study.

WHAT IS THE DEVICE BEING TESTED?

The treatment is delivered using the Revita System. This system consists of two parts, a tube and a console unit. The tube is attached to the console and is then introduced into the upper part of the gut (food pipe, stomach and duodenum). At the same time, a flexible camera called an endoscope is used to look inside the duodenum in a procedure called an endoscopy. First, the catheter is used to inject salt water (saline) into the inner layer of the gut wall to protect the underlying muscle. Second, the balloon at the end of the catheter is filled with hot water to heat the mucosa in the duodenum. This completes the treatment. The device is used to deliver a superficial and temporary injury (or ablation) to the surface layer of the duodenum before regrowth of this layer occurs.

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

Screening: If you agree to volunteer for this study you will first have a consultation with a doctor from the team that will ensure that you meet the inclusion criteria for the study and take a medical history, examine you, take basic blood tests (for example to look at your kidney and liver function), take a urine sample for a pregnancy test and perform an electrocardiogram to look at the structure/function of your heart. For this purpose your details will be registered with the Imperial College Healthcare NHS Trust. You will be asked also to do an oral glucose tolerance test. This involves drinking a sugar load of 75 grams, followed by blood sampling for 3 hours.

You will be asked to take Medroxyprogesterone to induce a menstrual bleed before the baseline visit. Medroxyprogesterone is used routinely in clinical practice for the same indication. The induction of the bleed will enable you to be studied at the same phase of your menstrual cycle as the other women taking part in the study.

Baseline visit: This will take place approximately 4 weeks before the intervention. Forty eight hours before you attend for the study visit you may be asked to refrain from alcohol and strenuous physical activity. This is so that they do not affect the results of the studies. You will be invited to attend the clinical research facility at Imperial London in the morning of your assessment. You will lie on a hospital bed for several hours while we test your body's ability to process glucose, and how sensitive your body is to insulin, using the "clamp" test. A nurse or doctor will use needles to insert a

tube (called a “cannulae”) into each arm vein; the needles will be removed but the cannulae will remain inside your veins and will be used to either draw blood samples from your arms or inject an isotope, dextrose (a sugar) and insulin into your body over the course of approximately 8 hours. The isotope is not radioactive and will not affect you in any negative way; it helps us determine how much glucose is being processed by your body. We will aim to keep your blood sugar in the normal range. In the very unlikely event that your blood sugar goes low, we will be able to correct this promptly by infusing more dextrose. In total up to 180 mls of blood will be taken (~36 teaspoonful). At the end of the study you will be given something to eat and drink, the cannulae will be removed and you can go home.

During/around the time of your stay with us we will also:

measure your body weight and body fat

measure your blood pressure and pulse

collect a blood and urine sample to analyse your metabolic and reproductive profile

do a urine pregnancy test

ask you to complete a food diary 3 days before the visit and return to the investigators

All patints enrolled in the study will also be offered to have an optional metabolic study at the Human Metabolic Research Unit (HMRU) at the University Hospital of Coventry and Warwickshire NHS Trust for body composition, energy expenditure and sleep study. You will be asked to attended fasting since 22:00 pm previous night.

On your visit, you would be invited to have a measurement of body fat in a special capsule (‘BodPod’) which only takes a couple of minutes to perform. Following initial fasting samples, a small plastic tube will be inserted into a vein in the arm (so that blood samples can be taken from this during the study). These samples taken during HMRU will be anonymised. They will be stored for 10 years and analysed by the clinical research team.

You would be invited to stay in the room for 24 hours. During this time, blood tests will be taken at pre-defined time-points and you will be monitored continuously (pulse and blood pressure). You will be able to leave the room whenever you want during this time, but leaving the room would invalidate the experiment and the study would therefore need to be discontinued. There will be at least one member of the research team on the Human Metabolism Research Unit throughout each study, and you will be free to communicate with them at any time using the in-built intercom device. Following the 24-hour study, you will be free to leave.

The research room on the Human Metabolism Research Unit is specially-designed, and hermetically-sealed to the outside environment. From analysing the air moving into and out of the research room, we will measure the gases that you breathe in and out. This will provide us with data on metabolic rate. All meals will be provided whilst you are inside the research room, and any dietary requirements will be respected and catered for. You would be invited to undertake some mild physical exercise for a few minutes whilst inside the research room, although this will only be requested if you are willing and able to perform this. In addition, we would also assess your sleep quality with a portable sleep machine whilst you are inside the research room, and our research nurse will explain to you how this works and train you on the use of the portable sleep machine before you enter the room. You will be also provided with theatre scrubs to ensure clothing standardisation during the study, and you will be asked to have two urine pregnancy tests. During the 24 hours that you are within the room, you will also be asked to collect urine produced in that time in a specific container to allow accurate measurement of your metabolic activity.

This metabolic study will take place at baseline and 6 months post-intervention follow-up.

Intervention

At your baseline visit you will be randomised (allocated randomly by a computer programme) to undergo either the duodenal mucosal resurfacing or the sham procedure. The intervention will be performed by a skilled endoscopist at Imperial College London NHS Trust or King's College Hospital NHS Trust. If the procedure takes place at King's College Hospital NHS Trust you will also be

registered there. The research and clinical team will not know which type of intervention did you have, unless clinical need and procedure dictates the un-blinding of the clinical team (e.g. development of a complication). You will not know which group you are in until the end of the trial.

You will be asked to fast the night before the procedure. Prior to the procedure you will need to sign a separate consent form which we do for any patient coming for an endoscopy. The procedure will be done under general anaesthesia with an anaesthetist present. In the first stage of the procedure, an endoscope (a small camera) will be passed through your mouth and stomach into the duodenum. The doctor performing the procedure will make sure there are no abnormalities in these areas that would prevent the procedure from being possible. These include things like ulcers, narrowing (strictures) or anatomy that would make the procedure too difficult or unsafe to perform.

If suitable, a guide-wire will be placed under X-ray guidance into the duodenum before the DMR catheter is placed over the wire and into your duodenum. You will be draped with a lead vest during the procedure to reduce the exposure to radiation. If you are in the DMR group, the catheter is used to inject liquid into the inner wall of the duodenum. The balloon at the end of the catheter is then filled with hot water to heat (or ablate) the mucosal surface of the duodenum to complete the treatment. If you are in the "sham" procedure group, the DMR catheter will be placed in the small intestine but no liquid will be injected and the lining of the small intestine will not be heated by water.

After the procedure you will be monitored for a few hours and then allowed to go home. You should either use a taxi or have someone give you a lift home. You should not drive or use public transport.

Following the procedure, you will need to follow a low-calorie diet for two weeks. This will be a liquid diet and you will be given advice on how to consume it by the research team. After the two weeks you will undergo a lifestyle modification programme that will be delivered by a dietician and psychologist for 6 months. They will see you every month in group or individual sessions and offer advice on how to change your nutrition in order to lose weight.

Early post-operative visit: This will take place ~14 days after the endoscopy. The following assessments and procedures will be performed:

Clinical assessment

Body weight and body fat

Blood pressure and pulse

Blood tests: routine blood tests like the ones you underwent during screening

do a urine pregnancy test

Oral glucose tolerance test

Complete a food diary 3 days before the visit and return to the investigators

Adverse events

12-week mechanistic visit: The following assessments and procedures will be performed:

Clinical assessment

Body weight and body fat

Blood pressure and pulse

Blood tests: routine blood tests like the ones you underwent during screening

do a urine pregnancy test

Euglycaemic hyperinsulinaemic clamp as described above

Oral glucose tolerance test

Ask you to complete a food diary 3 days before the visit and return to the investigators

Adverse events

Reproductive assessments weeks 12-24

During the duration of the study, information about self-reported menstrual bleeding will be collected.

The reproductive assessments that will be performed from weeks 12 to 24 will include:

Weekly pelvic ultrasound scans: The ultrasounds will be either transabdominal or transvaginal depending on views obtained and your preference. If transabdominal, you will be asked to lie down on an examination couch and to lift your clothes to uncover your abdomen. The radiologist will put a clear gel on your skin. He/she will then move the transducer firmly but slowly across the skin of your abdomen. You should not feel any pain during the abdominal ultrasound, but you may feel some discomfort if you have a full bladder. In case of being transvaginal, the ultrasound will be probed about two or three inches into your vaginal canal. When the transducer is inserted into your vagina, you will feel pressure and in some cases discomfort. The discomfort should be minimal and should go away once the procedure is complete. If something is extremely uncomfortable during the exam be sure to let the doctor know.

Measure serum reproductive hormones 7-10 days later.

Once-weekly blood test to analyse the reproductive profile.

If you are recruited at University Hospitals Coventry and Warwickshire NHS Trust you will be offered to have this follow-up done at locally.

6-month clinical visit

The clinical assessments will include:

Body weight and body fat

Blood pressure and pulse

Blood tests: routine blood tests like the ones you underwent during screening

do a urine pregnancy test

Adverse events

The energy expenditure study follow-up will be conducted for those patients who have opted to have it done at University Hospitals Coventry and Warwickshire NHS Trust.

WHAT ARE WE TESTING?

We are trying to find out if the Fractyl Revita System™ in women with polycystic ovarian syndrome is better for insulin resistance and reproductive profile than the sham procedure and if so we want to understand the underlying mechanisms.

WHAT ARE THE SIDE EFFECTS AND RISKS OF TAKING PART?

There are certain residual risks associated with the use of the Fractyl Revita System™ and the DMR procedure.

Common:

discomfort and bruising at the cannulae insertion sites.

abdominal bloating and discomfort following the procedure.

sore throat after endoscopy.

diarrhoea

Infrequent:

Narrowing of the small bowel after the procedure which would require another endoscopy to treat.

Failure of the equipment leading to cancellation of your procedure.

Your blood sugar can go low during the clamp test; this will be promptly treated by the research team if it happens

Rare or theoretical risks:

Perforation (a tear in your GI tract).

Pancreatitis (inflammation of your pancreas).

Low blood sugars after treatment – your doctor will go through symptoms of this.

the procedure of guiding the wire uses x-ray radiation. Radiation can damage the DNA and might cause cancer. The risks associated to this procedure are equivalent to the exposure of about one

year of natural background radiation; the risk for inducing cancer is very small (1 in 12,500 or 0.008%) compared to the natural occurrence of cancer of 1 in 4.

General anaesthesia risks: These are rare, occurring in less than 1 in every 10,000 cases. They include a serious allergic reaction to the anaesthetic (anaphylaxis), an inherited reaction to the anaesthetic that causes breathing difficulties, waking up during your operation – but this is rare, and the amount of anaesthetic given will be continuously monitored to help ensure this does not happen, and death – this is very rare, occurring in 1 in every 100,000 to 1 in every 200,000 cases

During the study, experienced doctors will be available at any time should you have any concerns. You will be provided with a mobile number that you can call 24 hours a day 7 days a week in case you develop any unusual severe symptoms and want to speak urgently to a member of the team (07710067018). If you suffer from any ill effects during the study you should report these to the doctors immediately. You may withdraw from the study at any time, without providing any explanation. If there are any unexpected side effects, the study will be stopped.

CAN I TAKE PART IF I AM PREGNANT?

Pregnant women must not take part in this study. All participants will be asked to have a pregnancy test at the beginning of each study visit in order to ensure that they are not pregnant before the study visit commences. Volunteers should have adequate contraception (e.g. “barrier” methods, intrauterine device (non-hormone releasing), or abstinence) for the duration of the study. Any woman who finds that she has become pregnant while taking part in the study should immediately tell her research doctor.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

You will benefit from frequent direct contact with our specialist team and an intensive lifestyle intervention. You will see our specialist dietician either in group sessions or as a one-to-one for a period of 6 month and be given information on healthy eating, reducing food intake and increasing physical activity. This will be provided to both groups to help them lose weight start having periods. In addition, if you have had the sham procedure you will be offered the DMR after they have completed the trial. Also, you will learn a lot about your body and reproductive pattern by taking part in the special tests of the trial. The data obtained from this study would help the researchers to

understand more about your condition and eventually this could help other patients with the same condition.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Alternatively, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

WHAT WOULD HAPPEN IF I LOST THE ABILITY TO CONSENT DURING THE COURSE OF THE STUDY?

In the unlikely event that during the course of the study you were no longer able to give your consent because you had lost the capacity to do so, the research team would withdraw you from the study and not perform any further testing on you. However, they would retain body fluid samples and personal data collected previously and would continue to use it for the purposes which you had already consented.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?

Once the study has finished, the results of the study will be made available to you and/or your GP should you wish. If you have any problems immediately following the study, then you should contact one of the research doctors (07710067018).

WHAT IF SOMETHING GOES WRONG?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Chief Investigator or the study team (07710067018). The normal National Health Service complaints

mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Imperial College London as Sponsor of this study will follow The EU General Data Protection Regulation ("GDPR") that became law in all EU member states on 25 May 2018. Please read the attached transparency document for more information (Version 1.0; Date 3rd July 2018).

If you wish to raise a complaint about how the Sponsor has handled your personal data, you can contact the Sponsor's Data Protection Officer, who will investigate the matter. If you are not satisfied with their response or believe the Sponsor is processing your personal data in a way that is not lawful, you can complain to the Information Commissioner's Office (ICO).

The Sponsor's Data Protection Officer is Robert Scott and you can contact him at robert.scott@imperial.ac.uk.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results are likely to be published within the 12 months following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results can be made available to you and/or your GP should you wish.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

This study is being organised by the Department of Investigative Medicine, Imperial College London. Fractyl® is funding this study through its investigator initiated study programme.

WHO HAS REVIEWED THE STUDY?

This study has been peer reviewed by the Imperial College Peer Review Office (Imperial College Healthcare NHS Trust) and reviewed and approved by the London-Dulwich Research Ethics Committee.

CONTACT FOR FURTHER INFORMATION

If you experience any problems during the study, you may withdraw at any stage. You will also have direct emergency access, 24 hours a day, to one of the doctors involved in the study through mobile

number 07710067018. The doctors may also be contacted through Dr. Alexander Miras secretary (020 8383 3242) during office hours. The hospital switchboard (020 8383 1000) holds the home and mobile phone numbers for all the doctors involved in the study and can contact them at any time outside normal working hours if necessary.

PAYMENT

You will receive £300 upon completion of the study as a reimbursement for your time and your travel expenses. If you are based outside the M25 you will also have your travel expenses reimbursed.

Department of Investigative Medicine

Imperial College London

6th Floor Commonwealth Building

Imperial College London at Hammersmith Campus

Du Cane Road, London W12 ONN, UK

Tel: +44 (0)20 8383 3242 Fax: +44 (0)20 8383 8320

Consent form

Investigation of the metabolic effects of DuOdenal resurfacing on

The participant should complete the whole of this sheet him or herself

(please initial each statement if it applies to you)

☐

I have read the Information Sheet for Research Participants

Version ……., dated ……..

☐

I have been given the opportunity to ask questions and discuss this study

I have received satisfactory answers to all my questions ☐

I have received enough information about the study ☐

I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing and without affecting my future medical care ☐

I agree to take part in this study ☐

I agree that my GP will be informed that I am taking part in the study ☐

I understand that the NHS, Imperial College London as sponsor and regulatory authorities may review records as part of audit process ☐

I agree that my samples will be kept for 10 years and may be used for further analysis or in futu ethically approved research projects ☐

I agree that my samples may be sent for analysis outside Imperial College London, the United Kingdom, to the European Union, USA or commercial companies. Anonymised samples of isotopic enrichment quantification will be transferred to the University of Surrey for analysis. ☐

I am aware that, in the course of the study, if I were to lose the capacity to consent I would be withdrawn from the study. However, the body fluid samples and personal information collected prior to this would continue to be used for the purposes to which I have consented. (This could include further research on the samples after the current project has ended.) ☐

I consent to being registered at the Imperial College Healthcare NHS Trust or King's College Ho NHS Trust and for my personal information being kept securely on NHS Trust computers. This information will only be accessible to researchers directly involved in the study and staff processing reimbursement. ☐

I am aware that Imperial College London as Sponsor of this study will follow The EU General Da Protection Regulation ("GDPR") ☐

One copy of the consent form will be given to the patient, one copy will be stored in the trial master folder and another copy will be stored in the medical records.

Participant's

signature……Date……..

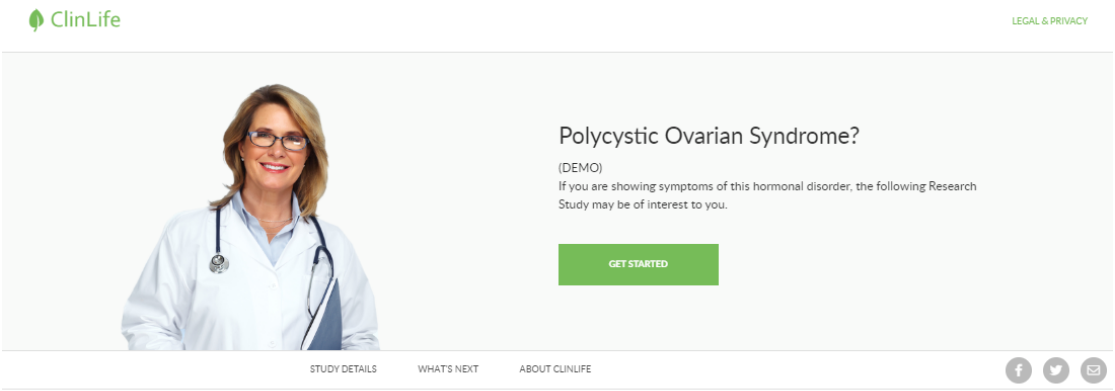
(NAME IN BLOCK CAPITALS) ……

Investigator's

signature……..Date……

(NAME IN BLOCK CAPITALS) ……

6.3 DOMINO Trial On-line Recruitment Website



Imperial College London

What is PCOS?

Polycystic ovarian syndrome (PCOS) is a common hormonal disorder that affects women of reproductive age. It causes infrequent or prolonged menstrual periods or excess male hormone (androgen) levels. The ovaries may develop numerous small collections of fluid (follicles) and fail to regularly release eggs.

Oftentimes the signs and symptoms of PCOS show firstly at puberty around the time of the first menstrual period. Sometimes it develops later, e.g. in response to substantial weight gain.

PCOS Symptoms

- Irregular periods (Infrequent, irregular or prolonged menstrual cycles are the most common sign)
- Excess androgen (Elevated levels of male hormone resulting in physical signs as excess facial and body hair, and severe acne and male-pattern baldness.)
- Polycystic ovaries (Ovaries are enlarged and contain follicles that surround the eggs; resulting in the ovaries failing to function regularly.)

Obesity can worsen complications of the disorder and is an associated symptom of PCOS.

Purpose of the study

The Fractyl Revita System ™ has achieved CE-marking and a number of studies support its safety and effectiveness in improving insulin sensitivity (reductions in blood glucose) for patients with Type 2 Diabetes.

Up to 70% of women with PCOS have poor insulin sensitivity making them more likely to develop glucose intolerance and Type 2 diabetes.

The DOMINO trial will investigate both whether this non-invasive device indeed increases insulin sensitivity using gold-standard methodologies but also whether it can help women of reproductive age start menstruating.

The procedure is performed with a camera test and does not involve any form of surgery. It does not require participants to take any form of medication to lower their sugar and no device is left in the patient (click here for further information).

If successful, the DOMINO trial can have a substantial impact on millions of women around the world. The technology could be used as a one-off treatment to enable women with PCOS to get pregnant without exposing them to the side effects of long-term medications or the complications of invasive pelvic interventions.

Publication about the study

https://www.express.co.uk/life-style/health/964287/infertility-pcos-diabetes-women-pregnant-baby-research

Who can participate in this study?

- You may qualify for this research study, if the following applies to you:
- You are a woman aged between 18-45 years
- Have PCOS (Polycystic ovarian syndrome) symptoms like: Irregular periods, Excess androgen, Polycystic Ovaries

OR

- Have been diagnosed with PCOS (Polycystic ovarian syndrome) by a medical professional

If you are affected by Type 1 - or type 2 Diabetes Mellitus, unfortunately, this study is not suitable for you.

DOMINO study is being conducted at Imperial College London. Patients can choose to have some study visits at University Hospitals Coventry and Warwickshire NHS Trust.

There are other eligibility requirements. A final decision on whether this research study may be suitable for you will be made after you speak with the study doctor at the research site. The study doctor will explain the benefits and risks of participation in this research study.

Additional info

If you participate in the DOMINO research study, you will be asked to attend the study doctor's office for study-related check-ups and evaluations.

You will receive all study-related evaluations, treatment and the procedure at no cost. You will also be reimbursed for transportation expenses incurred while participating in this study. The study doctor will inform you about the amount of expenses in a personal interview.

ClinLife

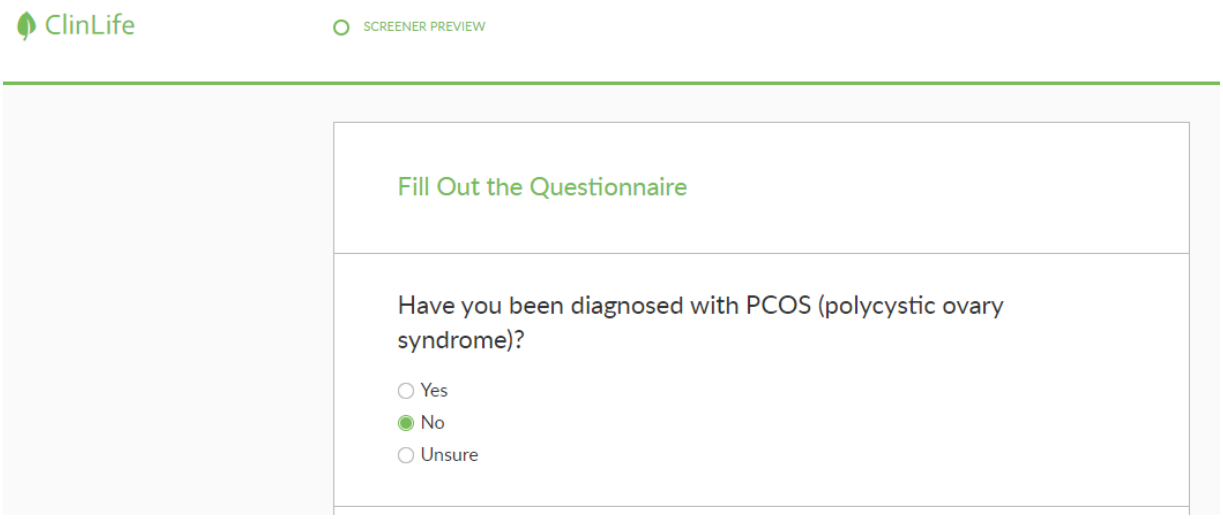
SCREENER PREVIEW

Fill Out the Questionnaire

Are you currently pregnant or breastfeeding?

- ○ Yes
- ○ No

NEXT


ClinLife
SCREENER PREVIEW
Fill Out the Questionnaire
Have you been diagnosed with PCOS (polycystic ovary syndrome)?
Yes
No
Unsure

ClinLife SCREENER PREVIEW

Do you experience any of the following?

Please select all that apply:

- [ ] Problems with acne
- [ ] Excess facial hair (e.g. visible hair growth on upper lip or chin)
- [x] Excess body hair (e.g. on chest, buttocks, around the navel)
- [x] Problem with weight gain (especially in the abdomen and upper body)
- [x] Thinning of hair or hair loss from the head
- [ ] Difficulties getting pregnant
- [ ] None of the above

Do you experience irregular periods (e.g. menstrual cycles lasting more than 35 days, missed periods or generally menstrual cycles of varying length)?

- (•) Yes
- ( ) No

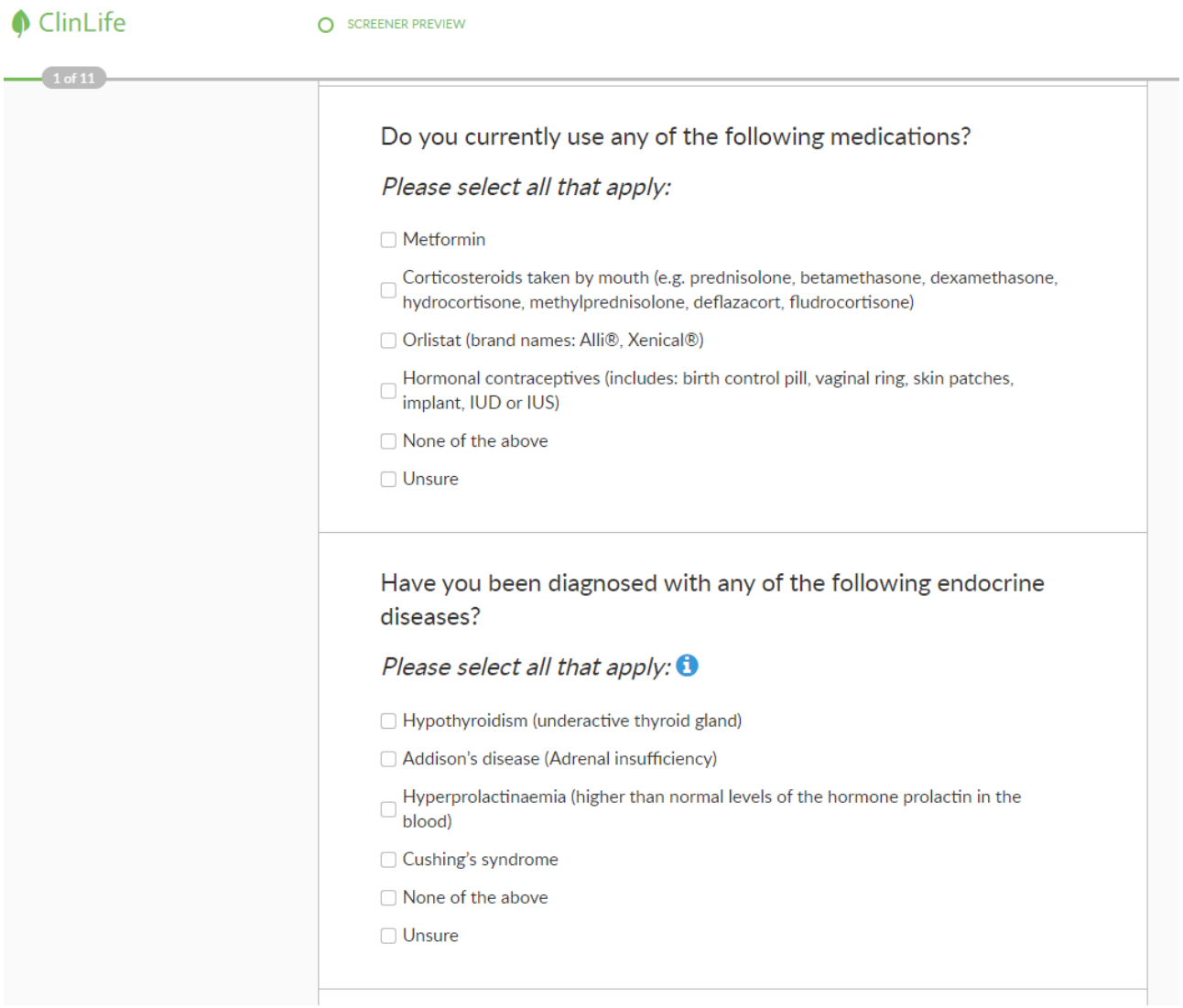
Please think about the previous 12 months, how many menstrual bleedings (periods) did you experience during that time?

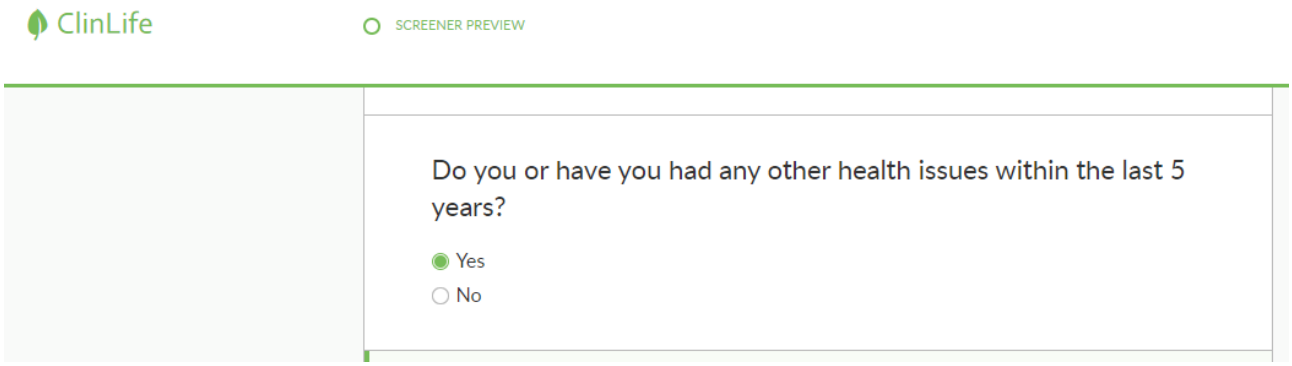
- ( ) No period
- ( ) 1 - 3 periods
- (•) 4 - 6 periods
- ( ) 7 - 9 periods
- ( ) 10 or more periods

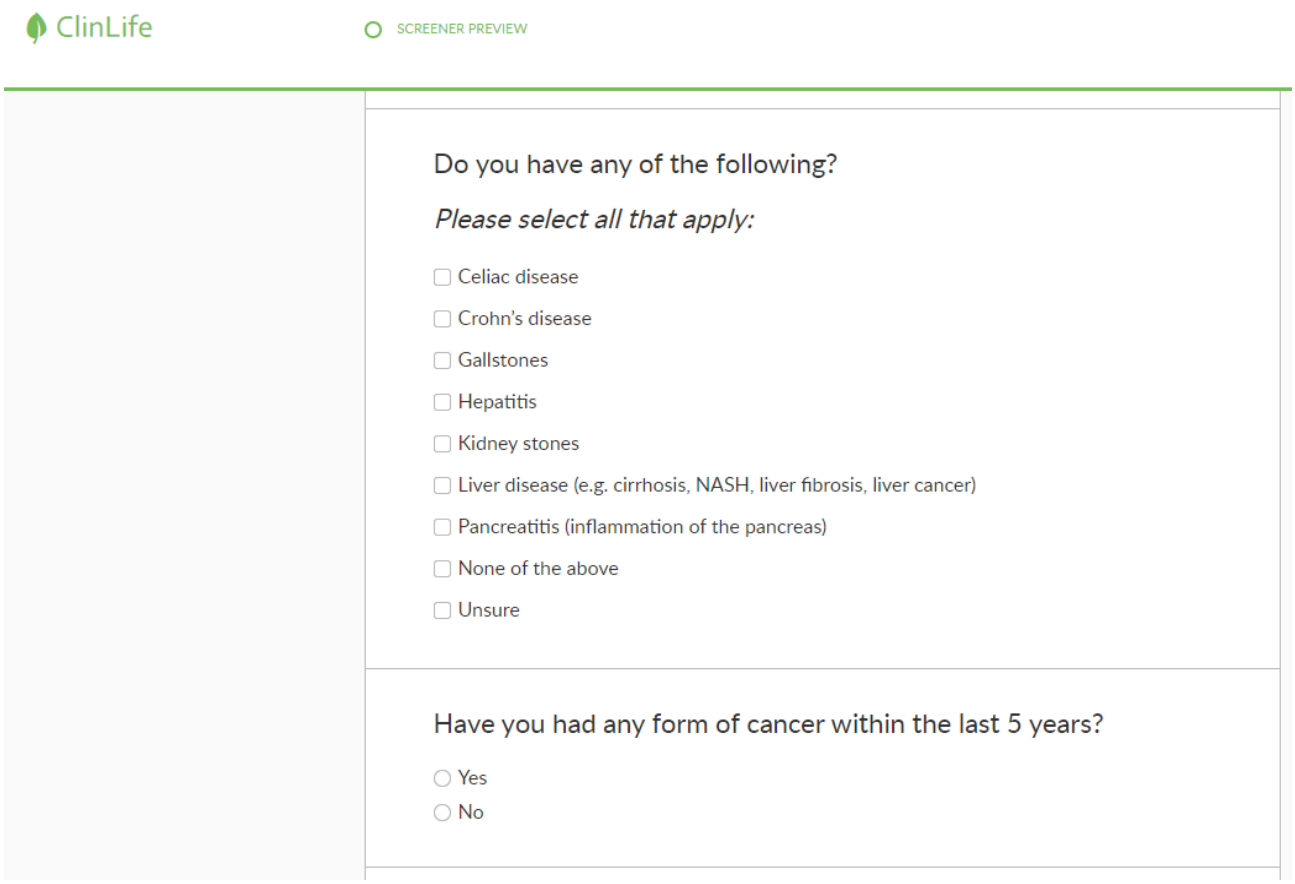
ClinLife SCREENER PREVIEW

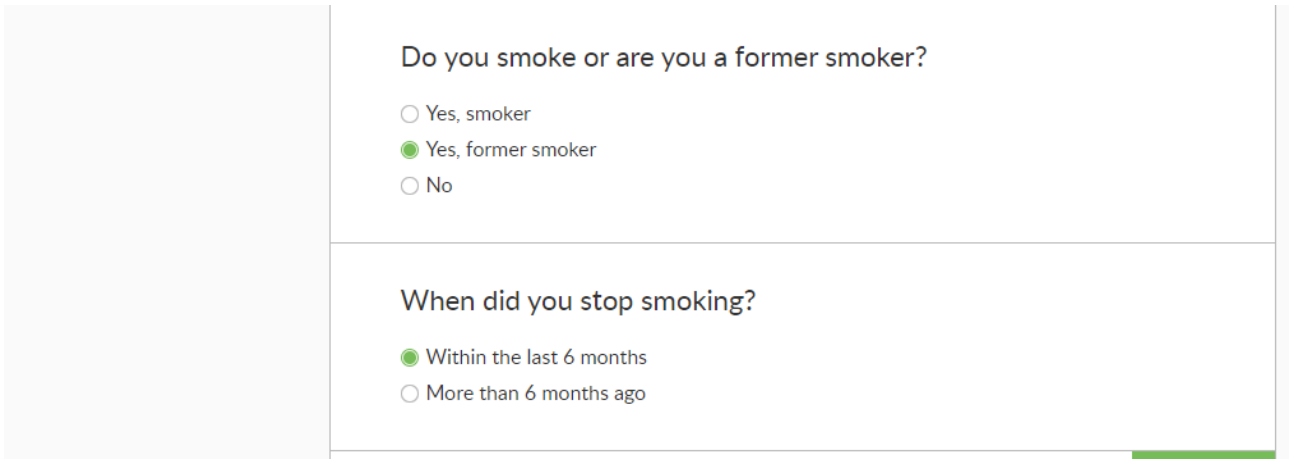
Are you diabetic?

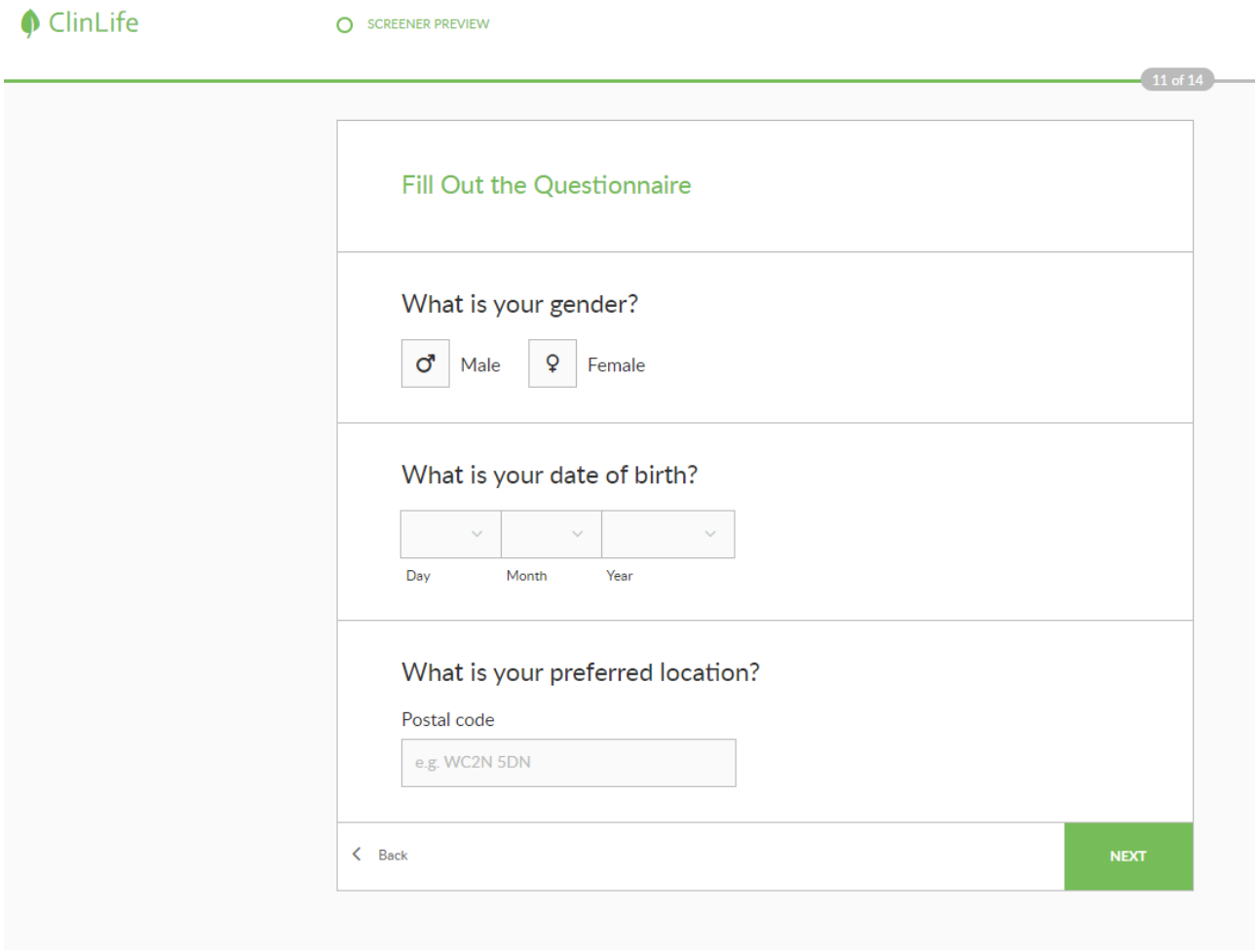
- ( ) Yes
- (•) No


ClinLife
SCREENER PREVIEW
1 of 11
Do you currently use any of the following medications?
Please select all that apply:
Metformin
Corticosteroids taken by mouth (e.g. prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone, deflazacort, fludrocortisone)
Orlistat (brand names: Alli®, Xenical®)
Hormonal contraceptives (includes: birth control pill, vaginal ring, skin patches, implant, IUD or IUS)
None of the above
Unsure
Have you been diagnosed with any of the following endocrine diseases?
Please select all that apply:
Hypothyroidism (underactive thyroid gland)
Addison's disease (Adrenal insufficiency)
Hyperprolactinaemia (higher than normal levels of the hormone prolactin in the blood)
Cushing's syndrome
None of the above
Unsure


ClinLife
SCREENER PREVIEW
Do you or have you had any other health issues within the last 5 years?
Yes
No


ClinLife
SCREENER PREVIEW
Do you have any of the following?
Please select all that apply:
Celiac disease
Crohn's disease
Gallstones
Hepatitis
Kidney stones
Liver disease (e.g. cirrhosis, NASH, liver fibrosis, liver cancer)
Pancreatitis (inflammation of the pancreas)
None of the above
Unsure
Have you had any form of cancer within the last 5 years?
Yes
No


Do you smoke or are you a former smoker?
Yes, smoker
Yes, former smoker
No
When did you stop smoking?
Within the last 6 months
More than 6 months ago


ClinLife
SCREENER PREVIEW
11 of 14
Fill Out the Questionnaire
What is your gender?
Male
Female
What is your date of birth?
Day
Month
Year
What is your preferred location?
Postal code
e.g. WC2N 5DN
Back
NEXT

6.4 DOMINO Trial Statistical Analysis Plan

STATISTICAL ANALYSIS PLAN

Investigation of the metabolic effects of DuOdenal resurfacing on insulin resistant woMen with polycystic ovariaN syndrOme

The DOMINO Trial

Principal Investigator

Dr Alexander Dimitri Miras

Sponsor

Imperial College London

23rd September 2019

Department of Investigative Medicine

Imperial College London

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1 Abbreviations

Abbreviation	Definition
AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BBA	Boston Biomedical Associates
BMI	Body Mass Index
CRF	Case Report Forms
CSR	Clinical Study Report
DHEAS	Dehydroepiandrostenedione
DMR	Duodenal Mucosal Resurfacing
FSH	Follicle-Stimulating Hormone
HbA1c	Glycated Hemoglobin
HOMA-IR	Homeostatic Model Assessment – Insulin Resistance
ITT	Intent-To-Treat Population
LH	Luteinizing Hormone
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-To-Treat Population
MMRM	Mixed Model Repeated Measures
NHS	National Health System
NIH	National Institutes of Health
PCOS	Polycystic Ovary Syndrome
PP	Per-Protocol Population
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SHBG	Sex Hormone Binding Globulin
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
UADE	Unanticipated Adverse Device Effect
USS	Ultrasound Scan

2 Study Design

This is a randomised double-blinded sham-controlled prospective investigation of women with PCOS, insulin resistance and oligo/amenorrhoea.

1:1 randomised, double blinded (subject and endocrinologist) trial comparing DMR treatment to sham procedure

In addition to the allocated procedure, all patients will be provided with standard NHS lifestyle advice for 6 months.

Key Inclusion criteria

Diagnosis of PCOS based on the NIH criteria

Insulin resistance as defined by a 2 hour oral glucose tolerance test glucose concentration of 7.8 mmol/l and/or HOMA-IR ≥ 3.0

BMI ≥ 30 Kg/m^2

Key Exclusion criteria

Other causes of anovulation

More than 6 menses in the previous 12 months

Medications affecting insulin sensitivity

Pregnant or breastfeeding at screening or 6 months previously

Type 2 diabetes mellitus

Key Assessments

Reproductive

Hormone profile: plasma/serum reproductive hormones

Weeks 0 to 24: Self-reported menses

Weeks 12 to 24:

Weekly USS to track development of ovarian follicles

Measure serum progesterone 7-10 days later (i.e. mid-luteal phase)

Metabolic

Oral glucose tolerance test to measure glucose and indices of insulin secretion and sensitivity. Will take place pre-intervention, within 2 weeks and then at 3 months post-intervention.

Insulin clamp: hepatic and peripheral insulin sensitivity will be measured using the gold-standard two-step euglycaemic hyperinsulinaemic clamp combined with a constant infusion of [6, 6 $^{2}H_{2}$] glucose pre and at 3 months post-intervention.

Fasting blood glucose and insulin to measure HOMA-IR pre-intervention, within 2 weeks, 3 and 6 months post-intervention.

Unblinding to occur after the last patient reaches the 24 week visit.

2.1 Interim Analysis

There will be no interim analysis for this study.

2.2 Final Analyses and Reporting

2.2.1 Final Double-Blind Analysis

The data through 24 weeks will be locked and all final planned analyses of primary and key secondary endpoints identified in the protocol and in this SAP, will be performed after the last subject has completed the 24-week visit.

3 Study Objectives and Endpoints

3.1 Study Objective

To demonstrate the efficacy of the Fractyl DMR Procedure using the Revita System compared to a sham procedure for the treatment of women with PCOS, insulin resistance and oligo/amenorrhoea.

0 - 24 Week Double-Blind Phase Objective: To study the effect of DMR on mechanistic and clinical endpoints 24 weeks post-procedure.

3.2 Study Endpoints

3.2.1. Primary Efficacy Endpoint

Primary Efficacy:

1. The change from baseline in total insulin sensitivity at 12 weeks. Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity as assessed by insulin clamp.

2. The change from baseline in insulin sensitivity at 24 weeks as assessed by HOMA-IR.

3. The number of menses during 24 weeks

3.2.2.Secondary Efficacy Endpoints

Change in hepatic insulin sensitivity from baseline at 12 weeks

Change in peripheral insulin sensitivity from baseline at 12 weeks

Number of ovulatory cycles defined by an increase in serum progesterone and / or ultrasound evidence of ovulation followed by menstrual bleeding between weeks 12-24

Change in the area under the curve (time 0-180 minutes, full and area above fasting value of the variable) of the concentrations of glucose, insulin and c-peptide at the OGTT from baseline at 12 weeks

Change in the Matsuda and Disposition indices at the OGTT from baseline at 12 weeks

Change from baseline in insulin sensitivity at 12 weeks as assessed by HOMA-IR

Change in the area under the curve (time 0-180 minutes, full and area above fasting value of the variable) of the concentration of glucose, insulin and c-peptide at the OGTT from baseline at 2 weeks

Change in the Matsuda and Disposition indices at the OGTT from baseline at 2 weeks

Change in glycated haemoglobin from baseline at 24 weeks

Change in free androgen index from baseline at 24 weeks

Change in concentrations of liver function tests from baseline at 24 weeks

Change in concentrations of the enhanced liver fibrosis test from baseline at 24 weeks

% body weight loss from baseline at 24 weeks

3.2.3.Exploratory Endpoints

Change from baseline in the following at 12 weeks

Energy expenditure

Body composition

Plasma lipid concentration

Arterial blood pressure

Change from Week 12 to Week 24, and change from Week 12 to each visit, for the following:

Endometrial thickness

Ovarian volume

Follicle number

Diameter of largest follicle in each ovary

Serum LH

Serum FSH

Serum Oestradiol

Serum SHBG

Serum Testosterone

Serum DHEAS

Serum Androstenedione

4 Sample Size

Assumptions of effect size for the primary efficacy endpoints in the treatment arm were derived from previous publications in which insulin sensitising medications were administered in similar groups of women [1, 2] and also takes into account information from previous studies on DMR.

It is assumed that:

(a) a difference in mean change in total insulin sensitivity (as assessed by insulin clamp) between treatment and control of 2.79 µmol/kg.min at 12 weeks with equal variance in both groups (standard deviation of 4.50), which gives a standardized effect size of 0.62 (= 2.79/4.50). Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity.

(b) a standardized effect size of 0.91 for the change from baseline in insulin sensitivity at 24 weeks as assessed by HOMA-IR.

(c) a difference in the number of menses between treatment and control of 1.0 over 24 weeks with equal variance in both groups (standard deviation of 1.0), which gives a standardized effect size of 1.0.

The Hochberg procedure to adjust for multiple endpoints is described in Section 6.5. Under this procedure 24 randomised subjects (12 per group) provides approximately 82.8% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.050 significance level, and provides approximately 72.9% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.025 significance level.

Thirty patients will be randomised to account for potential patients lost to follow up prior to the primary endpoint assessment. Patients who for technical reasons cannot have the DMR will be replaced.

5 Analysis Populations

5.1 Intent to Treat Population (ITT)

The intent-to-treat (ITT) population for this study includes all randomised subjects.

5.2 Modified Intent to Treat Population (mITT)

The mITT population includes all randomised subjects in whom the study procedure (DMR or sham) is attempted and who have a baseline measurement for at least one primary endpoint. The procedure is attempted when all endoscopic exclusion criteria are verified, the catheter is introduced into the subject, and at least one ablation is performed. Subjects will be analysed according to their randomised group assignment. The mITT population is the primary analysis population for both the primary and secondary efficacy endpoints.

5.3 Per-Protocol Population (PP)

The Per-Protocol (PP) analysis population includes the subset of mITT subjects who received the treatment to which they were randomised, and excludes any subjects with major protocol deviations, which include those DMR cases that did not undergo the full DMR procedure. The full details on "major protocol deviations" that lead to patients being excluded from the PP population are discussed in Section 6.4. This is a secondary analysis population for efficacy.

5.4 Safety Population

This analysis population includes all treated subjects, and these subjects are analysed by actual treatment received.

6 General Issues for Statistical Analysis

6.1 Analysis Software, GENERAL METHODS OF ANALYSIS AND ASSESSMENT OF NORMALITY

Analysis data sets, statistical analyses and associated output will be generated using SAS version 9.3 or later, SPSS Software version 24 or later or Prism version 6.0 or later.

Variables are presented by treatment group using various descriptive statistics. Nominal and ordinal variables for each time period are presented using frequencies and percent of patients in each category. For variables collected at multiple follow-up time periods, tables which include appropriate descriptive statistics of change from baseline are presented by treatment group at each follow-up interval.

Statistical tests for the efficacy endpoints will be carried out using the 5.0% one-sided significance level, as well as using the 2.5% one-sided significance level unless otherwise specified.

For each primary and secondary efficacy endpoint which is a continuous variable normality will be assessed. This assessment will be conducted by producing Q-Q plots and histograms of residuals from an ANCOVA model (including only patients with non-missing values for both baseline and the relevant post-baseline visit). This ANCOVA model (used only for the

purpose of assessing normality) will include terms for the baseline value and treatment. For primary and secondary endpoints that are assessed at more than one timepoint the assessment of normality will be based on the endpoint at the latest of these timepoints, e.g., assessment of normality for change from baseline to Week 24 in HOMA-IR will also determine whether change from baseline to Week 12 in HOMA-IR is to be analysed on the rank or original data scale.

If for a given endpoint either the Q-Q plot indicates non-normality or the histogram indicates non-normality then analysis for that endpoint will be based on the rank scale (using modified ridits), i.e., the response variable and the baseline value will both be converted to the rank scale before the formal analysis.

If for a given endpoint the Q-Q plot and the histogram are both consistent with normality then analysis will be carried out with the response variable on the original scale. In this case the raw baseline variable will also be assessed for normality to determine if this should be included in the formal analysis as a variable on the original scale or on the rank scale (using modified ridits).

6.2 Disposition of Subjects and Withdrawals

All subjects who provide written informed consent will be accounted for. The number and percentage of ITT and mITT subjects who discontinued the study prior to Week 24 will be presented by treatment group, overall and by reason of discontinuation (adverse event, discontinued by investigator, withdrawn consent/request to terminate, lost-to-follow-up, death, other). Percentages will be based on the number of ITT and mITT subjects.

6.3 Methods for Missing Data

All efforts will be made to prevent the occurrence of missing data. Nevertheless, it is anticipated that withdrawals will occur and hence there will be missing data on primary and secondary efficacy endpoints

For the measures of insulin sensitivity based on insulin clamp there are only assessments at baseline and at Week 12, i.e., there are no post-baseline values that could be used for

imputation. Therefore, in analysis of hepatic insulin sensitivity change will be missing if either the baseline value or the Week 12 value is missing. Likewise, change in peripheral insulin sensitivity will be missing if either the baseline or Week 12 value is missing. For the first primary endpoint of change from Baseline to Week 12 in Total Insulin Sensitivity based on insulin clamp, this will be missing if either of the two baseline values, or either of the two Week 12 values are missing.

Insulin sensitivity based on HOMA-IR is assessed at baseline, Week 2, Week 12, and Week 24. For the HOMA-IR change from baseline to Week 24 primary endpoint as described in Section 8.1, missing data will be accounted for by using a mixed model repeated measures (MMRM) analysis.

For number of menses during the 24 weeks after randomization, patients that do not have data recorded for any of Week 21-24 will have imputation carried out as follows: Let W represent the last week for which menses (yes, no) was recorded, let Y represent W rounded up to a multiple of 4 (so that Y can take the values 4, 8, 12, 16, 20, or 24), then X the number of menses recorded in the first W weeks after randomization is multiplied by (24/Y) prior to analysis to allow for the missing data on the last (24-W) weeks since randomization, e.g., if a patient's last week at which menses (yes, no) are recorded is Week 15 (so that W=15, Y=16) and where two menses (so that X=2) have been recorded since randomization, then the value X=2 is replaced by the imputed value of 3.0 (=2x[24/16]).

For continuous secondary efficacy endpoints that are measured at more than one post-baseline visit missing data will be taken into account by using the MMRM approach. For continuous secondary efficacy endpoints that are measured at only one post-baseline visit patients will be excluded if they are missing baseline or have a missing value at the post-baseline visit.

6.4 Protocol Violations

Protocol violations will be summarised in the CSR. This summary will include the number and percent of subjects with each violation type. Major violations in this study may be those that are related to:

Informed consent deviation

Inclusion/Exclusion criteria, if such protocol violation is likely to impact one of the two primary endpoints

Device or equipment not used per protocol

Device not returned to sponsor

DMR Procedure/laboratory assessment incomplete or not done

DMR Procedure/laboratory assessment not done per protocol

Participant not complying with trial protocol

The main reason for assessing the incidence of major violations during the study is to determine which patients are in the per-protocol population (the per-protocol population excludes “major” violations). Prior to database lock and un-blinding, all protocol violations will be reviewed in a blinded manner and patients who have had major violations will be noted and excluded from the per-protocol population.

6.5 Multiple ENDPOINT ADJUSTMENT

The trial will be viewed as positive if statistical significance is obtained on one or more of the three primary endpoints. Multiple endpoint adjustment will be carried out using the Hochberg[3] procedure so as to control the overall type 1 error at the required level.

Let P1, P2, and P3 denote the one-sided p-values for the three primary endpoints, where in each case the one-sided alternative hypothesis represents the beneficial effect of DMR over Sham. The Hochberg procedure will be first carried out using an overall α =0.050 one-sided. It would proceed as follows:

(i) if P1, P2, and P3 are all $\leq$ 0.050 then statistical significance is demonstrated for all 3 primary endpoints;

(ii) if (i) is not satisfied but any two of P1, P2, or P3 are ≤ 0.025 [=0.050/2] then statistical significance is demonstrated for the two primary endpoints with the lowest p-values;

(iii) if (i) and (ii) are not satisfied but the smallest of P1, P2, or P3 is ≤ 0.050/3 then statistical significance for that endpoint only is demonstrated; or

(iv) if none of (i)-(iii) are satisfied then none of the primary endpoints is statistically significant at this overall α = 0.050 one-sided significance level.

A symbol "*" will be used to denote statistical significance for the primary endpoints using the overall α = 0.050 one-sided significance level in the procedure described above.

The Hochberg procedure would then be repeated but using an overall α =0.025 one-sided, i.e., comparing vs. 0.025 in (i), comparing vs. 0.0125 in (ii) and comparing vs. 0.025/3 in (iii). A symbol "**" will be used to denote statistical significance for the primary endpoints using the overall α = 0.025 one-sided significance level.

If a primary endpoint qualifies to be flagged as both "**" and "*" then it will be flagged using "**", i.e., will be flagged to denote the strongest significance level.

There will be no adjustment for the multiple secondary endpoints.

7 Demographics and Other Baseline Characteristics

7.1 Demographics

Demographics will be summarised by randomised treatment group for the mITT and the safety analysis populations. There will be no formal statistical comparisons between treatment groups on demographic variables. The continuous variables will be summarised by treatment group using sample size, mean, standard deviation, minimum and maximum. For the categorical variables of gender, race, and ethnicity the number and percentage of patients in each category will be presented for each randomised treatment group.

7.2 Baseline Medical History

The medical history of all mITT and the safety analysis population subjects will be summarised in a table by treatment group. Specifically, for each condition, the number and percent of subjects who currently have the condition will be presented.

7.3 Baseline LABORATORY MEASUREMENTS

A table presenting descriptive statistics (sample size, mean, standard deviation, median, min and max) of laboratory variables by treatment group at baseline will be provided for the mITT analysis set. If the baseline value is missing for a given variable and patient, the screening value will be used in its place prior to calculating the descriptive statistics.

8 Efficacy Analyses

8.1 Primary Efficacy Variables

The primary efficacy variables are:

1. The change from baseline at 12 weeks in total insulin sensitivity, as assessed by insulin clamp;

2. The change from baseline at 24 weeks in insulin sensitivity as assessed by HOMA-IR; and

3. The number of menses during 24 weeks.

The primary analysis for the change from baseline to Week 12 in total insulin sensitivity endpoint (based on insulin clamp) will be performed in the mITT analysis population comparing treatment groups with an Analysis of Covariance (ANCOVA) model with terms for baseline total insulin sensitivity and treatment. Before conducting this analysis, assessment of normality for this endpoint will be carried out as described in Section 6.1 and a determination will be made as also described there on whether the primary analysis is to be based on raw data or on the rank scale (using modified ridits). If the primary analysis is based on the rank scale then a secondary analysis will be carried out with response variable of change from baseline to Week 12 in total insulin sensitivity using ANCOVA with terms for baseline total insulin sensitivity and treatment. If the primary analysis is on the raw data scale then this secondary analysis will instead be carried out using ANCOVA with response variable

of rank of change from baseline to Week 12 in total insulin sensitivity with terms for rank of baseline total insulin sensitivity and treatment.

The primary analysis for change from baseline to Week 24 in insulin sensitivity as assessed by HOMA-IR will be carried out using MMRM in the mITT analysis population. The methodology as described in Section 6.1 will be used to assess normality and determine whether in the primary analysis the response variable is to be in terms of raw data or based on the rank scale (using modified ridits). If the response variable in terms of change from baseline to Week 24 needs to be on the rank scale then in the MMRM analysis change from baseline to Weeks 2 and 12 will also be based on the rank scale. The MMRM analysis will include explanatory variables of baseline HOMA-IR (on the original scale or on the rank scale as determined using the approach described in Section 6.1), visit (as a categorical variable taking values 2, 12, or 24), treatment, and treatment-by-visit interaction as fixed effects with subject as a random effect in the model. This primary endpoint will be tested by deriving the contrast for comparing treatment groups at Week 24. An unstructured within patient covariance structure will be assumed, but if the model does not converge then a compound symmetry covariance structure will be assumed instead. A secondary analysis for this primary endpoint will be conducted using the other scale (original data, or rank based data using modified ridits) to that used in the primary analysis.

The primary analysis for the number of menses during the first 24 weeks after randomization will be performed in the mITT analysis population comparing treatment groups with an Analysis of Covariance (ANCOVA) model on rank of measured number of menses over 24 weeks with terms for rank of number of reported number of menses in the 12 months before randomisation and treatment.

For each of the analyses of the primary endpoints, least square means will be presented together with their SEs and two-sided confidence intervals.

For each primary endpoint corresponding analyses will also be conducted in the PP analysis population.

For each analysis, tables of descriptive statistics of the primary endpoint will include n, mean, standard deviation, least squares mean, standard error of least squares mean, median, quartiles, and minimum and maximum for each treatment group. Two-sided 90% and 95% confidence intervals of the difference between treatment least square means will be presented. Tables will be complimented by graphs as necessary.

8.2 Secondary Efficacy Variables

The following secondary endpoints will be compared between treatment groups on the mITT and PP analysis populations.

3. Change in hepatic insulin sensitivity from baseline at 12 weeks
4. Change in peripheral insulin sensitivity from baseline at 12 weeks
5. Number of ovulatory cycles defined by an increase in serum progesterone and / or ultrasound evidence of ovulation followed by menstrual bleeding between weeks 12-24
6. Change in the area under the curve (time 0-180 minutes, full and area above fasting value of the variable) of the concentrations of glucose, insulin and c-peptide at the OGTT from baseline at 12 weeks
7. Change in the Matsuda and Disposition indices at the OGTT from baseline at 12 weeks
8. Change from baseline in insulin sensitivity at 12 weeks as assessed by HOMA-IR
9. Change in the area under the curve (time 0-180 minutes, full and area above fasting value of the variable) of the concentration of glucose, insulin and c-peptide at the OGTT from baseline at 2 weeks
10. Change in the Matsuda and Disposition indices at the OGTT from baseline at 2 weeks
11. Change in glycated haemoglobin from baseline at 24 weeks
12. Change in free androgen index from baseline at 24 weeks
13. Change in concentrations of liver function tests from baseline at 24 weeks
14. Change in concentrations of the enhanced liver fibrosis test from baseline at 24 weeks
15. % body weight loss from baseline at 24 weeks

Secondary efficacy endpoints will be compared between treatments at a one-sided 0.050 level of significance with the direction of the alternative hypothesis favoring DMR over control. There will be no adjustment for the multiple secondary endpoints. Missing data for secondary endpoints is discussed in the Section 6.3 above. Analyses for the number of ovulatory cycles in Weeks 12-24 will be carried out in a similar manner to the analyses of number of menses as described in Section 8.1. Analyses for continuous secondary efficacy endpoints that are assessed at more than one post-baseline visit will be carried out in a similar

manner to the analyses of HOMA-IR change from baseline to Week 24 as described in Section 8.1. Any continuous secondary efficacy endpoints that are assessed at only one post-baseline visit will be carried out in a similar manner to the analyses of total insulin sensitivity change from baseline to Week 12 based on insulin clamp, as described in Section 8.1.

Tables will be complimented by graphs as necessary.

8.2.1 Exploratory Endpoints

16. Change from baseline in the following at 12 weeks
    - Energy expenditure
    - Body composition
    - Plasma lipid concentration
    - Arterial blood pressure

17. Change from Week 12 to Week 24, and change from Week 12 to each visit, for the following:
    - Endometrial thickness
    - Ovarian volume
    - Follicle number
    - Diameter of largest follicle in each ovary
    - Serum LH
    - Serum FSH
    - Serum Oestradiol
    - Serum SHBG
    - Serum Testosterone
    - Serum DHEAS
    - Serum Androstenedione

All analyses for exploratory endpoints will be based on the mITT analysis population. There will be no formal statistical comparisons between randomised treatment groups for exploratory endpoints, and there will be no imputation of missing data. Results will be presented by treatment group in terms of summary statistics of the change (from baseline, or from Week 12, as applicable). For endpoints that are measured weekly from Week 12 to Week 24 the actual values and the change from Week 12 values will also be displayed graphically by treatment group.

9 Safety Analyses

The analysis of safety data during the 24-week randomized double-blind period will be based on the Safety population, within which patients will be summarized by treatment administered.

9.1 Primary Safety Variable

The primary safety endpoint is the incidence rate of the device or procedure related Serious Adverse Events (SAEs) and Unanticipated Adverse Device Effects (UADEs) through the 24 weeks post treatment initiation. The safety endpoint summary will include the number and percentage of subjects in each of the categories overall and by MedDRA System Organ Class (SOC) and Preferred Term (PT); for the AEs occurring during the randomised phase, these numbers and percentages will be presented within each treatment group. A formal hypothesis test comparing treatments is not planned. Further details on adverse event analyses are provided below.

9.2 Secondary Safety Variables

Physical Examination Vital Signs: Observed measurements and changes in physical exams and vital signs from baseline to post-baseline study time points will be descriptively summarised for each treatment group. For each vital sign, descriptive statistics of each vital sign will be presented at each visit for each treatment group; descriptive statistics of the change from baseline to each visit will also be presented. Listings of abnormal physical examination results will be presented; included in the listing will be subject id, body system where the abnormality occurred, study visit, and the physical examination results for all visits (i.e., not just the visit where the abnormality occurred) for the given body system.

Clinical Laboratory Tests: Descriptive statistics of observed measurements in blood chemistry analysis and changes from baseline to each study time point in the double-blind phase will be presented for each treatment group. All laboratory values are compared to normal ranges; for each laboratory value, shift tables of normality status (low/normal/high, or normal/abnormal if the assignment of low and high does not apply) from baseline to each

post-baseline visit will be presented for each treatment group. All data will also be presented in listings.

Adverse Events: AEs, SAEs and UADEs are coded using Medical Dictionary for Regulatory Activities (MedDRA) and summarised through 24 weeks for each treatment by number and percentage of patients with at least one adverse event overall and by primary System Organ Class (SOC) and Preferred Term (PT). Detailed listings of subjects that experience AEs and SAEs will be provided. The incidence of AEs will also tabulated (frequencies and percentages) by severity and relationship to procedure or device as outlined below. In tabulating the severity of AEs on a per subject basis, the greatest severity will be assigned to a subject should there be more than one occurrence of the same AE with different reported severities. Relationship is categorised as no, possibly, probably, and definitely. The highest level of association is reported for subjects with different relationships for the same AE. Details of AE analyses are provided below.

9.3 Adverse Events

All adverse events (AEs) will be coded using the standardised MedDRA central coding dictionary, version 19.1 or greater. Adverse event analyses will be performed on the Safety analysis population.

9.3.1 All Adverse Events

The number of treatment emergent adverse events (TEAEs) and the number and percent of subjects with at least one TEAE will be presented overall by SOC and PT. A TEAE is an event starting or worsening in severity at or after initiation of the index procedure for the randomised treatment. For subject counts, subjects experiencing a given event more than once will be counted only once for that event. For TEAEs occurring in the randomised phase, results will be presented by treatment group.

The proportion of patients with at least one TEAE of special interest (TEAESI) with at least one procedure-related TEAE, and with at least one device related TEAE in the double-blind phase will be plotted by time point (peri-procedure, 0-1 week, 1-4 weeks, 4-8 weeks, …, 20-24 weeks) for each treatment group.

A listing of all adverse events will include the subject number, AE number, days since index procedure, the investigator description of the AE, the AE SOC and PT, the severity of AE, whether or not the AE is classified as serious (SAE), the relationship of the AE to the investigational device or procedure, the action taken, the outcome, and the adjudication status.

9.3.2. Adverse Events Leading to Withdrawal

A summary of number of TEAEs and of the incidence rates (number and percentage of subjects) of TEAEs leading to study withdrawal, by SOC and PT will be presented in a similar manner as discussed above (with the exception of the plot). A data listing of TEAEs leading to withdrawal will also be provided, displaying details of the event(s) captured on the CRF.

9.3.3.Serious Adverse Events

Summaries of serious TEAEs will be conducted in the same manner as for all TEAEs discussed above.

9.3.4 Device and Procedure Related Adverse Events

Summaries of device or procedure related TEAEs will be conducted in the same manner as for all TEAEs discussed above; patients with the occurrence of more than one TEAE within a given SOC or PT will be counted only under the maximum severity/relationship experienced for that SOC or PT, respectively.

9.3.5 Unanticipated Adverse Device Effects

Summaries of treatment emergent unanticipated device TEAES will be conducted in the same manner as for all TEAEs discussed above.

9.3.6 Adverse Events of Special Interest (AESIs)

- Specific events that may be related to the mechanism of action of the DMR procedure (eg, hypoglycemia)
- Potential adverse consequences of the procedure (e.g., gastrointestinal adverse events)

- Rare events that may or may not be related to the DMR procedure/device, but are of interest to the Sponsor (e.g., unexplained fever)

Events of Special Interest are:

- Hypoglycemia
- Diarrhea
- Abdominal pain, nausea, vomiting
- Gastrointestinal bleeding
- Unexplained fever
- Stenosis (GI)

The number of events and incidence of AESIs will be presented.

9.3.7 Deaths

If a death occurs during the course of the trial, relevant information (including study day of death relative to index initiation, cause of death, and adverse event leading to death) will be supplied in a data listing.

10 Other Planned Analyses

10.1 Planned Subgroup Analyses

The following sections list the planned subgroup analyses. Additional subgroup analyses may be performed for exploratory purposes and will be identified as exploratory in the final report.

Treatment comparisons on the primary efficacy endpoints will be presented for the mITT population patients within each of the following subgroups :

A. Body Mass Index (BMI; (<Median, ≥Median)
B. Baseline total insulin sensitivity (<Median, ≥Median)

The purpose of the subgroup analysis is not to assess significance of the difference between treatments within subgroups, but to assess the consistency of treatment effect across subgroups.

11. REFERENCES

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2. Rautio, K., et al., *Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study.* HumReprod., 2006. **21**(6): p...1400-7. Epub 2006 Feb 24.

3. Hochberg, YA. Sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 1988; 75:800 - 802.