Impact of Insulin Sensitising Interventions on Women with Polycystic Ovary Syndrome and Healthy Humans

This thesis is submitted to Imperial College London in fulfilment of the requirement for the degree of Doctor of Philosophy

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i) Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrinology condition in premenopausal women, with a prevalence of 8%-12% in this population. The presentation can vary between individuals, and although obesity is not part of the current diagnostic criteria, rates of obesity can range between 50%-80% in this patient population. PCOS is a common cause of anovulatory infertility, which an increased body mass index can compound. Lifestyle interventions with or without pharmacotherapy remain the mainstay of treatment, with bariatric surgery currently considered an experimental treatment.

The first part of this thesis will address the impact of insulin sensitiser pharmacotherapy on metabolic and reproductive outcomes in women with PCOS who have overweight and obesity through a comprehensive systematic review, meta-analysis and meta-regression. Following this, the BAMBINI randomised controlled clinical trial will compare the effectiveness of medical care to bariatric surgery in improving the number of ovulatory cycles over 52 weeks in women with PCOS who have obesity.

The use of an insulin sensitiser in women with PCOS who have overweight, or obesity resulted in a significant improvement in metabolic outcomes and some reproductive hormones; however, there was a lack of data for hard reproductive outcomes.
Bariatric surgery proved superior to medical care in improving anthropometric, metabolic, and reproductive outcomes in women with PCOS who have obesity. Further

randomised controlled trials are needed to investigate this effect and its impact on pregnancy outcomes.

The second part will focus on the melanocortin system and its role in glucose homeostasis through melanocortin receptor agonism. Animal studies have shown that α-melanocyte stimulating hormone (α-MSH) – a melanocortin receptor agonist, increases skeletal muscle glucose uptake following a glucose load. In this first-in-human, double-blind, randomised, cross-over experimental study, healthy volunteers received α-MSH (initially at three different doses) and saline during an oral glucose tolerance test and subsequent euglycaemic hyperinsulinaemic clamp. The oral glucose tolerance tests were repeated in a different group of healthy volunteers with high dose α-MSH and saline. Infusion with α-MSH significantly reduced mean plasma glucose and serum insulin concentrations compared to saline.

ii) Statement of Original Authorship

I certify that the submitted work is my own and was completed whilst registered as a candidate for the degree of Doctor of Philosophy. Based on the research presented in this submitted work, I have not obtained a degree elsewhere.

Dr Suhaniya Samarasinghe

iii) Copyright Declaration

iv) Publications

Type 2 diabetes prevention goes digital. S. N. S. Samarasinghe and A. D. Miras. The Lancet Regional Health - Europe 2023 Vol. 24 Pages 100538

Simple tool to prioritize access to bariatric surgery for people living with obesity during the COVID-19 pandemic. S. Samarasinghe, A. Sudlow, G. K. Dimitriadis, A. R. Ahmed, S. Purkayastha, C. Tsironis, et al. Br J Surg 2021 Vol. 108 Issue 4 Pages e179-e180

Manuscripts under review

Suhaniya Samarasinghe, Eduard Ostarijas, Matthew Long, Simon Erridge, Sanjay Purkayastha, Georgios Dimitriadis, Alexander D. Miras. Impact of insulin sensitisation on metabolic and fertility outcomes in women with polycystic ovary syndrome and overweight or obesity – a systematic review, meta-analysis, and meta-regression.

Manuscripts in Preparation

Patrick Swan, Brett Johnson, **Suhaniya Samarasinghe**, Ludmilla Pessanha, Giuseppe de Vito, Michael A. Cowley, Carel W. le Roux, Alexander D. Miras, Neil G. Docherty. Alpha-Melanocyte Stimulatory Hormone Improves Post-Prandial Glucose Tolerance in Healthy Humans.

v) Acknowledgements

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vi) Abbreviations

17-OHP	17-hydroxyprogesterone
ACTH	Adrenocorticotrophic hormone
ADA	American Diabetes Association
AE	Adverse event
AGB	Adjustable gastric banding
AgRP	Agouti related protein
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMH	Anti- Müllerian hormone
AR	Androgen receptors
ARC	Arcuate nucleus
ART	Assisted reproductive technology
ASIP	Agouti-signalling protein
ASMBS	American Society for Metabolic and Bariatric Surgery
AUB	Abnormal uterine bleeding
AUC	Area under the curve
BAT	Brown adipose tissue
BMI	Body mass index
BMR	Basal metabolic rate
CC	Clomiphene citrate
CI	Confidence interval
CLBR	Cumulative live birth rate
CVD	Cardiovascular disease
DHEAS	Dehydroepiandrosterone
DKA	Diabetic ketoacidosis
DNA	Deoxyribonucleic acid
DSS	Diabetes Surgery Study
EMA	European Medicine Agency
FAI	Free androgen index
FHA	Functional hypothalamic amenorrhoea
FPG	Fasting plasma glucose
FSH	Follicle-stimulating hormone
FSHB	FSH subunit beta
FSHR	FSH receptor
GABA	Gamma-aminobutyric acid
GC	Granulosa cells
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic polypeptide
GIR	Glucose infusion rate
GLP-1	Glucagon-like peptide 1
GLP-1 RA	GLP-1 receptor agonist
GLUT	Glucose transporter
GnRH	Gonadotrophin-releasing hormone

GTT	Glucose tolerance test
GWAS	Genome-wide association studies
HA	Hyperandrogenism
HbA1c	Glycated haemoglobin
HCD	Hypocaloric diet
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment for insulin resistance
HOMA-R	Homeostasis model assessment formula
HPG	Hypothalamic-pituitary-gonadal
HTN	Hypertension
iAUC	Incremental area under the curve
ICSI	Intracytoplasmic sperm injection
IQR	Interquartile range
IVF	*in vitro* fertilisation
KNDy	Kisspeptin, neurokinin B, and dynorphin
LAGB	Laparoscopic AGB
LDL-C	Low-density lipoprotein cholesterol
LEPR	Leptin receptor
LH	Luteinising hormone
LHB	LH subunit beta
MA	Meta-analysis
MC3R	Melanocortin 3 receptor
MC4R	Melanocortin 4 receptor
MCR	Melanocortin receptor
MDI	Multiple daily injections
MetS	Metabolic syndrome
MR	Meta-regression
MRAPs	Melanocortin receptor accessory proteins
MYO	Myoinositol
NCAH	Nonclassical adrenal hyperplasia
NICE	National Institute for Health and Care Excellence
OD	Ovulatory dysfunction
OGTT	Oral glucose tolerance test
OPK	Ovulation prediction kit
OSA	Obstructive sleep apnoea
PCOM	Polycystic ovary morphology
PCOS	Polycystic ovary syndrome
PG	Plasma glucose
POMC	Proopiomelanocortin
PPT	Post prandial thermogenesis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PYY	Peptide YY
QoL	Quality of life
RCT	Randomised controlled trial
RNA	Ribonucleic acid
RP3V	Third ventricle

RYGB	Roux-en-Y gastric bypass
SA	Secondary amenorrhoea
SAE	Serious adverse event
SBP	Systolic blood pressure
SD	Standard deviation
SG	Sleeve gastrectomy
SGLT	Sodium-glucose like co-transporter
SHBG	Sex hormone binding globulin
SMD	Standardised mean difference
SOS	Swedish Obese Subjects
SR	Systematic review
SST	Short synacthen test
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
TG	Triglycerides
TG	Triglycerides
VSG	Vertical sleeve gastrectomy
WAT	White adipose tissue
WC	Waist circumference
WHO	World health organisation
WHR	Waist-hip ratio
α-MSH	Alpha-melanocyte stimulating hormone

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1 PART ONE - INTRODUCTION

1.1 Presentation of Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) remains the most common endocrinology condition in women of reproductive age[1, 2], with a prevalence between 8%[1] to 12% in this group[2]. The highest reported prevalence of PCOS in the UK was 52% in South Asian immigrants, of whom 49% had menstrual irregularity[3]. Clinical or biochemical androgen excess, oligo/amenorrhoea or ovulatory dysfunction, and imaging demonstrating polycystic ovarian morphology are the three key features associated with PCOS. The 1990 National Institute of Health-National Institute of Child Health and Human Development Conference on PCOS recommended that clinical or biochemical androgen excess and olio- or amenorrhea be used as diagnostic criteria following the exclusion of other endocrinopathies[4]. The Rotterdam consensus expanded the diagnostic criteria in 2003 to require at least two of the three features listed above[4]. The Rotterdam criteria generate 4 PCOS phenotypes[4] as listed in table 1.1:

Type	**Phenotypes**
A	Hyperandrogenism or hirsutism (HA) + oligo/amenorrhoea (ovulatory dysfunction, OD) + polycystic ovary morphology (PCOM)
B	HA + OD
C	HA + PCOM
D	OD + PCOM

Table 1.1 PCOS phenotypes

PCOS phenotypes

The 1990 National Institutes of Health criteria defined two phenotypes – phenotype A (hyperandrogenism + oligo-anovulation + polycystic ovarian morphology) and phenotype B (hyperandrogenism + oligo-anovulation, but not polycystic ovarian morphology)[1]. Phenotype A is frequently considered the "complete" PCOS phenotype but both A and B are often referred to as "classic" PCOS[1]. The 2003 Rotterdam criteria included two further phenotypes – phenotype C which is the "ovulatory" PCOS and phenotype D which is "hyperandrogenic"[1]. The 2012 National Institutes of Health executive summary on PCOS recommended that the 2003 Rotterdam be used but that the specific PCOS phenotypes once identified, be noted[2].

Prior to making a diagnosis of PCOS, screening for disorders causing oligo-anovulation such as hyperprolactinaemia, hyper- and hypothyroidism should be excluded with laboratory investigations. In patients with evidence of androgen excess (confirmed by laboratory measurement of total and free testosterone), basal 17-hydroxyrogesterone (17-OHP) in the morning during the follicular phase will exclude 21-OH-deficiency in nonclassical adrenal hyperplasia (NCAH). For patients with an elevated 17-OHP level (>6.0 nmol/L at Imperial College NHS Healthcare Trust), the next step is a short synacthen test (SST)[5]. A marked rise in 17-OHP after adrenocorticotrophic hormone (ACTH) stimulation (>30 nmol/L) – dependent on if the patient is homozygous or heterozygous, is in keeping with a diagnosis of NCAH[5]. Other endocrine conditions such as Cushing's syndrome, androgen-secreting neoplasms, and disorders of severe insulin resistance (e.g. so-called hyperandrogenic-insulin resistance-acanthosis nigricans syndrome) should also be

excluded[1].

An important differential diagnosis for women presenting with secondary amenorrhea (SA) is functional hypothalamic amenorrhea (FHA). FHA is present in 1%-2% of women with SA[6]. The Endocrine Society recommends the following criteria for FHA: a menstrual cycle interval persistently exceeding 45 days or amenorrhea for longer than 3 months, psychological stressors or vigorous exercise, the presence of hypogonadotrophic hypo-oestrogenism (typically <184 pmol/L)[6, 7]. PCOS is a slightly more common cause of SA between 2%-13%[7].

Approximately 60% to 70% of daughters born to women with PCOS will manifest their PCOS phenotype during adolescence and early adulthood, making PCOS strongly familial and highly heritable[8]. The genetic aetiology of PCOS has previously been established[9], with symptoms reported in mothers and sisters of women with PCOS and abnormally elevated luteinising hormone (LH)/follicle-stimulating hormone (FSH) ratio in some male relatives (similar to levels seen in the women). Gonadotrophin-related genes have been implicated in the aetiology of PCOS, including those for FSH subunit beta (FSHB), the FSH receptor (FSHR), LH subunit beta (LHB), and the LH/choriogonadotrophin receptor[10]. A large body of published data has identified chronic anovulation in women with PCOS and obesity[11]. Genome-wide association studies (GWAS) have implicated gonadotrophin secretion and actions, androgen biosynthesis, metabolic regulation and ovarian ageing in PCOS pathogenesis[12]. A study analysing data from previous GWAS identified a metabolic subtype of PCOS characterised by higher body mass index (BMI), glucose and insulin levels with relatively low sex hormone binding globulin (SHBG) and LH levels[12].

Physiology of the menstrual cycle and Ovulation

Menstruation is the cyclic, orderly sloughing of the uterine lining in response to follicle-stimulating hormone (FSH), luteinising hormone (LH) from the anterior pituitary, and oestrogen and progesterone from the ovaries. It is divided into the follicular (proliferative) and luteal (secretory) phases. The number of days between the first day of menstrual bleeding in one cycle and the onset of bleeding in the next is the length of the menstrual cycle. On average, this is between 28 – 35 days. The luteal phase lasts 14 days and is relatively constant in all women; however, the follicular phase can range from 10 to 16 days. FSH acts on the ovary to stimulate the maturation of a follicle – the follicular cells, in turn, secrete increasing quantities of oestrogen. On days 1-5, a lack of signal from a fertilised egg causes a drop in oestrogen and progesterone production which results in sloughing off the endometrial lining (menstrual flow). This bleeding usually lasts around 3 – 5 days. Days 6-14 are known as the proliferative phase, during which a drop in oestrogen and progesterone stimulates the secretion of FSH from the anterior pituitary. FSH stimulates the maturation of an ovum with a Graafian follicle. Towards the end of this phase, there is an increase in LH which results in the release of the ovum (ovulation). The secretory phase (days 15-28) which is characterised by high levels of LH, which causes the empty Graafian follicle to develop into the corpus luteum. The corpus luteum releases progesterone, increasing blood supply to the endometrial in anticipation of fertilisation. If fertilisation does not occur, progesterone secretion stops, and the endometrial lining sheds resulting in menstruation. Figure 1.1 by Draper et al., demonstrates changing concentrations of female sex hormones according to menstrual cycle phase[13].

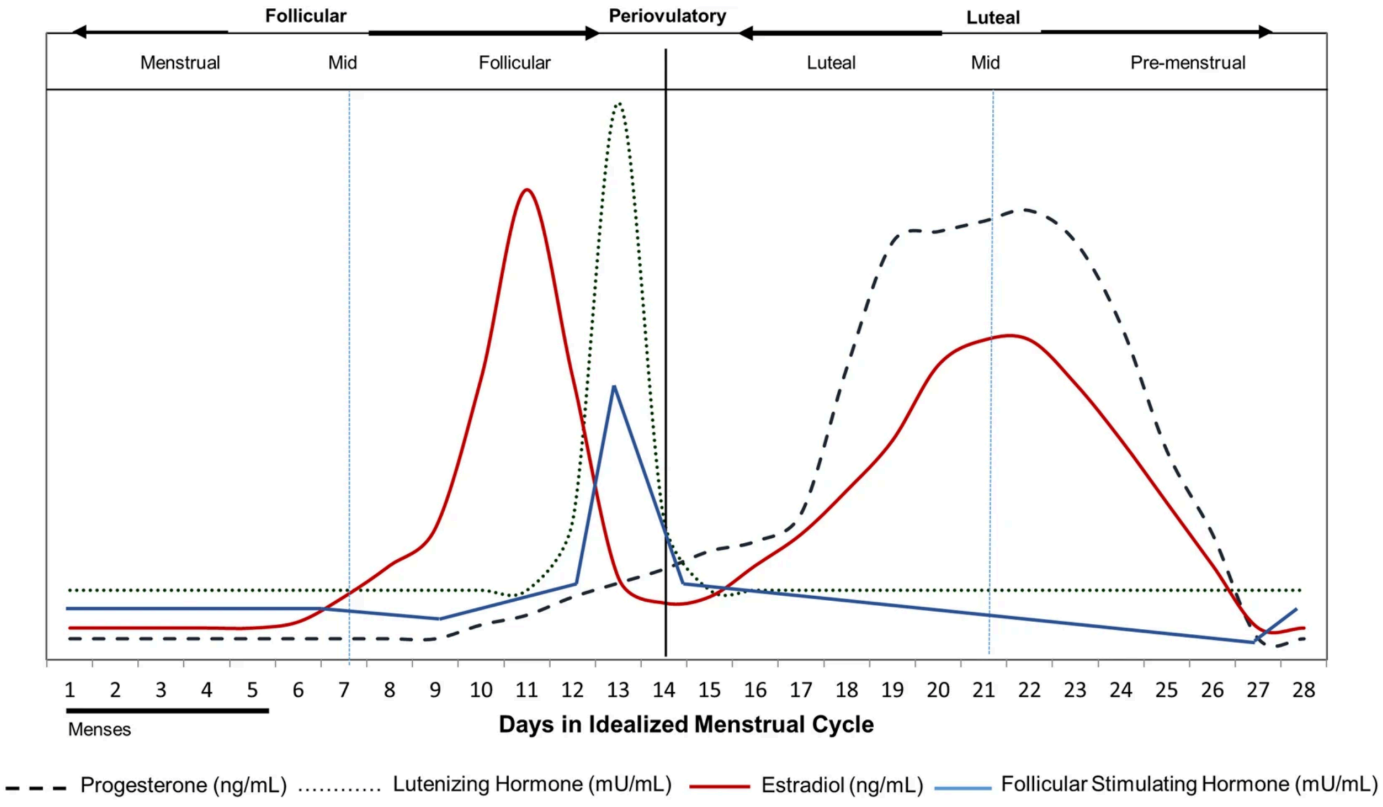


Figure 1.1 Changing concentrations of female sex hormones according to menstrual cycle phase**[13]**

An overview of the steroid biosynthesis pathway

Steroidogenesis initiates with converting cholesterol to pregnenolone within the mitochondria; pregnenolone is then catalysed into other steroids. There are five groups of steroid hormones: glucocorticoids, mineralocorticoids, androgens, oestrogens and progesterones. In humans, cortisol is the primary glucocorticoid, with the adrenal gland being the major source of glucocorticoids and mineralocorticoids. Androgens (e.g., testosterone) and oestrogens (e.g., oestradiol) and progestogens are synthesised by the gonads (testes and ovaries) and placenta. Figure 1.2 provides an overview of the steroid biosynthesis pathway.

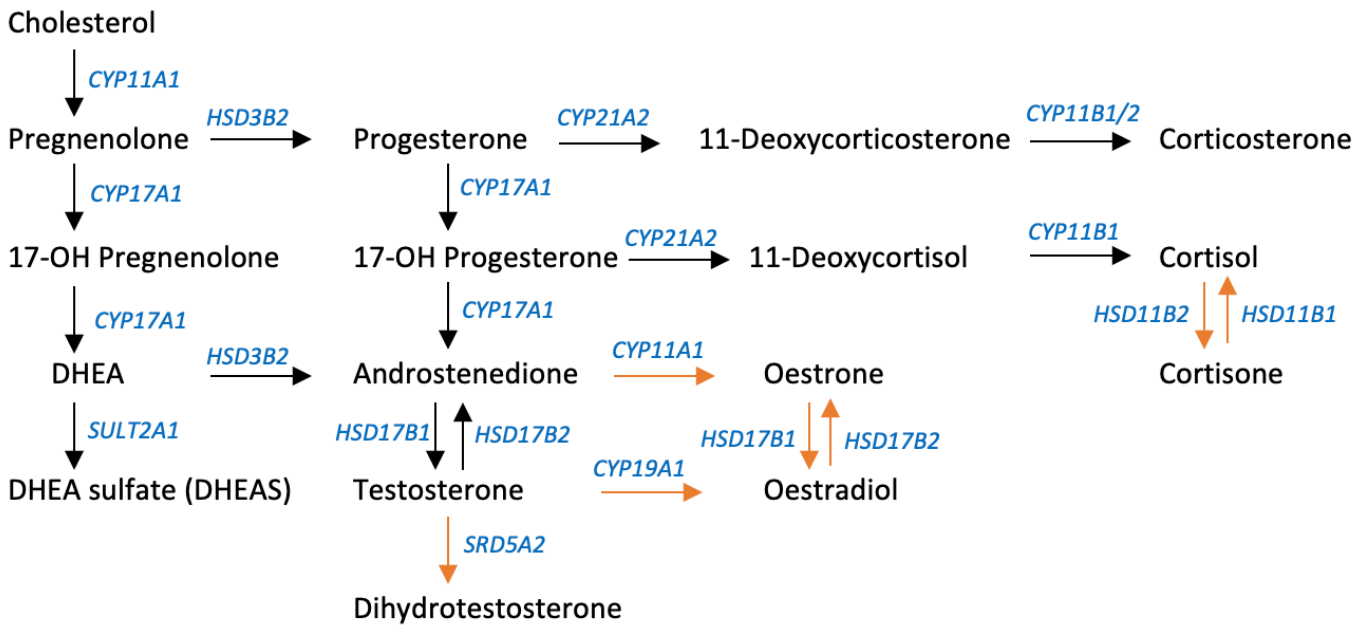


Figure 1.2 An overview of the steroid biosynthesis pathway in humans.

Arrows are labelled with the oxidative enzyme. Orange arrows signify peripheral metabolism.

Hypothalamic-pituitary-gonadal (HPG) axis

Gonadotrophin-releasing hormone (GnRH), the key regulator of the reproductive axis, is part of the hypothalamic-pituitary-gonadal (HPG) axis. Stimulation and secretion of GnRH by the hypothalamus are mediated by a neuropeptide called kisspeptin (also produced in the hypothalamus), encoded by the *KISS1* gene, which was initially isolated from the human placenta[10]. There are two significant populations of kisspeptin-producing neurons located in the rostral periventricular region of the third ventricle (RP3V) and arcuate nucleus (ARC; in animals)[14]. Kisspeptin neurons are under positive feedback (in the RP3V) and negative feedback (in the ARC) by gonadal sex steroids, and it is now commonly accepted that neurons in the ARC act as the GnRH pulse generator[14]. Whether or not plasma or serum kisspeptin concentration is higher in women with PCOS than in non-PCOS remains inconclusive[15]. Still, a study in adolescent girls with PCOS found increased

plasma kisspeptin levels positively correlated with LH and testosterone levels[16].

GnRH acts on the anterior pituitary to stimulate the secretion of LH and FSH. LH and FSH, in turn, produce both sex steroids and gametes from the gonads. Authors of the scientific statement on aspects of PCOS wrote, "A consistent feature of PCOS is disordered gonadotrophin secretion with elevated mean LH, low or low normal FSH, and a persistently rapid frequency of GnRH pulse secretion"[17]. Positive and negative feedback regulates the HPG axis. There are two distinct modes of GnRH secretion: pulsatile and surge model[17]. In PCOS, increased GnRH pulsatility (by approximately 40%) is characteristic and leads to increased LH secretion and a subsequent increase in ovarian androgen production[14]. High GnRH pulses favour LH production, while low GnRH pulses favour FSH production[18]. For this reason, persistently elevated GnRH pulsatility in PCOS results in an increased LH:FSH ratio. The exact mechanisms underlying the increased pulsatility of GnRH remain unclear. While high GnRH pulse frequency in PCOS partly reflects anovulation (i.e. infrequent progesterone secretion from corpora lutea), relative resistance to sex steroid negative feedback also plays a vital role as oestradiol and progesterone do not appropriately restrain GnRH pulse generator activity in PCOS[17]. The result is a vicious cycle of androgen excess contributing to poor negative feedback suppression of GnRH pulsatility, leading to gonadotrophin abnormalities that promote additional hyperandrogenaemia and ongoing ovulatory dysfunction[17]. The neuropeptide kisspeptin has been implicated as an essential component of the GnRH pulse generator by directly stimulating the GnRH-releasing neurons[19]. Published research has highlighted a potential relationship between kisspeptin and insulin resistance. Rodent cell-based studies found that kisspeptin could inhibit insulin secretion at physiological concentrations of glucose[20]. Human *in vivo*

studies in healthy male volunteers identified a beneficial role for kisspeptin in insulin secretion[20]. Yet, there is no published data on the role of kisspeptin (if any) on insulin resistance in women with PCOS with or without obesity.

Animal studies in mice have demonstrated a potential stimulant role of Anti-Müllerian hormone (AMH), produced by the granulosa cells in pre-antral and small antral ovarian follicle on direct GnRH secretion. Recent research (animal models) has identified that alterations in gamma-aminobutyric acid (GABA), kisspeptin, neurokinin B and dynorphin (KNDy), and AMH brain-specific signaling are likely involved in GnRH neuron hyperactivity in PCOS[21]. LH-mediated ovarian androgen production is the leading cause of androgen hypersecretion in women with PCOS, with ACTH-regulated adrenal androgen excess contributing approximately 25%[22]. There is also intrinsic dysfunction of ovarian theca cells, causing theca cell hyperandrogenism.

The effects of increased body weight on the reproductive axis are not fully understood. However, the association of obesity with lower gonadotrophins and lower levels of sex steroids has previously been established[23]. A large epidemiological study of 848 women during one menstrual cycle observed longer follicular phases, lower LH and FSH, lower oestradiol metabolites and lower progesterone metabolites in women who have overweight or obesity compared with normal-weight controls[24]. Women with a higher BMI have been observed to secrete significantly smaller amplitude LH pulses (0.8 ± 0.1 and 2.0 ± 0.3 IU/L) compared with controls (1.6 ± 0.2 and 3.4 ± 0.2 IU/L; $P < 0.01$) but a similar pulse frequency to normal-weight women[11]. In women with PCOS, a markedly elevated mean serum LH concentration and LH pulse amplitude throughout the follicular phase, as well as relative suppression of FSH, has been reported[22]. BMI significantly negatively

impacted 24-h mean LH pulse amplitude and the peak increment of LH in response to GnRH stimulation in women with PCOS (compared to normal cycling women)[25]. The 24-h LH pulse frequency was also uniformly increased in women with PCOS (independent of BMI)[25], with a blunting pulse amplitude with increasing BMI. Due to the variability of LH and FSH levels during random blood sampling in clinical practice, gonadotrophins are not currently part of the diagnostic criteria for PCOS[26].

Polycystic ovaries tend to have an increased number of antral follicles, ovarian stroma and theca cell hyperplasia. In both ovulatory and anovulatory women with PCOS, there is a higher density of primary follicles and a reciprocal decrease in the proportion of primordial follicles compared to normal ovaries[27]. AMH, secreted by the granulosa cell, is the major hormonal paracrine inhibitor of primordial follicle progression[28]. Serum AMH is an indicator of the number of growing follicles.

Under normal circumstances, testosterone production from the ovaries and adrenal glands is similar – the ovaries and adrenal glands secrete around 50% of testosterone, and the remainder is from peripheral conversion of circulating androstenedione (approximately equal secretion from ovaries and adrenal glands)[22]. Unlike oestradiol and cortisol, androgen secretion is not controlled by negative feedback[22]; the androgen production from the ovaries and adrenal glands responds to LH and ACTH (respectively). In both the gonads and adrenal glands, the formation of steroid hormones in response to trophic hormones (LH and ACTH in this case) is controlled by the enzyme cytochrome P450scc[22]. The formation of androgens in the ovaries and adrenals is controlled by cytochrome

P450c17, whose expression is dependent on stimulation by trophic hormones[22]. Androgen receptors (AR) are expressed in theca cells, granulosa cells and the oocyte of the follicle and throughout most stages of follicular development[29] in human ovaries. *In vivo* and *in vitro* studies have identified the critical role that androgens play (via the AR) in normal follicle development and function[30]. Androgen excess increases follicular development and antral follicles' dysfunctional formation, leading to PCOS. At the same time, low androgen levels may be associated with abnormalities of follicular growth, low functional ovarian reserve and primary ovarian insufficiency, negatively impacting female fertility[31]. The increase in follicular development is partly due to the up-regulation of FSH receptors on granulosa cells (GC), which in turn augment FSH induction of GC LH receptors leading to luteinising GCs and sensitising them to both gonadotrophins[32]. This luteinising process is further amplified by insulin. In PCOS, the ovaries remain sensitive to insulin despite IR in other tissues, which increases the effects of hyperinsulinaemia in the ovaries[12].

Around 80% to 90% of women with oligomenorrhoea have raised circulating androgen levels, of which free testosterone accounts for the majority[22]. SHBG levels are usually reduced in PCOS due to the effects of insulin and testosterone on the hepatic production of SHBG[33]. A report published in 1999 estimated that around 22%-25% of women with PCOS will also have elevated dehydroepiandrosterone (DHEAS) levels[34]; DHEAS is an adrenocortical steroid precursor.

The theca cells in the ovaries are the predominant source of androgen secretion in

response to LH and insulin, with the adrenals being the second source. The arrest of follicular development in PCOS (failure to proceed beyond the mid-antral stage) is likely multifactorial. Previous studies have established a potential role for exogenous FSH administration in anovulatory PCOS[35].

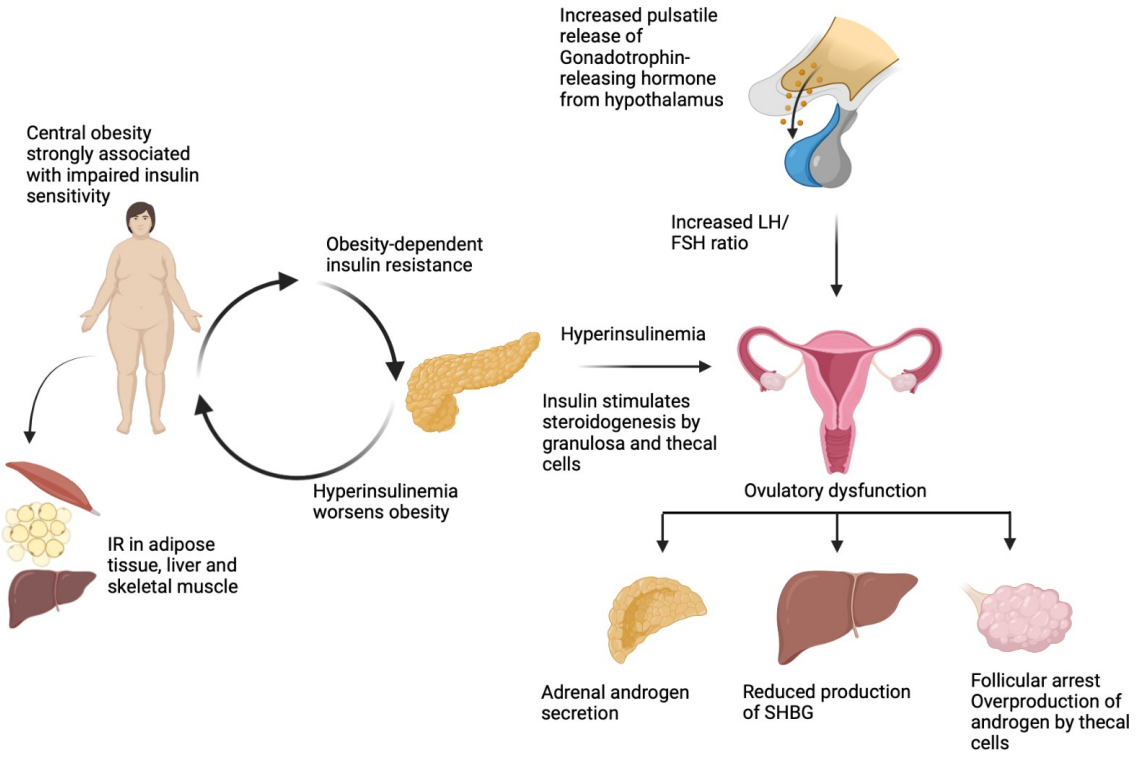


Figure 1.3 The pathophysiology of PCOS

Luteinising hormone = LH, Follicle-stimulating hormone = FSH, Sex hormone binding globulin = SHBG; Insulin resistance = IR

Summary

- PCOS is the most common endocrine disorder in women of reproductive age
- Diagnosis is based on the Rotterdam criteria
- Increased GnRH pulsatility leads to increased LH secretion and increased ovarian

androgen production.

- LH-mediated ovarian androgen production is the leading cause of androgen hypersecretion.
- There is an association between obesity and lower gonadotrophins
- The ovaries remain sensitive to insulin despite insulin resistance in other tissues

1.2 Complications of PCOS

The National Institute for Health and Care Excellence (NICE, revised February 2022) advised that women with PCOS should be informed about the possible long-term metabolic complications, including type 2 diabetes mellitus (T2D) and cardiovascular disease (CVD)[36]. Women with PCOS who have overweight or obesity should be offered advice on weight loss strategies or referred to a specialist dietician[36]. Those with risk factors – BMI ≥25 kg/m^2 or more, BMI <25 kg/m^2 but additional risk factors (e.g. older than 40 years of age), of non-Caucasian ethnicity, should be offered annual screening for T2D and IR. The cumulative incidence rates of T2D in women with normoandrogenic and hyerperandrogenic PCOS has been reported as 4.4% and 14.2% respectively[37].

CVD risk should be assessed with a detailed medical history, clinical examination and anthropometric measurements[36]. Multiple systematic reviews and meta-analyses comparing the CVD risk in women with PCOS and healthy controls have shown an increased risk of hypertension (HTN), T2D and total cholesterol in both reproductive and nonreproductive aged women[10, 38, 39]. One systematic review (SR) and meta-analysis (MA) assessed cardio-metabolic risk factors in young women with a diagnosis of infertility

(all causes included PCOS) and found that these women have a higher incidence of cardiometabolic risk factors when compared with women without an infertility diagnosis[40]. Women with PCOS tend to have an atherogenic serum lipoprotein profile and dyslipidaemia which is evidenced by higher levels of triglycerides (TG) and low- density lipoprotein cholesterol (LDL-C)[40]. The table below (table 1.2) contains relevant systematic reviews and meta-analyses assessing CVD risk in women with PCOS:

Author. year	**Number of studies**	**Hypertension**	**Type 2 diabetes (T2D)**	**Total cholesterol**	**Non-fatal cardiovascular events**	**Non-fatal cerebrovascular events**
Wekker (2020)[38]	23	RR 1.75 (95% CI 1.48 to 2.33)	RR 3.00 (95% CI 2.56 to 3.51)	MD 7.14 (95% CI 1.58 to 12.70)	RR 1.78 (95% CI 0.99 to 3.23) (no difference)	RR 1.41 (95% CI 1.02 TO 1.94)
Tehrani (2020)[41]	16	-	-	-	Reproductive age: pooled HR 1.38 (95% CI 1.12 to 1.71) Nonreproductive age: pooled HR 1.53 (95% CI 1.15 to 2.04)	
Amiri (2020)[39]	30	Reproductive age vs. control: Pooled P 0.15 (95% CI 0.12 to 0.18) vs 0.009 (95% CI 0.08 to				

		0.10) Nonreproductive age vs control: 0.49 (95% CI 0.28 to 0.70) vs 0.40 (95% CI 0.22 to 0.57)			
Mulder (2020)[40]	7			MD/SMD 12.61 (95%CI 3.37 to 21.85)	

Table 1.2 Systematic reviews and meta-analyses of the CVD risk in PCOS

RR = risk ratios, CI = confidence interval, MD = mean differences, SMD = standardised mean difference, HR = hazard ratio

Obstructive sleep apnoea (OSA)

OSA is considered one of the most significant sleep disorders and has a prevalence of 9% - 17%[42]. It is caused by a complete or partial obstruction of the upper airway resulting in apnoeic episodes of shallow breaths[43]. Obesity is a major risk factor for OSA[43]. Although OSA is not a component of metabolic syndrome (MetS), there is evidence to suggest that it may exacerbate CVD in patients with MetS[42]. A meta-analysis and review of the literature conducted in 2017 to assess the risk of OSA in adult women with PCOS found a significant association between the two – the risk was 9.74 times higher in this group[44]. This association has been published in another meta-analysis of 648 participants which found that 35% of women with PCOS had OSA[45].

Malignancy

Initial reports of an association between PCOS and cancer were in relation to endometrial disease[46-48] but more recently, the possibility of an increased risk of breast and ovarian cancer has also been suggested[49]. A systematic review and meta-analysis of 919 women with PCOS found a significantly increased risk of endometrial cancer but that the risk of ovarian and breast cancer was not significantly increased[49].

Mental health

It has been reported that in women with PCOS, there is “an increased prevalence of mild depressive and anxiety symptoms or an increase in”[50] the mean depression and anxiety

scores[51]. Numerous systematic reviews and meta-analyses have found increased odds of both depressive and anxiety symptoms[51-54] and lower quality of life (QoL) scores[52, 54]; women with PCOS and obesity had higher odds of depression[55]. Results are presented in the table below:

Author, year	**Number of patients with PCOS**	**Depressive symptoms**	**Anxiety symptoms**	**Emotional QoL**
Cooney (2017)[51]	3050	OR 3.78 (95% CI 3.03-4.72)	OR 4.18 (95% CI:2.68-6.52)	-
Veltman-Verhulst (2012)[54]	2384	SMD 0.60 (95% CI 0.47 to 0.73)	SMD 0.49 (95% CI 0.36 to 0.63	SMD -0.66 (95% CI -0.92 to 0.41)
Yin (2012)[52]	9265	SMD 0.64 (95% CI 0.50 to 0.78)	SMD 0.63 (95% CI 0.50 to 0.77)	SMD -0.55 (95% CI -0.69 to 0.40)
Brutocao 2018[53]	172,040	OR 2.79 (95% CI 2.23 to 3.50)	OR 2.75 (95% CI 2.10 to 3.60)	
Wang (2021)[55]	2316	Prevalence 42% (95% CI 33 to 52%)	Prevalence 37% (95% CI 14 to 60%)	

Table 1.3 Systematic reviews and meta-analyses of mental health symptoms in women with PCOS
OR = odds ratio, CI = confidence interval, SMD = standardised mean difference.

1.3 Impact of PCOS on fertility

PCOS is often associated with reproductive compromise, particularly anovulatory infertility, as previously mentioned[56]. In women with PCOS, the total number of pregnancies in their

lifetime tends to be lower than in those without PCOS, with higher rates of treatment of infertility (40.9 vs 4.6%) and miscarriage (11.1 vs. 6.1%); they were more likely to require IVF (17.2 vs 2.0%)[57]. For important neonatal outcomes stratified by maternal PCOS diagnosis, preterm birth (OR 1.54, 95% CI 1.43-1.66), Apgar score at 5 minutes <7 (OR 1.46, 95% CI 1.10-1.93), perinatal mortality (OR 1.49, 95% CI 1.02-2.18) were significantly higher in women with PCOS[58].

Due to conflicting ideas regarding the treatment of PCOS, the optimal treatment for women with PCOS and infertility has yet to be defined. A group of experts reached a consensus on infertility treatment related to PCOS and identified lifestyle modifications as the first-line treatment[59]. Through preconception counselling, healthcare professionals should be able to identify risk factors for reproductive failure, e.g., the presence of obesity, and attempt to correct them before initiating treatment[59]. Obesity is associated with anovulation, pregnancy loss and late pregnancy complications (preeclampsia, gestational diabetes, etc.); it is also associated with either failure or a delayed response to treatments such as clomiphene citrate (CC) and gonadotrophins[59]. A meta-analysis which assessed patient predictors for the outcomes of gonadotrophin ovulation induction in women with normogonadotrophic anovulatory infertility (World health organisation group II) and PCOS reported a positive association between obesity and the total amount of gonadotrophin[60]; four studies reported an association between obesity and ovulation rate (pooled odds ratio [OR] 0.44, 95% CI 0.31-0.61)[60]. There is little published data about the positive impact of weight loss on live-birth rates in women with obesity, but multiple observational studies have reported an improvement in spontaneous ovulation rates in women with PCOS with pregnancies reported with a modest weight loss of 5%[59].

The proportion of patients with PCOS undergoing *in vitro* fertilisation (IVF) is very high[56] and the clinical data shows that women with PCOS are more likely to seek infertility consultations and undergo assisted reproductive technology (ART) more often than matched controls without PCOS[56]. A comparison of cumulative live birth rate (CLBR) between PCOS and age- and BMI-matched controls with tubal factor infertility in IVF/intracytoplasmic sperm injection (ICSI) cycles reported a higher CLBR over a two-year period in women with PCOS[61]. At the end of their reproductive span, women with PCOS had the same number of children[62] and a similar prevalence for at least one child when compared to matched controls[56]. This could imply that women with PCOS have a good fecundity[62]. An observational study with nine thousand sixty-eight women with PCOS identified an association with subfertility but found that fertility rates are restored to those of the background population following diagnosis[63]. This study also reported an increased prevalence of pregnancy complications and adverse neonatal outcomes for women with PCOS, independent of obesity[63].

Genomic studies have demonstrated alterations in oocyte competence and development; increased oxidative stress can also increase the incidence of meiotic abnormalities[64]. Chromosome karyotyping of the first trimester miscarried chorionic villus from women with PCOS identified more frequent chromosomal aberrations in conceptuses compared with controls (61.3% v. 47.8%) and identified PCOS as a risk factor for an embryo/foetus to be chromosomally abnormal[65].

Finally, primary abnormalities in the endometrium, which include abnormal expression of proteins involved in cell cycle regulation, cellular transport and signalling, deoxyribonucleic

acid (DNA) repair, apoptotic processes and mitochondrial metabolism, have been detected in women with PCOS independent of ovulation and/or normal menstrual cyclicity[64]. Abnormal expression of oestrogen, progesterone, and androgen receptors and their co-regulators have been reported in the endometrium of women with PCOS[64]. Progesterone resistance may also be exacerbated by increased expression of ARs in endometrial epithelial cells, hypothalamic/pituitary dysfunction and increased circulating levels of oestrogen[64]. The endometrial epithelial cells of women with PCOS exhibit higher AR mRNA and protein expression compared to healthy controls, with enhanced upregulation of the AR in hyperandrogenic women[64]. This endometrial overexpression of AR coactivators is also present in ovulatory women with PCOS[64]. There is a sparsity of published data comparing live-birth rates in women with ovulatory PCOS or following ovulation induction versus healthy matched control without PCOS. For this reason, the strategies suggested by international guidelines[59] for treating women with PCOS and infertility should be employed where possible.

Measurement of ovulation

The NICE clinical guidelines on the assessment and treatment of fertility problems state that "women undergoing investigations for infertility should be offered a serum progesterone in the mid-luteal phase of their cycle (day 21 of a 28-day cycle) to confirm ovulation"[66]. In women with irregular menstrual cycles, serum progesterone may need to be requested later in the cycle and repeated weekly until the next menstrual cycle[66]. The guidelines suggest a serum progesterone value "from 16 to 28 nmol/L as the lowest limit indicative of ovulation"[66]. A secondary analysis of an observational European multicentre study identified that a random serum progesterone level ≥5 ng/ml (15.9 nmol/L) confirms ovulation with a specificity of 98.4 (95% CI 96.0-99.5) and sensitivity of

89.6 (95% CI 85.2-92.9)[67].

In research, the measurement of ovulation varies in the literature with some studies using daily ovulation prediction kits (OPK) with serum progesterone measured seven days after a positive result (if negative OPK, then serum progesterone measured on cycle day 21 or 22)[68], mid-luteal progesterone serum progesterone[69, 70] and menstrual diaries[69], serial transvaginal ultrasound scans until visualisation of a dominant follicle with confirmation of ovulation using serum progesterone[71, 72]. There is currently no validation for daily progesterone measurements in ovulation monitoring. Ovulation prediction kits measure urine LH and identify the LH peak which occurs around the time of ovulation; however, they do not predict the day of ovulation. In women with PCOS, OPKs might be of little value due to the overproduction of LH.

1.4 Insulin resistance

Obesity

The development of T2D because of insulin resistance in obesity has been recognised for decades. The term "insulin resistance" includes resistance to the effects of insulin on glucose uptake, metabolism or storage[73]. There are currently no consensus criteria for the definition of insulin resistance, but clinical criteria include a BMI >27 kg/m^2, a waist- to-hip ratio greater than 0.85, or the presence of acanthosis nigricans[74]. Insulin resistance is a key aspect of the aetiology of T2D and has been linked to other components of the MetS such as HTN and hyperlipidaemia[73]. The exact mechanism by which adipose tissue causes systemic insulin resistance is not fully understood but it has to do with the adipo-insulin axis[73]. Adipocytes are one of the most highly insulin-responsive cell types, and insulin in

turn is a critical mediator of all aspects of the adipocyte biology[73]. Insulin stimulates differentiation of preadipocytes to adipocytes and within adipocytes, it promotes lipogenesis[73]. Skeletal muscle is responsible for around 80% of postprandial glucose uptake[75] with adipose tissue uptake being considerably less. In obesity, insulin resistance manifests as decreased (insulin-stimulated) glucose transport and metabolism in adipocytes and skeletal muscle and reduced suppression of hepatic glucose output[76]. Other mechanisms of insulin resistance include reduced expression of some insulin signaling molecules in skeletal muscle in people with morbid obesity[77] and downregulation of glucose transporter type 4 (GLUT4) expression in adipocytes[78]. Adipose tissue is classified into two types: white adipose tissue (WAT) which is non-thermogenic, and brown adipose tissue (BAT), which is thermogenic. WAT and BAT are distinguishable by their colour. In lean humans, skeletal muscle mass is greater than WAT mass, but glucose transport into BAT is higher than in many muscle groups (despite the overall mass of BAT being small)[73]. The risk of MetS increases with increased body fat content; however, central adiposity (which tends to be WAT) is linked to an adverse metabolic profile e.g., insulin resistance[79]. Peripheral insulin resistance leads to increased insulin secretion from the pancreatic B cells in the pancreas. Non-insulin-dependent diabetes mellitus develops when the increase in insulin is insufficient to maintain euglycemia.

Insulin resistance in PCOS

Although not part of the current diagnostic criteria, insulin resistance in women with PCOS has been identified in the literature as far back as the 1980s. It was first described in 1921 as the association between disordered carbohydrate metabolism and hyperandrogenism and called “the diabetes of bearded women”[80]. Insulin resistance and the resulting

hyperinsulinaemia affects many women with PCOS (65%-70%), with 70%-80% of obese and 20%-25% of lean women exhibiting signs and symptoms[81]. Hyperinsulinemia is also caused by reduced insulin clearance. The measurement of insulin to C-peptide ratios is increased in women with PCOS, suggesting both increased secretion of insulin and reduced hepatic excretion[82]. Fasting hyperinsulinemia is commonly seen in women with PCOS[81]. It directly increases ovarian androgen secretion by activating the insulin receptor on ovarian thecal cells and stimulating testosterone biosynthesis[22] while decreasing SHBG production in the liver[83]. Insulin also acts as a co-gonadotrophin and enhances the effect of increased LH, which is frequently seen in women with PCOS[81].

In PCOS, insulin resistance often presents as normal fasting glucose levels sustained by hyperinsulinemia – this presentation is suggestive of reduced insulin sensitivity[84]. In women with PCOS, skeletal muscle and myotubes display impaired insulin responsiveness, whereas isolated adipocytes display impaired sensitivity but normal responsiveness[84]. A study from 1980 reported that women with PCOS had increased insulin responses during oral glucose tolerance testing, which was not accounted for by obesity[85]. They also demonstrated that hyperandrogenism correlates with hyperinsulinism[85]. A study from 1989 analysing *in vivo* insulin action using a euglycaemic glucose-clamp technique found that women with PCOS had significantly reduced in vivo insulin-stimulated glucose disposal, independent of obesity and a specific disorder of insulin-action independent of obesity or glucose tolerance[86]. The most likely cause of insulin resistance in PCOS is a defect in the insulin receptor and post-receptor signal transduction[80]. The reduction in insulin sensitivity could also be associated with a significantly decreased concentration of GLUT-4 glucose transporter content in adipocyte membranes in PCOS, independent of obesity[87]. There is conflicting data around insulin resistance in PCOS, with the consensus

being that women with PCOS and obesity are insulin resistant[88]. Several studies have identified insulin resistance only in women with hyperandrogenism and anovulation[88].

The clinical manifestations of insulin resistance, such as acanthosis nigricans, have been identified in obese and lean women with PCOS[80], which is also in keeping with insulin resistance being both dependent and independent of obesity.

Within the endometrium during decidualisation, progesterone binds to progesterone receptors and regulates the expression of insulin receptors necessary for insulin signalling. Insulin resistance and the resultant hyperinsulinaemia can negatively impact the implantation process[64]. Hyperinsulinemia could also act via inflammatory pathways to worsen the process[89].

Obesity in PCOS

The World Health Organisation (WHO) declared obesity a major public health problem and a global epidemic[90], with approximately 650 million people living with obesity in 2016[90]. Since then, the number of people who have overweight and obesity has continued to increase among adults and children. Although initially considered a problem in high-income nations, in recent years, it has been on the rise in low- and middle-income countries[91]. The recent COVID-19 pandemic saw a further increase in people living with obesity due to an unhealthy diet or sedentary lifestyle[92]. In 2016, 15% of women lived with obesity (compared to 11% of men), and this figure continues to rise[91]. The UK National Bariatric Surgery Registry Third Registry Report 2020 found that women accounted for the majority of obesity surgery in keeping with the predominance of women undergoing this type of surgery worldwide[93].

Women with PCOS frequently have one or more MetS-related conditions such as obesity, T2D, and HTN[94]. Insulin resistance in PCOS is seen in around 10%-15% of lean women and 20%-40% of women living with obesity[95]. A meta-analysis published

in 2020 concluded that “PCOS per se does not have a causal relationship with type 2 diabetes, CHD, or stroke”[96]; a meta-analysis of 12 studies reported a positive association of PCOS with T2D (OR 2.87, 95% CI 1.44-5.72) although there was significant heterogeneity[96]. The prevalence rate of T2D in (premenopausal) women with PCOS is around 5%-10%[96]. Insulin resistance is central to the pathogenesis of PCOS and is exacerbated, at least in part, by obesity. Obesity rates in this patient population can range between 50% and 80%, depending on ethnicity and study population[97]. Obesity is associated with an increased time to pregnancy[98] and impairs fecundability with a 4% lower pregnancy rate per BMI unit increase[56, 99]. It also appears to impair endometrial function in women with and without PCOS[89]. A 1992 study conducted in 7 subjects with PCOS (obese and lean) identified significantly reduced postprandial thermogenesis (PPT) compared to matched controls but no significant decrease in energy expenditure[100]. Reduction in PPT was statistically related to the reduced insulin sensitivity and may (in part) explain the obesity often seen with PCOS[100]. Basal metabolic rate (BMR) was reported to be significantly lower in women with PCOS compared to age- and BMI-matched controls[101]. More recently, an animal model of PCOS using prenatally programmed PCOS-sheep (gestational androgenization model) found a significantly increased body weight in adulthood and significant reduction in PPT in PCOS-sheep compared to controls[102]. A systematic review and meta-analysis of 15129 women found that women with PCOS had an increased prevalence of overweight, obesity and central obesity compared to women without PCOS[103]. However, a systematic review of fourteen studies comparing weight management interventions in women with and without PCOS did not find a significant difference in weight loss following the intervention between the two groups[104].

To optimise health, strategies need to be implemented to help with weight loss which will ultimately improve the metabolic and hormonal profile in these women. In addition to the negative impact of obesity on conception, pregnancy and live-birth rates, women with obesity have a reduced response to medications used for ovulation induction and assisted reproduction[105]. Pregnancy tends to exacerbate underlying IR and women with PCOS and obesity are therefore at a higher risk of gestational diabetes mellitus (GDM)[105].

Summary

- Possible long-term complications of PCOS include T2D, cardiovascular disease, OSA, malignancy and mental health conditions
- Anovulatory infertility is often seen in PCOS
- Women with PCOS are more likely to seek help with fertility
- Excess adipose tissue causes insulin resistance and has been identified in women with PCOS
- Obesity rates in women with PCOS can vary between 50%-80%

1.5 Obesity and bariatric surgery

Obesity is associated with life-threatening diseases and premature death with a subsequent reduction in life expectancy of 5 – 20 years[106]. The 2007 Swedish Obese Subjects (SOS) study reported that bariatric surgery reduced overall mortality by 29% over a mean follow-up period of 10.9 years[106]. Unfortunately, despite published data reporting the beneficial impact of bariatric surgery, only a minority of eligible patients undergo the procedure. In a follow-up study to the SOS study assessing overall mortality and life expectancy over three decades, in the surgery group, there was a mean BMI reduction of

approximately 11, which was observed 12 months after surgery; this was followed by gradual weight regain until around eight years post-operatively[106]. After this time, BMI stabilized at approximately 7 less than the baseline BMI[106]. Median life expectancy was 2.4 years longer in the surgery group compared to the control group; mortality was also lower after surgery[106].

The 5-year Diabetes Surgery Study (DSS), which compared Roux-en-Y gastric bypass (RYGB) with intensive medical management for the control of T2D found that in those with mild to moderate obesity and T2D, RYGB in conjunction with lifestyle modification and intensive medical management led to an increase likelihood of achieving a glycated haemoglobin HbA1c <7.0%, LDL-C <100 mg/dl and systolic blood pressure (SBP) <130 mmHg[107]. Sub-group analyses of 174 772 patients in a one-stage meta-analysis of patient-level data showed that both individuals with and without baseline diabetes who underwent bariatric surgery had lower rates of all-cause mortality; the treatment effect was significantly greater in those with diabetes[108]. Patients with diabetes in the surgery group had a median life expectancy of 9.3 years longer than the non-surgical group[108]. Overall, using results from this study and other published data, the authors estimated that every 1% increase in bariatric surgery utilisation rates among suitable candidates both with and without diabetes, could lead to 5.1 million and 6.6 million potential life years, respectively[108].

The three most commonly performed procedures are laparoscopic RYGB, vertical sleeve gastrectomy (VSG) and adjustable gastric banding (AGB)[109]. In RYGB, the stomach size is reduced to an upper stomach pouch of around 15-30 ml in volume, leaving a gastric remnant which is not exposed to food. This upper stomach pouch is anastomosed to the

mid-jejunum through a gastrojejunal anastomosis in a Roux-en-Y fashion[109]. Gastric, pancreatic, and biliary secretions flow through the biliopancreatic limb to join the alimentary limb via a jejuno-jejunal anastomosis, forming the common limb. A systematic review found that the majority of the benefits are from a reduction in calorie intake and in the early period following surgery, malabsorption only contributes around 10%-11% of the entire calorie deficit[110]. Although there are significant alterations to glucose kinetics, there is little carbohydrate or protein malabsorption following RYGB[110].

VSG was first recognised by the American Society for Metabolic and Bariatric Surgery (ASMBS) as an acceptable primary bariatric procedure in 2012[111]. It involves removal of 70%-80% of the stomach, therefore, reducing gastric capacity and food intake; this increases early satiety[112]. Removal of the fundus of the stomach decreases ghrelin production; ghrelin increases hunger through its action on the hypothalamus[112]. Studies have found that VSG also increases glucagon-like peptide 1 (GLP-1) production – delaying gastric emptying and that the action of peptide YY and pancreatic polypeptide are exaggerated, leading to a reduction in hunger and food intake[112].

The laparoscopic AGB (LAGB) was first described in 1993 and was initially one of the most common bariatric surgery procedures in the world, but its popularity has waned over the last few years[113]. In LAGB, an adjustable plastic and silicone ring is placed around the proximal aspect of the stomach immediately below the gastro-oesophageal junction, thereby creating a small proximal pouch[109]. Through a subcutaneous port, fluid can be injected to adjust the fluid volume in the band[109]. AGB likely works through reducing hunger and increasing satiation, potentially through neural mechanisms (i.e. vagal signaling)[109].

Mechanisms of weight loss after bariatric surgery

Bariatric surgery results in anatomical rearrangement of the gut and altered signalling to the brain, liver, pancreas and adipose tissue, which results in a reduction in hunger, increase in satiety, change in food preferences (away from high-calorie foods), increase in diet-induced thermogenesis, improved glycaemic control and reduced inflammation[109]. GLP-1 is another hormone secreted by L cells of the distal small bowel. Following RYGB and VSG[114], there is an increase in GLP-1 which binds to receptors in different areas of the brain and reduces gastric emptying, inhibits glucagon release and increases insulin secretion from the pancreas[115]. Ghrelin is a peptide hormone produced in the stomach and one of its main functions is to stimulate food intake – levels tend to decrease following a meal. Ghrelin levels decrease after VSG[116], increase after AGB[117] and may either decrease, increase or remain the same following RYGB[76. There is no formal consensus on the role of mechanical factors e.g. size of the gastric pouch and stoma in RYGB and volume of the gastric sleeve in VSG in food intake and body weight[109]. Postprandial concentrations of anorexigenic hormone peptide YY (PYY) is significantly higher after RYGB[118] and VSG[114] but not after AGB[109]. Following a meal, PYY is released from the L cells of the distal small intestine and acts via the hypothalamus to reduce food intake; it has also been reported to increase energy expenditure and delay gastric emptying[109].

Changes in food preferences following bariatric surgery has been reported in the literature – patients who have undergone RYGB preferentially choose food which is low in fat and/or sugar compared to those who underwent vertical or anterior gastric banding[109]. This change in food preference has also been reported after VSG but not AGB[119]. However, several other studies have reported no changes in food preference after RYGB and VSG[120, 121]. The magnitude and lasting effects of these changes to food preference remain a matter of debate. The effects of bariatric surgery on resting energy expenditure in

both human and animal models have been reported in the data with the majority of studies reporting an overall reduction which is most likely due to a decrease in both fat- free mass and fat mass[122, 123].

Despite advances in pharmacotherapy, bariatric surgery remains the most effective treatment for weight loss and long-term maintenance[109].

1.6 Management of PCOS

Lifestyle interventions are typically used as the first line of treatment in women with PCOS who have overweight or obesity to improve their metabolic and hormonal profiles; however, these interventions have only been studied in small cohorts of women with PCOS, yielding inconclusive evidence. Losing even 5-10% of total body weight can reduce central fat by up to 30% leading to an improvement in insulin sensitivity and restoration of ovulation[124] Long-term adherence to diet and physical activity recommendations is a significant challenge for most patients. A systematic review and meta-analysis of 17 studies into the effects of exercise or exercise and diet for the management of PCOS found that compared with controls, exercise had a statistically significant effect on metabolic outcomes (fasting insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), lipid profile, waist circumference, body fat percentage) which was greater for women who have overweight or obesity[125]. There were no obvious differences in outcomes between combined diet and exercise or diet alone[125]. Haqq et al reported similar findings in their meta-analysis of significant improvements in body composition and cardiorespiratory fitness in women who received lifestyle intervention when compared with standard care[126]. A systematic review and meta-analysis of 14 studies involving 617 women with PCOS found that exercise interventions improved

metabolic outcomes (lipid profiles, fasting insulin, systolic blood pressure) but the impact on reproductive functions remains unclear[127]. Two meta-analyses which assessed the effect of lifestyle intervention on women with PCOS and overweight or obesity both reported an improvement in anthropometric markers[128, 129] but conflicting results for metabolic outcomes. Lim et al reported no significant reduction in metabolic outcomes and free androgen index (FAI)[128] and Khatlani et al reported that short-term lifestyle intervention can effectively improve weight, insulin resistance and lipid profile and could therefore be recommended as first-line therapy[129]. A modest weight loss of event 5%-10% is considered clinically significant and has been associated with a reduction in impaired glucose tolerance, the prevalence of MetS and the risk factors for CVD and T2D in the general population[130, 131]. As previously mentioned, non-surgical approaches to weight loss interventions based on lifestyle modification and pharmacotherapy have shown limited success, with rapid weight regain and high attrition rates[132]. In a systematic review to assess the effectiveness of long-term (12 months) nonsurgical weight loss interventions in women with PCOS and obesity, lifestyle interventions improved weight loss outcomes but with relatively poor completion rates[133]. Although initial weight loss is a positive predictive factor of long-term success, most studies show limited ability to lose large amounts of excess body weight greater than 10 kg[134].

A prospective 8-week National Institute of Child Health and Human Development funded study in women with PCOS who have overweight and obesity found that a diet low in dairy and carbohydrates significantly reduced body weight and testosterone, and improved insulin sensitivity[135]. The general consensus is that energy restriction is a requirement for weight loss, but there is little agreement on what constitutes the optimal diet for women with PCOS[136]. Multiple studies have examined different dietary approaches, but

two randomised controlled trials (RCT)[137, 138] comparing the effect of different diets in women with PCOS did not show that a particular diet significantly impacted weight loss and reproductive outcomes. Currently, the recommended diet for women with PCOS and obesity is a hypocaloric diet (500 Kcal/day deficit) with a reduced glycaemic load[59].

The table below lists RCTs conducted in women with PCOS who have overweight or obesity comparing hypocaloric diets (HCD) to other treatments:

Author, year	Number of participants	Intervention	Findings
Nadjarzadeh (2021)[139]	64	Hypocaloric standardized diet + fennel (A) Hypocaloric high-protein diet + fennel (B) Hypocaloric standardized diet + placebo (C) Hypocaloric high protein diet + placebo (D) (HCD 500 kcal deficit/day)	No significant difference in anthropometric data, testosterone, SHBG, FAI
Mehrabani (2012)[140]	60	Conventional hypocaloric diet (A) Modified hypocaloric diet (high-protein, low-glycaemic load) (B) (HCD 500-1000 kcal deficit/day)	No significant difference in weight loss between groups; modified HD caused significant increase in insulin sensitivity

Palomba (2010)[72]	96	Structured exercise training (SET) + hypocaloric diet (A) Observation + CC therapy (B) SET + hypocaloric diet, one cycle of CC (C) (HCD 1000 kcal deficit/day)	Ovulation rate significantly higher in group C; significant improvement in hyperandrogenism and insulin sensitivity in groups A and C (compared to B)
Florakis (2007)[141]	59	Hypocaloric diet + sibutramine (A) Hypocaloric diet only (B) (HCD 600 kcal deficit/day)	Significant reduction in BW in both groups but higher in group A
Thomson (2008)[142]	94	Diet only (A) Diet + aerobic exercise (B) Diet + aerobic-resistance exercise (C) (Diet of 5000-6000 kJ/day)	Significant weight loss, reduction in lipid profile, metabolic and reproductive outcomes in all groups
Stamets (2004)[138]	35	High protein diet (A) High carbohydrate (B) (HCD 1000 kcal deficit/day)	Significant weight loss in both groups but no difference between groups (including hormonal, lipid, metabolic outcomes)
Esfahanian (2012)[143]	40	Metformin (A) Hypocaloric diet (B) (Group B- aim for 5-10% reduction in BW)	Significant reduction in BMI, WC, insulin resistance and sensitivity, TT in both groups
Kasim-Karakas (2008)[144]	33	Hypocaloric diet + protein (A) Hypocaloric diet + simple sugars (B)	More weight loss in and fat mass loss in group A

		(HCD 700 kcal deficit/day)	

Table 1.4 RCTs conducted in women with PCOS who have overweight or obesity comparing hypocaloric diets (HCD) to other treatments

Medical intervention with pharmacotherapy should be considered when patients do not respond to lifestyle interventions. The choice of pharmacotherapy depends on the treatment indication (fertility or non-fertility). The International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018[145] categorised pharmacological treatment for adult women with PCOS into non-fertility and fertility indications summarised in the table below:

Non-fertility indication	**Drug and recommendation**
Hyperandrogenism and/or irregular menstrual cycles	Combined oral contraceptive pill (COCP) alone
Metabolic features where COCP and lifestyle changes do not achieve desired goals; high metabolic risk groups; androgen related alopecia	COCP + metformin
In addition to lifestyle for treatment of weight, hormonal and metabolic outcomes	Metformin
In addition to lifestyle for management of obesity	Anti-obesity agents
Where COCPs are contraindicated or poorly tolerated	Anti-androgen agents

Considered an experimental therapy	Inositol
Fertility indication	**Drug and recommendation**
Ovulation induction (first line) in women with PCOS with anovulatory infertility	Letrozole
Anovulatory infertility	Clomiphene citrate and/or metformin
Ovulation induction (second line) in women with PCOS with anovulatory infertility	Gonadotrophins
Experimental therapy	Anti-obesity agents

Table 1.5 Pharmacological treatment for adult women with PCOS

The NICE guidelines on the management of PCOS state that treatment with insulin-sensitising drugs such as metformin should not be initiated in primary care[146]. There is no formal consensus on who should be referred for consideration of these drugs[146] but metformin is no longer recommended in routine management of anovulatory PCOS[124]. A review into the use of metformin for the treatment of PCOS found that 4-6 months of metformin therapy may restore regular, ovulatory menses in most women[74]. However, a 2006 study conducted on women with PCOS who have obesity (mean BMI 38 kg/m^2) reported no significant difference between metformin and placebo with regards to weight loss; both groups had increased menstrual cyclicity in those who lost weight[147]. In women with PCOS and obesity, metformin in conjunction with a low-calorie diet may be associated with greater weight loss than a low-calorie diet alone[74].

Inositols, of which myoinositol (MYO) is the most commonly distributed in nature, are biosynthesized from glucose[148] and available as nutritional supplements. D-chiro-inositol deficiency and an imbalance with its precursor myoinositol have been linked to

insulin resistance in molecular and animal studies[149]. MYO is an important part of the follicular microenvironment, and analysis of human follicular fluids have demonstrated that higher concentrations of MYO are associated with better quality oocytes[149]. The International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018 recommendations for inositol (in any form) are that it should "be considered an experimental therapy in PCOS "with further research needed[145].

Women with obesity have lower fertility rates in both natural and assisted insemination[150]. Pregnancy in women with obesity carries significant risks and is associated with higher rates of "congenital anomalies (neural tube (OR 3.5), omphalocele (OR 3.3) and cardiac defects (2.0)), miscarriage, gestational diabetes, hypertension and problems during delivery"[151]. The UK Confidential Enquiry into Maternal Health reported that of the 262 deaths between 2000 – 2002, 35% of women had obesity when compared with 23% of women in the general population, with more than a quarter having a BMI greater than 35 kg/m^2[152].

A study of 500 women receiving treatment with donor sperm reported a 30% reduction in the rate of conception with every 0.1 point increase in waist-hip ratio (WHR)[150]. Obesity and hyperandrogenaemia are linked with a poor response to clomiphene citrate-induced ovulation, gonadotrophins and ART[153]. It is suggested that women with these features consider weight loss as a first-line intervention for infertility[154]. However, in severe obesity, lifestyle interventions have limited efficacy[154]. The International evidence-based guideline for the assessment and management of PCOS 2018 states that "Bariatric surgery should be considered an experimental therapy in women with PCOS…"[145]. A published document on "The management of anovulatory infertility in women with

polycystic ovary syndrome…" recommended full fertility investigations and assessment of IR in women in whom ovulation is not detected[155]. Women with overweight or obesity should be offered lifestyle intervention and/or bariatric surgery with the goal of treatment being improvement of the endocrine profile and increased likelihood of ovulation and a better response to ovulation induction therapy[155].

There is currently limited evidence about fertility and pregnancy outcomes following bariatric surgery, with all the published data from nonrandomised clinical trials. A single centre cohort study of two-hundred and sixteen premenopausal women undergoing bariatric surgery identified increased pregnancy and fertility rates in women with PCOS and obesity, with few maternal and neonatal complications[156]. More recently, a meta-analysis of 21 studies and 552 patients found that following bariatric surgery (operation type was not limited), there was a significant improvement in the symptoms of PCOS, menstrual irregularity, metabolic outcomes and infertility[157]. There was also a substantial improvement in depression 12 months after surgery[157]. Below is a list of published studies of bariatric surgery in women with PCOS who had obesity:

Author	Type of study	Intervention	Number of patients	Outcomes	Results
Abiad (2018)[158]	Prospective case-control	SG	PCOS = 6 Non PCOS = 19	Anthropometric markers, inflammatory markers	Anthropometric markers p<0.05 Lipid profile, adiponectin, insulin, fasting blood glucose p<0.05 (12m)
Ahmed (2017)[159]	Prospective cohort	SG	8	BMI, fertility	Unable to access paper
Bhandari (2016)[160]	Prospective observational	SG	43	Change in serum AMH, menstrual function, estimated body weight loss	BMI p<0.0001 AMH p<0.001 (6m)
Chiofalo (2017)[161]	Retrospective case-control	SG; RYGB	14	Serum AMH, T, androstenedione, DHEAS	AMH reduction p=0.02 T, androstenedione, DHEAS reduction p <0.05 (12m)
Christ and Falcone (2018)[162]	Retrospective case-control		44	BMI, dyslipidaemia, HbA1c	BMI p<0.001; TG, HDL-c, VLDL-c p<0.05, TT p<0.05, irregular menses p<0.001

Christinajoice (2020)[163]	Retrospective case-control	LSG, RYGB, LAGB	29	BMI, reproductive	Amenorrhoea p=0.001 Excess BMI loss 76.6% (3 yr)
Dixon and O'Brien (2002)[164]	Prospective cohort	LAGB	30	SHBG, Testosterone, FAI	(Combined with non PCOS) anthropometric, biochemical hyperandrogenism, fasting blood glucose, insulin p <0.05
Eid (2005)[165]	Retrospective case-control	LRYGB	24	BMI, resolution of T2D, hirsutism, menstrual abnormalities	T2DM 100% resolution, HbA1c 62% change, hirsutism 79% change, menstrual dysfunction 100% (12m)
Eid (2014)[166]	Prospective cohort	LRYGB	14	Testosterone, FSH, LH, insulin, fasting blood glucose, lipid levels, BMI, menstrual pattern	Testosterone, fasting blood glucose, insulin, reduced (P<0.05), reduction in BMI; regular menses in all patients (12m)

Escobar-Morreal (2005)[167]	Prospective cohort	LGB, BPD	12	Weight loss, menstrual abnormality, biochemical hyperandrogenism, insulin, HOMA-IR	Weight loss P<0.0001, restoration of regular menses, normalisation of testosterone and DHEAS, decrease in insulin and HOMA-IR (7 – 26 m)
Jamal (2012)[168]	Retrospective case-control	RYGB	20	Menstrual abnormality, hirsutism, type 2 diabetes	Resolution of menstruation in 82%, hirsutism in 29%, type 2 diabetes in 77.8% (46.7 m)
Kyriacou (2014)[169]	Prospective cohort	GB	48	Weight loss, metabolic outcomes	Significant reduction in BMI HbA1c P<0.0001 (24 m)
Machado Junior, Ribeiro (2019)[170]	Prospective cohort	SG	18	Oestradiol, fasting insulin, LH, FSH, LH/FSH ratio, BMI	Oestradiol higher (not significant), fasting insulin and LH/FSH ratio reduced (P <0.05), BMI

					reduced (P <0.001) (3 m)
Turkmen (2015)[171]	Prospective cohort	RYGB	13	BMI, metabolic and hormonal outcomes; metabolic dysfunction	Improvement in metabolic outcomes and normalisation of menstrual cycle incomplete at 6 m
Wang (2015)[172]	Prospective cohort	SG	24	Weight loss, menstruation, improvements in hirsutism and metabolic symptoms	Significant reduction in BMI, androgen levels and restoration of normal menstrual cycles (6 m)
Singh (2020)[173]	Prospective cohort		18	Anthropometric data, menstrual cyclicity, markers of hyperandrogenism	Restoration of normal menstrual cyclicity, resolution Of metabolic syndrome and hirsutism, reduction in testosterone (P<0.01) (12 m)

Benito (2020)[156]	Prospective cohort	RYGB, AGB, SG	49	BMI, metabolic and hormonal markers, pregnancy rate	Significant reduction in BMI, metabolic and hormonal outcomes; higher pregnancy rates (P<0.05) (
Hu (2022)[174]	Prospective cohort	LSG	45	PCOS remission rate, free testosterone, ovary morphology	78% remission rate, significant reduction in total testosterone, improvement of regular menstruation

Table 1.6 Published studies of bariatric surgery in adult women with PCOS who had obesity. Adapted from Tian et al[157]. LAGB = laparoscopic adjustable gastric banding, RYGB = Roux-en-Y gastric bypass, LSG = laparoscopic sleeve gastrectomy, LRYGB = laparoscopic gastric bypass

In a prospective cohort study of women seeking fertiliy published by Benito et al with long-term follow-up, “pregnancy rates were 95.2% in PCOS and 76.9% in controls (P=0.096) and live birth rates were 81.0% and 69.2%, respectively (P=0.403)”[156]. In women seeking fertility, pregnancy rates and live birth rates were both higher (not significant) than in controls[156]. There was no significant difference in pregnancy outcomes in live births between PCOS and controls; however, mean birth weight (g) was significantly lower (2763 ± 618 vs. 3155 ± 586), and the need for intensive care (due to low birth weight), higher in the PCOS group[156].

More recently, Hu et al conducted a prospective non-randomised trial in women with

PCOS and obesity, dividing participants into drug (combined oral contraceptive pill for initial 6 months and metformin for 12 months) and surgical groups according to the patients' intentions for 12 months. In addition to the expected improvements in anthropometric and metabolic outcomes, they found the complete remission rate of PCOS was five times higher in the surgical group[174]. A significantly higher proportion of patients from the surgical group maintained regular menstruation post-operatively[174].

Although lifestyle intervention with or without pharmacotherapy remains the first-line treatment for women living with PCOS, it has limited efficacy in women with PCOS and obesity. Bariatric surgery has, albeit only through prospective and non-randomised trials, been shown to significantly improve metabolic and reproductive outcomes in this group of patients. However, due to limited evidence in the form of published RCTs on the effects of bariatric surgery in women with PCOS and obesity, it "should be considered an experimental therapy in women with PCOS, for the purpose of having a healthy baby…"[145].

Summary

- Bariatric surgery reduces overall mortality in people with obesity
- The three most performed procedures are laparoscopic RYGB, VSG and AGB
- Lifestyle intervention is the first line of treatment in women with PCOS who live with overweight or obesity
- Pharmacotherapy should be considered when patients do not respond to lifestyle interventions
- Bariatric surgery is considered an experimental treatment in women with PCOS

due to the absence of RCTs.

PART TWO - INTRODUCTION

1.7 Definition of diabetes mellitus

The American Diabetes Association (ADA) "Standards of Medical care in Diabetes" classifies diabetes into the following general categories[175]:

1. Type 1 diabetes mellitus (T1D) is due to autoimmune (T-cell-mediated) destruction of pancreatic β-cells and usually leads to absolute insulin deficiency, including latent autoimmune diabetes of adulthood
2. Type 2 diabetes mellitus (T2D) is due to the progressive loss of adequate β-cell insulin secretion, often on the background of insulin resistance
3. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (neonatal diabetes and maturity-onset diabetes of the young), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation)"
4. "Gestational diabetes mellitus (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation)"

T1D and T2D are heterogenous diseases with varying clinical presentation and disease progression[175], but classification is important for determining treatment. T2D accounts for around 80-90% of diabetes as previously referred to as "adult-onset diabetes". Typically, patients have overweight or obesity and insulin resistance. Children

with T1D usually present with a hallmark symptoms of polyuria/polydipsia, and approximately a third present with diabetic ketoacidosis (DKA)[175]. This presentation is more variable in adults, and they may not present with the symptoms typically seen in children.

The National Institute for Health and Care Excellence (NICE) criteria for the diagnosis of type diabetes defines persistent hyperglycaemia as[176]:

- HbA1c of 48 mmol/mol (6.5%) or more.
- Fasting plasma glucose level of 7.0 mmol/L or more.

  Random plasma glucose of 11.1 mmol/L or more in the presence of symptoms or signs of diabetes.

NICE guideline for Type 1 diabetes in adults: diagnosis and management, March 2022 recommends treatment with insulin replacement and the active management of other cardiovascular risk factors, such as hypertension and dyslipidaemia, to reduce the risk of long-term complications[177]. Insulin therapy can either be in the form of multiple daily injections (MDI) of basal and prandial insulin or continuous subcutaneous insulin infusion through an insulin pump. Continuous subcutaneous insulin infusion appears to be a more physiological way to deliver insulin when compared with MDI in that the delivery of insulin can be adjusted according to circadian variations in insulin sensitivity[178]. The current goal of insulin therapy in people with T1DM is to aim for >70% of time spent in the target glucose range of 3.9–10 mmol/L while reducing the burden of hypoglycaemia[179]. Complications of insulin therapy can be acute such as hypoglycaemia, or medium- to long-term, such as excessive weight gain and lipodystrophy.

1.8 Alpha melanocyte-stimulating hormone

The alpha-melanocyte stimulatory hormone (α-MSH) has well-recognised central effects on the control of food intake primarily through signals to several regions in the brain involved in downstream signaling[180]. Animal studies have demonstrated a significant reduction in food intake following chronic central administration of α-MSH[181]. This action is primarily through binding to the melanocortin 3 receptor (MC3R), and melanocortin 4 receptor (MC4R) found in the hypothalamic nuclei (in particular, the paraventricular nucleus)[181]. Administration of Setmelanotide (an MC4R agonist) in individuals with genetic obesity (either proopiomelanocortin (POMC) or leptin receptor (LEPR) deficiency) and a BMI of at least 30 kg/m^2 or a body weight of more than the 95th percentile for age (paediatric patients) for 52 weeks was associated with significant weight loss[182]. In patients with POMC deficiency, Setmelanotide was associated with a significant reduction in fasting plasma glucose[182]. Following this, in June 2022, the United States Food and Drug Administration approved Setmelanotide (an MC4R agonist) injection for chronic weight management in adults and paediatric patients 6 years of age and older with obesity due to three genetic conditions: POMC deficiency, proprotein subtilisin/kexin type 1 (PCSK1) deficiency, and LEPR deficiency. The deficiency must be considered pathogenetic and confirmed by genetic testing.

More relevant to this study is the effect of α-MSH on skeletal muscle glucose uptake. The two most physiologically relevant factors affecting glucose transport in skeletal muscle are insulin and exercise[180]. Insulin released by the pancreatic β-cells in response to rising blood glucose levels stimulates whole-body glucose uptake in skeletal muscles (predominantly), liver and adipose tissue. Plasma insulin increases lipid storage from free

fatty acids and *de novo* lipogenesis from glucose[183].

The melanocortin system

The central melanocortin system plays a pivotal role in energy homeostasis[184], which is driven by neurons expressing POMC and agouti related protein (AgRP)[180]. It is one of the most complex hormonal systems in vertebrates[142]. POMC undergoes cleaving to form melanocyte-stimulating hormones (α-, β- and ɣ-MSH) and ACTH)[180]. The active form of α-MSH is the 13-amino acid acetylated peptide hormone (Ac-SYSMEHFRWGKPV-NH2). α-MSH binds non-preferentially to the G protein-coupled receptors MCR (melanocortin receptor) 1, 3, 4 and 5, with ACTH being the primary agonist for MC2R[180]. Enriori et al. confirmed that only MC5R was expressed in mice skeletal muscle[185]. A group of transmembrane proteins called melanocortin receptor accessory proteins (MRAPs) further regulate the signalling and pharmacological profile of the MCRs[186], see table 1.7 below:

Receptor	Agonists/Antagonist	Accessory protein	Main tissue expression	Main function
Melanocortin peptides, receptors and accessory proteins				
MC1R	MSHs, B-defensin 103/ASIP	MRAP1	Skin	Melanogenesis
MC2R	ACTH	MRAP1	Adrenal gland	Stress response
MC3R	MSHs/AgRP	MRAP2	Brain	Energy balance
MC4R	MSH/AgRP	MRAP2	Brain	Energy balance
MC5R	MSHs	MRAP2	Non-specific	Exocrine secretion

Table 1.7 Agonists and antagonists of melanocortin receptors.

MCR = melanocortin receptor, MSH = melanocyte-stimulating hormone, ASIP = agouti-signalling protein, ACTH = adrenocorticotropic hormone, AgRP = agouti-related protein

Animal studies

α-MSH has recently been shown to exert control over insulin and glucose homeostasis through a novel pathway involving skeletal muscle MC5R agonism in animals[181, 183]. In primates fed a high-caloric diet (after an overnight fast), α-MSH levels increased significantly compared to fasting[185]. Investigators found that independent of direct hypothalamic control, the anterior pituitary gland secretes α-MSH into the bloodstream in response to a post-prandial glucose excursion[185]. This was further supported by investigating α-MSH levels in patients with low- or non-functioning pituitaries, panhypopituitarism, and in patients with craniopharyngioma after surgery which established the presence of very low plasma α-MSH levels (70% lower than healthy subjects with normal pituitary function)[185]. Plasma α-MSH levels peaked at 15 minutes after glucose administration during glucose tolerance tests (GTT) in healthy and obese children, lean and obese monkeys, and lean and diet-induced obese mice[185]. There was also a strong correlation between α-MSH and both plasma glucose and serum insulin levels which supports the hypothesis that glucose and/or insulin regulate pituitary release of α-MSH *in vivo*[185].

Administration of systemic α-MSH infusion in fasted lean mice did not alter basal euglycaemia; however, administration of either 3-hour saline or α-MSH infusion (varying doses) in mice during intraperitoneal GTTs found a significant improvement in glucose tolerance in a dose-dependent manner[185]. These findings further support the idea that the main effect of α-MSH is on glucose disposal in skeletal muscle[185]. During the hyperinsulinaemic-euglycaemic clamp, mice treated with α-MSH required a high glucose infusion rate (GIR) to maintain normal glucose levels; there was also significant glucose uptake in skeletal muscles when compared to other metabolically active tissues[185].

Interestingly, the administration of α-MSH in obese mice did not lead to any improvement in glucose tolerance[185]. Employing genetic knockout models and MC5R agonists, Enriori et al further went on to establish the effects of peripheral MCR agonism on glucose homeostasis are mediated by MC5R in animals.

It is thought that the impact of α-MSH on insulin resistance depends on the route of exogenous administration[181]. Animal studies in mice have shown that α-MSH stimulates glucose uptake in skeletal muscle which was independent of the upstream molecular signals normally associated with insulin-induced glucose uptake[184]. For example, in rodent muscle cells α-MSH did not induce GLUT4 translocation to the plasma membrane[184]. Unpublished work conducted by Professor Cowley's laboratory performed in a Streptozotocin treated mouse model of T1D showed that α-MSH reduced fasting blood glucose concentrations from the elevated baseline and diminished the rise in blood glucose after an intraperitoneal glucose tolerance test.

1.9 Adjuvant treatments for T1D

Currently, in Europe, only insulin and its analogues have been approved for the treatment of T1D, however, in the United States, the amylin analogue pramlintide is approved for adjuvant use with insulin[187]. β Cells of the pancreas co-secrete insulin and amylin and in T1D, there are deficiencies in both insulin and amylin[188]. Amylin reduces plasma glucose concentrations by three primary mechanisms – 1. Activation of

amylin receptions in the nucleus accumbens and dorsal vagal complex reduces food intake and depresses gastrointestinal motility, 2. Binding to pancreatic β cells may inhibit insulin release; suppression of postprandial glucagon from pancreatic α cells, and 3. Reduced gastrointestinal motility and gastric emptying[189]. Pramlintide acetate, a synthetic analogue of amylin, reproduces the amylin agonist activity and effectively decreases immediate postprandial hyperglycaemia[188]. Bolus injection of pramlintide acetate caused decreased glucose excursions and a greater delay in gastric emptying and glucagon suppression in adolescents with T1D[188]. The commonly reported side effects of treatment are reduced appetite, vomiting and nausea[189].

In 2019, the European Medicines Agency (EMA) approved the use of sodium-glucose like co-transporter (SGLT) inhibitors dapagliflozin and sotagliflozin as an adjunct to insulin in certain patients with T1D[187], however this approval has now been withdrawn.

More recently, a *Schistosoma japonicum*-egg tip-loaded asymmetric microneedle patch (STAMP) system was able to significantly reduce blood glucose and attenuate the pancreatic injury seen in T1D mice by balancing the Th1/Th2 immune responses[190]. Immunotherapy remains an experimental treatment; co-stimulation modulation with Abatacept in patients with recent-onset T1D (double-blind RCT) slowed the decline of beta cell function over two years[191]. In the follow-up study which monitored patients receiving abatacept treatment in addition to insulin therapy vs. patients receiving insulin therapy alone, abatacept treatment improved C-peptide levels - which indicates greater insulin production by the pancreas, and lower overall HbA1c levels[191].

Unpublished human myotube data

Unpublished data by Swan et al at University College Dublin using primary human myotubes and commercially obtained human skeletal muscle tissue ribonucleic acid (RNA) identified expression of MCRs with MCR1 being the most abundant, followed by MCR3/4; MC5R was detected at very low levels. They also found that α-MSH induced glucose uptake in human primary myotubes without insulin, which points to its insulin-independent actions. However, co-incubation with α-MSH and insulin significantly increased glucose uptake over incubation with insulin alone. The melanocortin receptor profile of skeletal muscle in rodents has been well-characterised and identified a high abundance of MC5R and low levels of MC4R[141, 143] (in contrast with human skeletal muscle). Incubating these myotubes with PG-901, a MC5R specific agonist, caused a glucose uptake effect akin to that observed after incubation with insulin or α-MSH. This suggests, like published animal data, α-MSH induced glucose uptake in human muscle may be mediated by MC5R.

1.10 Glucose homeostasis and methodologies

Glucose is the primary source of metabolic energy for most body cells and is especially critical for brain functioning. It is also the key regulator of insulin secretion from the pancreatic beta cells. Once plasma glucose levels rise above 3.9 mmol//L, there is stimulation of insulin synthesis, which occurs primarily by enhancing protein translation and processing; GLUT1 transports glucose in humans. Neuroendocrine cells in the gastrointestinal tract release incretins following the ingestion of food; incretins amplify glucose-stimulated insulin secretion and suppress glucagon secretion. Around 50% of the insulin secreted into the portal venous system is removed and degraded by the liver. The remaining insulin enters the systemic circulation, where it binds to receptors in target

sites, e.g. skeletal muscle, which is the leading target site for glucose utilisation and disposal (around 80%). The binding of insulin to its receptor leads to a complex cascade of phosphorylation and dephosphorylation reactions resulting in insulin's widespread metabolic and mitogenic effects. One of these effects is the translocation of a facilitative glucose transporter to the cell surface which is crucial for glucose uptake in skeletal muscle and fat.

Insulin is secreted in a pulsatile fashion, and two distinct modes of postprandial insulin secretion have been identified in response to glucose stimulation[192]. The first is the stimulated (postprandial) state, and the second is the basal (postabsorptive) state. The transient first-phase insulin secretion occurs immediately as a response to increased plasma glucose levels and stops within the first few minutes. This is followed by a sustained second phase of insulin secretion, which is low and continued, plateauing within 1 – 3 hours and lasting longer. In contrast to the first phase, the second phase is independent of the extracellular glucose level[192].

Techniques for measuring glucose homeostasis

The fasting plasma glucose concentration is determined by balancing hepatic glucose production and whole-body glucose utilisation. The liver provides around 90% of glucose in the fasting state, most of which is utilised by non-insulin-dependent tissues. The remainder is used by insulin-dependent tissues – mostly skeletal muscle and the liver. Elevated fasting glucose or insulin levels are indicative of insulin resistance. The most common index of insulin resistance in fasting is that arising from the HOMA-IR. HOMA-IR is derived from a mathematical model of the glucose-insulin homeostatic system[193].

Another index of fasting insulin sensitivity is the quantitative insulin sensitivity check index, QUICKI[194]. The information obtained with the two indices is virtually the same.

Insulin sensitivity from dynamic tests

Insulin has various effects on different cell types, but its main metabolic action is anabolic, affecting glucose, lipid, and protein metabolism. The secretion of insulin is very closely linked to the availability of glucose in the plasma. Insulin stimulates glucose uptake in insulin-sensitive tissue, oxidative and nonoxidative metabolic pathways, and inhibits lipid oxidation. Insulin can regulate glucose homeostasis in both the fed and fasted states through these metabolic pathways.

Following the administration of a glucose load, there is consequent hyperglycaemia and hyperinsulinemia; insulin-mediated glucose uptake plays a fundamental role in glucose clearance. Sustained hyperglycaemia may be due to reduced insulin secretion or insulin resistance, lasting longer than normal conditions. To evaluate insulin sensitivity, the glucose/insulin system must be challenged in dynamic conditions by administering exogenous glucose or insulin and relating glucose disappearance to insulin levels. One of the most common experimental procedures is the oral glucose tolerance test (OGTT). Plasma glucose levels peak 30 – 60 minutes after ingesting a glucose load (usually 75 g) and return to pre-load values after two to three hours.

Oral Glucose Tolerance Test

The OGTT remains the most used method to evaluate whole-body glucose tolerance *in vivo*[195]. However, it is difficult to derive information about whole-body insulin sensitivity from an OGTT. The product of the glucose area under the plasma glucose curve (AUC) and

the insulin area under the plasma insulin curve has been used as an index of insulin resistance[195]. During a standard 75 g OGTT, participants are asked to attend the visit following an overnight fast and having avoided strenuous exercise for the preceding 24-48 hours. Baseline blood samples (usually five or more) are collected over 2-3 hours, and the typical sampling times are 0 (pre-load), 30-, 60-, 90- and 120-minutes post-glucose load. Measurement of glucose, insulin and sometimes C-peptide are performed during the time points.

Euglycaemic hyperinsulinaemic clamp

Whole-body insulin sensitivity can be measured with the euglycemic insulin clamp technique, and it is considered the 'gold standard' under insulin-stimulated conditions[195]. This euglycaemic hyperinsulinaemic clamp method was developed by Andres et al. in 1966 and then further developed and studied in 1979 and worked by breaking the physiologically operating feedback loop between blood glucose concentration and pancreatic insulin secretion[196]. This "breaking of the loop" is achieved through the maintenance of supraphysiological levels of insulin (~100 uU/mL) via a fixed dose of intravenous insulin for several hours. Under these conditions, the subject's endogenous glucose production is suppressed, and their peripheral tissue's insulin response is maximal. A variable intravenous infusion of glucose is administered throughout the test to maintain euglycaemia. The rate of glucose infusion is then adjusted until the subject is "clamped" in a euglycaemic steady state. In this steady state, the intravenous GIR is matched to the glucose taken up by the body, and as endogenous glucose production is suppressed this GIR can used to quantify whole-body glucose uptake. During the postabsorptive state in individuals without diabetes, the rate of endogenous glucose output originating from hepatic (~90%)[197] and renal (~10%)[198] glucose release matches the whole-body

glucose utilisation rate. This, in turn, maintains constant glycaemia. Under these conditions, administering exogenous insulin will reduce endogenous glucose output and increase whole-body glucose utilisation, resulting in a decline in blood glucose concentration. This reduction in blood glucose concentration can be prevented by intravenous administration of an exogenous glucose load which matches the insulin-induced glucose flux out of the glucose space into glucose-utilising cells and the reduction of endogenous glucose output.

There remains an unmet clinical need for adjunct therapies in T1D, which work independently of insulin and human studies to understand the physiological action of melanocortin receptor agonism on glucose homeostasis by peripheral administration of α-MSH is a critical next step towards managing blood glucose levels.

2 Insulin sensitisation improves metabolic and fertility outcomes in women with polycystic ovary syndrome who have overweight or obesity- a systematic review, meta – analysis and meta-regression

Numerous clinical trials have looked at the use of direct insulin sensitisers in women with PCOS, that is, drugs that have a direct effect on insulin sensitivity while causing little or no weight loss. Biguanides (metformin) and thiazolidinediones (e.g., pioglitazone) are the two most studied drug classes[199]. Glucagon-like petitde-1 receptor agonists (GLP-1 RAs) (e.g., exenatide and liraglutide)[200], glucosidase inhibitors (acarbose), and lipase inhibitors (orlistat) are three other classes of drugs of interest in the treatment of PCOS that can indirectly increase insulin sensitivity through weight loss. Naltrexone/bupropion and Phentermine/topiramate are two other indirect insulin sensisiters which decrease energy intake resulting in weight loss. Only a few systematic reviews and meta-analyses have been conducted on the use of insulin sensitisers in women with PCOS who have overweight or obesity. These have primarily focused on metformin, pioglitazone, and, to a lesser extent, GLP-1 RAs, with weight loss as the desired outcome and little information on reproductive parameters[201, 202]. These meta-analyses included open-label studies, which increased the possibility of bias.

In this systematic review (SR), meta-analysis (MA) and meta-regression (MR), the aim was to assess the highest quality evidence on the impact of insulin sensitisers on key metabolic and reproductive outcomes in women with PCOS who have overweight or obesity.

2.1 Methods

Selection criteria and search strategy

I followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement when reporting this systematic review, meta-analysis, and meta- [12]. The protocol was prospectively registered on PROSPERO (CRD42021236556). A comprehensive search of four databases, including MEDLINE via Ovid and EMBASE, for articles published between inception and June 29, 2021, yielded eligible randomised controlled trials (RCTs). In the search process, myself, and another independent clinician used the MeSH terms (polycystic ovary syndrome) AND ((overweight) OR (obesity)) AND ((biguanide) OR (thiazolidinediones) OR (glucagon-like peptide 1 receptor agonist) OR (lipase inhibitor) OR (alpha glucosidase inhibitor)) OR (naltrexone/bupropion) OR (phentermine/topiramate) and relevant terms.

Each database was searched using the same terms, and studies were imported into the Covidence systematic review management tool for screening and data extraction. Human studies in adult females, a diagnosis of PCOS, BMI greater than or equal to 25 kg/m2, double-blind RCTs, and metabolic and reproductive outcomes of interest were the inclusion criteria. PCOS was diagnosed using the 2012 National Institute of Health criteria[2] or the Rotterdam Consensus Criteria (2003)[203].

Exenatide, liraglutide, metformin, orlistat, pioglitazone, rosiglitazone, troglitazone, acarbose, naltrexone/bupropion or phentermine/topiramate versus placebo or other agents, such as ovulation induction agents, were included in the intervention. Despite being withdrawn from clinical care, relevant studies of rosiglitazone and troglitazone were

included to assess the impact of their mechanism of action on PCOS. All the studies that were chosen included the following information: demographic characteristics, PCOS diagnosis criteria, drug dosages, and treatment duration. Studies that used two or more drugs in combination were excluded as it was impossible to distinguish which medication influenced the primary and secondary outcomes. The primary outcomes were a difference from baseline in BMI, fasting blood glucose, and menstrual frequency. Secondary outcomes included change from baseline in fasting insulin, HOMA-IR, plasma concentrations of AMH, SHBG, total testosterone, and the Ferriman-Gallwey Scale for hirsutism. Other outcomes of interest included DHEAS, FSH, LH, and pregnancy as a fertility marker. The results are presented as SMD with a 95% confidence interval.

For studies that met the inclusion criteria, the corresponding author was contacted if missing data was required. The RCTs that met the inclusion criteria were identified and selected (Table 2.1). Disagreements between reviewers were resolved through discussion, with the senior author making the final decision. The risk of bias was assessed using the Cochrane Collaboration risk of bias framework[204], which included seven distinct domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each domain is classified as “low,” “unclear,” or “high.” Individual patient-level data was sought if the summary estimates were not provided in the desired format. Table 2.1 depicts the characteristics of the studies included in the MA and MR.

Literature search

Eligible RCTs were identified through a comprehensive search of databases, including PubMed, MEDLINE via Ovid and EMBASE, for articles published on 29th June 2021. Two reviewers applied the following MeSH terms – (polycystic ovary syndrome) AND ((overweight) OR (obesity)) AND ((biguanide) OR (thiazolidinediones) OR (glucagon-like peptide 1 receptor agonist) OR (lipase inhibitor) OR (alpha glucosidase inhibitor) OR (naltrexone/bupropion) OR (phentermine/topiramate)) in the search process. Search terms were applied to each database, and studies were imported into Covidence for screening and data extraction. Study authors were contacted for missing data if necessary for studies that met the inclusion criteria. Individual patient-level data was sought if the summary estimates were not provided in the desired way. The two independent investigators performed a risk assessment using the Cochrane Collaboration risk of bias[204] which comprised of seven distinct domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each domain is 75ensitizer75ed as “low”, “unclear”, or “high” risk (Figure 2.1).

2.2 Data analysis

I, and the other independent clinical extracted data for the study using an excel spreadsheet that recorded the study author and year of publication, the number of study participants, the intervention (insulin 75sensitiser agent), and the study outcomes. I contacted the corresponding author for clarification if data was missing or unclear. The results reported in two studies[205, 206] were inconsistent with data from other studies and were excluded from the numerical analysis due to a high risk of bias and

poor performance in influence analysis. Data analysis was performed by a statistician using the inverse variance method and a mixed-effects (plural) model with a restricted maximum-likelihood (REML) estimator for τ^2. The model assessed within-subgroup studies using the random- effects model, while the between-subgroup level was estimated using a fixed-effects model (ISBN: 9780367610074)[204]. SD, if not available for estimation using confidence intervals, were approximated using the average SD of similar studies, as suggested by the Cochrane Handbook for Systematic Reviews of Interventions (ISBN: 9781119536604)[204]. The results are SMD with their respective 95% CI. Heterogeneity was assessed and quantified with χ^2 and I^2 tests, respectively, with a cut-off value set at $\alpha = 0.1$.

Egger's test of intercept was used to assess potential publication bias in all outcomes with a minimum of ten studies, which was confirmed visually with funnel plot asymmetry. A meta-regression analysis using bubble plots also examined study duration as a potential moderator of result significance (figure 2.3). All meta-analytical calculations, including forest and bubble plots, were performed using the R statistical software (v4.1.2) and the packages meta (v5.1-0) and dmetar (GitHub commit 3e7ef5f). Meta-regression (minimum of 10 studies) was performed to assess if the study results are significant despite differences in the study duration (figure 2.4).

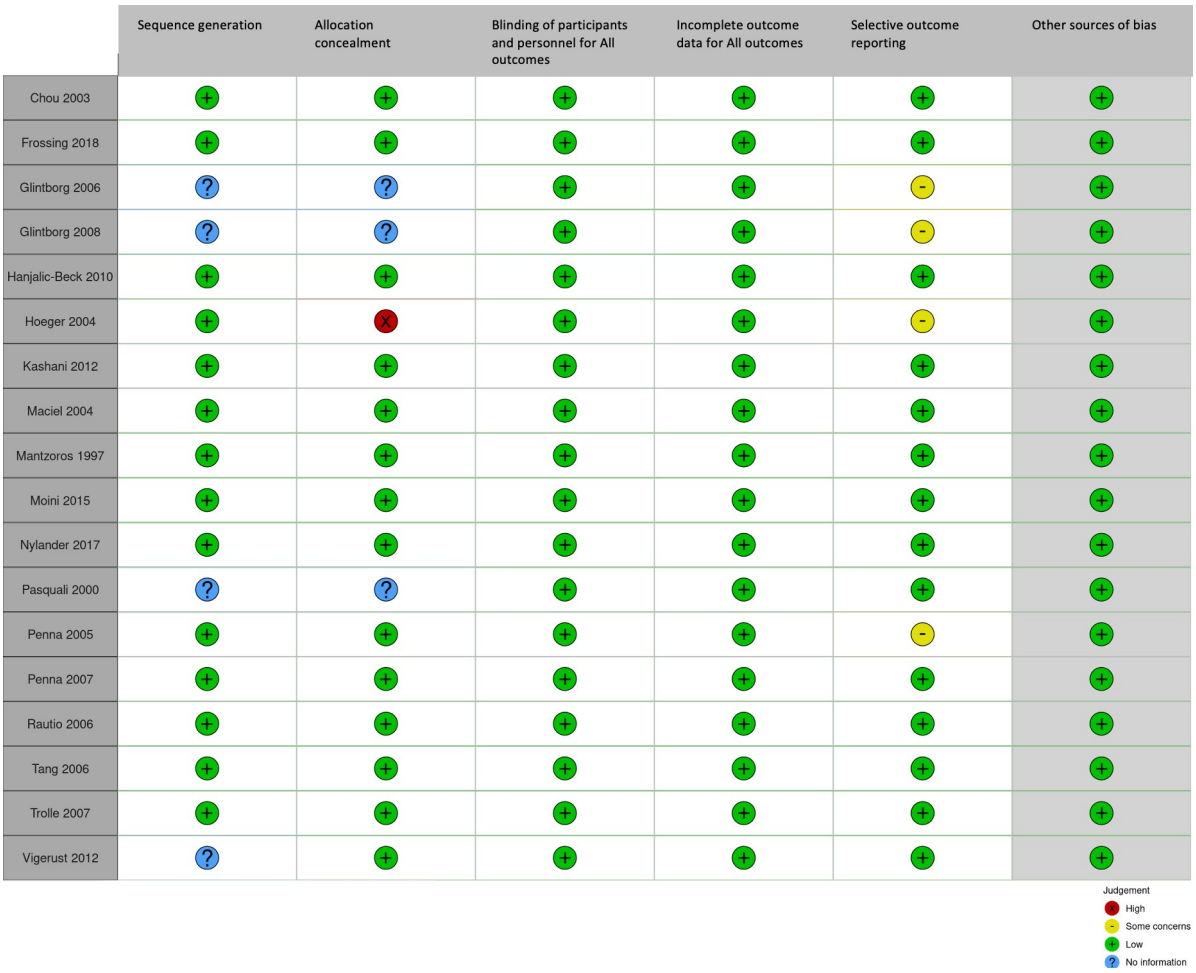


Figure 2.1 Risk of bias results for included studies

The characteristics of the studies included in the meta-analysis are presented in figure 2.2.

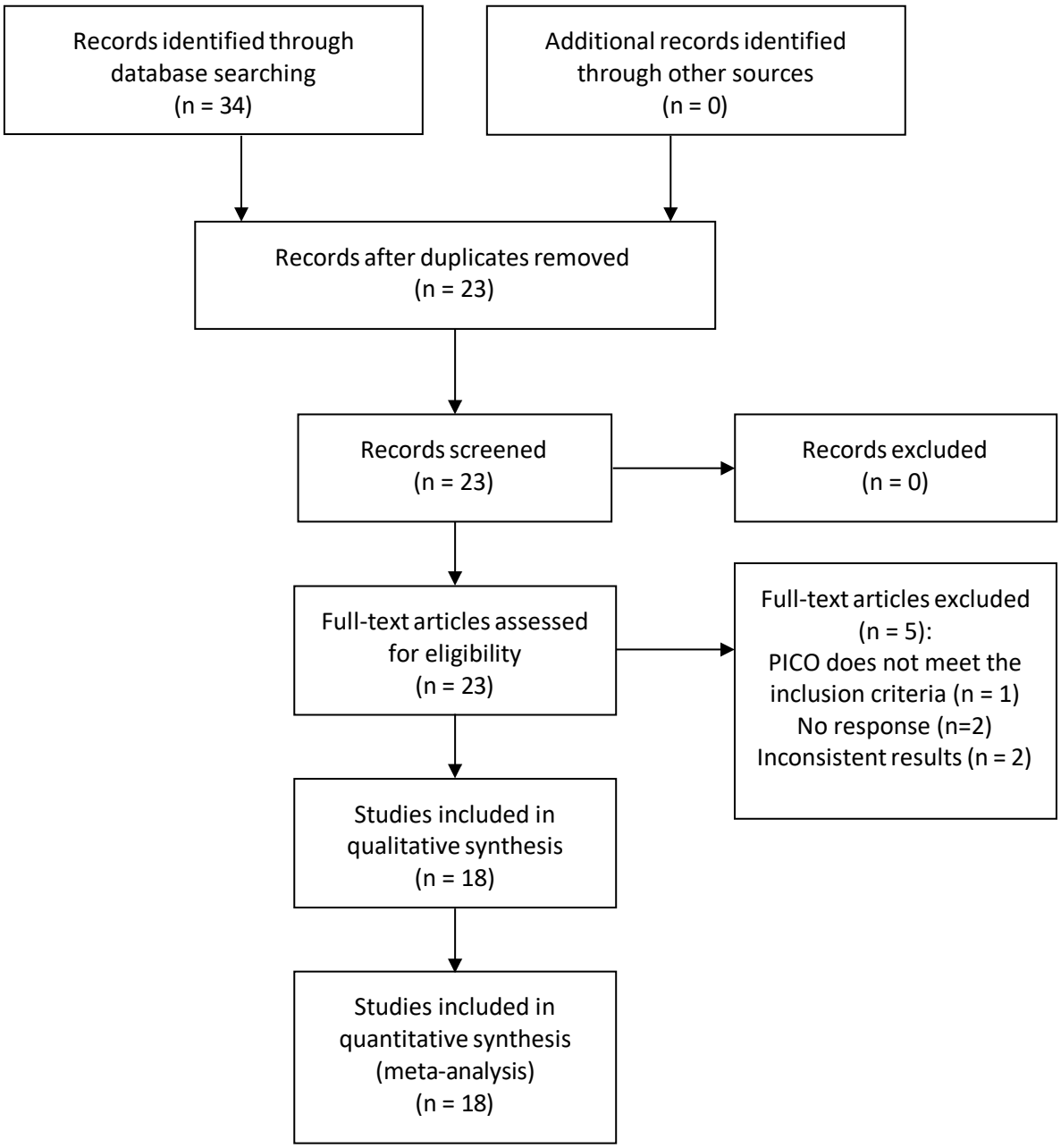


Figure 2.2 PRISMA flow diagram of systematic reviews modified from Page et al[207]

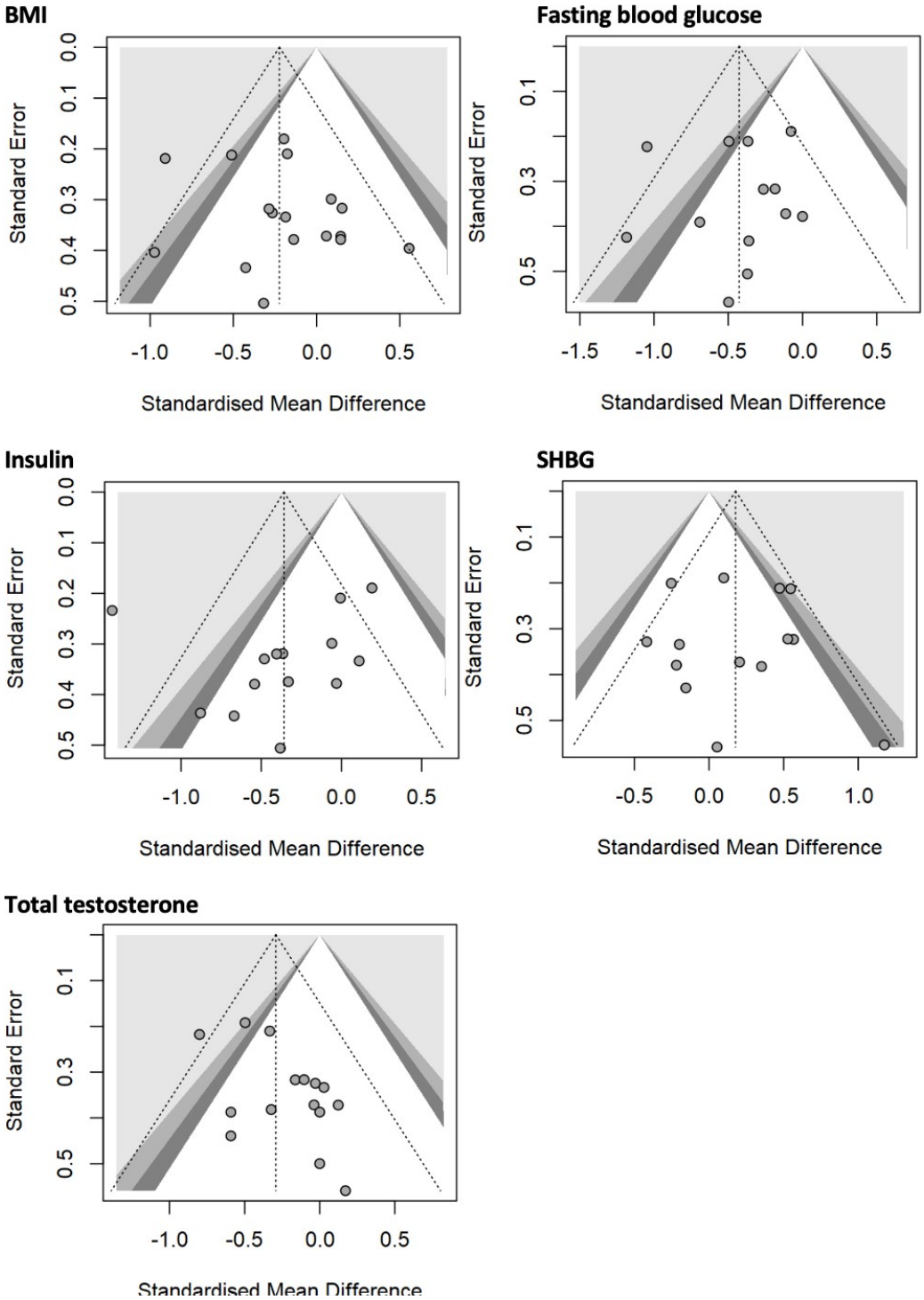


Figure 2.3 Egger's test and funnel plots calculated for all outcomes where the number of studies was at least 10

First author (year)	Country	Diagnostic criteria	Sample size for intervention group (n)	Intervention	Comparator	Dose	Body mass index (kg/m^2)	Duration (weeks)
Chou et al (2003)[208]	Brazil	NICHD	14	Metformin	Placebo	500 mg TDS, PO	35.60 (4.90)	13
Frossing et al (2018)[209]	Denmark	ESHRE/ASRM	48	Liraglutide	Placebo	1.8 mg OD, S/C	33.30 (5.10)	26
Glintborg et al (2006)[210]	Denmark	NICHD	15	Pioglitazone	Placebo	30 mg OD, PO	32.20 (30.70-36.60)	16
Glintborg et al (2008)[211]	Denmark	ESHRE/ASRM	15	Pioglitazone	Placebo	30 mg OD, PO	33.40 (27.30-40.60)	16
Hanjalic-Beck et al (2010)[212]	Germany	NICHD	19 18	Metformin vs. Acarbose	Metformin or acarbose	850 mg, TDS, PO 100 mg, TDS, PO	35.50 (5.62) 33.50 (5.50)	12
Hoeger et al (2004)[213]	USA	NICHD	9	Metformin	Lifestyle modification + metformin, lifestyle modification + placebo, placebo alone	850 mg, BD, PO	37.10 (4.90)	48
Kashani et al (2012)[214]	Iran	ESHRE/ASRM	20	Metformin vs. Pioglitazone	Metformin or pioglitazone	750 mg BD, PO vs. 15 mg BD, PO	32.98 (3.51) 33.15 (3.12)	6
Maciel et al (2004)[215]	Brazil	NICHD	8	Metformin	Placebo	500 mg TDS, PO	37.20 (1.70)	26
Mantzoros et al (1997)[216]	USA	NICHD	24	Troglitazone	Troglitazone 400 mg or 200 mg + placebo	200 mg or 400 mg OD, PO	42.89 (1.23)	13
Moini et al (2015)[217]	Iran	ESHRE/ASRM	50	Orlistat + low energy diet	Low energy diet + orlistat, low energy diet + placebo	120 mg TDS, PO	29.01 (2.09)	13
Nylander et al (2017)[218]	Denmark	ESHRE/ASRM	48	Liraglutide	Placebo	1.8 mg OD, S/C	33.30 (5.10)	26
Pasquali et al (2000)[219]	Italy	NICHD	12	Metformin + low energy diet	Placebo	850 mg BD, PO	39.80 (7.90)	26
Penna et al (2005)[220]	Brazil	NICHD	15	Acarbose	Placebo	150 mg OD, PO	35.87 (2.60)	26
Penna et al (2007)[221]	Brazil	NICHD	15	Acarbose	Placebo	150 mg OD, PO	35.87 (2.60)	26
Rautio et al (2006)[222]	Finland	ESHRE/ASRM	12	Rosiglitazone		4 mg BD, PO	33.10 (1.70)	17
Tang et al (2006)[147]	UK	ESHRE/ASRM	69	Metformin	Placebo	850 mg BD, PO	37.60 (5.00)	26

Trolle et al (2007)[223]	Denmark	NICHD	50	Metformin	Placebo	850 mg BD, PO	35.20 (6.40)	26
Vigerust et al (2012)[224]	Denmark	NICHD	14	Pioglitazone	Placebo	30 mg OD, PO	32.20 (30.70-36.60)	16

Table 2.1 Characteristics of studies included in the meta-analysis.

NICHD = National Institute of Child Health and Human Development, ESHRE/ASRM = European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine, TDS = 3 times a day, PO = oral administration, OD = once daily, S/C = subcutaneously, BD = twice daily, data presented as mean (± SD) or median (IQR)

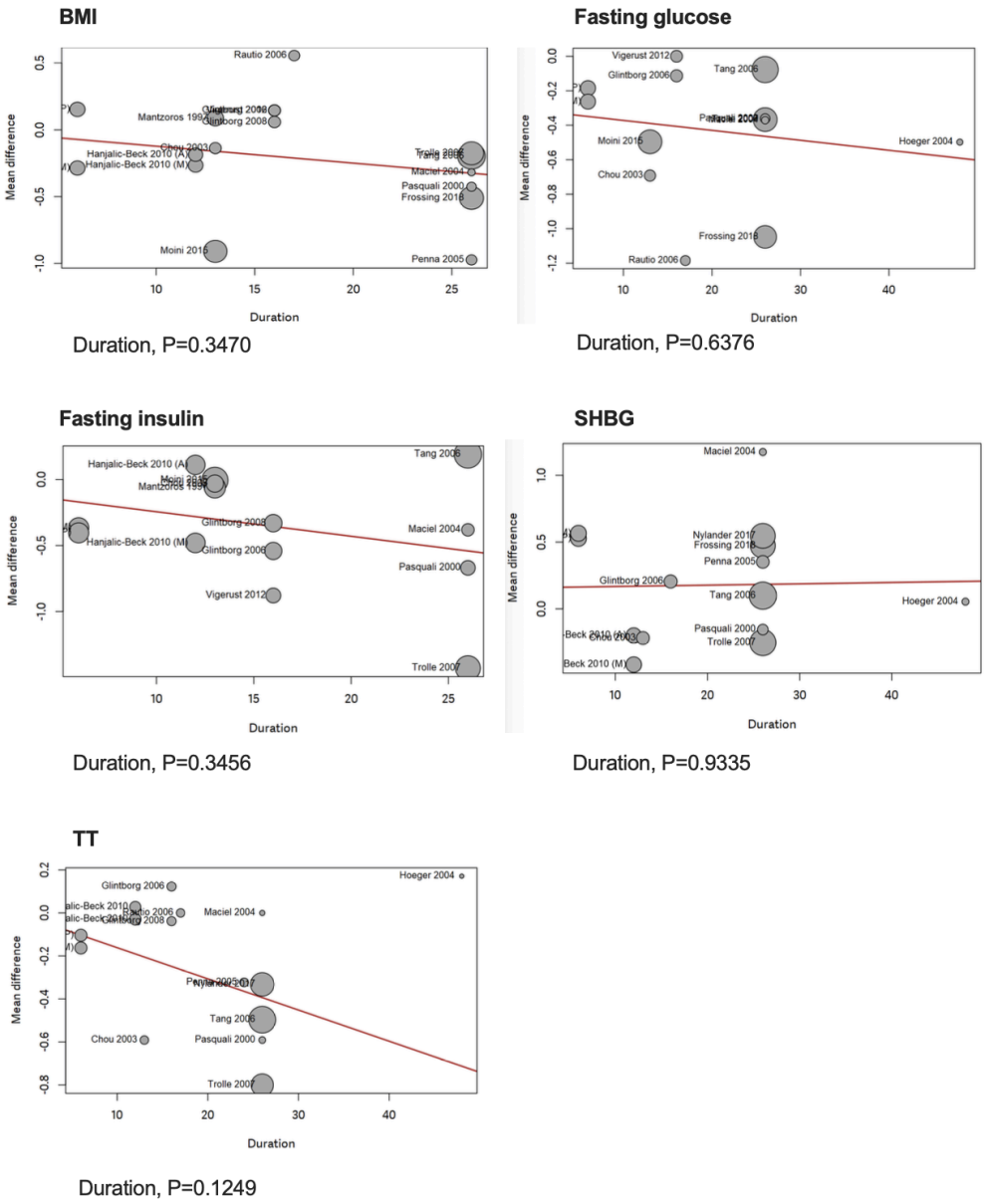


Figure *2.4* Bubble plots with a fitted meta-regression line of the relationship between mean difference (between experimental and control groups) and the duration of treatment.

2.3 Results

The search yielded 34 RCTs conducted in women with PCOS and overweight or obesity (Figure 2.1). After removing duplicates and excluding studies that did not meet the PICO (Patient, Intervention, Comparison, Outcome) or had inconsistent results, the MA and MR included 18 studies that met the eligibility criteria. All studies included information on the criteria used to diagnose PCOS, sample size, intervention and dosing schedule, baseline BMI, and treatment duration. The length of treatment varied between studies, but this did not have a significant effect on the overall results for BMI, fasting glucose, fasting insulin, SHBG or TT (figure 2.4).

Primary outcomes

Metformin significantly reduced BMI (SMD -0.22; CI -0.43 to -0.02) with low heterogeneity between studies (I^2 = 0%). One RCT on liraglutide and one on orlistat also significantly reduced BMI. Neither acarbose nor thiazolidinediones had a significant effect on BMI (figure 2.5), although use of thiazolidinediones tended towards an increase in BMI.

Similar results were seen for fasting blood glucose (figure 2.6) with metformin causing a significant reduction in fasting blood glucose (SMD -0.28; CI -0.50 to -0.06); this effect was also seen in the single studies for liraglutide and orlistat. Thiazolidinediones did not have a significant effect on fasting blood glucose. Although menstrual frequency was a pre-defined primary outcome, no relevant data was identified in the literature. An RCT comparing liraglutide to placebo demonstrated that the use of liraglutide resulted in a significant increase in the number of menstrual bleeds measured using bleeding diaries during the study period[218]; there was n o significant reduction in anti-Mullerian hormone

concentrations[218]. An RCT comparing metformin to placebo demonstrated a significant increase in menstrual frequency in both groups, but no significant differences between groups[147].

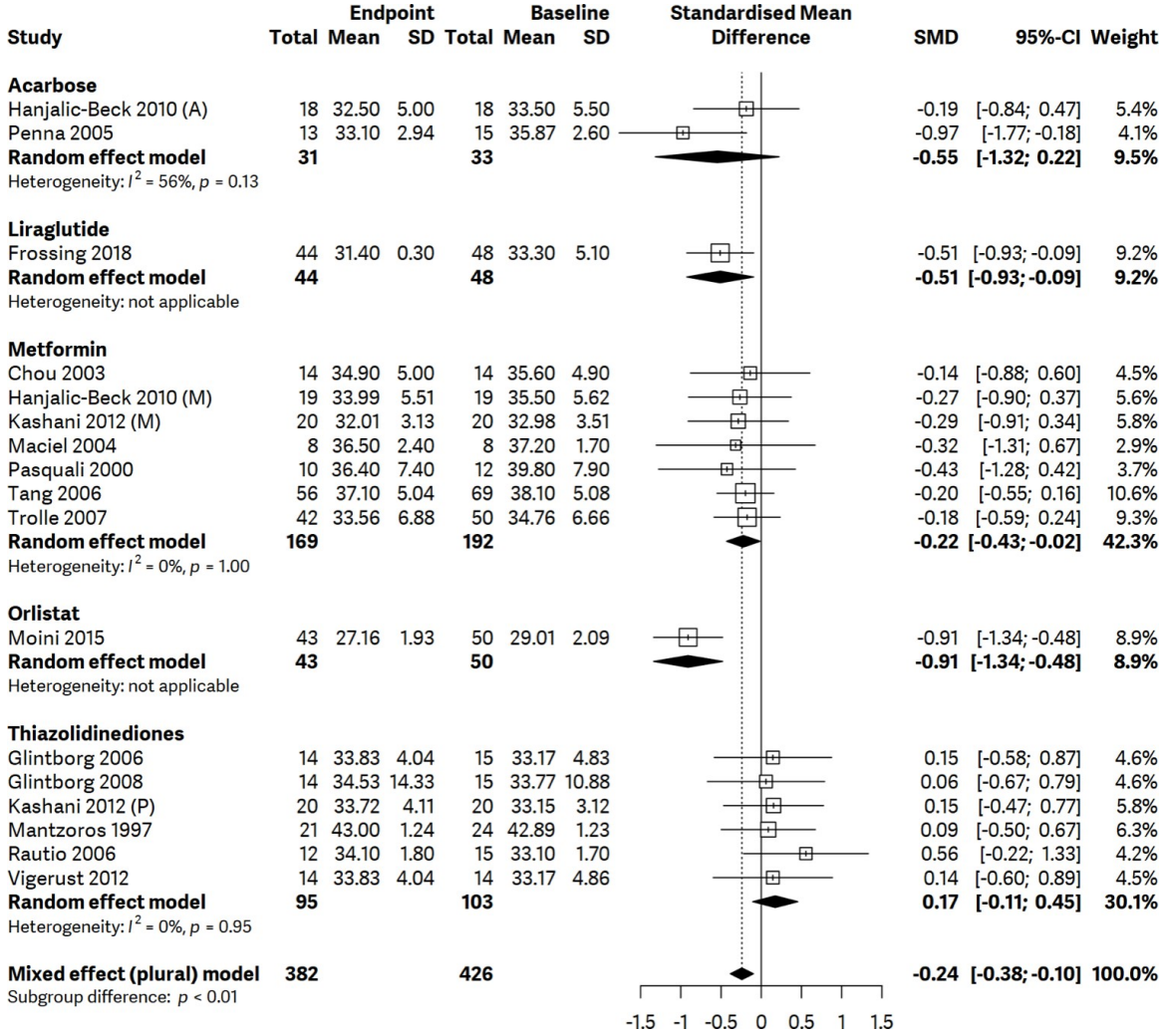


Figure 2.5 Forrest plot comparing BMI outcomes between insulin sensitiser therapies

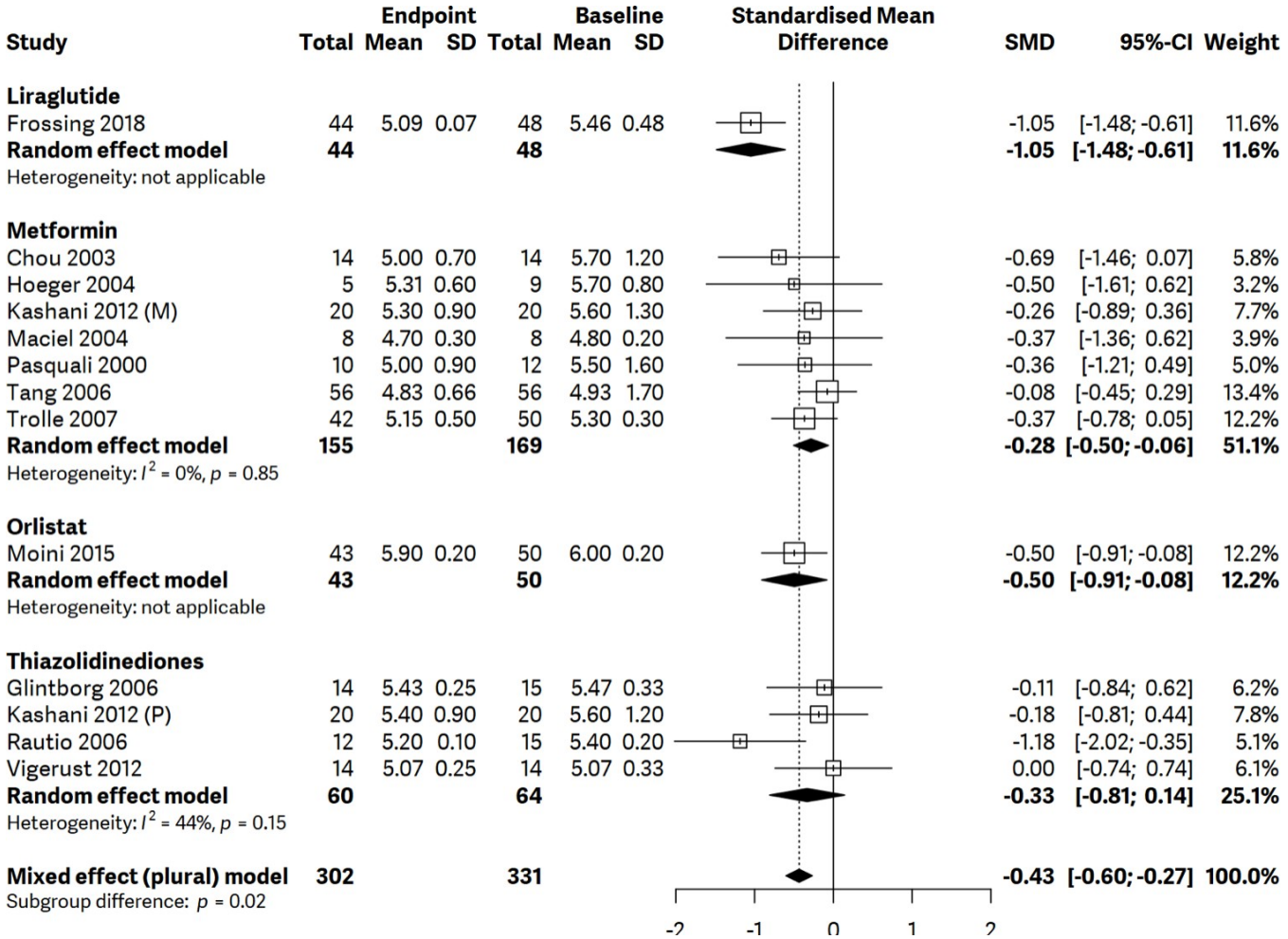


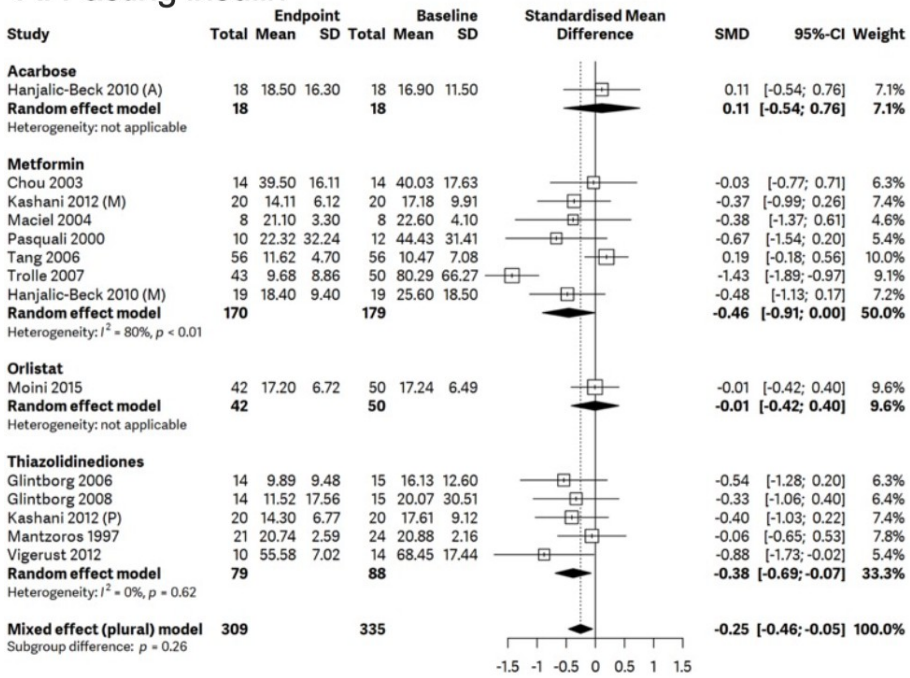
Figure 2.6 Forrest plot comparing fasting blood glucose outcomes between insulin sensitiser therapies

Secondary outcomes

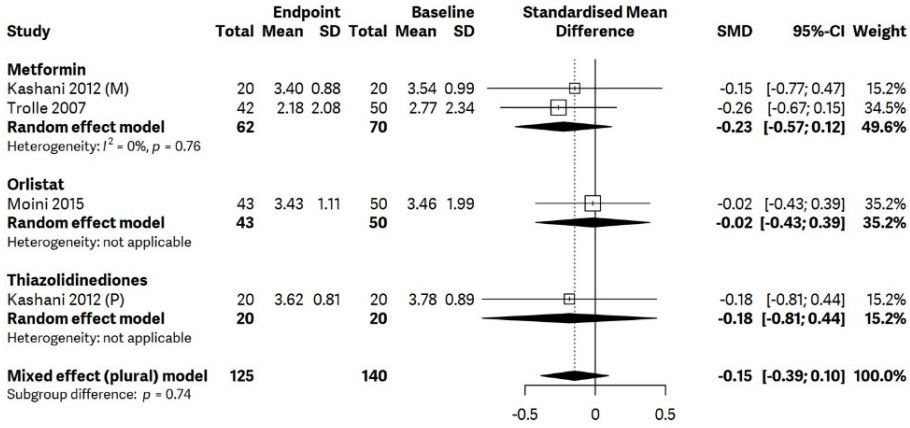
Metformin (SMD -0.46; CI -0.91 to 0.00) and thiazolidinediones (SMD -0.38; CI -0.69 to -0.07) both reduced fasting insulin levels significantly (figure 2.7A). There was, however, significant heterogeneity (I2 = 80%) among the metformin studies. Acarbose and orlistat did not show a significant effect on fasting insulin in single studies. Metformin, orlistat, and thiazolidinediones had no effect on HOMA-IR (SMD -0.15; CI -0.39 to 0.10). (figure 2.7B). There was no information available for the other medications studied. SHBG was significantly increased by liraglutide (SMD 0.51; CI 0.22 to 0.80); acarbose, metformin, or thiazolidinediones may cause an increase in SHBG (figure 2.7C). Only metformin reduced total testosterone significantly (SMD -0.42; CI -0.66 to -0.18). (figure 2.7D). Acarbose, liraglutide, and thiazolidinediones had no effect on total testosterone concentrations. The Ferriman-Gallwey hirsutism score was not significantly reduced by acarbose, metformin, or

thiazolidinediones (figure 2.7E). There was no information available about AMH levels.

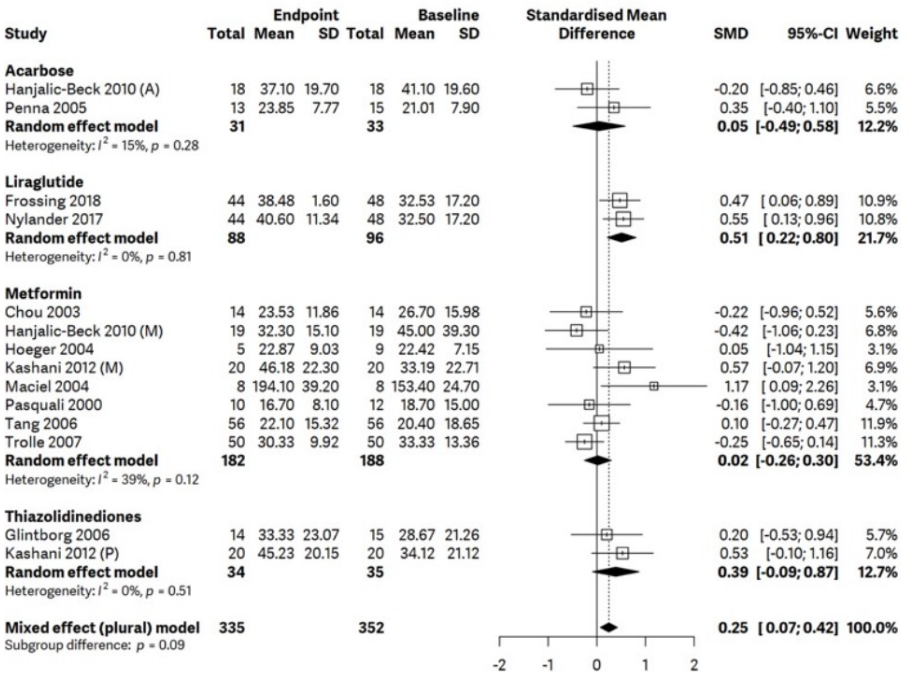
A. Fasting insulin



B. HOMA-IR



C. Sex hormone binding globulin



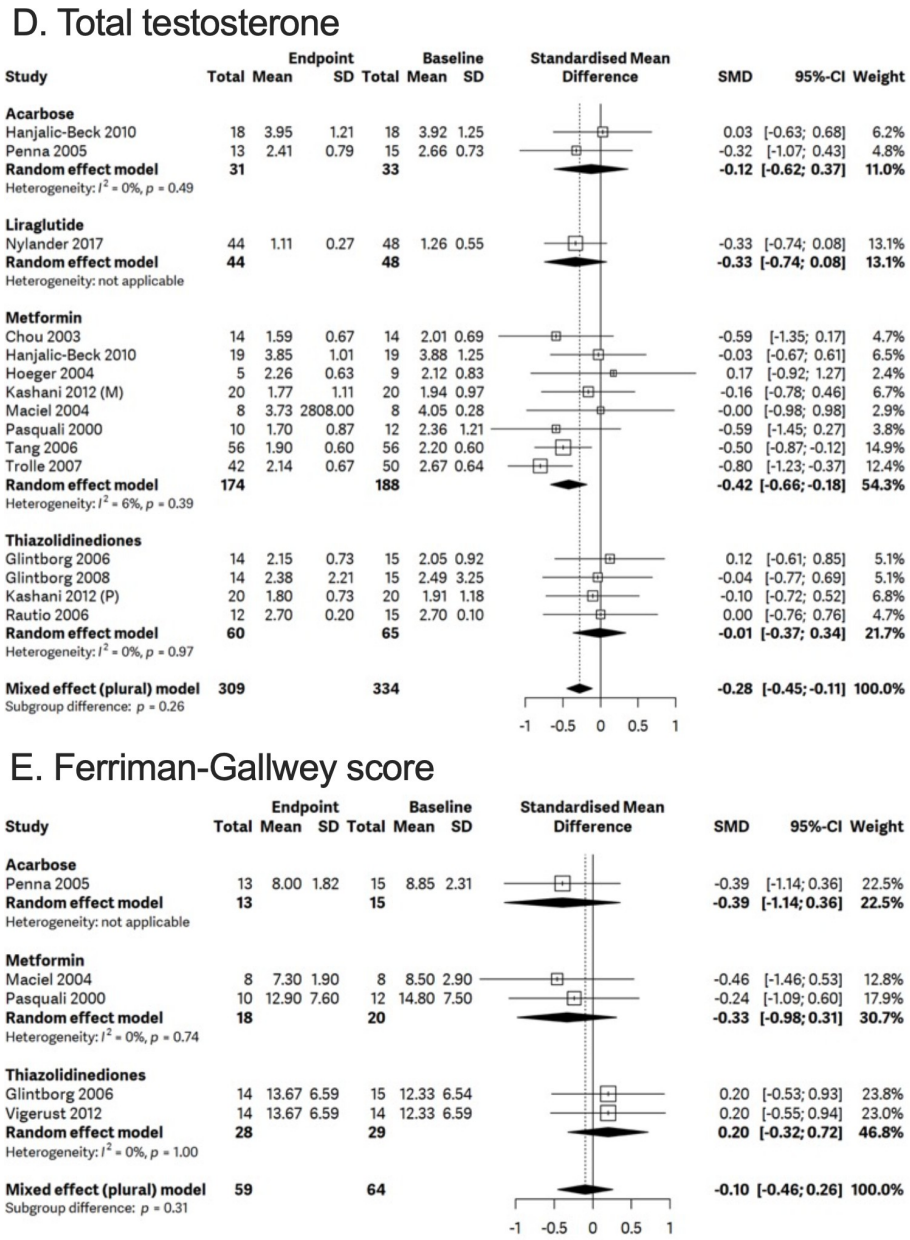


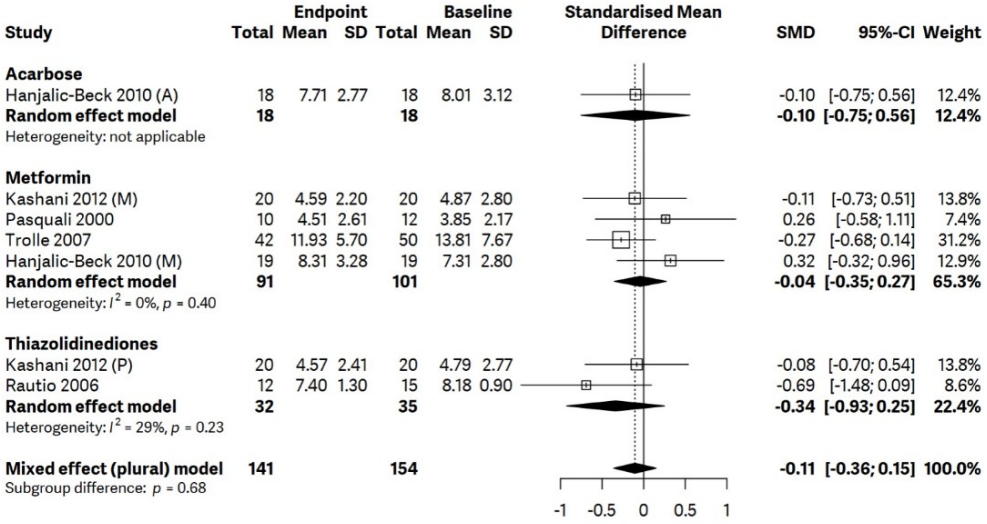
Figure 2.7 Forrest plots comparing secondary outcomes between insulin sensitiser therapies

A. Fasting insulin, B. HOMA-IR, C. Sex hormone binding globulin, D. Total testosterone, E. Ferriman-Gallwey score

Other clinically significant outcomes

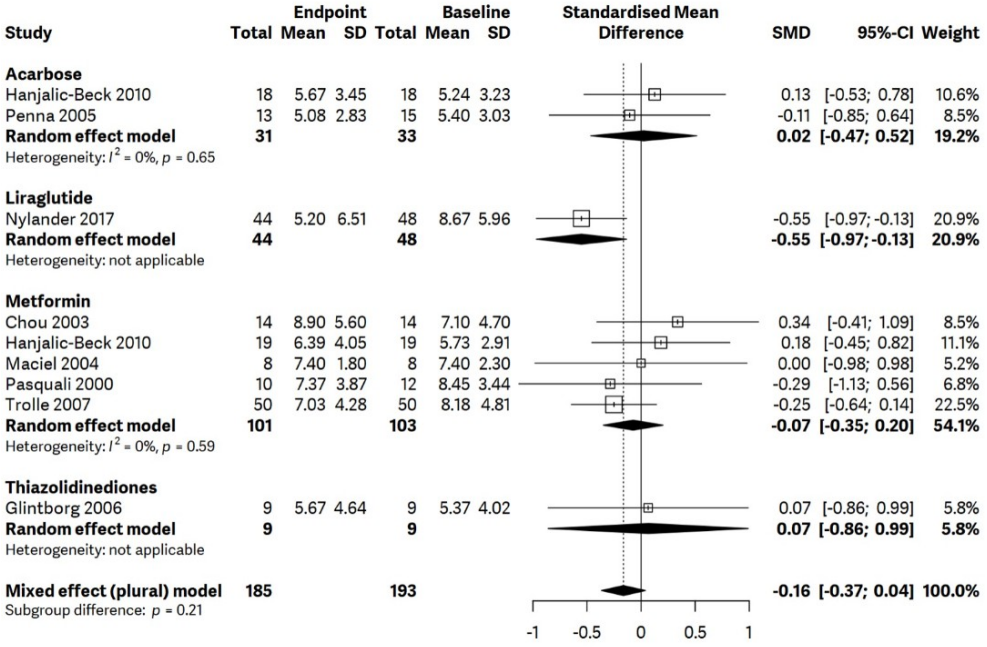
Acarbose, metformin, and thiazolidinediones did not affect DHEAS (SMD -0.11; CI -0.36 to 0.15). (figure 2.8A). A single liraglutide RCT revealed a significant reduction in LH (SMD -0.55; CI -0.97 to -0.13) (figure 2.8B); there was no significant reduction in FSH (SMD 0.02; CI -0.21 to 0.25) (figure 2.8C). There were no pregnancy statistics available.

A. DHEAS



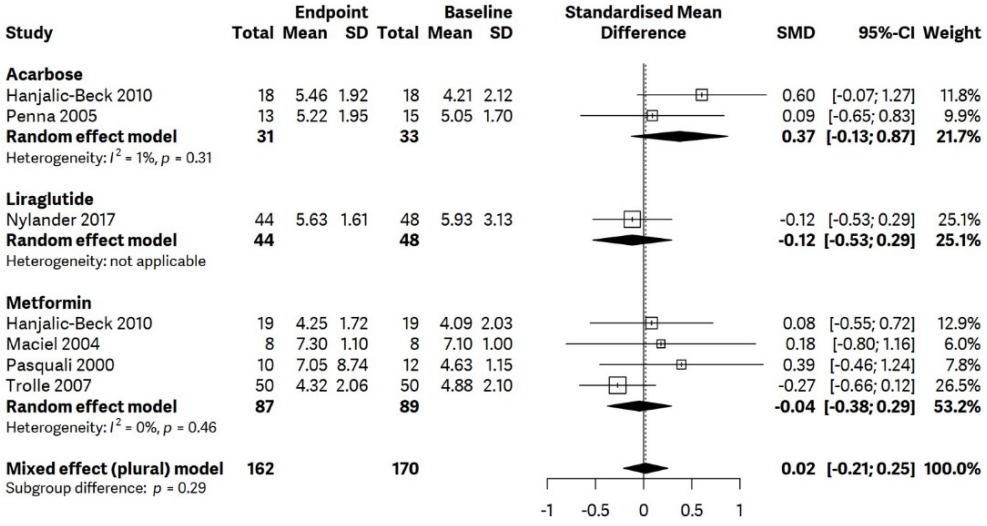
Study	Endpoint Total	Mean	SD	Baseline Total	Mean	SD	SMD	95%-CI	Weight
Acarbose									
Hanjalic-Beck 2010 (A)	18	7.71	2.77	18	8.01	3.12	-0.10	[-0.75; 0.56]	12.4%
Random effect model	**18**			**18**			**-0.10**	**[-0.75; 0.56]**	**12.4%**
Heterogeneity: not applicable									
Metformin									
Kashani 2012 (M)	20	4.59	2.20	20	4.87	2.80	-0.11	[-0.73; 0.51]	13.8%
Pasquali 2000	10	4.51	2.61	12	3.85	2.17	0.26	[-0.58; 1.11]	7.4%
Trolle 2007	42	11.93	5.70	50	13.81	7.67	-0.27	[-0.68; 0.14]	31.2%
Hanjalic-Beck 2010 (M)	19	8.31	3.28	19	7.31	2.80	0.32	[-0.32; 0.96]	12.9%
Random effect model	**91**			**101**			**-0.04**	**[-0.35; 0.27]**	**65.3%**
Heterogeneity: I^2 = 0%, p = 0.40									
Thiazolidinediones									
Kashani 2012 (P)	20	4.57	2.41	20	4.79	2.77	-0.08	[-0.70; 0.54]	13.8%
Rautio 2006	12	7.40	1.30	15	8.18	0.90	-0.69	[-1.48; 0.09]	8.6%
Random effect model	**32**			**35**			**-0.34**	**[-0.93; 0.25]**	**22.4%**
Heterogeneity: I^2 = 29%, p = 0.23									
Mixed effect (plural) model	**141**			**154**			**-0.11**	**[-0.36; 0.15]**	**100.0%**
Subgroup difference: p = 0.68									

B. Luteinising hormone



Study	Endpoint Total	Mean	SD	Baseline Total	Mean	SD	SMD	95%-CI	Weight
Acarbose									
Hanjalic-Beck 2010	18	5.67	3.45	18	5.24	3.23	0.13	[-0.53; 0.78]	10.6%
Penna 2005	13	5.08	2.83	15	5.40	3.03	-0.11	[-0.85; 0.64]	8.5%
Random effect model	**31**			**33**			**0.02**	**[-0.47; 0.52]**	**19.2%**
Heterogeneity: I^2 = 0%, p = 0.65									
Liraglutide									
Nylander 2017	44	5.20	6.51	48	8.67	5.96	-0.55	[-0.97; -0.13]	20.9%
Random effect model	**44**			**48**			**-0.55**	**[-0.97; -0.13]**	**20.9%**
Heterogeneity: not applicable									
Metformin									
Chou 2003	14	8.90	5.60	14	7.10	4.70	0.34	[-0.41; 1.09]	8.5%
Hanjalic-Beck 2010	19	6.39	4.05	19	5.73	2.91	0.18	[-0.45; 0.82]	11.1%
Maciel 2004	8	7.40	1.80	8	7.40	2.30	0.00	[-0.98; 0.98]	5.2%
Pasquali 2000	10	7.37	3.87	12	8.45	3.44	-0.29	[-1.13; 0.56]	6.8%
Trolle 2007	50	7.03	4.28	50	8.18	4.81	-0.25	[-0.64; 0.14]	22.5%
Random effect model	**101**			**103**			**-0.07**	**[-0.35; 0.20]**	**54.1%**
Heterogeneity: I^2 = 0%, p = 0.59									
Thiazolidinediones									
Glintborg 2006	9	5.67	4.64	9	5.37	4.02	0.07	[-0.86; 0.99]	5.8%
Random effect model	**9**			**9**			**0.07**	**[-0.86; 0.99]**	**5.8%**
Heterogeneity: not applicable									
Mixed effect (plural) model	**185**			**193**			**-0.16**	**[-0.37; 0.04]**	**100.0%**
Subgroup difference: p = 0.21									

C. Follicle-stimulating hormone



Study	Endpoint Total	Mean	SD	Baseline Total	Mean	SD	SMD	95%-CI	Weight
Acarbose									
Hanjalic-Beck 2010	18	5.46	1.92	18	4.21	2.12	0.60	[-0.07; 1.27]	11.8%
Penna 2005	13	5.22	1.95	15	5.05	1.70	0.09	[-0.65; 0.83]	9.9%
Random effect model	**31**			**33**			**0.37**	**[-0.13; 0.87]**	**21.7%**
Heterogeneity: I^2 = 1%, p = 0.31									
Liraglutide									
Nylander 2017	44	5.63	1.61	48	5.93	3.13	-0.12	[-0.53; 0.29]	25.1%
Random effect model	**44**			**48**			**-0.12**	**[-0.53; 0.29]**	**25.1%**
Heterogeneity: not applicable									
Metformin									
Hanjalic-Beck 2010	19	4.25	1.72	19	4.09	2.03	0.08	[-0.55; 0.72]	12.9%
Maciel 2004	8	7.30	1.10	8	7.10	1.00	0.18	[-0.80; 1.16]	6.0%
Pasquali 2000	10	7.05	8.74	12	4.63	1.15	0.39	[-0.46; 1.24]	7.8%
Trolle 2007	50	4.32	2.06	50	4.88	2.10	-0.27	[-0.66; 0.12]	26.5%
Random effect model	**87**			**89**			**-0.04**	**[-0.38; 0.29]**	**53.2%**
Heterogeneity: I^2 = 0%, p = 0.46									
Mixed effect (plural) model	**162**			**170**			**0.02**	**[-0.21; 0.25]**	**100.0%**
Subgroup difference: p = 0.29									

Figure 2.8 Forrest plot comparing other clinically significant outcomes between insulin sensitiser therapies

A. DHEAS, B. Luteinising hormones, C. Follicle-stimulating hormone

Overall, the use of an insulin sensitiser in women with PCOS who have overweight or obesity caused a significant improvement in metabolic outcomes BMI (SMD -0.24; CI -0.11 to -0.10), fasting blood glucose (SMD -0.43; CI -0.60 to -0.27), fasting insulin (SMD -0.25; CI -0.46 to -0.05) as well as some elements of the reproductive profile, SHBG (SMD 0.25; CI 0.07 to 0.42) and total testosterone (SMD -0.28; CI -0.45 to -0.11).

2.4 Discussion

The significant effect of metformin on most metabolic outcomes and the reproductive profile is one of the key findings of this systematic review, meta-analysis, and meta-regression. Thiazolidinediones reduced fasting insulin levels as expected. The lack of data for hard reproductive outcomes such as menstrual frequency and pregnancy was an unexpected finding.

This study concentrated on pharmacotherapy, which has been shown to reduce insulin resistance either directly or indirectly, such as through weight loss. Metformin is the most used drug to treat PCOS's metabolic and reproductive symptoms because it is safe, widely available, and inexpensive. The International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018[145] reviewed RCTs assessing metformin and its impact on metabolic and reproductive outcomes and discovered that metformin causes a significant reduction in BMI, plasma testosterone, and cholesterol when compared to placebo. Metformin resulted in a significant decrease in BMI, fasting glucose, and insulin without affecting any other metabolic or reproductive outcomes in the RCTs we

included. However, there was significant heterogeneity among studies for fasting insulin, so the findings should be interpreted with caution. While fasting glucose and insulin levels were significantly lower, they may not have been low enough to result in a significant decrease in HOMA-IR.

In 2020, a network meta-analysis of fourteen trials involving 619 women found that combination therapy with metformin and GLP-1 receptor agonists (RA) or metformin and thiazolidinediones was superior to metformin monotherapy in terms of improving hyperandrogenism[225]. When compared to GLP-1 RA alone, combination therapy with metformin and GLP-1 RA improved fasting glucose. The authors discovered that pioglitazone and rosiglitazone were less effective than metformin in lowering fasting blood glucose levels, which is consistent with our findings. In contrast to the findings of this network meta-analysis, we discovered that thiazolidinediones cause a significant reduction in fasting insulin. This is consistent with its mechanism of action, which is to increase peripheral insulin sensitivity[226].

Although the use of thiazolidinediones resulted in a significant reduction in fasting blood glucose, there was no significant reduction in fasting insulin. This could be because the RCTs included in this meta-analysis had short treatment durations. There wasn't a significant increase in BMI with thiazolidinediones[226], which could be attributed to the brief duration of treatment. Pioglitazone has produced mixed results in clinical trials, with some studies observing significant weight gain[227, 228] and others observing moderate weight gain[229, 230]. Rosiglitazone and troglitazone are no longer used because they have been linked to an increased risk of cardiovascular events and liver toxicity, respectively. They were, however,

included in this systematic review and meta-analysis to determine the efficacy of PPAR-agonism as a mechanism of action in PCOS women.

Trials of GLP-1 RA therapy in women with PCOS and obesity revealed that both liraglutide and exenatide are effective in weight loss as monotherapy or in combination with metformin[178-180] which is consistent with these findings. In women with PCOS and obesity, exenatide treatment significantly improved first-phase insulin responses to oral glucose administration[178, 180]. When exenatide alone or in combination with metformin was studied, both monotherapy and combination therapy significantly reduced BMI and HOMA-IR. Menstrual frequency, testosterone, and the free androgen index were all increased by monotherapy and combination therapy[231]. A network meta-analysis comparing liraglutide, liraglutide and metformin, and metformin and orlistat in women with PCOS and overweight or obesity discovered that liraglutide alone resulted in the greatest reduction in body weight[232]. Combination therapy with liraglutide and metformin also resulted in a significant reduction in these two outcomes, albeit at a lower level, possibly due to a lower liraglutide dose in the combination treatment[232]. The significant increase in SHBG observed in our meta-analysis with liraglutide is most likely due to increased insulin sensitivity with weight loss. Despite the increase in SHBG, there wasn’t a significant reduction in total testosterone, likely due to only one study's inclusion. A randomised, double-blind, placebo-controlled trial comparing liraglutide 3 mg to placebo for reduction of body weight and hyperandrogenism in women with PCOS and obesity found that liraglutide was superior to placebo[233]. This RCT was not included as it was published after the study concluded.

Orlistat causes only minor weight loss and has no systemic side effects[225]. The results of a single RCT on orlistat demonstrated a significant reduction in fasting blood glucose and BMI. This finding is consistent with previous meta-analyses which reported that orlistat reduces anthropometric and metabolic outcomes such as BMI and HOMA-IR as well as testosterone[234] in women with PCOS[232, 234]. Orlistat and metformin had similar positive effects on BMI, insulin resistance, insulin, and testosterone[183].

Acarbose is an alpha-glucosidase inhibitor that is now rarely used to treat type 2 diabetes. It works by delaying glucose absorption in the intestine, which reduces postprandial insulin secretion[220] and increases GLP-1 secretion[235]. Large amounts of undigested carbohydrates reach the distal small intestine in the presence of acarbose, where a high density of L-cells produce GLP-1[236]. A study of chronic acarbose therapy in newly diagnosed T2D patients revealed a significant increase in both fasting and postprandial active GLP-1[236]. In the context of PCOS, this mechanism of action is critical. Following carbohydrate consumption, the rise in blood glucose causes insulin secretion, which over time, worsens insulin resistance, exacerbating the pathology of PCOS. As acarbose lowers postprandial plasma glucose and insulin concentrations, it may be beneficial in PCOS[236]. A potential weight loss is likely due to decreased insulin secretion and increased GLP-1 secretion.

A meta-analysis discovered that acarbose caused a significant decrease in total testosterone, despite significant heterogeneity between studies in the meta-analysis[237]. There was no significant reduction in BMI in that meta-analysis. An RCT published in 2005 found that a low dose of acarbose reduced free androgen index and BMI while increasing SHBG, which was

accompanied by improvement in clinical hyperandrogenism as measured by the Ferriman-Gallwey scale[220]. The findings presented here differ from previous research, most likely due to the small number of acarbose double-blind RCTs included in this study.

Strengths

The main strength of this systematic review, meta-analysis and meta-regression is many studies included overall. Only double-blind RCTs were included in this study to reduce risk of bias and increase confidence in the findings. Other strengths include performing a meta-regression (Egger's test), using internationally accepted diagnostic criteria for PCOS in the study population, and having low heterogeneity between studies. Previously published meta-analyses on the metabolic and reproductive effects of pharmacotherapy on women with PCOS and overweight or obesity included both non-randomised studies and RCTs and focused on specific types of commonly used pharmacotherapies such as metformin, orlistat, and GLP-1 receptor agonists. This is the first study to include all insulin sensitiser pharmacotherapy, both direct and indirect.

Limitations

The main limitation was the inclusion of a small number of studies for GLP-1 receptor agonists, orlistat, and acarbose. Although several other studies on the use of GLP-1 RA in women with PCOS who have overweight or obesity have been conducted, most of them were either open-label or single-blind and thus of lower methodological quality. Another unexpected limitation was the lack of data for hard reproductive outcomes such as ovulation and pregnancy.

In conclusion, while lifestyle modification is the recommended first-line treatment for women with PCOS who are overweight or obese, the use of insulin sensitizer pharmacotherapy has been shown to improve metabolic outcomes and, to a lesser extent, reproductive hormonal profile. More research is needed to evaluate the impact of modern obesity pharmacotherapy on hard outcomes like menstrual cyclicity and fertility in women with PCOS who are overweight or obese.

Future work

Conducting a systematic review and meta-analysis into the effects of insulin sensitiser pharmacotherapy in this group of women but changing the inclusion criteria to include single-blind and open-label studies will likely increase the number of studies for acarbose, orlistat and the GLP-1 RAs. Another option is the inclusion of studies which used combination therapy e.g., metformin and pioglitazone, metformin and a GLP-1RA and comparing this with monotherapy for the same primary and secondary outcomes.

Summary

- Although lifestyle intervention is the first-line treatment, there is a role for insulin sensitiser pharmacotherapy in treating women with PCOS who have overweight or obesity.
- Insulin sensitisers predominantly affect metabolic outcomes and to a lesser extent, some reproductive hormones.
- There is a paucity of data from RCTs pertaining to the effects of insulin sensitiser pharmacotherapy on harder reproductive outcomes such as ovulation, menstrual frequency, and pregnancy rates.

3 Bariatric surgery vs. Medical care for obesity and polycystic ovarian syndrome related infertility: The BAMBINI randomised-controlled clinical trial

PCOS is the most common cause of anovulatory infertility[238] and accounts for more than 75% of cases[239]. Clinical data has shown that women with PCOS are more likely to seek infertility consultations and undergo assisted reproductive technology[56]. In women with PCOS, the total number of pregnancies in their lifetime tends to be lower than in those without PCOS[58]. As previously mentioned, lifestyle intervention with or without pharmacotherapy is considered the first-line treatment for women with PCOS and obesity. However, long-term adherence to specialist dietary advice and a minimum level of physical activity can be difficult to adhere to. Obesity remains the most common medication condition in women of reproductive age[240], and its impact on fertility outcomes e.g. menstrual cyclicity, pregnancy and live-birth rates, has been well documented in the literature[240]. In addition to the reduction in fertility and increase in time taken to conceive, pregnant women with obesity are at increased risk of first-trimester pregnancy loss, congenital foetal malformations, delivery of large for gestational age infants, shoulder dystocia, spontaneous and medically indicated premature birth, and stillbirth[241]. For women living with PCOS and obesity, the metabolic and reproductive implications are two-fold higher.

This is the first randomised clinical trial to assess the effectiveness and safety of bariatric surgery to medical care in women with PCOS and obesity. The effectiveness of surgery will

be assessed using clinical markers of ovulation (serum progesterone, menstrual periods, pregnancy). Although obesity does not form part of the diagnostic criteria for PCOS and is neither necessary nor sufficient for the PCOS phenotype[242], affected women will often have higher visceral adiposity when compared to age and BMI matched controls[243]. This is evidenced by studies looking at women with and without obesity and PCOS versus age and BMI matched controls[244]. Lifestyle interventions which reduced weight by as little as 5% of total body weight have been shown to have health metabolic, reproductive and psychological benefits[137]. Currently, treatment aims to optimise a healthy weight through lifestyle modification and pharmacotherapy. Non-surgical weight loss and metformin are advocated as first-line treatments, but the long-term weight loss from lifestyle modification with caloric restriction is around 5%-10% of initial weight and is often not maintained in the long-term[245].

Hypothesis

Obesity surgery is superior to medical care in increasing the number of ovulatory cycles in women with PCOS, obesity and oligomenorrhoea or amenorrhoea.

Objectives

To perform an RCT comparing the safety and efficacy of standard medical care versus obesity surgery for women with PCOS, obesity and oligomenorrhoea or amenorrhoea.

3.1 Methods

Study design

In this prospective open-label RCT, 80 women with PCOS, obesity and oligomenorrhoea or amenorrhoea were recruited from Imperial College Healthcare NHS Trust and University Hospitals Coventry & Warwickshire NHS Trust. They were eligible for obesity surgery based on NICE CG189[246]. Patients were randomised at a ratio of 1:1, stratified by BMI and trial site to either medical care (n=40) which was a combination of a lifestyle intervention ± pharmacotherapy (metformin, orlistat) *or* bariatric surgery (n=40) which was a standard laparoscopic VSG. I conducted the recruitment, screening, and trial visits for Imperial College Healthcare NHS Trust participants. A dedicated research team conducted recruitment, screening, and trial visits at University Hospital Coventry & Warwickshire NHS Trust. During the trial, we had bi-monthly online meetings with research team members at both sites to discuss any concerns regarding the trial. We hosted online patient support sessions for both groups every six months to discuss concerns and listen to patient feedback.

Sample processing

100 uL aprotinin (Trasylol) was added to the (green) gut hormone collection tubes using a syringe before blood sampling; 20 mcl 0.8M HCl was added to the ghrelin cryovials. During the visit, the green (gut hormone and ghrelin) tubes were placed on ice immediately after collection and centrifuged within 15 minutes. Gold tubes (C-peptide and insulin) were allowed to clot for 30 minutes at room temperature and centrifuged within one hour. Samples were centrifuged at 4 C for 10 minutes at 2590rcf (4000 rpm) and separated into at least 0.5 mL aliquots according to colour-coded cryovials, which were stored in a -20 C day freezer and then moved to a -80 C freezer at the end of the study day. Adipose tissue samples were collected using an aseptic non-touch technique and placed in BRAND tm Bio Cert TM RNase-, DNAse-, DNA- free microcentrifuge tubes and transported to a -80 C freezer for storage immediately.

Sample analysis

Plasma glucose samples were analysed by Imperial College Healthcare NHS Trust pathology laboratory at Hammersmith Hospital using the Abbott Alinity platform with an intra-assay imprecision of <1.9% and an inter-

assay imprecision of <1.2%. Insulin and adipose tissue samples have not been analysed yet.

Lifestyle intervention

This was delivered by a registered bariatric dietician and was structured around:

- Regular eating patterns and review of portion sizes
- Macronutrients
- Mindful eating and reduction of emotional eating
- Goal setting
- Pharmacotherapy – metformin and/or orlistat

In addition, patients were advised to perform at least 30 minutes of moderate-intensity physical activity daily. Although this was actively promoted through weekly group meetings with the dietician for the first eight weeks, followed by monthly sessions for the remainder of the trial, adherence was not formally assessed.

Inclusion Criteria

Pre-menopausal women ≥18 years old were recruited if they had a BMI ≥ 35 kg/m^2 with obesity-related complications and a diagnosis of PCOS based on international evidence-based

guidelines for the assessment and management of polycystic ovary syndrome 2018 that requires two of the following[145]:

i. for women > 3 years post menarche to perimenopause: < 21 or > 35 days or < 8 cycles per year

ii. Hyperandrogenism
    a. clinical hirsutism (Modified Ferriman-Gallwey hirsutism score ≥ 4-6) or male pattern alopecia (positive Ludwig visual score)] or
    b. biochemical (raised free androgen index or free testosterone)

iii. Polycystic ovaries on ultrasound: Using transvaginal ultrasound transducers with a frequency bandwidth that includes 8MHz, the threshold for PCOM should be on either ovary, a follicle number per ovary of > 20 and/or an ovarian volume ≥ 10ml, ensuring no corpora lutea, cysts or dominant follicles are present.

Exclusion Criteria

Women were excluded if they had a past medical history of type 1 or type 2 diabetes mellitus, specific contraindications to obesity surgery, previous obesity surgery, inability to maintain adequate contraception, medications affecting reproductive function (e.g. oral steroids, hormonal contraceptives) at screening or 3 months previously), other causes of anovulation (e.g. untreated hypothyroidism, adrenal or pituitary disorders), current pregnancy or breastfeeding, 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, without access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study.

Adverse Events

An adverse event (AE) was defined as any untoward medical occurrence in a patient or clinical study subject. Serious adverse event (SAE) was defined as any untoward and unexpected medical occurrence or effect that resulted in death, is life-threatening, requires hospitalisation, or prolongation of existing inpatients' hospitalisation, results in persistent or significant disability or incapacity, is a congenital anomaly or birth defect. Participants were asked to use non-hormonal contraception for the duration of the study (intra-uterine device, barrier method, vasectomised partner, abstinence). This was especially important for those in the surgical arm as pregnancy in the first 12 months following obesity surgery should be avoided due to the risk of nutritional deficiencies and other potential complications.

Study Days

An overview of study visits is presented in figure 3.1 below.

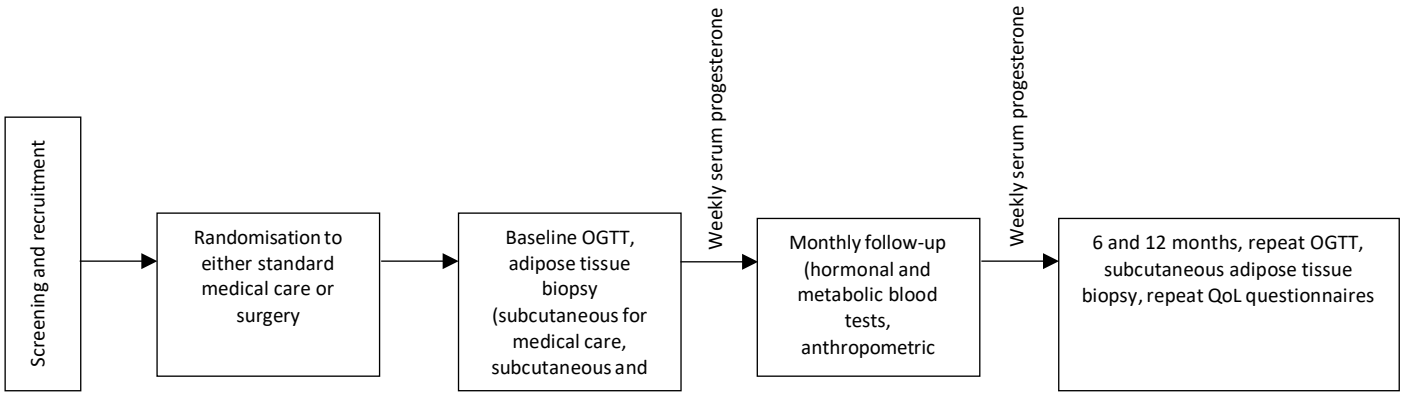


Figure 3.1 Study overview

Study outcome measures

Primary outcome

The number of ovulatory cycles within the 12-month follow-up period. The effectiveness of bariatric surgery on ovulation was assessed using an objective clinical marker of ovulation (serum progesterone measurements) rather than patient-reported measures. Ovulation was defined as a rise in serum progesterone ≥ 16 nmol/L[67].

Secondary outcomes

Change from baseline to 12 months. For each endpoint, temporal changes, mean levels and peak levels will be analysed as appropriate:

- Number of reported menses – participants were asked to document menstrual periods using a phone application. A menstrual bleed was defined as vaginal bleeding lasting 7-9 days as per The International Federation of Gynaecology and Obstetrics (FIGO) systems 2018 revisions[247]
- Anthropometric data (body weight, waist circumference, body composition)
- Metabolic outcomes (plasma lipid concentration, liver function tests, HbA1c)
- Arterial blood pressure
- Reproductive hormones (serum LH, FSH, oestradiol, SHBG, testosterone, free androgen index, DHEAS, androstenedione, AMH)
- Glucose concentrations at the OGTT
- Psychosocial outcomes (Hospital Anxiety and Depression Scale, Multidimensional Health Profile: Health Functioning questionnaire, Social Functioning Questionnaire and PCOS Health-Related Quality of Life scores)
- Clinical hyperandrogenism outcomes (Modified Ferriman-Galwey hirsutism, Ludwig visual, Savin Alopecia Scale scores and Cardiff Acne Disability Index)
- Number of medications

- Adverse events
- Pregnancy rates

Justification of sample size

Based on the available data on the effect size of lifestyle interventions[137] and obesity surgery[248] on ovulation, I estimated that women in the standard medical care group would have a mean of 7 and women in the obesity surgery group a mean of 10 ovulatory cycles in the 12-month follow-up period. With a standard deviation of 3.3 around both means, I would need 33 women in each group to have a 95% power to detect significant differences between the groups at α of 0.05. Therefore 40 patients would need to be recruited into each group to account for a 15-20% drop-out rate based on rates in similar trials have conducted in this field.

3.2 Statistical analysis

This was performed using Prism (GraphPad) 9. For the primary outcome, which was the number of ovulatory cycles at 12 months, a Mann- Whitney test was used as the data was discrete and not normally distributed. All the secondary outcomes were analysed using two-way ANOVAs (or mixed model). To calculate the mean difference and 95% confidence interval (CI) between the groups at 12 months, an unpaired t test was performed.

Participants were given the option to participate in an additional sub-study which involved collection of subcutaneous and omental fat biopsies either intraoperatively (for participants randomised to surgery) or under local anaesthetic in the Clinical Research Facility. Follow-up

abdominal subcutaneous biopsies were performed at 6- and 12-months post-intervention.

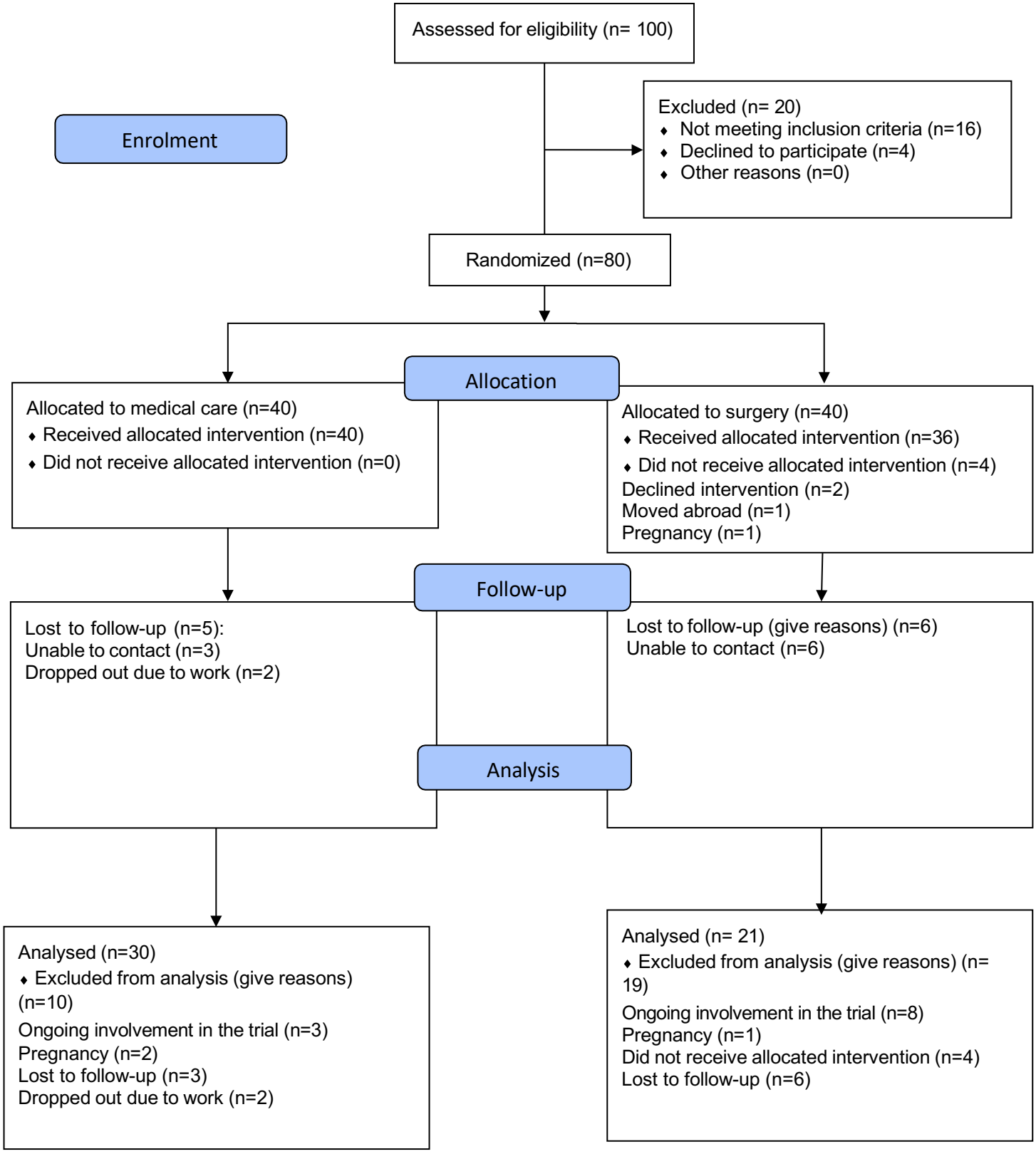


Figure 3.2 Consort 2010 flow diagram

[249]

Baseline characteristics

	Medical care n=40	**Surgery n=40**
Age (years)	31.65 ± 6.2	31.2 ± 5.7
BMI (kg/m^2)	42.6 ± 5.43	45.9 ± 6.04
Weight (kg)	116 ± 18	125 ± 19.5
Waist Circumference (cm)	121 ± 13	126 ± 13
% Body fat	46.6 (44.1 – 50.0)	47.9 (44.5 – 52.4)
Systolic blood pressure (mmHg)	126 ± 12.3	126 ± 11.6
Diastolic blood pressure (mmHg)	79.2 ± 8.91	80.5 ± 8.65
Fasting plasma glucose (mmol/l)	5.1 ± 0.9	5.1 ± 0.4
Fasting insulin (mU/L)	21 (14.1 - 30.5)	21 (14.1 - 30.5)
HbA1c (mmol/mol)	37.3 ± 3.96	37.1 ± 3.57
HOMA-IR	4.296 (2.710 - 6.286)	4.438 (2.955 - 6.059)
Alanine aminotransferase (IU/L)	30.0 (20.8 – 50.8)	29.5 (20 – 44.5)
Alkaline phosphatase (U/L)	74.5 (58.0 – 97.0)	81.0 (67.0 – 100)
HDL cholesterol (mmol/L)	1.10 (1.00 – 1.23)	1.10 (1.00 – 1.30)
LDL cholesterol (mmol/L)	3.18 ± 0.77	3.29 ± 0.79
Triglycerides (mmol/L)TG	1.60 (1.10 – 1.90)	1.30 (1.00 – 1.83)
Sex Hormone Binding Globulin (nmol/L)	23.3 (19.3 – 33.8)	27.0 (20.0 – 34.0)
Testosterone (nmol/L)	1.85 ± 0.58	1.77 ± 0.69
Free Androgen Index	7.95 ± 3.95	7.14 ± 3.81
Dehydroepiandrosterone (umol/L)	6.44 ± 3.11	6.37 ± 3.00
Androstenedione (nmol/L)	6.30 (4.80 – 8.40)	5.80 (2.40 – 4.90)
Anti-Mullerian Hormone (pmol/L)	44 (22.6 - 57.9)	29.4 (15.2 - 42)
Reported menses in the preceding 12 months	3 (2 – 6)	4 (2 – 6)
Ferriman-Gallwey score	13.0 (7.00 – 15.8)	9.00 (7.00 – 13.8)
Depression score	6 (4 – 8)	6 (3.75 – 8.25)
Anxiety score	7.73 ± 3.71	8.03 ± 3.49
Metformin	20	N/A
Orlistat	7	N/A

Table 3.1 Baseline characteristics

Values are presented as mean ± SD, or median and IQR.

3.3 Results

Primary outcome

Number of ovulatory cycles	12 months
Median of Medical care (n=39)	1
Median of Surgery (n=32)	5
P value	P=0.0003

Table 3.2 Number of ovulatory cycles, Medical care vs. Surgery at 12 months

Parameter estimates	Variable	Estimate	95% CI (profile likelihood)	P value
β0 (off metformin)	Intercept	0.288	-0.246 to 0.741	0.250
β1 (on metformin)	0 for none, 1 for MTF [1]	0.900	0.373 to 1.49	0.001

Table 3.3 Number of ovulatory cycles on and off metformin, Medical care at 12 months

Secondary outcomes

	Medical care			Surgery			Medical care – Surgery at 12 months		
	Baseline n=40	**12 months n= 30**	**P value***	**Baseline n=40**	**12 months n= 21**	**P value***	**Mean difference ± SEM**	**95% confidence interval**	**P value†**
BMI (kg/m^2)	42.6 ± 5.43	43.5 ± 6.00	0.49	45.9 ± 6.04	32.5 ± 5.77	**<0.0001**	11.0 ± 1.66	7.65 to 14.3	**<0.0001**
Weight (kg)	116 ± 18	119 ± 18.8	0.57	125 ± 19.5	88.9 ± 17.4	**<0.0001**	29.9 ± 5.11	19.6 to 40.2	**<0.0001**
WC (cm)	121 ± 13	121 ± 13.4	0.98	126 ± 13	104 ± 14.8	**<0.0001**	17.1 ± 4.04	8.98 to 25.2	**0.0001**
% Body fat	46.6 (44.1 – 50.0)	48.2 (45.5 – 51.5)	**0.01**	47.9 (44.5 – 52.4)	36.9 (33.4 – 42.3)	**<0.0001**	10.1 ± 1.76	6.61 to 13.7	**<0.0001**
SBP (mmHg)	126 ± 12.3	126 ± 11.3	0.24	126 ± 11.6	113 ± 11.2	**<0.0001**	8.57 ± 3.24	2.057 to 15.08	**0.011**
DBP (mmHg)	79.2 ± 8.91	78.3 ± 8.11	0.89	80.5 ± 8.65	67.0 ± 9.23	**<0.0001**	11.3 ± 2.44	6.43 to 16.2	**<0.0001**
HbA1c (mmol/mol)	37.3 ± 3.96	38.3 ± 3.85	0.29	37.1 ± 3.57	33.1 ± 2.15	**<0.0001**	5.22 ± 0.879	2.06 to 15.1	**<0.0001**
HOMA-IR	4.296 (2.710 - 6.286)	4.715 (2.130 - 6.720)	0.65	4.438 (2.955 - 6.059)	1.530 (0.96 - 1.72)	0.13	3.77 ± 1.10	1.56 to 5.98	**0.001**
ALT (IU/L)	30.0 (20.8 – 50.8)	23.0 (17.0 – 34.0)	0.35	29.5 (20 – 44.5)	15.0 (12.0 – 20.0)	**0.001**	9.46 ± 5.22	-1.04 to 20.0	0.076
ALP (U/L)	74.5 (58.0 – 97.0)	77.0 (61.0 – 86.5)	0.80	81.0 (67.0 – 100)	64 (56.0 – 78.0)	**0.0004**	9.80 ± 6.96	-4.20 to 23.8	0.166

HDL-C (mmol/L)	1.10 (1.00 – 1.23)	1.22 (1.10 – 1.40)	0.71	1.10 (1.00 – 1.30)	1.29 (1.16 – 1.44)	**0.001**	0.0716 ± 0.164	-0.259 to 0.402	0.665
LDL-C(mmol/L)	3.18 ± 0.77	3.12 ± 0.82	0.82	3.29 ± 0.79	2.95 ± 0.66	**0.01**	0.175 ± 0.213	-0.253 to 0.602	0.416
TG (mmol/L)	1.60 (1.10 – 1.90)	1.54 (1.29 – 1.98)	0.29	1.30 (1.00 – 1.83)	0.97 (0.72 – 1.40)	**0.002**	0.656 ± 0.171	0.313 to 1.00	**0.0004**
SHBG (nmol/L)	23.3 (19.3 – 33.8)	24.9 (16.8 – 34.5)	0.74	27.0 (20.0 – 34.0)	52.5 (43.0 – 62.0)	**<0.0001**	-25.2 ± 4.21	-33.6 to -16.7	**<0.0001**
Testosterone (nmol/L)	1.85 ± 0.58	1.67 ± 0.59	0.09	1.77 ± 0.69	1.16 ± 0.48	**<0.0001**	0.513 ± 0.169	0.172 to 0.853	**0.004**
FAI	7.95 ± 3.95	7.61 ± 4.19	0.58	7.14 ± 3.81	2.65 ± 1.66	**<0.0001**	4.95 ± 1.04	2.86 to 7.05	**<0.0001**
DHEAS (umol/L)	6.44 ± 3.11	5.20 ± 3.11	**0.005**	6.37 ± 3.00	3.97 ± 2.21	**0.005**	1.22 ± 0.612	-0.00775 to 2.45	0.051
Androstenedione (nmol/L)	6.30 (4.80 – 8.40)	5.50 (4.40 – 7.23)	**0.001**	5.80 (2.40 – 4.90)	3.70 (2.40 – 4.90)	**<0.0001**	1.78 ± 0.620	0.532 to 3.03	**0.006**
AMH (pmol/L)	44 (22.6 - 57.9)	36.6 (24.6 – 66.0)	0.93	29.4 (15.2 - 42)	22.0 (9.53 – 34.0)	**<0.0001**	20.6 ± 9.18	1.96 to 39.1	**0.031**
Reported menses (/year)	3 (2 – 6)	5.50 (3.75 – 8)	**0.001**	4 (2 – 6)	8 (7.5 – 10)	**<0.0001**	-2.71 ± 0.754	-4.22 to -1.20	**0.001**
FG score	13.0 (7.00 – 15.8)	10.0 (6.50 – 17.0)	0.55	9.00 (7.00 – 13.8)	9.00 (3.00 – 15.0)	0.87	2.19 ± 1.96	-1.75 to 6.14	0.270

Depression score	6 (4 – 8)	7 (3.75 – 8.25)	0.99	6 (3.75 – 8.25)	2 (0 – 6.50)	**0.01**	2.61 ± 1.06	0.484 to 4.74	**0.017**
Anxiety score	7.73 ± 3.71	7.77 ± 3.32	0.93	8.03 ± 3.49	6.57 ± 3.83	0.20	1.20 ± 1.01	-0.83 to 3.22	0.241

Table 3.4 Baseline and 12-month results for secondary outcomes

Data presented as mean ± SD, or median ± IQR. *P values from Šídák's multiple comparisons test, †P values from a two-tailed unpaired t test

		Plasma Glucose	
		Mixed-effects analysis	
		F (DFn, DFd)	**P value**
Time		F (6, 173) = 34.57	P<0.0001
Follow up		F (1, 29) = 6.733	P=0.015
Time x Follow up		F (6, 173) = 8.109	**P<0.0001**
		Medical care - Surgery **Šídák's multiple comparisons test**	
Time point	Predicted (LS) mean diff.	95% CI of diff.	Adjusted P Value
-30	0.5313	-1.194 to 2.256	0.973
0	0.5058	-1.198 to 2.210	0.978
15	-0.3875	-2.092 to 1.317	0.996
30	0.3067	-1.397 to 2.011	0.999
60	1.429	-0.2755 to 3.133	0.157
120	3.786	2.082 to 5.490	**<0.0001**
180	1.853	0.1484 to 3.557	**0.025**

Table 3.5 Oral Glucose Tolerance Test, Medical care - Surgery at 12 months.

Data presented as mean ± SD

.

Oral Glucose Tolerance Test	**0-180 minutes** **Mixed-effects analysis**		
	P value	**F (DFn, DFd)**	
Treatment (between columns)	0.9672	F (2, 30) = 0.03337	
	Dunnett's multiple comparisons test		
	Mean Diff.	95% CI of diff.	Adjusted P Value
Baseline vs. 6 months	12.18	-103.9 to 128.3	0.955
Baseline vs. 12 months	15.14	-57.95 to 88.22	0.837

Table 3.6 Incremental Area Under the Curve during the oral glucose tolerance test(0-180), Medical care

Data presented as mean

Oral Glucose Tolerance Test	**0-180 minutes Mixed-effects analysis**		
	P value	**F (DFn, DFd)**	
Treatment (between columns)	0.0009	F (1.814, 25.39) = 9.910	
	Dunnett's multiple comparisons test		
	Mean Diff.	95% CI of diff.	Adjusted P Value
Baseline vs. 6 months	214.0	82.73 to 345.3	**0.002**
Baseline vs. 12 months	240.3	66.85 to 413.8	**0.009**

Table 3.7 Incremental Area Under the Curve during the oral glucose tolerance test(0-180), Surgery

Data presented as mean.

Event	**Medical care (n=32)**	**Surgery (n=30)**
Cardiovascular		
Fatal myocardial infarction	0	0
Stroke	0	0
Gastrointestinal		
Bowel obstruction	0	0
Stricture	0	0
Ulcer	0	0
Leak	0	0
Bleeding	0	0
Gastroesophageal reflux disease	0	0
Dumping syndrome	0	0
Gallstone disease	0	0
Urinary		
Nephropathy	0	0
Calculus	0	0
Incontinence	0	0
Neurological and psychiatric		
Memory loss	0	0
Depression	0	0
Nutritional and metabolic		

Intravenous treatment for dehydration		1
Anaemia	2	4
Hypoglycaemic episode	0	0
Severe hypoglycaemia requiring intervention	0	0
Hypoalbuminaemia	0	1
Excessive weight gain	6	0
Infectious		
Wound infection	0	4
Pneumonia	0	0
Sepsis	0	0
Pregnancy	2	1

Table 3.8 Adverse events and pregnancy at 12 months

Excessive weight gain is defined as a 5% increase in body weight over baseline at 12 months.

Pharmacotherapy	**Baseline (n)**	**12 months (n)**
Metformin	20	17
Orlistat	7	2
Both	0	1

Table 3.9 Number of participants on pharmacotherapy, Medical care at baseline and 12 months

Results figures

Primary outcome and reported menses

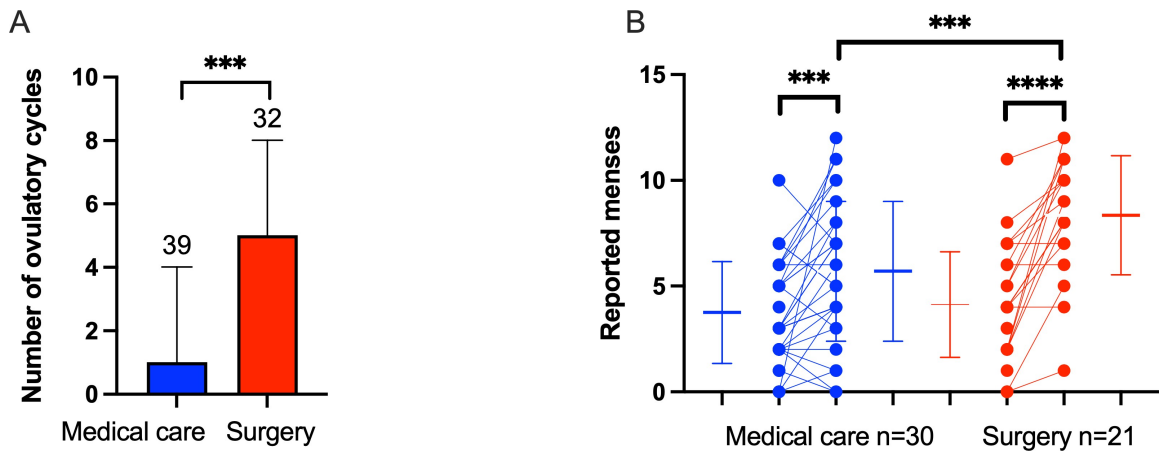


Figure 3.3 Primary outcome and reported menses

A. Number of ovulatory cycles over the 12 months, results are presented as bars with median ± IQR, B. Number of reported menses baseline vs.12 months. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

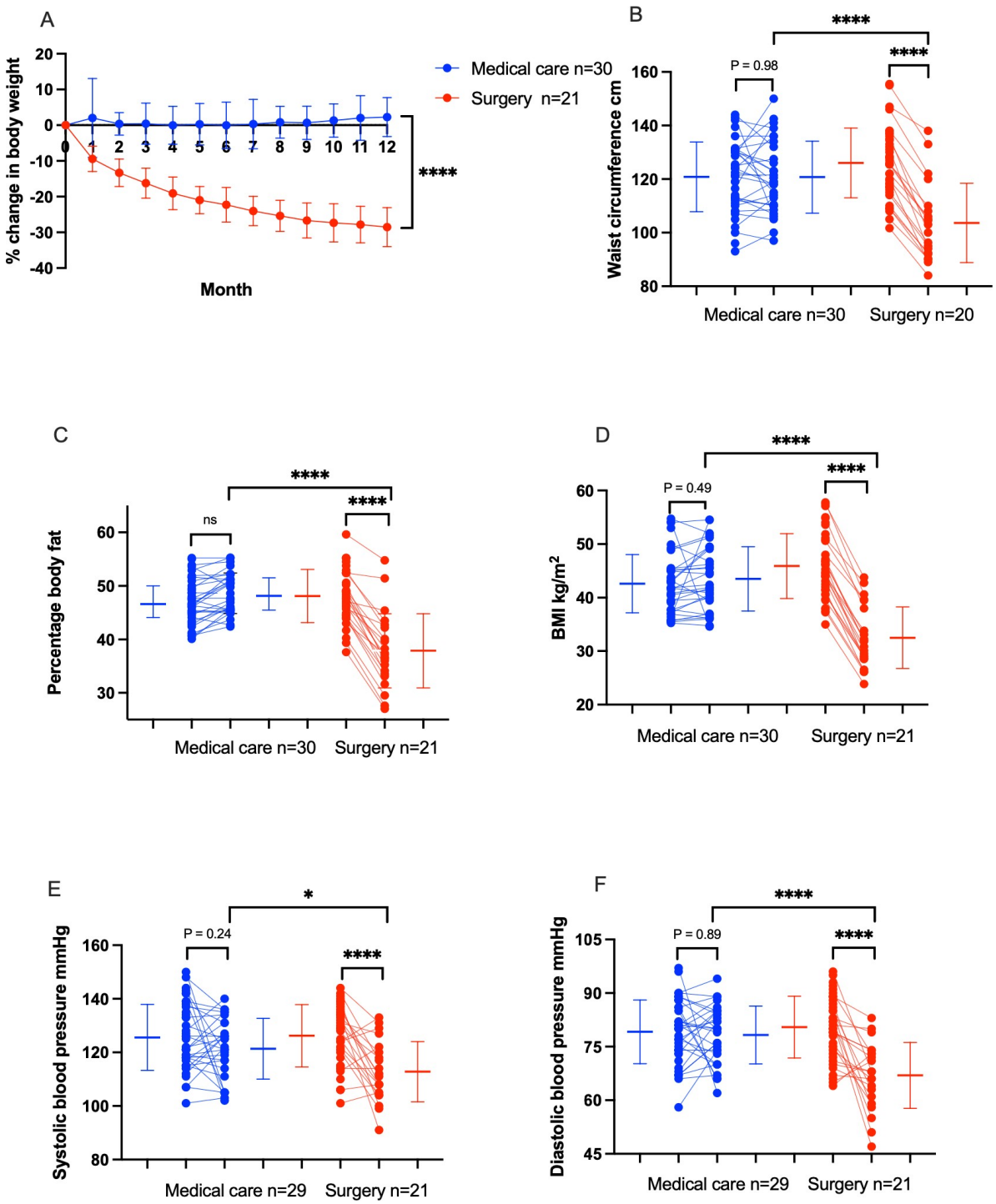


Figure 3.4 Anthropometric data and arterial blood pressure

A. Percentage change in body weight over 12 months, B. Change in waist circumference baseline to 12 months, C. Percentage change in body fat baseline to 12 months, D. Change in BMI baseline to 12 months, E. Change in systolic blood pressure baseline to 12 months, F. Change in diastolic blood pressure baseline to 12 months. Results are presented as before- after (baseline to 12 months) with floating error bars showing mean ± SD or median ± IQR. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

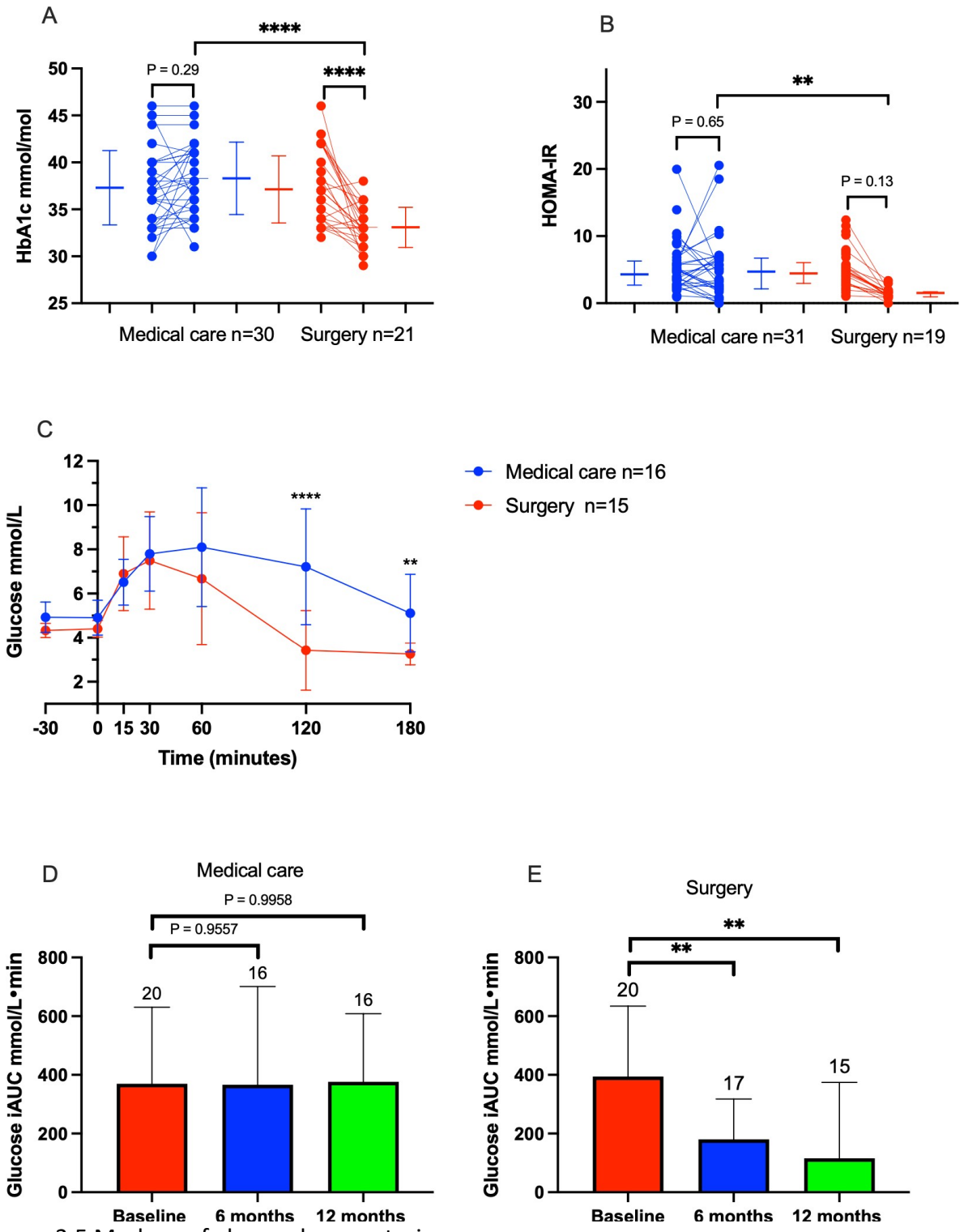


Figure 3.5 Markers of glucose homeostasis

A. Change in HbA1c baseline to 12 months, B. Change in HOMA-IR baseline to 12 months, C. Plasma glucose concentrations during the OGTT at 12 months, D. Incremental $AUC_{(0\text{-}180)}$ of plasma glucose concentrations at the OGTTs for medical care, E. Incremental $AUC_{(0\text{-}180)}$ of

plasma glucose concentrations at the OGTT for surgery. Results are presented as before- after (baseline to 12 months) with floating error bars showing mean ± SD or median ± IQR. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

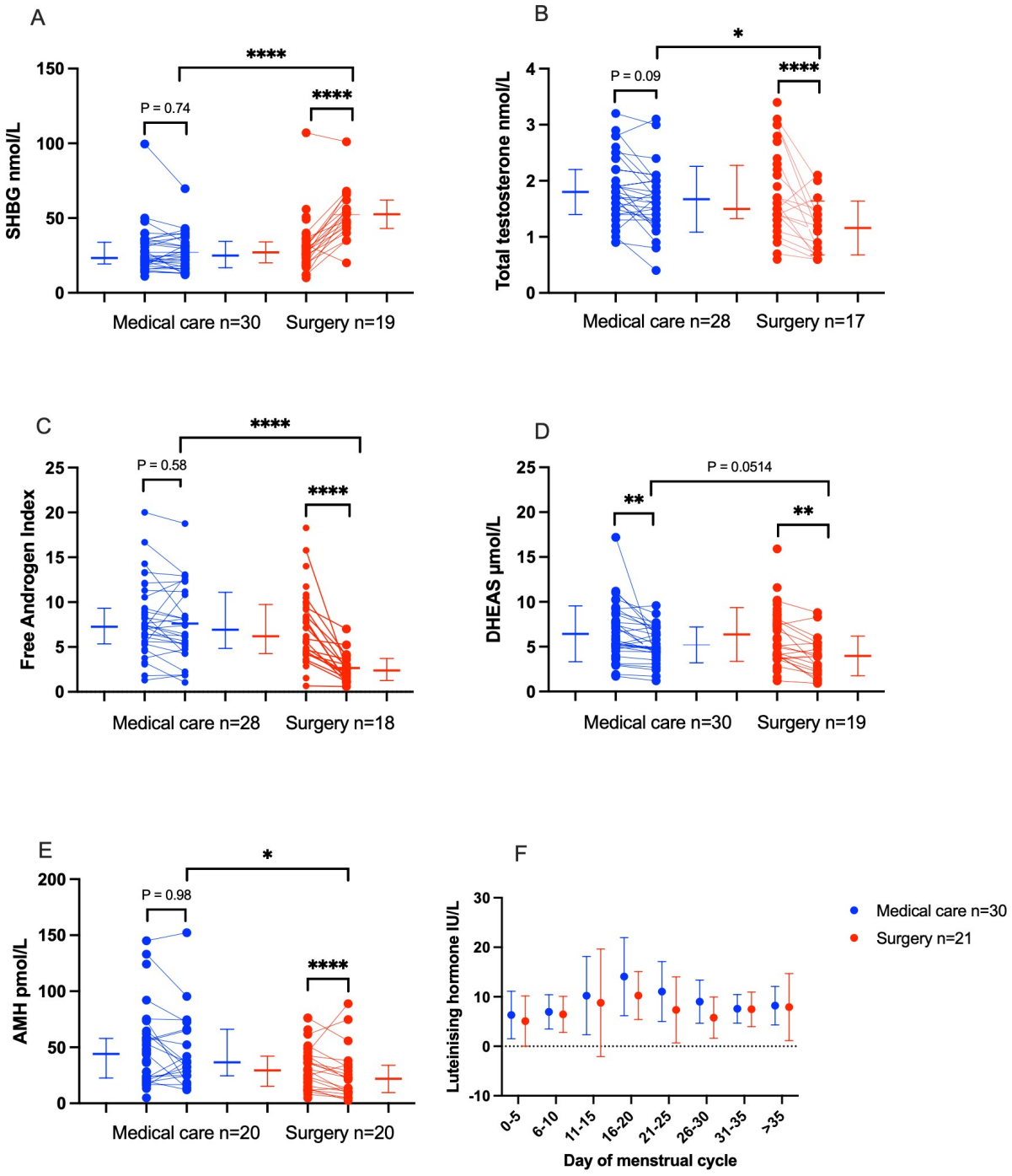


Figure 3.6 Reproductive hormones

A. Change in SHBG baseline to 12 months, B. Change in total testosterone baseline to 12 months, C. Change in Free Androgen Index baseline to 12 months, D. Change in DHEAS baseline to 12 months, E. Change in AMH baseline to 12 months, F. Luteinising hormone levels over the 12 months corresponding to cycle day. Results are presented as before-after (baseline to 12 months) with floating error bars showing mean ± SD or median ± IQR. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

Primary outcome

There was a significantly higher number of ovulatory cycles in the surgery group compared to the medical care group at 12 months (medical care 1 *vs*. 5 surgery group, P=0.0003). There was a statistically significant increase in the number of ovulatory cycles in patients on metformin compared to those not using metformin in the medical care group (P=0.0014).

Anthropometric data and arterial blood pressure

There was a significant difference in BMI, weight, waist circumference and percentage body fat between surgery and medical care (P <0.0001). There was a significant difference in both systolic (P=0.011) and diastolic blood pressure (P <0.0001) between surgery and medical care.

Markers of glucose homeostasis

There was a significant difference in both HbA1c (P <0.0001) and HOMA-IR (P=0.001) between surgery and medical care. At the 12-month OGTT, there was a significant difference in mean plasma glucose at 120 (P <0.0001) and 180 minutes (P=0.025 between surgery and medical care. There was no significant difference in glucose iAUC during the OGTTs for medical care at 6- or 12 months compared to baseline. There was a significant

difference in glucose IAUC during the OGTTs for surgery at 6- (P=0.002) and 12 months (P=0.009) compared to baseline.

Reproductive hormones

There was a significant difference in SHBG (P <0.0001), testosterone (P=0.004), FAI (P <0.0001), androstenedione (P=0.006) and AMH (P=0.031) between surgery and medical care. There was no significant difference in DHEAS (P=0.051). There was a significant difference in reported menses for both medical care (P=0.001) and surgery (P <0.0001) compared to baseline, between medical care and surgery at 12 months (P=0.001). There was no significant difference in the Ferriman-Gallwey score within or between the groups.

Hospital anxiety and depression score

There was a significant difference in depression scores (P=0.017) but no significant difference in anxiety scores (P=0.241) between medical care and surgery

Liver function tests and lipid profile

There was no significant difference in ALT (P=0.076), ALP (P=0.166), HDL-cholesterol (P=0.665), and LDL-cholesterol (P=0.416) between medical care and surgery. There was a significant difference (P=0.0004) in triglycerides between medical care and surgery.

Adverse events and pregnancy

There were more adverse events in the surgery group (intravenous treatment for dehydration, anaemia and hypoalbuminaemia) compared to the medical care group. One patient from the surgery group required an overnight hospital admission for intravenous

fluids at her 4-month follow-up due to ongoing nausea resulting in reduced oral intake. She did not require further admissions and her symptoms improved over time. Hypoalbuminaemia in the post-operative period was managed with increased dietary protein intake under supervision of the dieticians. For patients with anaemia, this was treated with either oral or intravenous iron replacement depending on the value of the serum haemoglobin. The incidence of post-operative wound infections was higher in the surgery group. Overall, there have been four reported pregnancies – two in the medical care group and two in the surgery group. In the medical care group, there was one pregnancy resulting in a live birth and one termination. In the surgery group, there is one ongoing pregnancy and there has been a termination. Six patients in the medical care group had excessive weight gain at 12 months. Wound infections following laparoscopic VSG in the surgery group were treated with a short course of oral antibiotics. None of the patients required admission for intravenous antibiotics.

The current drop-out rate for the trial (excluding patients removed from the trial due to pregnancy) is 18.75%. During the sample size calculation, recruitment of 40 participants in each group would allow for a 15-20% drop-out rate.

3.4 Discussion

This is the first randomised controlled trial to compare bariatric surgery to standard medical care in women with PCOS and obesity. For the primary outcome at 12 months, women receiving surgery had a significantly higher number of ovulatory cycles than those receiving medical care. Within the groups, however, there was a significant increase in the number of

ovulatory cycles between 3 and 12 months despite no significant weight loss. A potential reason for this could be the use of pharmacotherapy (metformin, orlistat) offered to all participants in the medical care group. Seventeen patients were on metformin and 2 on orlistat at a 12-month follow-up. Multiple linear regression showed a significant albeit slight increase of 0.8995 ovulatory cycles in participants on metformin compared to those not using metformin at 12 months. Published data has shown conflicting results with the use of metformin in women with PCOS and obesity, however, results from my unpublished systematic review and meta-analysis found that metformin had a significant impact on metabolic outcomes and to a lesser degree, reproductive hormones in this group of women. Participant-reported menses were included as a secondary outcome measure. Participants were asked to keep menstrual diaries which they would bring with them to their monthly follow-up. A systematic review of forty-nine smartphone applications for period tracking reported that these apps were user-friendly and can therefore be readily adopted into the routine tracking and management of periods[250]. Although both groups reported a significant increase in the number of reported menses, the number was significantly higher after surgery (P=0.0053).

There is limited data available on the effects of metformin therapy on ovulation and menstrual frequency; a systematic review and meta-analysis of fourteen trials reporting on 619 women reported that metformin might improve menstrual cyclicity and ovulation. Still, more importantly, it may increase the effects of ovulation-inducing agents[225, 251], although data in the literature is conflicting[252]. An RCT of metformin or orlistat for the management of 40 non-PCOS women[253] and 80 women with PCOS and obesity[254] showed no significant difference in ovulation rates between groups. The use of metformin in women with PCOS has been shown to successfully menstrual disturbance[255] and

ovulation rates[256] in women with PCOS. The results are conflicting, and other studies have reported no significant improvement in ovulation rate with metformin in women with PCOS[257, 258]. Patient-reported outcomes are very subjective; therefore, this was not part of the primary outcome. Menstruation occurs when an ovum – released from the ovary is not fertilised, and the uterus sheds its lining.

It is less likely to menstruate without ovulating, and in article for the ACP internist, Dr Ricardo Azziz stated that 'about 30£ of the patient with PCOS by the NIH criteria will actually have regular vaginal bleeding episodes, but they don't ovulate"[259].

However, as in this study, the discrepancy between patient-reported menses (subjective) and a serum progesterone value indicative of ovulation (objective) is most likely due to abnormal uterine bleeding (AUB) or anovulatory bleeding. AUB is non-cyclical uterine bleeding which is irregular[247].

A recently published systematic review and meta-analysis on the effects of metabolic surgery on patients with PCOS, which included fourteen prospective and retrospective case-control studies of 501 patients, reported that "metabolic surgery could reduce the incidence of menstrual abnormalities from 82% to 15%" ($P<0.001$)[260]. For hard fertility outcomes such as pregnancy, three studies reported that 31 out of 32 patients with PCOS successfully conceived following metabolic surgery[156, 165, 168], with Jamal et al[168] demonstrating no pregnancy or postpartum complications. A study by Benito at al found that live birth rates were 81% after surgery[156].

Successful long-term weight loss maintenance is defined as "intentionally losing at least 10% of initial body weight and keeping it off for at least 1 year."[261]. As expected, the percentage change in body weight was more significant after surgery than in medical care

(P<0.0001). Failure of the medical group to lose weight with structured lifestyle intervention delivered by a registered bariatric dietician has previously been reported in the literature.

Lifestyle intervention covers three principal components – diet, exercise, and behavioural therapy. The lifestyle intervention for this study was structured around mindful eating, goal setting and pharmacotherapy.

In addition, patients were advised to perform at least 30 minutes of moderate-intensity physical activity daily. Although this was actively promoted through weekly group meetings with the dietician for the first eight weeks, followed by monthly sessions for the remainder of the trial, adherence was not formally assessed. Elkind adopted a similar approach- Hirsch et al., which also included a calorie deficit of 500-800 kcal/day and showed similar results with mean percentage weight loss of only 1.4 +- 1.09[233]. The effects of lifestyle intervention on weight loss in women with PCOS and obesity varies between studies, but in general, they are ineffective at producing lasting weight loss[262]. The Diabetes Prevention program (DPP) provided evidence of the important health benefits of lifestyle intervention for weight loss; participants who were treated with lifestyle intervention lost around 7 kg at the end of 12 months with subsequent weight regain of around 1 kg a year in the following 3 years[263]. The data from published RCTs between 1974 and 2002, shows that in the short-term, patients can lose around 10 kg of their initial body weight in 30 weeks of treatment with lifestyle modification[264]. Long-term, weight regain following dietary and behavioural interventions for obesity is a significant problem, with patients regaining around 30-35% of their lost weight in the first year after treatment[264]. This weight regain slows down over time, but by 5 years, 50% or more of patients will likely return to their baseline weight[264].

The SOS study involving 4047 patients with obesity who underwent bariatric surgery (n=2010) or conventional treatment (n=2037) reported significant weight loss in the surgical groups (gastric bypass 32%, vertical-banded gastroplasty 25%, gastric-banding 20%)[265].

More relevant to this study, the Swiss Multicentre Bypass or Sleeve Study RCT compared sleeve gastrectomy (SG) and RYGB in patients with obesity; the percentage excess BMI loss for SG at one year was 72.4% vs 76.7% after RYGB[266]. Following SG, 61.5% of patients achieved remission or improvement (15.4%) of their T2D and HTN (remission 62.5%, improvement 25%), which is in keeping with the results presented in this study. Glucose metabolism was significantly higher during the OGTT at the 120- and 180-minute time points following bariatric surgery at both 6 and 12 months. This is due to an improvement in glucose homeostasis following significant weight loss[266]. Measurements such as waist circumference (WC) and waist-hip ratio (WHR) tend to correlate better with body fatness than percentage body fat and are more predictive of adverse metabolic effects[267]. Bariatric surgery tends to significantly reduce WC and WHR, thereby improving the metabolic profile – in particular insulin resistance in these patients[268]. In a cohort study of 211 patients who underwent bariatric surgery, a WC <100 cm was associated with a reversal of insulin resistance[268].

The significant improvement in some reproductive hormones, in particular total testosterone, SHBG and subsequently FAI, which is a more useful marker of biochemical hyperandrogenism has previously been reported in women following bariatric surgery[157, 269]. In a retrospective cross-sectional analysis of 125 premenopausal women with obesity, there was no significant difference in mean age between those with low and high levels; however, the group with low SHBG had a significantly higher BMI and WC than the group with high SHBG[270]. The low SHBG group also had a significantly higher FAI level, and further analyses identified that BMI and FAI were significant, independent predictors of SHBG concentration in premenopausal women[270]. The exact underlying mechanisms are

not fully understood. Still, in male obesity-associated secondary hypogonadism (MOSH), increased leptin levels in obesity were associated with a decrease in testosterone and SHBG levels[271]. The increased androgens frequently seen in PCOS are mainly due to the effects of LH on the ovaries and, to a lesser extent, the elevated response of adrenal steroids, which is sustained through adrenal stimulation of ACTH[272]. Significant weight loss, usually through bariatric surgery, is likely to resolve biochemical hyperandrogenism[167]. AMH which is secreted by the granulose cells of growing follicles is strongly correlated with antral follicle count and is a reliable marker of ovarian reserve[273]. Analysis of AMH levels in women with PCOS (normal and with overweight/obesity) and non-PCOS controls (normal and with overweight/obesity) reported significantly lower levels in women with overweight and obesity compared to normal-weight women[274]. AMH levels were higher in the group classed as “severe” PCOS compared to “ovulatory” PCOS, “mild” PCOS and the control group (respectively)[274]. In contrast, a narrative review of thirteen studies involving women with and without obesity and regular menstrual cycles did not find a consistent impact of obesity on AMH levels in women[275]. At present, the impact of obesity on AMH levels remains unclear. This study showed a significant reduction in the levels at 12 months following surgery compared to medical care, which is in keeping with results from other similar studies[161, 276]. This contrasts with the results reported following non-surgical weight loss intervention with a very low-energy diet which showed no significant change in circulating AMH levels despite significant weight loss[277].

Results from this open-label, RCT have demonstrated the superiority of bariatric surgery over medical care in significantly improving the metabolic, reproductive, and psychosocial outcomes in women with PCOS and obesity.

Strengths

This is the first RCT to directly compare standard medical care to bariatric surgery in the same cohort of patients with PCOS who have obesity. Oligo- or amenorrhoea in the preceding 12 months was part of the inclusion criteria and, therefore, automatically selected for more severe symptoms such as ovulatory dysfunction. The primary outcome - ovulation is a hard marker of reproductive function and was measured objectively using weekly serum progesterone measurements for 52 weeks. There is currently no consensus on how ovulation should be measured in research, and published studies tend to use a combination of patient-reported outcomes, i.e., reported menses, transvaginal ultrasound scans to identify the presence of a dominant follicle and mid-luteal serum progesterone measurements.

Patients in the medical care group benefited from close supervision from a trained bariatric dietician during the 12-month intervention. They were able to access one-to-one support if required during this time. To standardise care between the two sites, VSG was chosen as the surgery of choice for patients in the surgery group.

Limitations

One of the main limitations is the lack of weight loss in the medical care group, which has previously been reported in studies using lifestyle intervention without calorie restriction. Every patient in the medical care group was offered either metformin or orlistat at the beginning of the intervention as would be the case in clinical practice. Uptake was variable within the group with 20 patients starting metformin and 7 starting orlistat. However, at the 12-month follow-up, only 20 patients were still using pharmacotherapy (metformin n=17, orlistat n=2, both n=1). The main reason for discontinuation was gastrointestinal side

effects. Newer weight loss medications such as GLP-1 RAs were not available nationally available for the treatment of obesity without other comorbidities when the trial commenced.

Pregnancy was not defined as an outcome, as this would require longer-term follow-up. There has been a higher-than-expected drop-out rate in the surgery group mainly due to patients being unable to commit to the weekly serum progesterone measurements and monthly clinical follow-up. Drop-out rates in the medical care group were lower but for similar reasons.

In conclusion, this research provides strong evidence for bariatric surgery as a treatment option in women with PCOS and ovulatory dysfunction who have obesity. Further large RCTs of longer duration are needed to assess the impact of bariatric surgery on pregnancy outcomes in this group of women.

Future work

The BAMBINI clinical trial has shown that surgery is superior to standard medical care in increasing the number of ovulatory cycles in women with PCOS who have obesity. Since the trial was initially designed, newer obesity pharmacotherapy has become available. A future trial comparing these newer pharmacotherapy agents to a placebo to measure the effects on ovulation in women with PCOS who have obesity, and oligo-anovulation is important. Currently, obesity surgery is more cost-effective than GLP1-RA, but as more pharmaceutical companies manufacture these medications, the cost should reduce over time. A small proportion of patients will remain who do not lose more than 5% of their body weight with a GLP1-RA and for these patients, surgery will remain the best option. Currently, GLP1-Ras are not licensed for women actively trying to conceive, those who are pregnant or breastfeeding. The SURMOUNT-1 clinical trial demonstrated that Tirzepatide (a novel glucose-dependent insulinotropic polypeptide and GLP-1 RA) led to

significant and sustained reductions in body weight over 72- weeks[278]. It is not currently available for weight loss in the UK.

Summary

- In women with PCOS who have obesity, bariatric surgery led to a significantly higher number of ovulatory cycles when compared with medical care
- Bariatric surgery improved anthropometric, metabolic, and reproductive outcomes
- There was limited weight loss with standard medical care over 12 months
- Further studies are needed to investigate the impact of bariatric surgery on objective markers of reproductive function in women with PCOS who have obesity

Impact of the COVID-19 pandemic

As a result of COVID-19, which was declared a pandemic on 11 March 2020 by the World Health Organisation, Imperial College London suspended all non-urgent research from March 2020 until September 2020. This significantly impacted the BAMBINI clinical trial, as participants did not receive their allocated treatment until the study could resume. Fortunately, participants randomised to standard medical care could begin their 12-month lifestyle intervention online. All non-urgent surgery was postponed during the pandemic's peak and was slow to resume, with patients receiving care based on clinical need. This had a direct impact on the surgical arm of the trial, as our patients were deemed to be stable. Travel restrictions and personal circumstances (e.g., shielding) also affected participant retention. Despite these challenges, the trial continued, and the last patient last visit was conducted in April 2023.

4 A Physiological Study on the Effect of Alpha-MSH (α-MSH) on Glucose tolerance in Health Participants

The central melanocortin system plays a pivotal role in energy homeostasis[184] which is driven by neurons expressing POMC and agouti-related protein AgRP[180]. α-MSH has recently been shown to exert control over insulin and glucose homeostasis through skeletal muscle MC5R agonism in animals[181, 183]. Several observations in cell lines and animals led to the discovery that α-MSH also acts in skeletal muscle as part of a previously unrecognised neuroendocrine glucoregulatory axis. Human studies to understand the physiological action of melanocortin receptor agonism on glucose homeostasis by peripheral administration of α-MSH are a critical next step towards managing blood glucose levels.

Hypothesis

Exogenous α-MSH improves glucose clearance in healthy humans.

Study outcome measures

Primary outcome

- Difference in the baseline-adjusted area under the curve of glucose concentration at an OGTT during saline vs. α-MSH infusion between 0 and 180 minutes

Secondary outcomes

- Difference in the glucose infusion rate (M value) at the euglycaemic-hyperinsulinaemic clamp during saline vs. α-MSH infusion
- Difference in the baseline-adjusted area under the curve of insulin concentration at an OGTT during saline vs. MSH infusion between 0 and 180 minutes.

4.1 Dose-finding study

Methods

A dose-finding study was conducted to determine (i) whether acute administration of α-MSH lowers blood glucose in a dose-dependent manner, (ii) the effect size of the α-MSH dose with the greatest impact on glucose-lowering and (iii) whether the glucose-lowering effects of α-MSH are mediated through an increase in the uptake of glucose by skeletal muscle. I performed the screening, recruitment and supervision of the infusion visits as well as the statistical analysis for both the dose-finding and replication cohort.

Infusion visits

Oral Glucose Tolerance Tests visits 1, 2, 3, 4

Participants were asked to attend the CRF following an overnight fast and having abstained from strenuous exercise for the preceding 48 hours.

Preparation of α-MSH for infusion visit

Using 3.3 mg vial of α-MSH:

1. To the vial, add 1 mL of sterile water. Close and invert several times gently to ensure all the peptide is dissolved.
2. To a 50 mL falcon tube, add 1 mL of dissolved peptide, then 19 mL of 0.9% normal saline and invert gently several times. Label this as **STOCK.**

3. For a 15 ng/kg/hour (low) dose - add 455 uL of STOCK peptide to a cryovial, then 645 uL of 0.9% saline. Invert the tube several times to mix. In to a new cryovial, add 100 uL of the peptide solution and 900 uL of 0.9% saline and invert the cryovial several times to mix. To a 50 mL syringe, draw up 1 mL α-MSH from the cryovial, 2 mL gelofusine and 47 mL of 0.9% saline and invert several times to mix. Label appropriately, then aliquot 1mL of the syringe solution into a labelled cryovial for storage and dosage measurement before loading it into the syringe driver.
4. For a 150 ng/kg/hour (medium) dose – add 455 uL of **STOCK** peptide to a cryovial, then 645 uL of 0.9% saline. Invert the tube several times to mix. To a 50 mL syringe, draw up 1 mL α-MSH from the cryovial, 2 mL of gelofusine and 47 mL of 0.9% saline and invert several times to mix. Label appropriately, then aliquot 1mL of the syringe solution into a labelled cryovial for storage and dosage measurement before loading it into the syringe driver.
5. For a 1500 ng/kg/hour (high) dose – to a 20 mL syringe, add 4.55 mL of **STOCK** peptide, then 6.45 mL of 0.9% saline. Invert the tube several times to mix. To a 50 mL syringe, draw up 10 mL of α-MSH from the 20 mL syringe, 2 mL of gelofusine and 38 mL of 0.9% saline. Invert several times to mix. Label appropriately, then aliquot 1mL of the syringe solution into a labelled cryovial for storage and dosage measurement before loading it into the syringe driver.

An intravenous catheter was inserted into each anterior cubital fossa, and an infusion was initiated (T=-30, relative to glucose load) either saline, low-, medium-, or high dose α-MSH in a randomised, double-blind, crossover manner. At time point 0, the participant was asked to consume a 75 g oral glucose drink within 2 minutes and blood samples were taken at 15, 30, 60, 120 and 180 minutes. Participants were asked to complete visual analogue scales at time points -30, 0, 60 and 180 minutes. After the final time point, the infusion was stopped, intravenous catheters removed, and patient discharged following a meal.

An overview of the visit is presented below (figure 4.1):

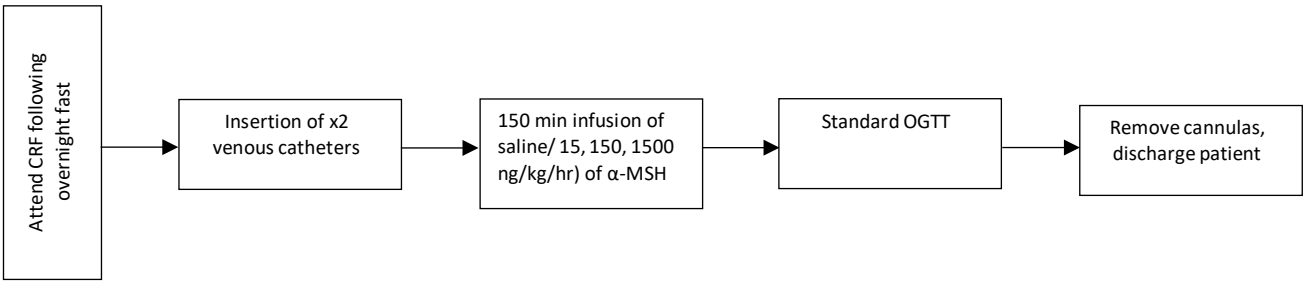


Figure 4.1 Oral glucose tolerance test visit overview

Euglycaemic-hyperinsulinaemic clamp visits 5 and 6

Euglycaemic-hyperinsulinaemic clamps were carried out with placebo and medium dose alpha-MSH. Participants were asked to attend the CRF following an overnight fast and having abstained from strenuous exercise for 48 hours prior to the visit. A modified design of the traditional single-stage, hyperinsulinaemic clamp was performed which included a monitoring period (1st stage) of 0-120 minutes, where an infusion of saline or α-MSH was initiated (randomised, double-blind, crossover manner) and blood glucose was measured every 10 minutes. A bag of 20% dextrose was connected to the patient via a separate intravenous line as per standard clamp protocol. Euglycaemia was maintained by infusing 20% dextrose at a variable rate. The 1st stage of the clamp was initiated to assess if an infusion of α-MSH would lead to a reduction in blood glucose levels in the basal state. At 120 minutes (2nd stage), an intravenous insulin infusion was initiated at a rate of 1 µU/kg/hr for 180 minutes, and the GIR increased as required to main the euglycaemic clamp. The 3rd stage of the clamp was between 270-300 minutes and is described as the steady state – this is the point at which the GIR equals

glucose uptake by all body tissues, and it is therefore considered a direct measure of whole-body insulin sensitivity.

An overview of the visit is presented below (figure 4.2):

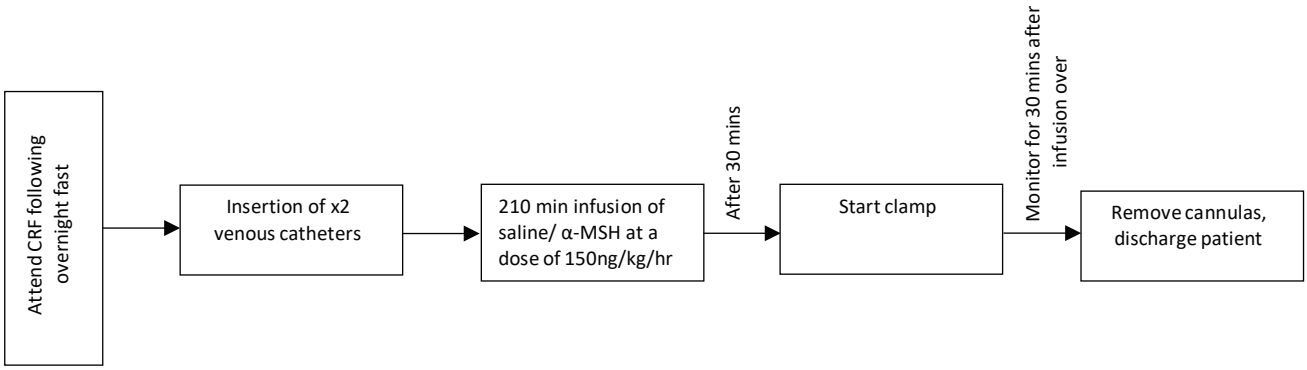


Figure 4.2 Euglycaemic hyperinsulinaemic clamp visit overview

Sample processing

100 uL aprotinin (Trasylol) was added to the (green) gut hormone collection tubes using a syringe. During the visit, the green (gut hormone) and lavender (α-MSH) tubes were placed on ice immediately after collection and centrifuged within 15 minutes. Gold tubes (C-peptide and insulin) were allowed to clot for 30 minutes at room temperature and centrifuged within one hour. Samples were centrifuged at 4 C for 10 minutes at 2590rcf (4000 rpm) and separated into at least 0.5 mL aliquots according to colour-coded cryovials, which were stored in a -20 C day freezer and then moved to a -80 C freezer at the end of the study day.

Sample analysis

Plasma glucose samples were analysed by Imperial College Healthcare NHS Trust pathology laboratory at Hammersmith Hospital using the Abbott Alinity platform with an intra-assay imprecision of <1.9% and an inter-assay imprecision of <1.2%. The insulin samples were analysed at University College Dublin by P. Swan using Alinity I Insulin Assay (04T7520) run on an Abbott Architect i2000SR with a CV of 2.4%. The α-MSH samples were shipped via international courier to

Monash University and analysed using a radioimmunoassay by Phoenix Pharmaceuticals with a CV of 12.5%. This CV is high but was due to the placebo samples being below the dynamic range of the standard curve and at the limit of the assay's detection.

Statistical analyses

The dose-finding study aimed to determine the effect size of the α-MSH dose with the highest glucose-lowering effect. Thus, due to the exploratory nature of the study, formal statistical comparisons were not performed.

The 1500 ng/kg/hr dose of α-MSH had the greatest glucose-lowering effect. Based on that, it was estimated that for the Replication study, 22 participants were required to provide 80% power to detect a statistically significant difference between α-MSH at a dose of 1500 ng/kg/hr vs. placebo at $\alpha=0.05$.

Baseline characteristics for the dose-finding cohort.

For the dose-finding cohort, fifteen healthy volunteers over 18 years of age with no major medical conditions were entered into the study if they passed screening. All participants

were metabolically healthy, with a BMI ≥18 <30 Kg/m2, normal fasting glucose (<5.6 mmol/L) and HbA1c (<48 mmol/mol).

	Dose finding (n=15)
Sex	8M, 7F
Age (years)	28 ± 8.4
Weight (kg)	72.9 ± 14.6
Body fat (%)	24.3 ± 5.9
BMI (kg/m^2)	23.9 ± 3.0
Fasting glucose (mmol/l)	4.5 ± 0.4
HOMA-IR	1.4 ± 0.6
HbA1c (mmol/mol)	32 ± 4.1

Table 4.1 Anthropometric and metabolic characteristics of healthy participants in the dose- finding cohort. Data presented as mean ± SD.

Results for the Dose-finding cohort

Dose-finding Cohort (n=15) Oral Glucose Tolerance tests							
	Incremental Area Under the Curve 0-60 minutes				**Mean difference vs. Saline (95% Confidence Interval)**		
	Saline	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**
Plasma glucose (mmol/l.min)	126.5 ± 43.59	120.7 ± 32.88	110.8 ± 43.34	92.47 ± 45.75	5.890 (-22.05 to 33.83)	15.78 (-17.92 to 49.49)	34.08 (3.060 to 65.10)
Serum insulin (µU/ml.min)	2564 ± 1569	2508 ± 1616	2785 ± 1649	1931 ± 927.8	56.43 (-277.3 to 390.2)	-221.2 (-486.1 to 43.70)	633.1 (189.7 to 1077)

Table 4.2 Oral glucose tolerance test in the dose-finding cohort – incremental Area Under the Curve(0-60)

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval) α-MSH was infused at 15 ng/kg/hr, low dose; 150 ng/kg/hr, medium dose; 1500 ng/kg/hr, high dose. Groups compared using RM one-way ANOVA.

Dose-finding Cohort (n=15) Oral Glucose Tolerance tests							
	Incremental Area Under the Curve 0-120 minutes				**Mean difference vs. Saline (95% Confidence Interval)**		
	Saline	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**
Plasma glucose (mmol/l.min)	237.4 ± 130.7	225.1 ± 104.5	210.8 ± 103.6	180.8 ± 126.8	12.33 (-69.74 to 94.39)	26.62 (-57.55 to 110.8)	56.57 (-26.03 to 139.2)
Serum insulin (µU/ml.min)	5611 ± 3358	5004 ± 2714	5792 ± 3889	4577 ± 2171	606.7 (-380.2 to 1594)	-181.5 (-836.0 to 472.9)	1034 (-111.0 to 2178)

Table 4.3 Oral glucose tolerance tests in the dose-finding cohort – incremental Area Under the Curve(0-120)

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval) α-MSH was infused at 15 ng/kg/hr, low dose; 150 ng/kg/hr, medium dose; 1500 ng/kg/hr, high dose. Groups compared using RM one-way ANOVA.

Dose-finding Cohort (n=15) Oral Glucose Tolerance tests							
	Incremental Area Under the Curve 0-180 minutes				**Mean difference vs. Saline (95% Confidence Interval)**		
	Saline	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**
Plasma glucose (mmol/l.min)	303.8 ± 131.0	281 ± 112.4	262.9 ± 114.2	241.5 ± 145.2	22.41 (-57.35 to 102.2)	40.89 (-32.59 to 114.4)	62.35 (-18.16 to 142.9)
Serum insulin (µU/ml.min)	6960 ± 4601	606**6** ± 3252	7172 ± 5424	5923 ± 2962	894.1 (-728.3 to 2516)	-212.2 (-1327 to 902.4)	1037 (-605.9 to 2680)

Table 4.4 Oral glucose tolerance test in the dose-finding cohort – incremental Area Under the Curve(0-180)

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval) α-MSH was infused at 15 ng/kg/hr, low dose; 150 ng/kg/hr, medium dose; 1500 ng/kg/hr, high dose. Groups compared using RM one-way ANOVA.

Dose-finding cohort (n=14) **Euglycaemic-hyperinsulinaemic clamps**			
	Saline	**Medium dose α-MSH**	**Mean difference (95% Confidence Interval)**
Glucose infusion rate (mg/kg/min)	6.7 ± 1.9	7.2 ± 2.2	-0.49 (-1.47 to 0.50)

Table 4.5 Euglycaemic-hyperinsulinaemic clamps

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval), n=14. α-MSH was infused at 150 ng/kg/hr, medium dose.

Dose-finding Cohort (n=15) **Adverse Events**							
Hypoglycaemia	**Infusion**				**Difference vs Saline**		
	Saline	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**	**Low dose α-MSH**	**Medium dose α-MSH**	**High dose α-MSH**
Plasma Glucose <3.9mmol/l Total number of events	3	2	2	4	1	1	-1
Visual Analogue Scale					**Mean difference vs. Saline (95% Confidence Interval)**		
Nausea (mm)	13.3 ± 8.7	9.3 ± 9.9	17.1 ± 9.9	6.5 ± 7.4	4.0 (-6.1 to 14.2)	-3.8 (14.9 to 7.3)	6.8 (-0.3 to 13.9)
Sleepiness (mm)	28.0 ± 20.6	28.2 ± 21.0	29.3 ± 17.4	26.1 ± 16.2	-0.1 (-14.7 to 14.5)	-1.2 (-20.3 to 17.8)	2.0 (-30.0 to 33.9)

Stress (mm)	11.0 ± 11.7	10.1 ± 12.5	10.4 ± 15.1	7.2 ± 11.1	1.0 (-6.2 to 8.1)	0.6 (-4.0 to 5.1)	3.8 (-9.5 to 17.1)	
Flushing (mm)	10.0 ± 9.7	10.3 ± 13.9	9.0 ± 12.8	7.0 ± 13.2	-0.2 (-11.6 to 11.1)	1.0 (-4.7 to 6.8)	3.1 (-13.3 to 19.4)	

Table 4.6 Adverse events at the oral glucose tolerance test in the dose-finding cohort

Data presented as absolute number of observed events across all OGTTs, mean ± SD and mean difference vs. Saline (95% Confidence Interval).

α-MSH was infused at 15 ng/kg/hr, low dose; 150 ng/kg/hr, medium dose; 1500 ng/kg/hr, high dose.

Results figures

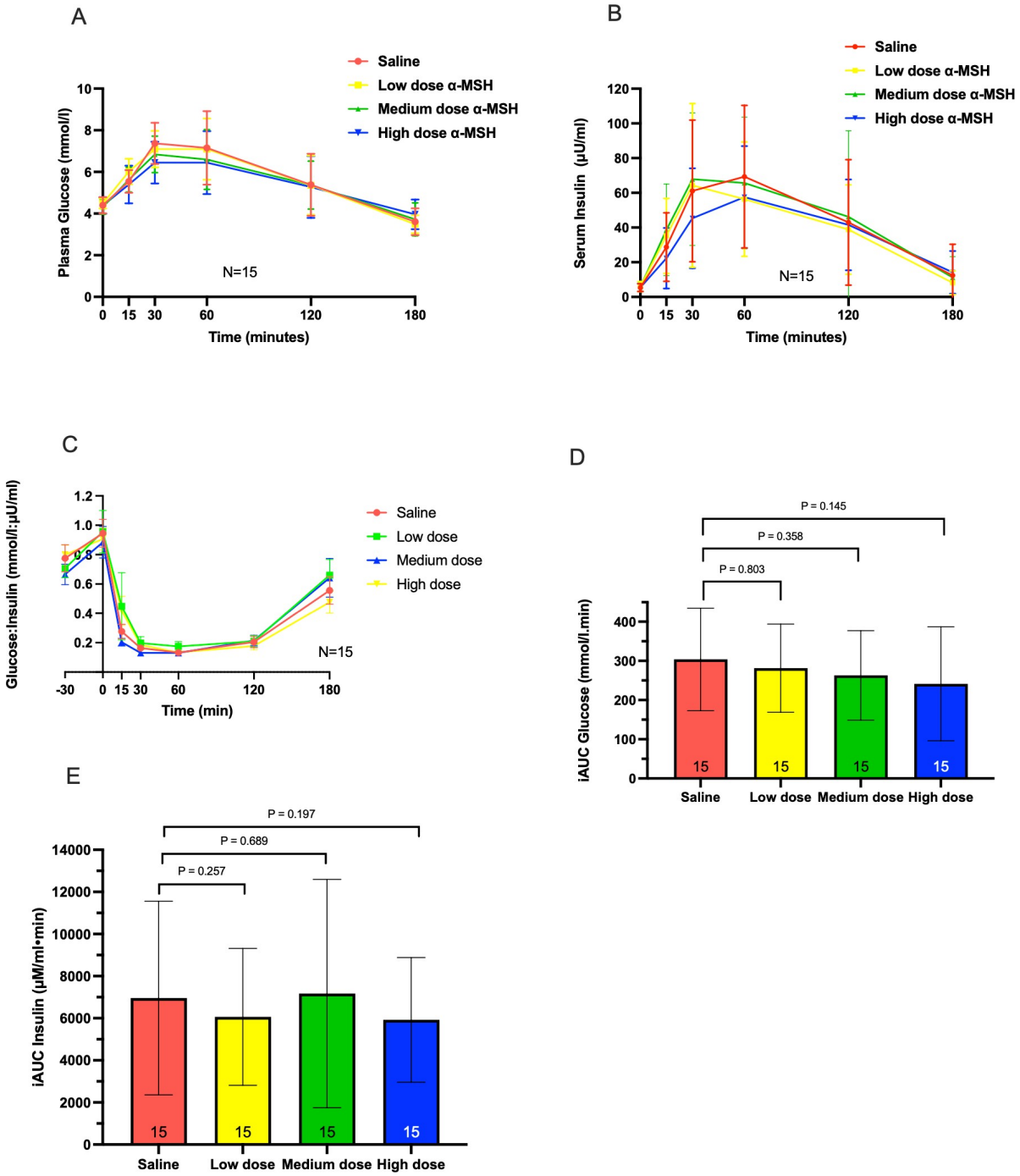


Figure 4.3 Oral glucose tolerance test in the dose-finding cohort

A. Plasma glucose concentrations during the OGTTs, B. Serum insulin concentrations during the OGTTs, C. Glucose:insulin ratios during the OGTTs, D. Incremental $AUC_{(0\text{-}180)}$ of plasma glucose concentrations at the OGTTs, E. Incremental $AUC_{(0\text{-}180)}$ of serum insulin

concentrations at the OGTTs. Data are presented as mean ± SD. α-MSH was infused at 15 ng/kg/hr, low dose; 150 ng/kg/hr, medium dose; 1500 ng/kg/hr, high dose.

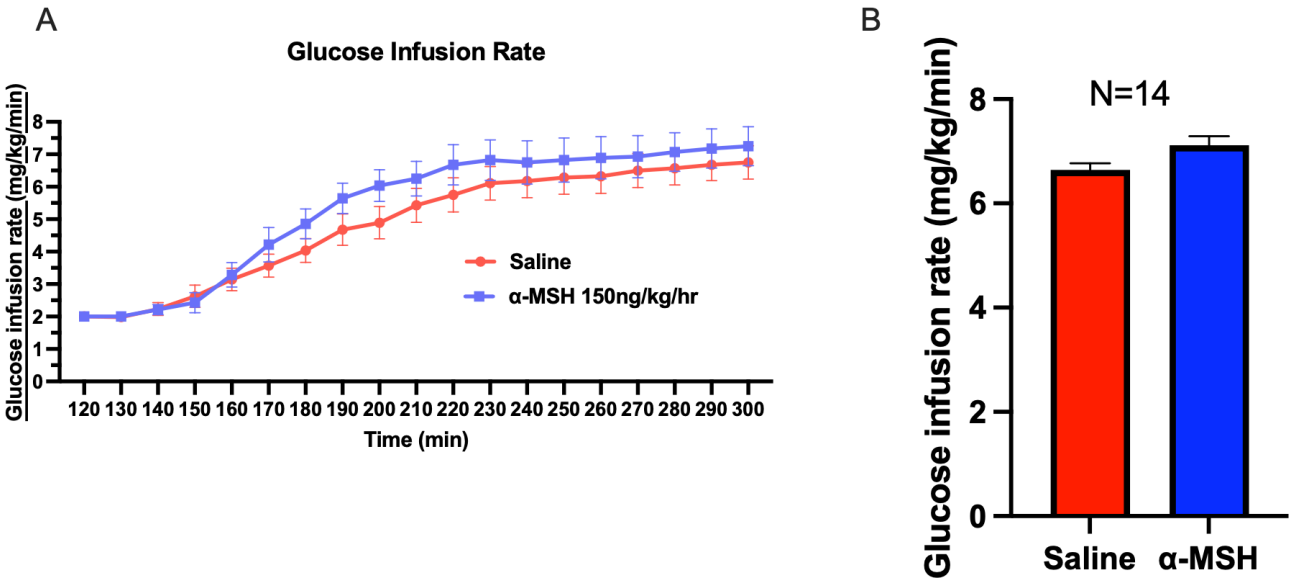


Figure 4.4 Euglycaemic hyperinsulinaemic clamps in the dose-finding cohort

A. Glucose infusion rate at the euglycaemic-hyperinsulinaemic clamps (second stage), B. Glucose infusion rate clamp third stage (steady state): 270 - 300 minutes. Data are presented as mean ± SD. α-MSH was infused at 150 ng/kg/hr, medium dose.

Oral glucose tolerance tests

Plasma glucose

There was a dose-dependent lowering of glucose, with the maximum reduction in $iAUC_{(0\text{-}180)}$ at the high dose of α-MSH, corresponding to a reduction of 20.5% compared to saline (Table 4.4).

Serum insulin

There was no apparent dose-dependent lowering of insulin. The greatest reduction in $iAUC_{(0-180)}$ was observed with the high dose of α-MSH, corresponding to a reduction of 14.9% compared to saline (Table 4.4).

Euglycaemic-hyperinsulinaemic clamp

There was a modest increase in GIR with α-MSH compared to saline (Table 4.5).

Safety

Infusion of α-MSH was well tolerated without evidence for a higher incidence in nausea, flushing, hypoglycaemia, or any other adverse events (Table 4).

Summary

- Dose-dependent lowering of glucose with α-MSH compared to saline
- No apparent dose-dependent lowering of insulin with α-MSH compared to saline
- There was a modest increase in GIR with α-MSH compared to saline

4.2 Replication study

This effect size from the dose-finding cohort was used to power the replication study, which compared the effect of acute administration of α-MSH vs placebo in a different cohort of participants (ISRCTN26265036). An additional cohort of 22 healthy human participants were recruited from the community. The same methods used in the dose-finding study were followed. Participants attended on two occasions for a standard OGTT whilst receiving 1500 ng/kg/hr of α-MSH or placebo in a double-blind, randomised manner.

Statistical analysis

This was performed using Prism (GraphPad) 9. For the incremental Area Under the Curve for plasma glucose and serum insulin at the different time points, groups were compared using a paired t-test or Wilcoxon test. Plasma glucose and serum insulin levels, arterial blood pressure and heart rate during the OGTTs were analysed using a two-way ANOVA. Post hoc comparisons were performed with Šídák's correction presented as the mean difference Saline - MSH (95% Confidence Interval).

Baseline characteristics for the Replication cohort.

For the replication cohort, twenty-two healthy volunteers over 18 years of age with no major medical conditions were entered into the study if they passed screening. All participants were metabolically healthy, with a BMI ≥18 <30 Kg/m2, normal fasting glucose (<5.6 mmol/L) and HbA1c (<48 mmol/mol).

	Replication (n=22)
Sex	11M, 11F
Age (years)	29.1 ± 9.1
Weight (kg)	70.3 ± 13.2
Body fat (%)	21.4 ± 7.7
BMI (kg/m^2)	22.7 ± 3.1
Fasting glucose (mmol/l)	4.5 ± 0.4
HOMA-IR	1.0 ± 0.5
HbA1c (mmol/mol)	33 ± 0.8

Table 4.7 Anthropometric and metabolic characteristics of healthy participants in the replication cohort

Data presented as mean ± SD.

Results

Replication Cohort (n=22) **Oral glucose tolerance tests**				
	Incremental Area Under the Curve 0-60 minutes			
	Saline	**High dose α-MSH**	**P value**	**Mean difference (95% Confidence Interval)**
Plasma glucose (mmol/l.min)	133.9 ± 65.54	85.88 ± 37.64	<0.0001	48.01 (27.51 to 68.51)
Serum insulin (µU/ml.min)	2454 ± 1475	1745 ± 1074	0.0008	709.1 (333.3 to 1085)

Table 4.8 Oral glucose tolerance test in the replication cohort – incremental Area Under the Curve(0-60)

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval) α-MSH was infused at 1500 ng/kg/hr, high dose. Groups compared using paired t-tests.

Replication Cohort (n=22) **Oral glucose tolerance tests**				
	Incremental Area Under the Curve 0-120 minutes			
	Saline	**High dose α-MSH**	**P value**	**Mean difference (95% Confidence Interval)**
Plasma glucose (mmol/l.min)	232.2 ± 148.9	124.4 ± 85.73	0.0006	107.8 (51.88 to 163.8)
Serum insulin (µU/ml.min)	5107 ± 3388	3282 ± 2093	0.0001	1825 (1010 to 2639)

Table 4.9 Oral glucose tolerance test in the replication cohort – incremental Area Under the Curve(0-120)

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval) α-MSH was infused at 1500 ng/kg/hr, high dose. Groups compared using paired t-test.

Replication Cohort (n=22) **Oral glucose tolerance tests**				
	Incremental Area Under the Curve 0-180 minutes			
	Saline	**High dose α-MSH**	**P value**	**Mean difference (saline – MSH) (95% Confidence Interval)**
Plasma glucose (mmol/l.min)	226.6 ± 181.6	109.9 ± 113.4	0.0053	116.7 (38.71 to 194.6)
Serum insulin (μU/ml.min)	6270 ± 4632[a]	3843 ± 2562[a]	<0.0001	2427 (1277 to 3576)[a]

Table 4.10 Oral glucose tolerance test in the replication cohort – incremental Area Under the Curve(0-180)

Data presented as mean ± SD and mean difference vs. Saline (95% Confidence Interval) α-MSH was infused at 1500 ng/kg/hr, high dose. Groups compared using paired t-test or [a]Wilcoxon test.

Replication cohort (n=22) **Oral glucose tolerance tests**				
Two-way ANOVA				
	Plasma Glucose		**Serum Insulin**	
	F (DFn, DFd)	**P value**	**F (DFn, DFd)**	**P value**
Time	F (6, 126) = 40.58	P<0.0001	F (2.357, 49.49) = 35.98	P<0.0001

Infusion	F (1, 21) = 25.64	P<0.0001	F (1, 21) = 21.06	P=0.0002
Time x Infusion	F (6, 126) = 7.271	P<0.0001	F (2.954, 62.03) = 4.365	P=0.0077
Post hoc comparisons with Šídák's correction (Saline – MSH)				
	Plasma Glucose		**Serum Insulin**	
Timepoint	**Mean difference (95% Confidence Interval)**	**P value**	**Mean difference (95% Confidence Interval)**	**P value**
0	0.1136 (-0.5063 to 0.7336)	>0.9988	0.1227 (-1.478 to 1.723)	>0.9999
15	0.4955 (-0.1245 to 1.115)	0.1728	12.02 (-4.601 to 28.64)	0.2678
30	1.155 (0.5346 to 1.775)	<0.0001	11.37 (-2.158 to 24.90)	0.1377
60	1.377 (0.7573 to 1.997)	<0.0001	18.63 (2.534 to 34.72)	0.0171
120	0.8455 (0.2255 to 1.465)	0.0021	18.81 (0.1894 to 37.43)	0.0467
180	-0.3136 (-0.9336 to 0.3063)	0.7290	1.245 (-3.010 to 5.501)	0.9702

Table 4.11 Oral glucose tolerance test in the replication cohort using two-way ANOVA

Post hoc comparisons with Šídák's correction presented as mean difference as Saline - MSH (95% Confidence Interval) α-MSH infused at 1500 ng/kg/hr, high dose.

Replication Cohort (n=22) **Adverse Events**			
Hypoglycaemia	**Infusion**		**Difference**
	Saline	**High dose α-MSH**	**High dose α-MSH**
Plasma Glucose < 3.9mmol/l Total number of event5	5	8	-3
Visual Analogue Scale ratings			**Mean difference (95% Confidence Interval)**

Nausea (mm)	3.1 ± 4.1	4.3 ± 6.3	-1.2 (-3.2 to 0.8)
Sleepiness (mm)	35.3 ± 22.1	30.2 ±27.1	5.0 (-4.5 to 14.5)
Stress (mm)	5.9 ± 9.1	5.3 ±6.8	0.6 (-3.5 to 4.8)
Flushing (mm)	5.7 ± 9.2	6.4 ± 11.0	-0.8 (-5.6 to 4.1)

Table 4.12 Adverse events at the oral glucose tolerance test

Data presented as absolute number of observed events across all OGTTs, mean ± SD and mean difference vs. Saline (95% Confidence Interval).

α-MSH was infused at 1500 ng/kg/hr, high dose.

Replication cohort **Arterial blood pressure – Saline vs MSH**						
Two-way ANOVA						
	Systolic blood pressure			**Diastolic blood pressure**		
	F (DFn, DFd)		**P value**	**F (DFn, DFd)**		**P value**
Time	F (3, 48) = 1.601		P=0.202	F (3, 48) = 1.703		P=0.1789
Infusion	F (1, 16) = 0.709		P=0.412	F (1, 16) = 0.209		P=0.654
Time x Infusion	F (3, 48) = 1.560		P=0.211	F (3, 48) = 0.956		P=0.421
	Post hoc comparisons with Šídák's correction (Saline – MSH)					
	Systolic blood pressure			**Systolic blood pressure**		
Timepoint	**Predicted (LS) mean diff.**	95% CI of diff.	**Adj. P value**	**Predicted (LS) mean diff.**	**95% CI of diff.**	**Adj. P value**
0	0.875	-17.84 to 19.59	>0.9999	-2.025	-13.24 to 9.192	0.984
60	-1.300	-20.01 to 17.41	0.9996	1.675	-9.542 to 12.89	0.992
120	-15.800	-34.51 to 2.912	0.1299	-5.450	-16.67 to 5.767	0.625

180	-0.150	-18.86 to 18.56	>0.9999	-0.575	-11.79 to 10.64	0.9999

Table 4.13 Arterial blood pressure at the Oral glucose tolerance test

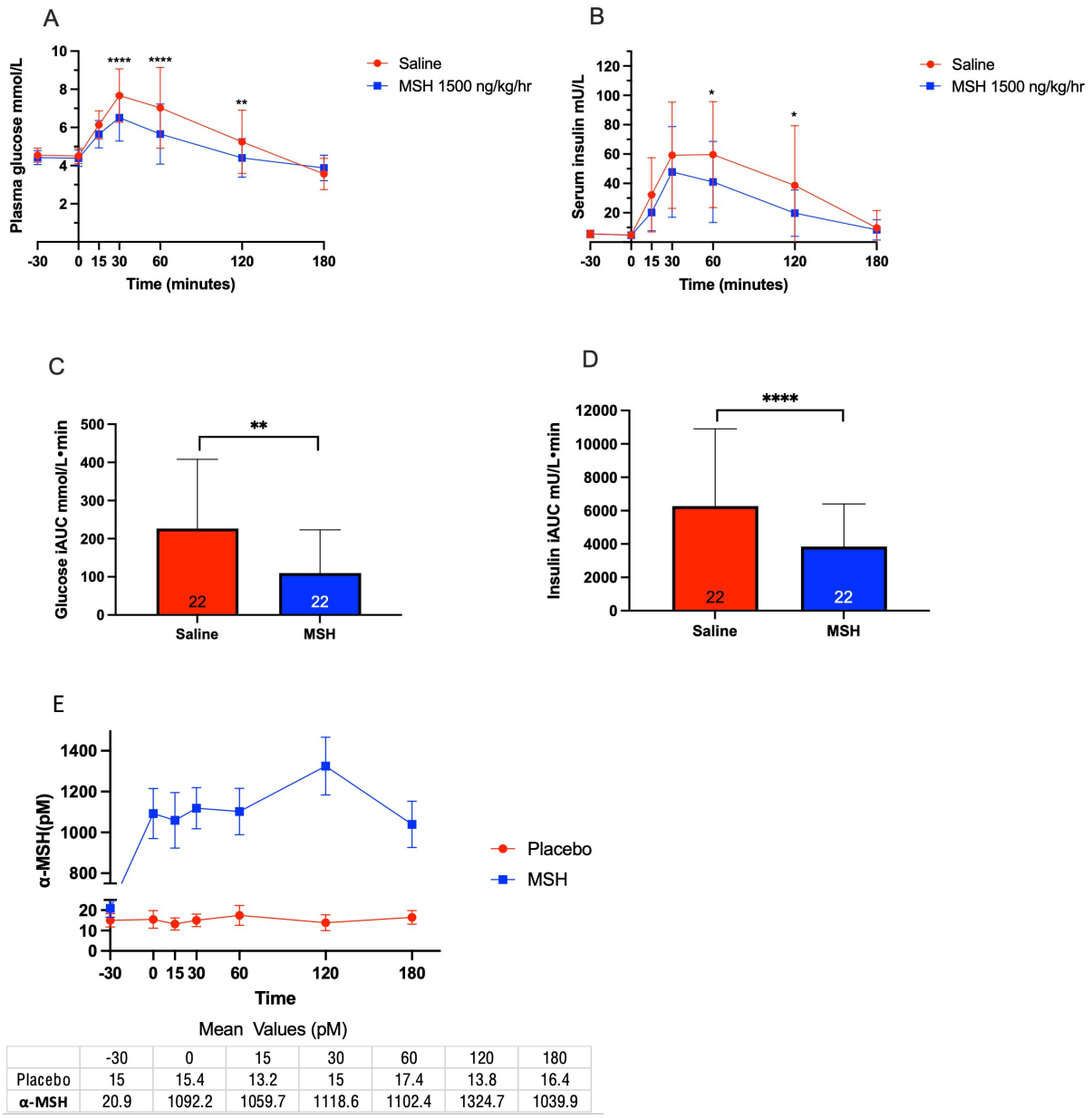
Post hoc comparisons with Šídák's correction presented as predicted (LS) mean difference, Saline - MSH (95% Confidence Interval) α-MSH infused at 1500 ng/kg/hr, high dose.

Replication cohort **Heart rate – Saline vs MSH**			
Two-way ANOVA			
	F (DFn, DFd)	**P value**	
Time	F (3, 48) = 0.940	P=0.429	
Infusion	F (1, 16) = 0.321	P=0.579	
Time x Infusion	F (3, 48) = 1.178	P=0.328	
	Systolic blood pressure		
Timepoint	**Predicted (LS) mean diff.**	**95% CI of diff.**	**Adj. P value**
0	-2.250	-18.80 to 14.30	0.995
60	0.425	-16.13 to 16.98	>0.9999
120	-5.325	-21.88 to 11.23	0.881
180	-6.375	-22.93 to 10.18	0.795

Table 4.14 Heart rate at the oral glucose tolerance test

Post hoc comparisons with Šídák's correction presented as predicted (LS) mean difference, Saline - MSH (95% Confidence Interval) α-MSH infused at 1500 ng/kg/hr, high dose.

Results figures



Mean Values (pM)

	-30	0	15	30	60	120	180
Placebo	15	15.4	13.2	15	17.4	13.8	16.4
α-MSH	20.9	1092.2	1059.7	1118.6	1102.4	1324.7	1039.9

Figure 4.5 Oral glucose tolerance test, replication cohort

A. Plasma glucose concentrations, B. serum insulin concentrations, C. Incremental AUC (0-180) of plasma glucose concentrations, D. Incremental AUC (0-180) of serum insulin concentrations, E. Plasma α-MSH levels at the OGTTs, n=22. Data are presented as mean ± SD. α-MSH was infused at 1500 ng/kg/hr, high dose. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

Plasma glucose

A significant reduction in plasma glucose iAUC at all three major time points was observed with high dose α-MSH compared to saline. The two-way ANOVA showed a significant interaction between time and infusion. Post hoc comparisons demonstrated significant reductions in plasma glucose at time points 30, 60 and 120 minutes (Table 4.1)).

Serum insulin

A significant reduction in serum insulin iAUC at all three major time points was observed with high dose α-MSH compared to saline (Table 4.10). The two-way ANOVA showed a significant interaction between time and infusion. Post hoc comparisons demonstrated significant reductions in serum insulin at 60 and 120 minutes (Table 4.1).

Safety

No signal was observed for a higher incidence of adverse events during infusion, including hypoglycaemia, nausea, or flushing (Table X). There was no significant difference in arterial blood pressure and heart rate between saline and high-dose α-MSH infusion.

4.3 Discussion

This is the first-in-human α-MSH infusion study to assess the impact of α-MSH on glucose homeostasis. The study consisted of two cohorts – a dose-finding cohort with fifteen healthy volunteers and a replication cohort with twenty-two healthy volunteers. For the dose-finding cohort, OGTTs were conducted by infusing saline, 15 (low), 150 (medium) and 1500 (high) ng/kg/hr α-MSH in a double-blind, randomised, cross-over manner. The α-MSH

infusion was started 30 minutes before administering the oral glucose load to allow for circulating α-MSH to equilibrate. Analysis of the primary endpoint (0–180-minute glucose iAUC) revealed improvements in glucose tolerance at all three doses, with the greatest effect size observed at high dose α-MSH. In normoglycaemic, insulin-sensitive individuals, the time to glucose peak usually occurs at or before 30 minutes, with a later time to peak of ≥ 60 minutes observed in adults with type 2 diabetes[279]. Using multiple comparisons, at time point 30 for high dose α-MSH versus saline, there was a significant mean difference in plasma glucose concentrations (0-0.9267, 95% CI -1.829 to -0.02435, adjusted P value 0.0431). This reduction in plasma glucose following a 75-g glucose load with α-MSH has previously been reported in pre-clinical models by Enriori et al[185]. Serum insulin samples collected and analysed for secondary endpoint analysis did not show a significant reduction with α-MSH versus saline overall, however, with high dose α-MSH, there was a significant difference between saline and α-MSH at 60 minutes. Interim unblinding was performed once ten participants from the dose-finding cohort had completed OGTTs with saline and all three doses of α-MSH. The data showed that the middle dose of 150 ng/kg/hr caused the greatest reduction in glucose iAUC. This dose was therefore used during the clamp studies. A modified design of the traditional single-stage, hyperinsulinaemic clamp was carried out, which included a monitoring period (1st stage) of 0-120 minutes where an infusion of saline or α-MSH was initiated, and blood glucose was measured every 10 minutes; a 20% dextrose bag was connected via a separate intravenous line as per standard clamp protocol. The dextrose infusion was started if a participant's blood glucose fell below the clamped euglycaemic range. This 1st stage of the clamp was initiated to assess if an infusion of α-MSH would reduce blood glucose levels in the basal state. Although there was a slight increase in GIR with α-MSH infusion, it was not physiologically significant and matched pre-clinical

findings in mice where basal euglycaemia was unaffected by a continuous α-MSH infusion[185]. At 120 minutes, intravenous insulin was administered at a rate of 1 μU/kg/hr for 180 minutes and the GIR increased as required to maintain the euglycaemic clamp. During the equilibration phase - between 180-270 minutes, there was a notable increase in GIR with the α-MSH infusion, however by the time the glucose infusion was equilibrated with whole body glucose uptake, the effect size between α-MSH and saline on GIR had diminished. One potential reason for this is receptor desensitisation to the peptide, as by the time equilibration phase was reached, the participants would have been receiving a continuous infusion of α-MSH for almost 5 hours.

Once all the OGTTs and clamp studies had been completed and the results analysed, high dose α-MSH (1500 ng/kg/hr) was identified to cause the greatest reduction in glucose compared to saline. The OGTTs were repeated in twenty-two healthy volunteers using two infusions – saline and high dose α-MSH (1500 ng/kg/hr). In the replication cohort, there was a significant reduction in glucose iAUC from 0-180 minutes ($P=0.0053$), and post hoc comparisons demonstrated a significant mean difference between saline and α-MSH between 30-120 minutes. Infusion of α-MSH led to a significant reduction in insulin iAUC from 0-180 minutes ($P<0.0001$) and post hoc comparisons demonstrated a significant mean difference from 60-120 minutes during the OGTTs.

Analysis of data gathered from both cohort's present evidence of a glucose-lowering physiological response to peripheral melanocortin receptor agonism. A concomitant reduction in insulin further suggests this improvement in glucose tolerance is not due to enhanced β-cell insulin secretion. In the context of data from animal studies, unpublished human myotube data and in-human clamps, this work is compelling early evidence for a

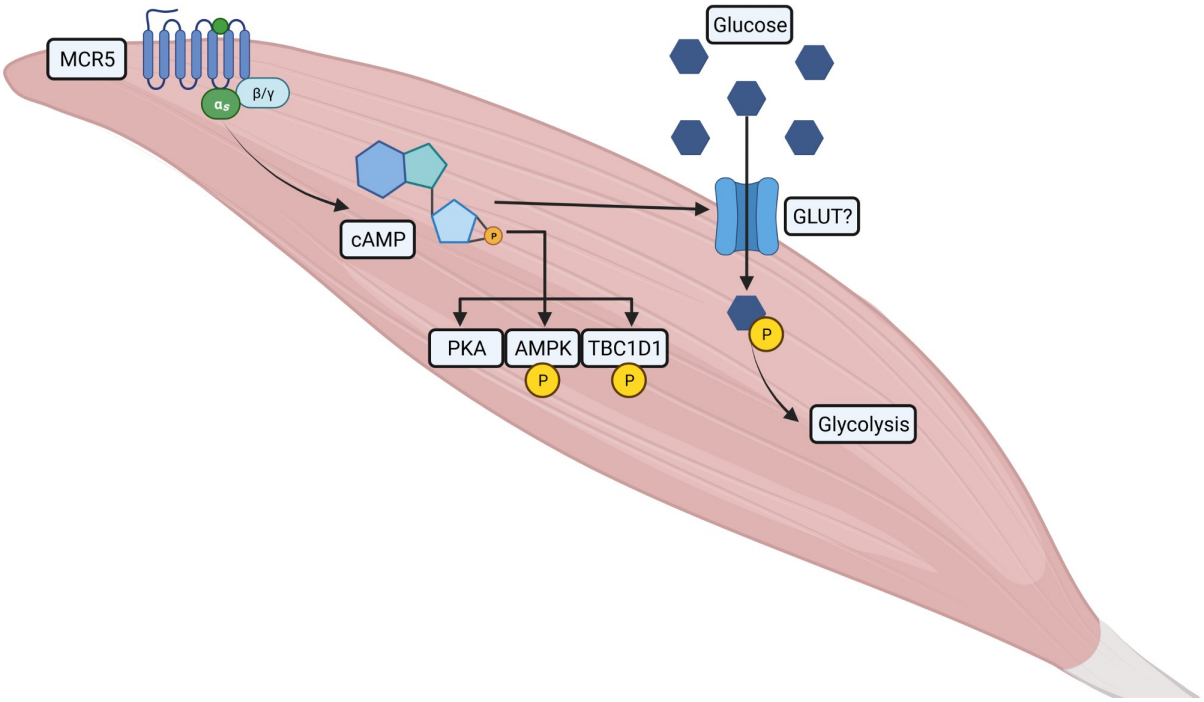


Figure 4.6 A molecular overview of MC5R-mediated glucose uptake in rodent skeletal muscle

novel pathway in which α-MSH works through skeletal muscle to increase glucose uptake in an insulin-independent manner (figure 4.6).

MC5R agonism leads to cAMP generation which results in translocation of an unknown GLUT transporter to the plasma membrane inducing glucose uptake and glycolysis. *Image courtesy of P. Swan.*

Animal studies identified that peripheral α-MSH increases temperature in skeletal muscles particularly, soleus and gastrocnemius muscles and significantly increases glucose uptake through activation of muscle MC5R and protein kinase A[185].

In the skeletal muscle of obese mice, there was blunting of α-MSH-induced cAMP levels and absence of MC5R and protein kinase A activation[185]. In a study of fifteen male subjects

with obesity (BMI > 26.4 kg/m^2), the plasma concentrations of α-MSH were significantly higher than non-obese controls and positively correlated with BMI ($P < 0.05$) and fasting insulin concentrations ($P < 0.05$) and GIR (a measure of insulin resistance)[280]. There was no significant difference in plasma concentrations of ACTH and cortisol between the groups which indicated that plasma concentrations of α-MSH did not reflect central nervous system levels[280]. There is a positive correlation between plasma concentrations of α-MSH and visceral fat, which may be a potential source of α-MSH secretion and induce insulin resistance[280]. Current published data in animal models and humans with obesity, albeit limited, point to a limited role of peripheral α-MSH in patients with T2D and obesity.

The OGTT results in the replication cohort identified that a dose of 1500 ng/kg/hr caused a significant reduction in glucose iAUC and in future clamp studies, this dose of α-MSH should be used. Additionally, muscle biopsies taken before and during the clamp will be an important method by which to identify if α-MSH does, in fact, increase skeletal muscle uptake of glucose as well as desensitisation of the peptide. Results from OGTTs performed during the dose-finding and replication cohort, when analysed with the GIR from the euglycaemic hyperinsulinaemic clamp studies, point to the likely effect of α-MSH on glucose uptake in skeletal muscle. The final 30 minutes of the clamp is usually described as a steady state, i.e., a balance between glucose uptake and exogenous glucose infusion after a prolonged period of hyperinsulinaemia, and insulin sensitivity is expressed as a mean of GIR once a steady state is achieved. Although muscle-specific glucose uptake was not measured - it is typically measured via tracer molecules and muscle biopsies, skeletal muscle accounts for around 80% of glucose uptake in the hyperinsulinaemic steady state[195].

This collaborative research project between Monash University, University College Dublin and Imperial College London, has identified that α-MSH, a selective MC5R agonist, improves whole-body glucose uptake following a glucose load. Pre-clinical animal studies conducted by Prof. Michael Cowley demonstrated that MCR5 agonism works in an insulin-independent role to improve glucose uptake in skeletal muscle. To date, there is no published data regarding this specific pathway in humans. As previously mentioned, infusion of α-MSH in obese rats did not lead to a significant reduction in glucose levels so it is unclear if it would be effective in directly lowering blood glucose levels in T2D. It appears that α-MSH is complementary to leptin in the endocrine circuity by regulating bodyweight, food intake and metabolic rate. This could indirectly improve insulin sensitivity in patients with T2D[281]. However, in T1D-induced rodents, there was a significant reduction in glucose levels during a GTT. The next step in this study is the infusion of the effective dose α-MSH in patients with T1D during an OGTT, which is currently underway. Once the OGTTs are complete, euglycaemic hyperinsulinaemic clamps will be performed using saline and α-MSH (randomised, double-blind, crossover method) with skeletal muscle biopsies taken before and during the clamp.

Although further work is required to identify the exact mechanism by which MC5R agonism leads to increased whole-body glucose uptake following a glucose load, the results highlighted in this thesis are very promising. The post-prandial effects of α-MSH on glucose levels in an insulin-independent manner will lay the foundations for future research and potential drug development employing MC5R agonism as an adjuvant therapy to insulin in patients with T1D.

Strengths

This is the first-in-human, randomised, double-blind, cross-over study to assess the effects of continuous peripheral α-MSH infusion on glucose homeostasis. Using data available from animal studies and dose conversion, the dose-finding cohort was able to identify the dose of α-MSH which caused the greatest reduction in mean plasma glucose during the OGTTs. Euglycaemic hyperinsulinaemic clamp studies performed in the dose-finding cohort identified a reduction in the glucose infusion rate with medium α-MSH 150 ng/kg/hr. The study was repeated in a replication cohort of n=22 participants and demonstrated a significant difference in mean plasma glucose and serum insulin concentrations during the OGTTs with high dose α-MSH (1500 ng/kg/hr) compared to saline.

MC3R is abundantly expressed in the hypothalamus which controls activity of the autonomic nervous system[281]. Stimulation of the posterior and lateral hypothalamic nuclei results in elevation of blood pressure and increase in heart rate[282]. Serial blood pressure and heart rate measurements during infusion with high dose α-MSH and saline did not significantly differ. Retention was very good throughout both parts of the study, with only one participant unable to complete the euglycaemic hyperinsulinaemic clamp studies due to a needle phobia.

Limitations

The two main limitations were initiation of the euglycaemic hyperinsulinaemic clamps once 10 participants had completed the OGTTs (instead of the total 15 participants) which meant that medium dose α-MSH (150 ng/kg/hr) was used for the clamp studies instead of high dose α-MSH (1500 ng/kg/hr) which was ultimately found to be the effective dose. Although there was a reduction in glucose infusion rate with medium dose α-MSH, the value was not

significant. As previously mentioned in the discussion, muscle biopsies were not performed before and during the clamp studies and so it is difficult to say with certainty that α-MSH exerts its effects on skeletal muscle by increasing glucose uptake.

In conclusion, this first-in-human experimental study infusing α-MSH in healthy volunteers demonstrated a significant improvement in both mean plasma glucose and serum insulin concentrations following an oral glucose load compared to saline. Further studies are needed to investigate the effects of melanocortin receptor agonism on glucose homeostasis in people with T1D.

Future work

Results in healthy human volunteers demonstrated that α-MSH caused a significant reduction in mean plasma glucose and serum insulin during an OGTT. With unpublished data from type 1 animal studies, the next step is an infusion of α-MSH in patients living with T1D during an OGTT. Once OGTTs are complete, repeating the OGTT visits with skeletal muscle biopsies taken during the time point with the greatest mean difference in mean plasma glucose between saline and α-MSH (during the OGTT) will help identify if α-MSH does cause an increase in skeletal muscle glucose uptake which is independent of insulin. The hypothesis that α-MSH works in an insulin-independent manner to lower glucose concentrations following a glucose load may favour treatment with melanocortin receptor agonists as adjuvant therapy to insulin in patients with T1D.

Other options for future work include performing mixed meal testing instead of OGTTs, as a mixed meal is more physiological and using a chronic infusion over 24 hours rather than an acute infusion.

Summary

- Acute intravenous infusion with high dose α-MSH (1500 ng/kg/hr) reduced mean plasma glucose and serum insulin concentrations following an oral glucose load in healthy adults.
- Acute intravenous infusion with high dose α-MSH did not cause hypertension or tachycardia in healthy adults.
- Further studies are required to assess the impact of α-MSH on patients with T1D.

5 Summary

The first part of my thesis focuses on treatment options for PCOS in women who have overweight or obesity. Lifestyle intervention remains the first-line treatment, but pharmacotherapy can be used as adjuvant therapy in patients who do not respond to this. Through a systematic review, meta-analysis and meta-regression to assess the impact of direct and indirect insulin sensitisers on metabolic and reproductive outcomes in women with PCOS who have overweight or obesity, I was able to demonstrate that the use of insulin sensitisers caused a significant improvement in important metabolic outcomes and some elements of the reproductive profile. However, this was primarily due to metformin and, to a lesser extent, thiazolidinediones. My review highlighted the lack of objective data for hard reproductive outcomes such as ovulation, menstrual frequency, and pregnancy, which was an unexpected finding. Future RCTs should look at both difficult reproductive outcomes and the impact and safety of modern obesity pharmacotherapy on women with PCOS. A recent single-blind, randomised, placebo-controlled prospective study comparing the effects of semaglutide to placebo in healthy women with PCOS and obesity found that semaglutide improved anthropometric and metabolic outcomes such as BMI, waist circumference, plasma glucose, and serum insulin[283]. Tirzepatide, a dual glucose-dependent insulinotropic polypeptide and GLP-1 RA approved by the Food and Drug Administration in May 2022 for adults with type 2 diabetes, is another potential treatment of interest. The SURMOUNT-1 study found that once-weekly tirzepatide treatment resulted in a significant and sustained reduction in body weight[278].

For women with ovulatory dysfunction secondary to PCOS who also have obesity and in whom lifestyle intervention with or without pharmacotherapy has not been successful, bariatric surgery remains an experimental treatment. Through a prospective open-label RCT conducted across two sites, I have demonstrated that bariatric surgery (VSG) is superior to medical care in improving anthropometric, metabolic, and reproductive outcomes. For this study, an objective reproductive outcome – ovulation, was monitored for 52 weeks from the intervention date. This is the first RCT to compare medical care to surgery in women with PCOS who have obesity. Although the results are promising, further RCTs are needed to strengthen the evidence base and potentially change clinical practice. RCTs with longer follow-up are required to assess the impact of bariatric surgery on pregnancy, miscarriage, live-birth rates, perinatal and maternal morbidity, and mortality. Future studies to compare newer pharmacotherapy agents for weight loss - GLP-1 and dual glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 RA to bariatric surgery will help tailor treatment and provide options for patients who do not want surgery. A recent double-blind RCT comparing liraglutide to placebo in women with PCOS who have obesity reported superior weight loss and improvement in androgenicity and cardiometabolic parameters[233]. A systematic review and meta-analysis of six studies (332 patients) comparing weight loss between GLP-1 RAs and bariatric surgery in adults with obesity found that bariatric surgery is superior to GLP-1 Ras for weight loss and reduction in BMI but led to similar improvements in glycaemic outcomes[284]. For now, bariatric surgery remains an experimental treatment to improve fertility in women with PCOS who have obesity.

The second part of my thesis was an experimental medicine study on the effect of melanocortin agonism on glucose homeostasis in healthy humans. This study was divided

into a dose-finding study and a replication cohort. Infusion of exogenous high dose α-MSH (1500 ng/kg/hour) significantly improved mean plasma glucose concentrations during an OGTT compared with saline. Future work will focus on the infusion of high-dose α-MSH and saline in a double-blind, randomised, cross-over manner in 22 participants with T1D. For the first stage, they will undergo OGTTs with saline or high dose α-MSH; once the OGTTs are complete, participants will be invited to attend for euglycaemic hyperinsulinaemic clamps during which muscle biopsies will be performed before and during the clamp. This study aims to assess the effects of α-MSH on mean plasma glucose concentrations in individuals with little or no endogenous insulin production. Participants will be asked to maintain the same rate of basal insulin infusion (insulin pump) or the dose of basal insulin (multiple dose injection therapy) for both visits. On completion of the OGTTs, the second part of the study will be euglycaemic hyperinsulinaemic clamps with skeletal muscle biopsies performed before and during the clamp. Muscle biopsies will help identify if α-MSH does, in fact, cause an increase in skeletal muscle glucose uptake which is independent of insulin. The hypothesis that α-MSH works in an insulin-independent manner to lower glucose concentrations following a glucose load may favour treatment with melanocortin receptor agonists as adjuvant therapy to insulin in patients with T1D.

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