



Tallaght
University
Hospital

An Academic Partner of Trinity College Dublin

Ospidéal
Ollscoile
Thamhlachta



Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

**Exploring the effects of time-restricted eating as a novel intervention in patients
with polycystic ovarian syndrome**

Submitted to Trinity College Dublin, the University of Dublin for the Degree of Master in
Science, Clinical Medicine

25th of April, 2024

Dr Ruairí Floyd, BSc, BMBS, MRCPI

School of Medicine

Trinity College Dublin, the University of Dublin

Under the supervision of Dr Lucy-Ann Behan and Dr Sinead N Duggan

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

A handwritten signature in black ink, appearing to read 'Ruairí Floyd', is written over a solid black horizontal line.

Dr Ruairí Floyd

Summary

Polycystic ovarian syndrome (PCOS) is the most common reproductive endocrinopathy in women of reproductive age. It is associated with hyperinsulinemia, insulin resistance and a high lifetime risk of developing type-2 diabetes mellitus. Hyperinsulinaemia has direct effects on hyperandrogenism, which affects ovarian function and the menstrual cycle. While many dietary interventions have been trialled and tested in PCOS to improve insulin resistance, there is minimal data on the effects of time-restricted eating on metabolic profiles in PCOS.

The dual aims of this thesis were to firstly explore the literature to determine the effects of time-restricted eating on insulin levels in PCOS (Chapter 1-3), and secondly, to conduct a feasibility study, with a randomised crossover design, to determine if time-restricted eating was an option for women with PCOS in terms of willingness to participate, compliance, side effects, and effects on metabolic parameters (Chapter 4-6). To achieve these aims, a systematic review was conducted to identify all literature available on fasting regimens for ameliorating insulin resistance in PCOS. Secondly, the overall feasibility of time-restricted eating in women with PCOS was examined in a randomised crossover study. The effect of time-restricted eating on insulin resistance, anthropometrics, androgens, lipids and dietary intake was examined.

The systematic review identified one study, focusing specifically on Ramadan fasting in PCOS. Despite the lack of evidence base for this intervention, time-restricted eating has gained traction among the PCOS community without clear evidence for recommendation [1]. Given the clear research gap, a feasibility study of time-restricted eating in PCOS was deemed necessary before larger RCTs could be carried-out, to assess the efficacy of time-restricted eating in PCOS.

After identifying this literature gap, a feasibility study (NCT05126199) was designed. Women with PCOS, aged 18 to 42, were recruited by detailed pre-screening with a strict inclusion/exclusion criteria. Patients were randomised to start with time-restricted eating and crossover to ad libitum eating, or vice-versa. Baseline metabolic, anthropometric, clinical symptoms and food diaries were assessed. Participants were educated on the time-restricted eating intervention which involved fasting for 18 hours with an unrestricted six hour eating window. Timings were determined by the patient with the majority choosing to eat between 12:00 and 18:00. Women were reviewed at six study visits and all parameters were re-assessed.

In terms of feasibility, time-restricted eating was found to be a feasible intervention with near-total compliance in those completing the intervention, with no significant adverse outcomes for those in the TRE group. However, recruitment was difficult due to the strict inclusion and exclusion criteria, crossover design and study commitment. In terms of secondary outcomes, there was no statistically significant difference in the changes between both the time-restricted eating and ad libitum groups for the insulin-related parameters. There was a significant reduction in weight, BMI, waist, and hip circumferences in the time-restricted eating group compared to the ab libitum eating group. There was a statistically significant weight gain seen in the ad libitum eating group. Regarding the androgen and lipid profiles, only the change in SHBG and Apolipoprotein A1 differed significantly between the groups. Those in the time-restricted eating group consumed significantly less energy (calories), carbohydrate, saturated fat, and calcium. There was no difference in consumption of sugar, protein, fat, or vitamin D.

In conclusion, time-restricted eating was safe and feasible with high compliance in the study participants. However, recruitment difficulties resulted in a small sample size,

meaning that the study was underpowered to detect clinical differences, precluding meaningful clinical conclusions. Nevertheless, these studies showed that, while there is a research gap in this area, the intervention was feasible and has potential to positively impact weight management in this group. Future work should further investigate the feasibility and clinical efficacy of this intervention in a larger cohort.

Acknowledgements

This research was an ambitious undertaking and it would not have been possible without the help of many individuals involved at various stages of the project but mainly my supervisors Dr Lucy Ann Behan and Dr Sinead Duggan. I couldn't have asked for more support throughout with a solution at every road block and an unrelenting passion for research and this subject. They are experts in their fields and it was a privilege to learn from their expertise in the areas of reproductive endocrinology and nutrition, respectively.

I would like to thank the Meath Foundation of Tallaght University Hospital for funding this research with a John Barragry Research Grant, as well as Trinity College Dublin for the Research Booster Award. This research could not have happened without this financial support.

My sincerest thanks to the patients that agreed to participate in the study to understand the effects of time-restricted eating in polycystic ovarian syndrome. I dedicate this thesis to them.

Thank you to Dr Lisa Owens and Dr Niamh Phelan for their help with recruitment of patients from St. James's Hospital. Thank you to Prof Carel Le Roux for his advice regarding the appetite and gut hormone aspects of the study, Dr Ana Rakovac for her expertise regarding lipids, Dr Fatimah Alawami for helping with TUH recruitment, Fiona Murphy for helping in the dietary analysis and Prof James Gibney for his expert input and review at various stages. Thank you to the TUH laboratory who processed many urgent samples in a timely fashion and the entire endocrinology department in Tallaght SIMS Building for the use of their facilities.

A big thank you to my family and partner Róisín who are always supportive in any of my academic endeavours. A special word of thank you to my friend Adam who provided invaluable time and support with various aspects of teaching laboratory techniques as well as providing education and advice on the statistical analysis of the thesis results.

Publications And Funding

Peer-reviewed Journal Publication:

Floyd R, Gryson R, Mockler D, Gibney J, Duggan SN, Behan LA. The Effect of Time-Restricted Eating on Insulin Levels and Insulin Sensitivity in Patients with Polycystic Ovarian Syndrome: A Systematic Review. *Int J Endocrinol*. 2022 Sep 16;2022:2830545. doi: 10.1155/2022/2830545. PMID: 36159086; PMCID: PMC9507776.

Trial Protocol Registration:

Ruairí Floyd, Tallaght University Hospital, Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (TimeMAP), clinicaltrials.gov, Identifier NCT05126199, Available at <https://clinicaltrials.gov/ct2/show/NCT05126199>

Abstract Publications:

R Floyd, J Gibney, L Owens, N Phelan, A Rakovac, C LeRoux, S Duggan, LA Behan (2022)

The Effect of Time-Restricted Eating on Insulin Levels in Polycystic Ovarian Syndrome: A Randomised Feasibility Study of Real-World Clinical Advice – An Interim Analysis. *Irish Journal of Medical Science*, 46th Annual Meeting of The Irish Endocrine Society

R Floyd, J Gibney, L Owens, N Phelan, A Rakovac, C LeRoux, S Duggan, LA Behan (2022)

The Effect of Time-Restricted Eating on Insulin Levels in Polycystic Ovarian Syndrome: A Randomised Feasibility Study of Real-World Clinical Advice – An Interim Analysis. *Irish Medical Journal*, 2022 Oral Presentations, Junior Obstetrics and Gynaecology Society Annual Scientific Meeting

Conference Proceedings:

R Floyd, J Gibney, L Owens, N Phelan, A Rakovac, C LeRoux, S Duggan, LA Behan (2023)

The Effect of Time-Restricted Eating on Insulin Levels in Polycystic Ovarian Syndrome: A Randomised Crossover Feasibility Study of Real-World Clinical Advice.

FIGO World Congress of Gynaecology and Obstetrics, 9-12th October 2023, Paris, France
International Conference Poster Presentation

R Floyd, J Gibney, L Owens, N Phelan, A Rakovac, C LeRoux, S Duggan, LA Behan
(2022)

The Effect of Time-Restricted Eating on Insulin Levels in Polycystic Ovarian Syndrome:
A Randomised Feasibility Study of Real-World Clinical Advice – An Interim Analysis.
Irish Endocrine Society Annual Scientific Meeting, Mater, Dublin, 11th-12th Nov 2022
National Conference Oral Presentation

R Floyd, J Gibney, L Owens, N Phelan, A Rakovac, C LeRoux, S Duggan, LA Behan
(2022)

The Effect of Time-Restricted Eating on Insulin Levels in Polycystic Ovarian Syndrome:
A Randomised Feasibility Study of Real-World Clinical Advice – An Interim Analysis.
Junior Obstetrics and Gynaecology Annual Scientific Meeting, RCPI, Dublin, 25th Nov
2022
National Conference Oral Presentation

*Awarded Clinch Medal for Best SHO Oral presentation

Funding Received

1. Meath Foundation award of Research Grant 'The John Barragry Research Grant'
€75,000
2. Trinity College Dublin Research Booster Award €3,000
3. Junior Obstetrics and Gynaecology Highly Recommended Travel Bursary Award
€200

Table of contents

List of Abbreviations	12
List of Tables	16
List of Figures	19
1. Chapter 1: Introduction and Background on the Effects of Time-Restricted Eating on insulin levels and insulin sensitivity in PCOS	
1.1. Describing the condition	22
1.2. Time-restricted eating and intermittent fasting	25
2. Chapter 2: A Narrative Review on the Effects of Time-Restricted Eating in PCOS	
2.1. Mechanism of insulin resistance in PCOS on a molecular levels	30
2.2. The effect of fasting on insulin resistance in PCOS on a cellular level	33
2.3. Proposed biochemical and metabolic effects of time-restricted eating on PCOS	35
2.4. Hyperinsulinaemia causes hyperandrogenism	36
2.5. Assessment for insulin resistance in practice	38
2.6. The effects of continuous eating on insulin levels and resistance	41
2.7. The effects of time-restricted eating on insulin levels and resistance	42
2.8. The effects of time-restricted eating on fertility and reproductive health	44
2.9. The effects of time-restricted eating on the gut microbiome	45
3. Chapter 3: Systematic Review on the Effects of Time-Restricted Eating on insulin levels and insulin sensitivity in PCOS	
3.1. Introduction	46
3.2. Methods	46
3.3. Results	50
3.4. Discussion	52
3.5. Implication for future research	54
3.6. Conclusion	54

4. Chapter 4: Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (TimeMAP): A feasibility study of real-world clinical advice	
4.1. Introduction and Research Rationale	56
4.2. Study Aims	57
4.3. Methods	58
4.4. Results	71
4.5. Discussion	82
5. Chapter 5: Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (TimeMAP): Analysis (Secondary) Clinical Outcomes	
5.1. Introduction	84
5.2. Methods	85
5.3. Results	86
5.4. Discussion	117
6. Chapter 6: Discussion, Conclusion & Future Direction	
6.1. Brief Chapter Summary	120
6.2. Key Findings of the Thesis	121
6.3. Limitations and Strengths	133
6.4. A Roadmap for Future Research	135
6.5. Conclusions	140
Appendices	141
References	166

List of Abbreviations

17-OHP	17-hydroxyprogesterone
Acetyl CoA	Acetyl Coenzyme A
ADI	Adiponectin Index
ALT	Alanine Transaminase
AMP	Adenosine Monophosphate
AMPK	Adenosine Monophosphate Activated Protein Kinase
ATP	Adenosine Triphosphate
AUCGlu	Glucose Area Under the Curve
AUCIns	Insulin Area Under the Curve
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
CI	Confidence Interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
Cm	Centimetre
CNAQ	Council of Nutrition Appetite Questionnaire
COCP	Combine Oral Contraceptive Pill
CRP	C-reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
DASH	Dietary Approach to Stop Hypertension
DHEA-S	Dehydroepiandrosterone Sulphate
E2	Oestradiol
EDTA	Ethylenediaminetetraacetic Acid
EMBASE	Excerpta Medica database
ERK	Extracellular-Signal-Regulated Kinase

FGF21	Liver Fibroblast Growth Factor 21
FOXO	Forkhead Box Os
FSH	Follicle Stimulating Hormone
GLP-1	Glucagon-Like Peptide 1
GLUT-4	Glucose Transporter Type 4
GnRH	Gonadotropin-releasing Hormone
GSH	Glutathione
HbA1c (DCCT)	Haemoglobin A1c (Diabetes Control and Complications Trial unit %))
HDLs	High Density Lipoproteins
HOMA-B	Homeostatic Model Assessment to Quantify Beta-Cell Function
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IF	Intermittent Fasting
IGF1	Insulin-like Growth Factor 1
IL-6	Interleukin 6
IQR	Interquartile Range
IRS-1	Insulin Receptor Substrate 1
Kg/m ²	Kilograms/metres squared
LDLs	Low Density Lipoproteins
LH	Luteinizing Hormone
MAPK	Mitogen-Activated Protein Kinase
mTOR	Mammalian Target of Rapamycin
mRNA	Messenger Ribonucleic Acid
NAD ⁺ /NADH	Nicotinamide Adenine Dinucleotide
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer

NO	Nitric Oxide
NRF2	Nuclear Factor Erythroid 2-Related Factor 2
OGTT	Oral Glucose Tolerance Test
PCOS	Polycystic Ovarian Syndrome
PGC-1 α	Proliferator-Activated Receptor γ Coactivator 1 α
PI3K	Phosphoinositide 3-kinase
PICO	Population Intervention Comparison Outcome
PKA	Protein Kinase A
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-
Analyses	
PYY	Pancreatic Peptide YY
QQ plots	Quantile-quantile plot
QUICKI	Quantitative Insulin-Sensitivity Check Index
Ras	Rat Sarcoma Gene
RDW	Red Blood Cell Distribution Width
RER	Rough Endoplasmic Reticulum
SD	Standard Deviation
SECA	Medical Measurement Systems and Scales Company
SHBG	Sex Hormone-Binding Globulin
SIRT6	Deacetylases Sirtuins
SNAQ	Simplified Nutritional Appetite Questionnaire
T2DM	Type 2 Diabetes Mellitus
TFTs	Thyroid Function Tests
TNF α	Tumour Necrosis Factor Alpha
TRE	Time Restricted Eating

TRF	Time Restricted Feeding
TTC	Trying to conceive
US	United States
WHO	World Health Organisation
WOS	Web of Science

List of Tables

Table 1.1: Summary of typical intermittent fasting / time-restricted eating regimens

Table 3.1: Reported study characteristics and outcome statistics of the study included in the review

Table 3.2: Reported study characteristics and outcome statistics of relevant studies which were excluded from the review

Table 4.1: Details of n=9 study participant characteristics at baseline. Data presented as median (interquartile range) or number (n) of participants

Table 4.2: List of participant dropouts, point of dropout and reasons for dropout

Table 4.3: Side effect profile of each individual participant while following a time-restricted eating protocol (6 hours eating window per day). Severity of adverse effects classified using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5. All side effects reported as detailed above were Grade 1 only with none requiring medical intervention.

Table 5.1: Median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the 0 week and 12 week timepoints are presented. The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p value for all insulin related parameters.

Table 5.2: Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased). The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p-value for change across 12-weeks for insulin related parameters.

Table 5.3: Results of multi-level linear regression of insulin resistance data showing beta coefficients and p values for each parameter. The β Coefficient gives a change in parameter

over time (per week in this case) and the *p* value indicates whether or not this change is significant.

Table 5.4: Median and interquartile ranges for participants for both the *ad libitum* and time-restricted eating groups at the 0 week and 12 week timepoints are presented. The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where *z* is test statistic and corresponding *p* value for all anthropometric parameters.

Table 5.5: Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased). The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where *z* is test statistic and corresponding *p*-value for change across 12-weeks for anthropometric parameters.

Table 5.6: Results of multi-level linear regression of anthropometric data showing beta coefficients and *p* values for each parameter

Table 5.7: Median and interquartile ranges for participants for both the *ad libitum* and time-restricted eating groups at the 0 week and 12 week timepoints are presented. The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where *z* is test statistic and corresponding *p* value for all reproductive hormone parameters.

Table 5.8: Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased). The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where *z* is test statistic and corresponding *p*-value for change across 12-weeks for the reproductive hormone parameters.

Table 5.9: Results of multi-level linear regression of androgen profile data showing β -coefficients and *p* values for each parameter

Table 5.10: Median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the 0 week and 12 week timepoints are presented. The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p value for all lipid parameters.

Table 5.11: Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased). The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p-value for change across 12-weeks for the lipid parameters.

Table 5.12: Results of multi-level linear regression of lipid profile data showing beta coefficients and p values for each parameter.

Table 5.13: Results of beta coefficients and p values for all parameters for assignment to assess if assignment had an effect on outcomes.

Table 5.14: Results of the Wilcoxon Rank Sum Tests for nutritional intake data showing means/medians and standard deviation/interquartile ranges as well as p values for each parameter. Overall is the average of each nutritional measurement, across the three visits while undertaking the ad libitum or time-restricted eating arm.

List of Figures

Figure 2.1: Illustration of the effect of hyperinsulinaemia on insulin receptors and stimulation of steroidogenesis caused by hyperinsulinaemia and increase LH levels.

Figure 2.2: Cellular metabolic pathways and responses to intermittent fasting showing downstream cascade effects of fasting and resulting beneficial outcomes

Figure 2.3: Hyperinsulinemia downstream effects on ovarian theca cell hyperproduction of androgens and subsequent pituitary feedback causing increased basal LH. Hyperandrogenism results in reduced SHBG (often used clinically for assessment of insulin resistance in PCOS)

Figure 3.1: PRISMA diagram of systematic review search

Figure 4.1: Randomised crossover design where participants were randomised to start with either 12 weeks of time-restricted eating followed by a four week window period prior to crossing over to 12 week ad libitum eating without dietary restriction allowing participants to return to their normal eating pattern, or vice versa.

Figure 4.2: Flow sheet representing recruitment and dropouts at various stages of the study with numbers for final analysis. N=9 subjects participated in the full study, completing both the time-restricted eating and ad libitum arms. Therefore, there are n=9 subjects for analysis in each arm.

Figure 4.3: Side effects of time-restricted eating reported during this aspect of the study

Figure 4.4: Improvements/benefits noted during the time-restricted eating regimen

Figure 5.1: Graphical display of the medians and interquartile ranges of the insulin sensitivity parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks.

Figure 5.2: Graphical display of the medians and interquartile ranges of the anthropometric parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks.

Figure 5.3: Graphical display of the medians and interquartile ranges of the reproductive hormone parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks

Figure 5.4: Graphical display of the medians and interquartile ranges of the lipid parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks.

Figure 5.5: Graphical display of the differences in the means and standard deviation of calorie intake (normally distributed) and medians and interquartile ranges of the macronutrient and mineral intake averages between participants following ad libitum and time-restricted eating regimens from 0-12 weeks

“The best of all medicines is resting and fasting”

(Benjamin Franklin)

Chapter 1

Introduction and Background on the Effects of Time-Restricted Eating on insulin levels and insulin sensitivity in PCOS

1.1 Describing the condition

Polycystic ovarian syndrome (PCOS) affects up to 10% of pre-menopausal women and is characterised by multiple ovarian cysts, hyperandrogenism causing hirsutism, hyperinsulinemia, insulin resistance, obesity, infertility, and mood disturbances [2]. Affected women have an increased risk of developing endometrial cancer, cardiovascular disease, hypercholesterolaemia and type-2 diabetes mellitus making early intervention and management of crucial importance [3]. Diagnosis is most commonly based on the Rotterdam criteria [4]. PCOS is classified as a WHO Group II Ovulation disorder due to hypothalamic-pituitary-ovarian dysfunction. Both pharmacological and non-pharmacological treatments are employed to manage the multi-system effects. Non-pharmacological therapies are based around weight loss, diet, and exercise, and are the foundation of PCOS management. Pharmacological therapies used clinically include metformin [5], spironolactone [6], eflornithine [7], clomiphene [8], GLP-1 agonists [9], thiazolidinediones such as rosiglitazone or pioglitazone [10, 11], N-acetyl cysteine [12], myo-inositol [13], statins [14] as well as various combined-oral contraceptive pill preparations [15] to counteract the various manifestations of PCOS [3, 16]. Metformin alone is considered first line treatment. Clomiphene alone is first line for fertility in the absence of insulin resistance. Ovulation induction with or without metformin can be used for infertility treatment [4].

1.1.2 The PCOS patient

The majority of women with PCOS have insulin resistance, independent of weight [17-19]. This is often subclinical and may not be reflected in biochemical tests, such as glycated haemoglobin (HbA1c), which are routinely done in other patient populations such as pre-diabetes and diabetes to assess for insulin resistance [20]. The clinical utility of HbA1c for diagnosing impaired glucose tolerance and type 2 diabetes in patients with PCOS in daily practice is low [21]. However, hyperinsulinemia and abnormalities in glucose homeostasis leads to cardiovascular and other systemic inflammatory changes [22]. Women with PCOS are more likely to have postprandial hyperglycaemia as a consequence of peripheral insulin resistance versus intact endogenous glucose production mechanism [23]. Overall, data suggest that one in three women with PCOS have impaired glucose tolerance and one in 10 have type 2 diabetes, with the majority (60%) being overweight or obese [2].

Hyperinsulinemia is also important in persistent hyperandrogenism and its clinical manifestations by directly driving excessive androgen production. Due to the relationship between increased insulin levels and androgens, it is important to treat hyperinsulinemia with pharmacologic and non-pharmacological approaches, notwithstanding the pre-clinical effects of high insulin levels and pre-disposition to a pre-diabetic state and diabetes. Insulin resistance has also been implicated in cravings for carbohydrates, subsequent overeating, and weight gain [24, 25]. As cravings and binge pattern eating can lead to weight gain, this has the potential to have confounding effects on overall risk of hyperinsulinemia, subsequent diabetes and cardiovascular disease. Obesity in PCOS is a key driver of deranged cardiometabolic parameters including insulin resistance, hyperandrogenism and dyslipidaemia. Obesity exacerbates these conditions compared to women with PCOS who have a normal BMI. In particular, central obesity is associated with severity and worsening of insulin resistance [26].

1.1.3 Dietary management of PCOS

Dietary interventions in PCOS are varied with many diets being unsustainable and largely ineffective. Studies on various dietary regimens have been trialled including energy (calorie) restriction [27, 28], Mediterranean diet [29], low carbohydrate diet [30], Dietary Approach to Stop Hypertension (DASH) diet [31], pulse-based diet [32], low starch diet [33], low dairy diet [34], ketogenic diet [35], and low glycaemic index diet [36]. Current dietary recommendations for patients with PCOS include regular eating and low glycaemic index foods with avoidance of sugar and carbohydrate-rich diets, as well as vitamin D and calcium supplementation [37]. While the restriction of calories and an increase in exercise improve insulin sensitivity in overweight patients with PCOS, sustained calorie-restricted diets over a prolonged period of time are difficult to sustain [38]. More recently, time-restricted eating [39] has been trialled in patients with PCOS. In contrast to diets that are based specifically on food restriction, time-restricted eating (where patients are asked to consume all energy within a restricted daily time-period) may offer more sustainable weight loss, cardiometabolic changes and may be more acceptable as a permanent lifestyle change [38].

1.2 Time-restricted eating and intermittent fasting

1.2.1 A description

Time-restricted eating, also referred to as intermittent fasting, involves a period of fasting during the day allowing a decrease in insulin levels, an improvement in insulin sensitivity and an improvement in glucose regulation. The short term benefits of time-restricted eating include putative increased cell metabolic and oxidative stress resistance [40]. A period of daily fasting depletes liver glycogen stores and switches energy sources to fatty acid and ketones. This bioenergetic challenge activates signalling pathways that strengthen mitochondrial function, stress resistance and upregulate autophagy of damaged molecules adopting a stress resistance mode [40]. This reduces insulin signalling and overall protein synthesis. During refeeding, following the daily ‘fast’, glucose levels rise and ketones are cleared with increased protein synthesis. This promotes growth and repair and allows more efficient cellular performance, leading to cellular resilience and disease resistance as a long term adaption [38]. A time-restricted eating regimen (where evening food intake is restricted) reportedly improves post-prandial insulin and glucose handling due to alignment of circadian rhythm with diurnal food intake [41]. In terms of public acceptability, the term ‘time-restricted eating’ may arguably be more acceptable than the term ‘intermittent fasting’ due to the heavily restrictive connotations associated with ‘fasting’. Table 1.1 summarises several variations of time-restricted eating and terms that exist.

Regimen	Description
Intermittent Fasting/Time-Restricted Eating	Most commonly involves fasting for 16-18 with an eating window of 6-8 hours, leveraging the natural circadian rhythm. Plain water and unsweetened fluids (black plain tea/coffee) allowed during the fasted period
Time-Restricted Feeding	Restriction of caloric intake to specific time periods of the day, typically 8-12 hours during day time hours. Term typically used in animal studies
Alternate Day Complete Fasting	No calorie intake on fasting days, followed by a day of ad libitum intake (eating to satiety)
Alternate Day Modified Fasting	Restricted calorie intake on 'fasting' days (<25% daily energy requirements), alternated with days of ad libitum intake (eating to satiety)
Periodic Fasting (5:2 or 6:1 protocols)	1-2 fasting days/week and 5-6 days normal caloric intake
Ramadan Fasting (religious fasting)	Fasting from dawn until sunset followed by ad libitum calorie intake after sunset to before dawn. Similar to time-restricted eating but conflicts with circadian rhythm No water or fluids permitted during fasting

Table 1.1: Summary of typical time-restricted eating/ intermittent fasting regimens

1.2.2 Potential benefits of time-restricted eating

Time-restricted eating causes a shift in fuel source from glucose to fatty acids during the fasting period [38]. Typically twelve hours into a fast, there is depletion of liver glycogen stores and fatty acids are mobilised and released as the main energy fuel[38]. By aligning meal times with the light-dark cycle, energy intake, weight control, glucose levels, and insulin levels may be optimised [42]. Shift workers, who contend against the concept of circadian rhythm and aligning of meals with the light-dark cycle, have higher rates of cardiometabolic dysfunction [42].

Time-restricted eating may also positively affect the gut microbiome [43]. The gut microbiome is heavily influenced by diet as well as circadian rhythm with time-restricted eating shown to improve intestinal bacteria microenvironment with increasingly favourable microbial profile. The downstream effects of this includes favourable metabolic regulation and reduction in inflammation [44].

One large prospective study (n=1,422) in which participants underwent a periodic Buchinger fast (named after Otto Buchinger, the physician who first postulated the therapeutic benefits of extended fasting) of between four and 21 days for one year showed overall positive effects on glucoregulation including increased insulin sensitivity, a significant decrease in blood glucose levels, increased overall ketonaemia, and a significant decrease in HbA1c [45]. However, differences in fasting regimens and rules make data on time-restricted eating somewhat heterogenous.

1.2.3 *A typical fast*

Low-calorie sweetened drinks are permitted during the ‘fasted’ period by many studies. Whilst artificial sweeteners are thought to have a negligible effects on insulin levels [46, 47], sucralose, aspartame and saccharin may have an adverse effect on the gut microbiome - which may indirectly lead to impaired glucose homeostasis and insulin resistance and contribute to metabolic disease [48]. While artificial sweetened and low calorie diets reduce food energy content and may promote satiety (and may therefore aid in weight reduction), their potential effect on insulin levels must also be considered given the lack of evidence on their interaction. Other ingredients in artificially flavoured drinks may also effect glucose and insulin levels. Arguably, a true fasted period should not include sweet-tasting beverages, and patients who must fast for biochemical testing are not permitted to take sweet drinks during the fast [49-51].

The optimum length of a time-restricted eating intervention to have a significant effect on insulin levels is undecided. On initially switching from standard dietary pattern to intermittent fasting, excess hunger, irritability and reduced concentration ability during the adaptation phase is expected [40]. These initial effects usually dissipate within one month, making this a reasonable minimum time-frame for an intervention. The ability to maintain time-restricted eating as an indefinite lifestyle measure is untested. High dropout rates have been shown in trials conducted in patients with obesity, PCOS and type 2 diabetes [39, 52]. A clear endpoint may be required as a motivational factor for patients undergoing time-restricted eating for a prolonged period such as prevention of type 2 diabetes, weight loss, return of fertility or amelioration of hyperandrogenism or menstrual symptoms of PCOS. Religious fasting such as Ramadan requires abstinence from food and drink from dawn to dusk for 30 days. Studies which have investigated the effect of Ramadan on insulin levels in PCOS over this 30-day time frame showed no statistically significant benefit [44, 53].

1.2.4 The aims of this thesis

Given its effects on insulin and other metabolic parameters, time-restricted eating represents a novel solution to aid in the control of insulin resistance in patients with PCOS in combination with already established non-pharmacological and pharmacological managements. The first aim of this thesis was to perform a background narrative on the current evidence available on time-restricted eating in PCOS. The second aim was to undertake a systematic review of the studies investigating the effect of time-restricted eating on insulin levels in women with PCOS. The third aim to was describe the feasibility of time-restricted eating in women with PCOS, and to investigate its effects on metabolic abnormalities with a randomised crossover design. Chapter 2 describes an in-depth narrative review of the potential benefits of time-restricted eating for patients with PCOS.

Chapter 2. A Narrative Review on the Effects of Time-Restricted Eating in PCOS

This narrative review aims to give an in-depth description of the mechanisms of insulin resistance in PCOS, and to suggest a potential mechanism of action for time-restricted eating to reduce hyperinsulinaemia and improve insulin resistance in this patient group.

2.1 Mechanism of insulin resistance in PCOS on a molecular level

On a molecular level, there are several reasons for insulin resistance in patients with PCOS.

2.1.1 Insulin receptor defect

There is a defect downstream of the insulin receptor after insulin binds resulting in marked decreases in insulin sensitivity. This defect is due to serine phosphorylation of the insulin receptor and Insulin Receptor Substrate 1 (IRS-1) secondary to intracellular serine kinases. This results in reduced Phosphoinositide 3-kinase (PI3K) activity downstream after insulin mediated activation and resistance to the metabolic actions of insulin. Activation of kinases in Extracellular-Signal-Regulated Kinase/Mitogen-Activated protein kinase (ERK/MAPK) mitogenic pathway in PCOS cause inhibitory serine phosphorylation of IRS-1 in skeletal muscle in patients with PCOS (Figure 2.1). These defects in the insulin receptor gene exist in patients with PCOS with extreme insulin resistance, although insulin receptor numbers and affinity is similar to patient without insulin receptor defects [54]. Hyperinsulinaemia downregulates insulin receptor expression and also encourages internalisation of insulin receptors in response (Figure 2.1). Steroidogenesis is stimulated by both hyperinsulinemia causing inositolglycan generation and increased basal luteinising hormone (LH) secreted from the anterior pituitary in response (Figure 2.1).

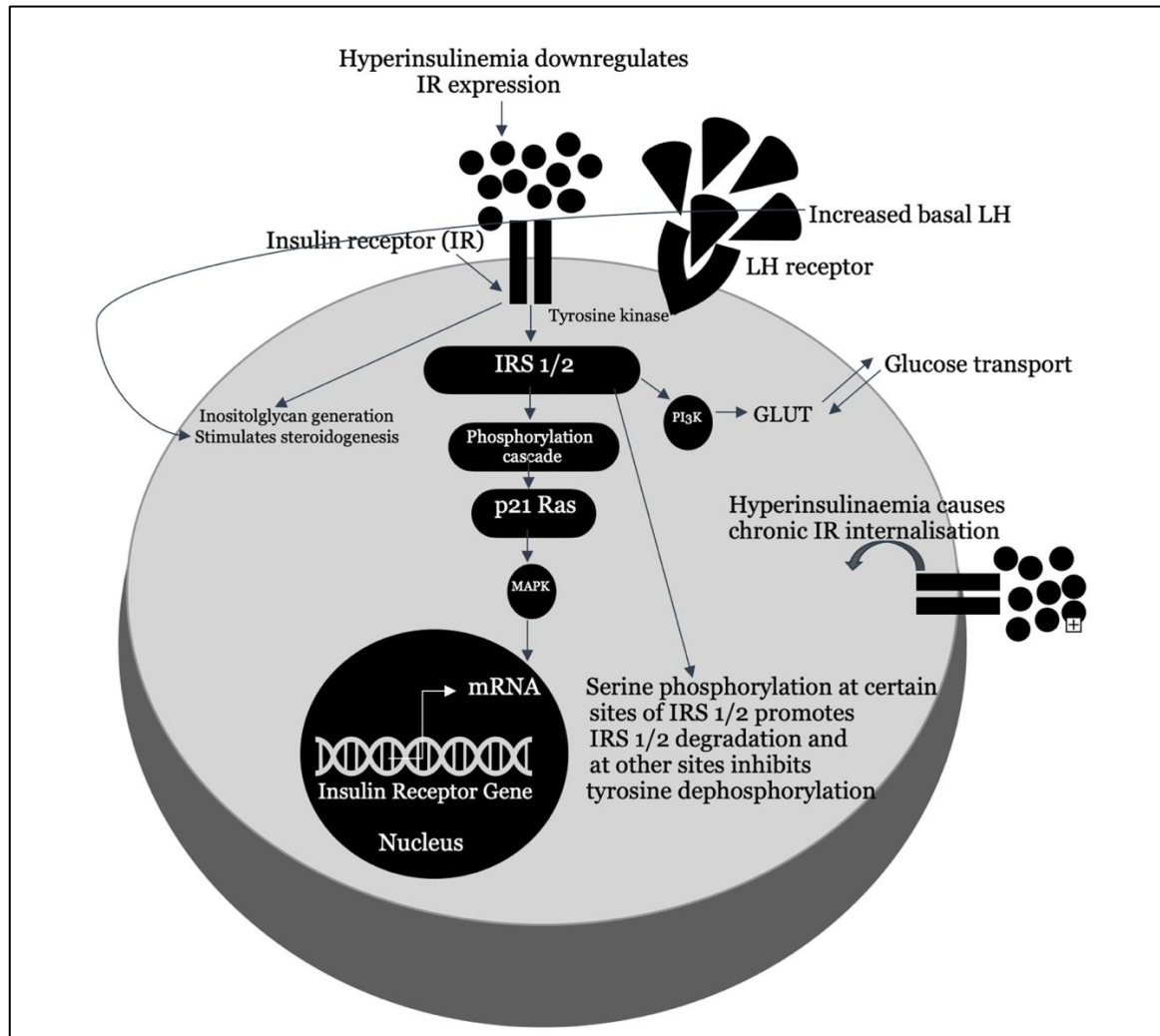


Figure 2.1: Illustration of the effect of hyperinsulinaemia on insulin receptors and stimulation of steroidogenesis caused by hyperinsulinaemia and increase luteinising hormone levels

IR insulin receptor, LH luteinising hormone, GLUT Glucose Transporter, IRS Insulin Receptor Substrate, Ras Rat Sarcoma gene, mRNA messenger ribonucleic acid, MAPK Mitogen-Activated Protein Kinase, PI3K Phosphoinositide 3-kinase

2.1.2 Beta cell dysfunction

Patients with PCOS may also have beta-cell dysfunction. In the setting of insulin resistance, there would normally be a compensatory increased insulin secretion. This hyperbolic function has high inheritability and is the most powerful factor at predicting diabetes risk. The interplay between environmental factors with genetics leads to beta cell overstimulation, with subsequent hyperinsulinemia [55]. This, in combination with obesity and insulin resistance in patients with PCOS, causes beta cell exhaustion and increases overall type 2 diabetes risk [56]. That said, beta cells are known to be dysfunctional in PCOS, independent of age and weight [55, 56]. This suggests that there is a defect in glucose-stimulated insulin secretion in PCOS. This phenomenon is found in early adolescence in women with PCOS with impaired glucose tolerance [57].

2.1.3 Reduced insulin clearance

Insulin clearance in PCOS may also be decreased due to decreased insulin receptor number and function [23]. This decreased hepatic insulin clearance combined with the basal hyperinsulinaemia results in overall increased fasting insulin levels [23]. Increased basal insulin secretion is one of the underlying mechanisms of hyperinsulinemia characteristic of PCOS as demonstrated in Figure 2.3. Clinically, this is seen in women with PCOS with an increased insulin response during OGTT, independent of age and obesity [23].

2.1.4 Secretion of adipokines from visceral adipose tissue

Adipokines, such as leptin, are pro-inflammatory causing chronic low-level inflammatory effects. Leptin participates in interleukin-6 (IL-6) production which promotes c-reactive protein (CRP) production in the liver and upregulates Tumour Necrosis Factor Alpha (TNF-alpha). Conditions associated with an increased BMI and insulin resistance including

metabolic syndrome [58, 59], diabetes [59, 60] and PCOS [61, 62] all show increased leptin levels. Adiponectin, in contrast, is anti-inflammatory by its interaction with Nuclear Factor Kappa-Light-Chain-Enhancer (NF- κ B) and TNF- α . It is beneficial to glucose homeostasis in that it promotes fuel switch to fatty acid breakdown in the liver, reduced gluconeogenesis and increased insulin sensitivity and glucose uptake. With increased BMI and adiposity, adiponectin levels decrease, as do their anti-inflammatory and insulin sensitisation effects. The leptin and adiponectin imbalance is involved in the metabolic alterations which increase the risk of type-2 diabetes [59, 60].

2.2 The effect of fasting on insulin resistance in PCOS on a cellular level

On a cellular level, time-restricted eating contributes to the fluctuating ratios of NAD⁺ to NADH, ATP to AMP and acetyl CoA to CoA. These have downstream activation of proteins, including transcription factors (FOXOs, PGC-1 α and NRF2); AMP kinase; and deacetylases such as sirtuins (SIRT6) [38, 40] (Figure 2.2). These help regulate cellular basic function and improve cellular stress resistance. AcetylCoA and NAD⁺ serve as co-factors for these SIRT6 epigenetic modifiers [38, 40]. SIRT6 deacetylate the above transcription factors and this promotes gene expression which aid with cellular stress resistance and effects (Figure 2.2).

Downregulation of the insulin-IGF-1 signalling pathway and reduced circulating amino acids with fasting reduces mTOR activity with subsequent inhibition of protein synthesis. The above processes allows activation of cellular repair and maintenance processes, stress resistance and mitochondrial biogenesis, cellular proteostasis and autophagy with overall improved cell survival. The ratio of AMP to ATP is increased by fasting, activation of AMPK again triggering repair and halting anabolic processes [40].

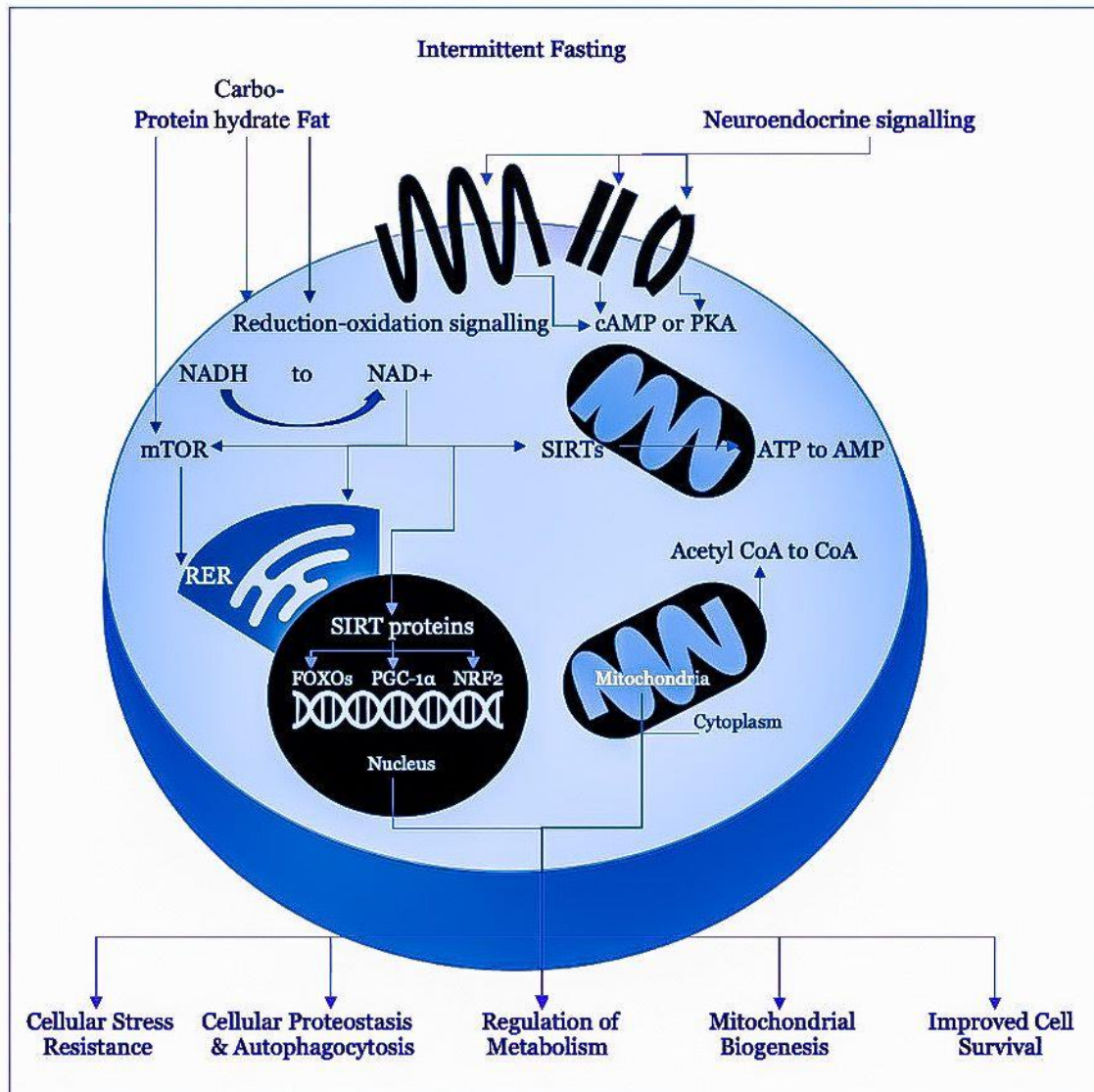


Figure 2.2: Cellular metabolic pathways and responses to time-restricted eating/fasting showing downstream cascade effects of fasting and resulting beneficial outcomes

Abbreviations: NAD⁺ nicotinamide adenine dinucleotide, FOXOs forkhead box Os, PGC-1α proliferator-activated receptor γ Coactivator 1α, NRF2 nuclear factor erythroid 2-related factor 2, AMP kinase AMPK, cAMP cyclic adenosine monophosphate, ATP adenosine triphosphate, SIRTs deacetylases sirtuins, mTOR mammalian target of rapamycin, IGF-1, insulin-like growth factor 1, PKA Protein Kinase A, RER Rough Endoplasmic Reticulum, Acetyl CoA Acetyl Co-enzyme A

2.3 Proposed biochemical and metabolic effects of time-restricted eating on PCOS

The association of PCOS with insulin resistance, along with the role of insulin as a reproductive hormone affecting ovulation, has led to interest in the use of insulin sensitising agents to reduce circulating insulin in women with PCOS. Due to its putative effects on reducing hyperinsulinaemia and improving insulin sensitivity, time-restricted eating has the potential to replace or complement the available pharmacological therapies. In combining the molecular effects of time-restricted eating (including improved efficiency of hepatic glucose management and shifting away from carbohydrate metabolism consequentially lowering required beta cell secretion of insulin) with the genetic and environmental mechanisms of insulin resistance seen in PCOS, the hypotheses of the effects of time-restricted eating on hyperinsulinemia in PCOS can be explored.

In summary, the biochemical effects of time-restricted eating in PCOS proposed include:

1. Increased recruitment of insulin receptors, internalised by the effects of persistent hyperinsulinemia
2. Upregulation of insulin receptor expression which are downregulated by hyperinsulinemia
3. Lowering insulin requirements with fasting. Fasting overcomes the issues seen in patients with PCOS including saturation of defected insulin receptors, beta cell dysfunction and reduced hepatic insulin clearance
4. Reduced leptin levels and increased adiponectin with the overall improved balance improving insulin resistance

2.4 Hyperinsulinaemia causes hyperandrogenism

In PCOS, there is an increased frequency of pulsatile gonadotropin-releasing hormone (GnRH) from the hypothalamus which increases overall LH secretion leading to increased basal LH (Figure 2.3), pathognomonic for PCOS. Increased basal LH leads to stimulation of the theca cells to produce testosterone. As there is low, muted or unchanged levels of follicle-stimulating hormone (FSH) due to this imbalance, testosterone is incompletely aromatised by the granulosa cells, stimulated by FSH under normal circumstances. An increase in steroidogenic enzyme activity in PCOS also increases androgens. A seminal study by Burghen *et al* in 1980 correlated hyperandrogenism with hyperinsulinism in PCOS. Insulin helps to regulate ovarian function with dysfunctional ovaries utilising excess insulin which leads to increased androgen production and subsequent anovulation. Anovulation occurs due to the halt in follicular maturation which is a sure sign that there is ovarian pathology.

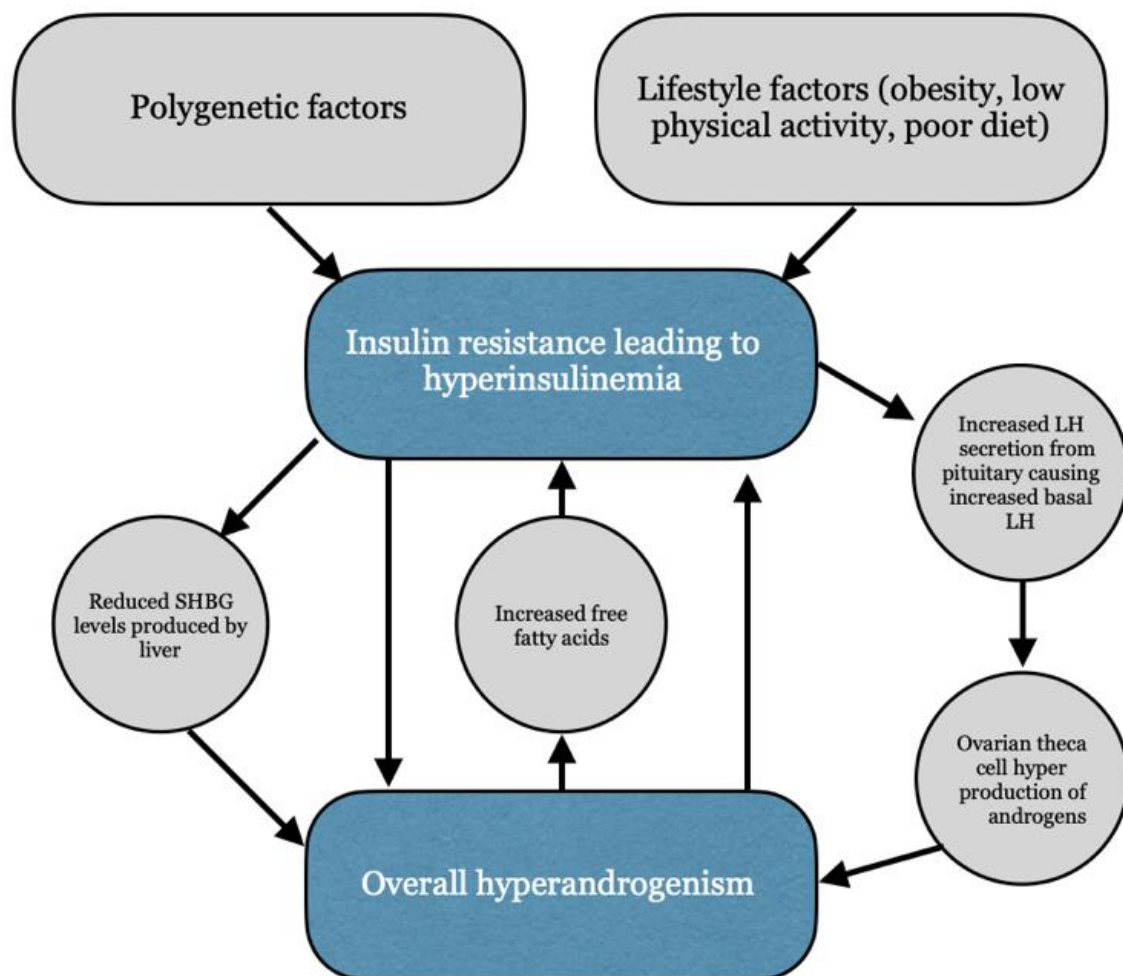


Figure 2.3: Hyperinsulinemia downstream effects on ovarian theca cell hyperproduction of androgens and subsequent pituitary feedback causing increased basal LH. Hyperandrogenism results in reduced SHBG (often used clinically for assessment of insulin resistance in PCOS)

Abbreviations: LH luteinising hormone, SHBG Sex Hormone Binding Globulin.

Insulin has direct action on ovarian IGF-I receptors (these are structurally and functionally similar to insulin receptors elsewhere in the body [54]). There is hyperstimulation and activation of the IGF-1 receptors by increased insulin levels in PCOS, which in turn increases theca cell steroidogenesis. This subsequently impacts the hypothalamic-pituitary-

gonadal axis by increasing LH secretion [54]. The overall increase in basal LH leads to these anovulatory cycles in PCOS. Insulin's action on steroidogenesis in granulosa and theca cells is mediated by the insulin receptor. It also appears that women with PCOS have theca cells that are more susceptible to insulin's androgen stimulating actions [63]. Insulin can act as a co-gonadotropin to enhance LH-induced luteinisation of the granulosa cells. LH and FSH can be stimulated by GnRH release caused by hyperinsulinaemia. Both of the above mechanisms then lead to increased basal LH levels [63]. Insulin sensitising medications used in PCOS are designed to treat hyperinsulinaemia and subsequent ovarian androgen hyperproduction with the aim of correcting sex hormone binding globulin (SHBG) levels [64]. An increase in SHBG can reduce the symptoms of PCOS by normalising hyperandrogenaemia, restoring normal menstrual cycles, and improved cardiometabolic parameters.

2.5 Assessment for insulin resistance in practice

2.5.1 Euglycaemic hyperinsulinaemic clamp method

The gold standard for accurate measurement of insulin resistance is the euglycaemic hyperinsulinaemic clamp method. It requires a continuous insulin infusion to raise and hold plasma insulin concentration at a pre-set level, such as 100 $\mu\text{U}/\text{ml}$. A continuous concurrent glucose infusion allows a steady glucose plasma concentration held constant at basal levels. Once balance with the steady-state is reached, the glucose infusion rate corresponds with glucose uptake, and therefore measures insulin sensitivity and subsequently resistance. While more accurate, this is more demanding to execute in practice than serum blood samples as well as being expensive, time consuming and labour intensive [65].

With many variations in practice worldwide on assessing insulin resistance, global consensus is illusive. Finding an accurate, but easily obtainable and minimally invasive, test is paramount. Insulin resistance can be assessed with a multitude of biochemical tests including fasting insulin and glucose levels which are used to calculate Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), OGTT and assessment of areas under the glucose/insulin curves, and SHBG levels [66]. The remainder of section 2.5 describes some of the more commonly used indirect methods of testing insulin resistance used in practice.

2.5.2 HbA1c

Based on a large retrospective study, the clinical utility of HbA1c for diagnosing insulin resistance and type 2 diabetes in PCOS is low, with low sensitivity when compared to OGTT [21].

2.5.3 HOMA-IR

The homeostatic model assessment (HOMA) index is calculated with fasting glucose and insulin to determine insulin resistance (termed HOMA-IR). It is frequently used in the literature when reporting outcomes relating to insulin resistance in PCOS patients [65, 67-70]. HOMA may also be evaluated alongside red blood cell distribution width (RDW) and together become a stronger diagnostic tool with minimal effect on cost [71]. However, HOMA-IR has also been shown to lack sensitivity in young, normal-weight patients with PCOS [72]. This study suggested that this subcategory of patient with PCOS should undergo 3-hour OGTT to assess glucose levels, which they suggested has better sensitivity to detect early insulin resistance. Shorter interval evaluation after was insufficient also in identifying all hyperinsulinaemic patients with PCOS, making this a diagnostically challenging subgroup [72].

2.5.4 QUICKI

The calculation of quantitative insulin sensitivity check index (QUICKI) is also used in conjunction with BMI and waist circumference as a simple, quick and sensitive assessment of insulin resistance in PCOS. It is derived using the inverse sum of the logarithms of fasting glucose and insulin and correlates well with the gold standard euglycaemic hyperinsulinaemic clamp. These three parameters are useful early markers in identifying insulin resistance [73, 74].

2.5.5 Adiponectin levels

Adiponectin and the adiponectin (ADI) index (adiponectin/fasting blood glucose x fasting insulin) is a good surrogate index and also correlates well with the euglycemic-hyperinsulinaemic clamp for the assessment of insulin resistance with good sensitivity and specificity [75, 76]. In PCOS, when serum adiponectin level is decreased, the degree of insulin resistance is increased with serum adiponectin level acting as an adequate marker for determining the degree of insulin resistance and risk of type-2 diabetes mellitus (T2DM) and metabolic syndrome [76].

2.5.6 SHBG

SHBG correlates with features of the metabolic syndrome and can be used as an index of insulin resistance in metabolic syndrome [77] and PCOS [78] to target individuals who may benefit from insulin sensitising interventions. However, it has been suggested that relationships between insulin parameters and SHBG are weak and that SHBG is a poor predictor of insulin resistance [79]. SHBG also has poor specificity in that low levels are also caused by hypothyroidism, obesity, metabolic syndrome, Cushing's syndrome, acromegaly, and androgens including anabolic steroids.

2.5.7 OGTT

Overall, quick, simple assessments such as HOMA-IR or OGTT represent the optimal method to assess women with PCOS as they give valuable information about both insulin resistance and glucose intolerance and allows patients to be followed up closely or targeted for early interventions [80]. The American Association of Clinical Endocrinologists suggest that a baseline OGTT for all patients with PCOS diagnosis should be carried out and repeated every 1-2 years based on risk factors for T2DM, and yearly in women with known insulin resistance [81].

2.5.8 Matsuda index

The Matsuda index is calculated by extrapolating data from OGTT results to give whole-body insulin sensitivity index combining both hepatic and peripheral tissue insulin sensitivity[82]. It can be used clinically and based on simple calculation from clinical data[82]. It has been shown to be very sensitive for detecting insulin resistance especially in women with PCOS and normal BMI [83].

2.5.9 Cederholm index

The Cederholm index is calculated after an oral glucose load and the results refer to peripheral insulin sensitivity and uptake of glucose by muscles given that glucose is largely metabolised in peripheral tissues [84]. It is a suitable assessment for insulin sensitivity for epidemiological and research purposes [84].

2.6 The effects of continuous eating on insulin levels and resistance

Long feeding windows (and therefore short fasting periods) are thought to perturb normal glucose metabolism. Eating habits have enormously changed with modern lifestyle. A

longitudinal study of dietary habit changes over a 40 year timespan in the US showed higher daily energy intake, later breakfast and lunch times, and shorter time between dinner and a post-dinner snack, with a significant increase in snacking among women [85]. There are few human studies on continuous eating and its effect on insulin levels. It could be hypothesised (based on longitudinal studies describing a change in dietary patterns with less fasting time) that an increasingly lengthy post-prandial state could increase the risk of insulin resistance and metabolic disorders. In animal studies comparing continuous ad libitum with time-restricted feeding, ad libitum feeding leads to increased rates of obesity, cancer, renal and cardiovascular disease and decreased overall survival [86].

2.7 The effects of time-restricted eating on insulin levels and resistance

Eating within a limited time-frame (either on a daily or weekly basis) is thought to alter cardiometabolic parameters including fasting glucose levels, insulin levels, weight, blood pressure, lipid profiles and markers of inflammation. The majority of studies that cite therapeutic benefits of fasting regimens in various patient populations also report weight loss, with most beneficial cardiometabolic effects as a result of the latter [87-90]. Intermittent fasting may arguably result in caloric restriction due to limited eating windows and resultant weight loss. This could represent an obvious confounding factor in the improvement of insulin resistance, making it difficult to isolate the fasting regimen in itself as being beneficial to insulin resistance. It is difficult to remove this confounding factor when designing studies on intermittent fasting, as asking patients to restrict their eating to a limited period may plausibly result in reduced calorie intake and subsequent weight loss. However, arguably this is an important outcome in itself. Whilst 'permanent' energy-restricted diets will result in weight loss, eating fewer calories appears to be virtually impossible in the long term. If time-restricted eating results in a sustainable, long term

reduction in energy intake due to its effect on appetite or satiety, then this would be of clear value for a myriad of patient types. Compliance has been shown to be similar for patients following a time-restricted eating regimen compared to traditional calorie restricted dietary regimens [87] although compliance has not been examined in large patient cohorts over prolonged time periods.

Weight loss surgical interventions result in significant alterations in insulin patterns as well as improvement in other hormone profiles important in obesity and metabolic health in women with PCOS [91, 92]. In addition to weight loss, sleeve gastrectomy surgery ameliorated hormone profiles including insulin and gonadotrophic hormones to more homeostatic levels conducive to successful reproduction in women with PCOS [92]. That said, the effects of bariatric surgery is thought to be equivalent to weight loss with a calorie-restricted diet in reducing glucose and insulin levels [93]. Animal studies have shown that time-restricted feeding, without reduction of calories, show protection against hyperinsulinemia and improves overall hepatic glucose metabolism [94-99]. Animal studies have also shown that animals who were fed a high fat, dairy, and sugary drinks-containing 'Western diet' within a time-restriction, had positive metabolic changes in the absence of weight loss [98]. A small study in pre-diabetic men showed that time-restricted eating (6 hour eating period) conferred cardiometabolic benefits including reduced blood pressure, reduced oxidative stress and reduced appetite as well as increased insulin sensitivity and β cell responsiveness independent of weight loss [93]. However, whether or not the benefits of time-restricted eating are independent of weight loss, particularly in patients with PCOS, is not known and has been insufficiently researched. Ideally, randomised human studies on patients with insulin resistance (including type-2 diabetes, metabolic syndrome or PCOS) should be carried out. A study design comparing time-

restricted eating with continuous eating, with both arms receiving isocaloric, meal frequency-matched diets would be of value but would be practically challenging.

2.8 The effects of time-restricted eating on fertility and reproductive health

Overweight and obese women experience increased rates of menstrual disorders, subfertility and gestational morbidities increasing the risk in pregnancy. PCOS is often associated with increased BMI as well as metabolic and reproductive disturbances. PCOS is the main cause of anovulatory infertility and seen in 72% of women with PCOS in comparison with a rate of 16% in the general population, and a 15 fold higher incidence of infertility [100]. The aetiology of reduced fertility in PCOS are multifactorial and include the effects of obesity, metabolic and endocrine abnormalities as well as increased inflammatory and increased sympathetic responses. These lead to impairment in ovulation, oocyte quality as well as endometrial receptivity. PCOS is an independent risk factor for miscarriage as well as being associated with advanced maternal age pregnancy as a result of long-standing infertility. PCOS also increases the risk of gestational morbidity, being associated with gestational diabetes, hypertensive disorders of pregnancy, preterm delivery and low birth weight and their associated complications [101].

This is associated with major economic cost and a large proportion of healthcare resources. Proving significant benefits of a dietary regimen in addition to the current recommended management would be major benefit both for women with PCOS with potential to improve clinical symptoms, metabolic profiles and fertility outcomes as well as reducing the risk of chronic diseases such as diabetes, cardiovascular disease, metabolic syndromes and high-risk pregnancy. In addition, this would clearly confer considerable health and economic benefits. Fasting during Ramadan has been shown to reduce the chronically overactive sympathetic system seen in patients with PCOS consequentially reducing stress response

hormones cortisol and noradrenaline which ultimately can effect fertility [53]. Interestingly, this study showed no significant difference in insulin levels between women undergoing Ramadan fasting and the non-fasting group (n=40). Fasting has the potential to correct ovulation responses in women with PCOS by altering FSH and LH levels and restoring normal menstrual cycles [102]. Fasting, independent of weight loss, is postulated to have these effects on neurohormonal regulation.

2.9 The effects of time-restricted eating on the gut microbiome

Previous literature suggested that those on long term calorie restricted diets may have more diverse and rich faecal microbiota. Preliminary data suggest that intermittent fasting enriches the contents of the gut microbiota, with favourable microbial groups including Bifidobacteriaceae, Lactobacillaceae and Akkermanisaceae [103, 104]. These gut microbiota have a significant role in glucose metabolism with effects on reducing gut permeability and enhancing tight junctions which effect and ameliorate endotoxemia. They effect glucose homeostasis and insulin resistance by increasing glycogen synthesis and improve GLUT-4 translocation and improve efficiency and effectiveness of glucose uptake by insulin among many other actions [105]. Diet is heavily influential on the make-up of an individual's diverse gut microbiome and this could partially account for the benefits of intermittent fasting in improving insulin resistance and glucose homeostasis [106]. Probiotic supplementation has been shown to have beneficial effects on weight, lipids, insulin resistance, androgen levels and clinical symptoms in patients with PCOS [107-109].

In Chapter 2, a narrative review of the potential effects of time-restricted eating in women with PCOS was provided. Chapter 3 describes a systematic review of studies investigating time-restricted eating to reduce insulin in women with PCOS.

Chapter 3

A Systematic Review on the Effects of Time-Restricted Eating on insulin levels and insulin sensitivity in PCOS

3.1 Introduction

Chapter 2 described the effect of time-restricted eating at a molecular and cellular level in PCOS and the various mechanisms that improve insulin resistance. The discussion then led to how this correlates clinically, in practice. Time-restricted eating represents a novel solution to aid in the control of insulin resistance in patients with PCOS. Despite the potential benefits of implementing time-restricted eating in women with PCOS, there has been no systematic review of this topic to date to assess available evidence. This study therefore aims to systematically review and meta-analyse intervention studies investigating the effect of time-restricted eating on insulin levels and insulin sensitivity in patients with PCOS.

3.2 Methods

This systematic review followed PRISMA reporting guidelines [110]. This review was registered with the PROSPERO registry under registration number CRD42021267268.

3.2.1 Review question

The PICO (Population, Intervention, Comparator, Outcomes) model was employed to expand the return from the review and applicability of data collected.

- **Population:** Over eighteen years of age, females diagnosed with polycystic ovarian syndrome using Rotterdam criteria.

- **Intervention:** Any time-restricted feeding regimen, alone or in-combination (pharmacological therapy, exercise, weight loss). To include 16:8 method, 18:6 method, 5:2 diet, alternate day fasting, intermittent fasting, Ramadan or other religious fasting methods deemed relevant.
- **Comparator:** Comparison to usual ad libitum diet or no dietary or fasting intervention, standard treatment-as-usual, or pharmacological therapy
- **Outcomes:** Metabolic parameters which represent effect on insulin levels, C-peptide, Glucagon, insulin-like growth factor-1 (IGF1), HbA1c, HOMA-IR, fasting blood sugar, SHBG or OGTT

3.2.2 Search strategy and study selection

A preliminary scoping review was conducted to identify the nature and extent of the research available. A systematic search strategy was constructed with oversight of a medical librarian. Five search strategies were created for Medline, Cumulative Index to Nursing and Allied Health Literature (CINAHL), EMBASE, Web of Science (WOS) and Cochrane Library (Appendix 1). The five databases searched yielded 6,340 potential papers: Medline (1,737), CINAHL (339), EMBASE (1,909), WOS (2,086) and Cochrane (268). After removal of duplicates, 2,739 papers remained. No further studies were identified by hand-reviewing citation lists of eligible studies, previous reviews, and field expert publications. Thirty-seven papers underwent full-text review, and one met the inclusion criteria (Figure 3.1).

Keywords used for searches included *'polycystic ovarian syndrome'*, *'PCOS'*, *'time-restricted feeding'*, *'TRF'*, *'time-restricted eating'*, *'TRE'*, *'insulin levels'*, *'metabolic changes'*, *'endocrine changes'*, *'diet'*, and *'Ramadan'* (full search details in Appendix 1).

Papers were selected for full-text review after dual-screening by two independent reviewers (another researcher and I) who made independent decisions on inclusion. All conflicting decisions were resolved by the thesis co-supervisor (SND). Further manual searches were carried out of the reference lists of all relevant papers for additional relevant papers. Studies were deemed to be excluded due to incorrect study design, incorrect intervention, incorrect setting, incorrect population, failure to meet inclusion criteria, duplicate not detected, ongoing studies, still recruiting, non-controlled trials and different outcome measured (Appendix 2). The following data were extracted from each paper: study design, patients lost to follow-up, completion of intention-to treat analysis, group allocation method, participant numbers, age-range, study population, country, baseline pre-intervention weight and insulin levels, intervention type, comparison, and outcome assessment.

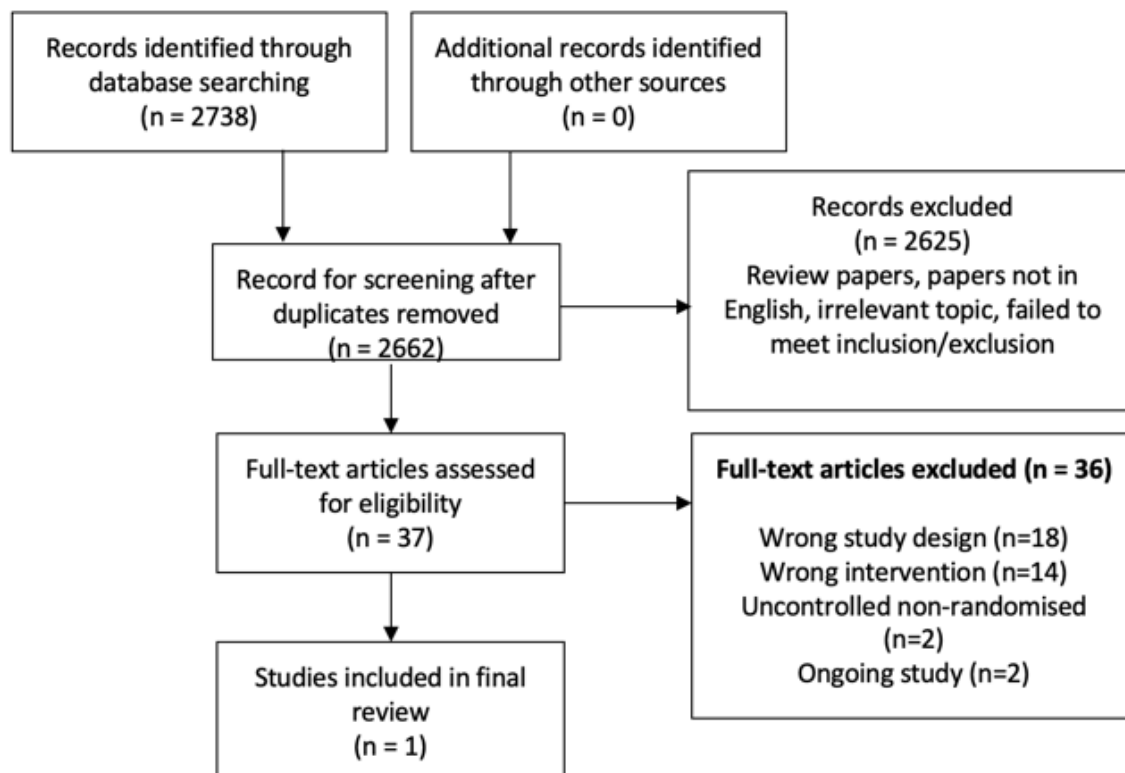


Figure 3.1: PRISMA diagram of systematic review search

3.2.3 Inclusion/exclusion criteria

Timeline: Published prior to 10th of May 2021 when final searches were run

Patients: Female patients with a diagnosis of PCOS according to the Rotterdam criteria [4].

Inclusion: The following studies were included: All study designs on human subjects published in peer-reviewed medical journals; studies involving time-restricted eating, Ramadan, and diets with any fasting regimen; studies where time-restricted eating / intermittent fasting combined with concurrent medication use (COCP, metformin, spironolactone etc.); Pilot studies assessed for inclusion if clear outcome effects detailed. Studies had to have baseline and post intervention measures of outcomes.

Exclusion: The following studies were excluded: Conference papers, unpublished reports, letters to the editor, papers not in English, studies considering administration of supplements, studies without a control group, studies without baseline compared to post intervention measures; non-human studies, pre-clinical and animal studies.

3.2.4 Assessment of results

Study characteristics were collected including details of participants, study methodology, intervention details and effect on insulin and other metabolic parameters. Analysis to assess study quality and risk of bias was conducted in accordance with Cochrane Handbook for Systematic Reviews of Interventions.

3.2.5 Analysis/data synthesis

The studies included in the review were inherently heterogeneous due to varying interventions and outcomes. As a result, a descriptive presentation of the studies was used without pooling of outcomes for analysis.

3.3 Results

3.3.1 Study identification and characteristics

Following application of the inclusion and exclusion criteria, n=1 study was retrieved in the search. This study was a non-randomised controlled study of women with PCOS with a Ramadan fasting regimen (Table 3.1). Due to the variety of fasting interventions and heterogeneity in study design, it was not possible to conduct a meta-analysis.

The single study retrieved recruited n=40 women with PCOS, n=20 of whom underwent Ramadan fasting for a mean of 26 days; they were compared to a non-fasting control group. Details of the length of fasting times were not included but, rather, details of eating patterns before sunrise, after sunset and between sunset and sunrise. There were no significant differences in levels of beta-endorphin ($p=0.543$), insulin ($p=0.818$), FSH, LH, testosterone or adrenaline after undergoing Ramadan fasting. Those who followed Ramadan fasting had significantly lower cortisol ($p=0.049$) and nor-adrenaline levels ($p=0.047$). Overall there was no benefit of Ramadan fasting shown in this study on insulin levels or glucose homeostasis [111]

Author, year Study details Country	n	Age Inc.	Mean Age	Outcome Measured	Significance (p)	95% CI	Effect size (d)*
Zangeneh 2015 Ramadan Fasting Non-Randomised Control Trial Duration: 4 weeks Iran	40 (40 completed and analysed)	20-40	29	Weight	0.221	Not reported	Not reported
				Infertility duration	0.445	Not reported	Not reported
				Acne	0.723	Not reported	Not reported
				Hirsutism	0.451	Not reported	Not reported
				Irregular menstrual cycles	0.231	Not reported	Not reported
				FSH	0.542	Not reported	Not reported
				LH	0.827	Not reported	Not reported
				Testosterone	0.683	Not reported	Not reported
				Insulin	0.818	Not reported	Not reported

Table 3.1: Reported study characteristics and outcome statistics of the study included in the review

**Effect sizes classified as follows: small effect: $d=0.2$, medium effect: $d=0.5$, large effect: $d=0.8$.*

Abbreviations: FSH Follicle stimulating hormone, LH Luteinising Hormone, CI Confidence Interval.

3.4 Discussion

3.4.1 Summary of findings

This was the first study to systematically review the literature to identify studies investigating the effect of time-restricted eating on insulin parameters in women with PCOS [112]. Only one study was retrieved. The review showed that no randomised controlled trials assessing the effects of time-restricted eating on insulin levels in patients with PCOS had been conducted on this topic to date. Overall, the study showed that Ramadan fasting had no effect on insulin levels or glucose homeostasis in individuals with PCOS.

3.4.2 The potential effect of Ramadan

An additional study on Ramadan fasting in women with PCOS was excluded from the search as it was non-controlled and did not meet the inclusion criteria. Asemi et al looked at the effects of Ramadan fasting in 27 women who fasted for a mean period of 16.5 hours a day for 29 days. There were no significant differences in weight ($p=0.439$), BMI ($p=0.646$), fasting blood glucose ($p=0.183$), insulin ($p=0.474$), HOMA-IR ($p=0.364$), HOMA-B ($p=0.736$), QUICKI ($p=0.308$), CRP levels ($p=0.072$), or lipid profiles. Significant effects were shown of Ramadan fasting on nitric oxide levels ($p=0.003$) and total glutathione levels ($p=0.011$). Overall, this study demonstrated that Ramadan fasting exerted no benefit on parameters of glucose homeostasis, but had modest benefits on markers of inflammation and cardiovascular health (Table 3.2) [113].

Ramadan fasting has previously been shown to have positive benefits on insulin levels and glucose homeostasis in small studies of non-PCOS populations. Ramadan fasting improved HbA1c levels in 29 patients with type-2 diabetes [114], as well as demonstrating a significant decline in glucose levels in 80 healthy subjects examined [115]. There appears

to be modest benefits on hormonal markers of stress (cortisol and nor-adrenaline levels) [111], inflammation (CRP levels) and cardiovascular health (NO and GSH levels) [113]. The underlying mechanism of these benefits remain to be defined and elucidated.

3.4.3 Studies on fasting in PCOS that were excluded from the search

Li et al looked at the 16:8 fasting regimen in 18 women with PCOS who completed a 16 hour fast daily for five weeks, preceded by a one week baseline weight stabilisation period. This was not a randomised controlled trial and therefore failed to meet the inclusion criteria of the systematic review. Three of the 18 participants (16.67%) dropped out of the trial. Significant positive effects were shown of 16:8 on fasting insulin levels ($p=0.017$), insulin area under the curve (AUCIns, $p=0.007$), ratio of AUCIns/Glucose area under the curve (AUCGlu) ($p=0.001$), HOMA-IR ($p=0.0025$), SHBG ($p<0.001$), body weight ($p<0.001$), BMI ($p<0.001$), body fat mass ($p<0.001$), percentage free androgen index ($p=0.001$), total testosterone ($p=0.048$), CRP ($p=0.040$), ALT ($p=0.027$), and IGF-1 ($p=0.006$). There were no significant differences in fasting glucose ($p=0.614$), AUCGlu ($p=0.516$), lipid profiles, gonadal parameters including LH, FSH and LH/FSH ratio. Overall, this study demonstrated the 16:8 fasting regimen exerts benefit on parameters of glucose homeostasis and weight, summarised in Table 3.2 [39]. There was significant weight loss reported making it difficult to interpret the positive results on parameters of glucose homeostasis with the confounding factor of concomitant weight loss. Li et al observed significant results in a short 6-week time-restricted eating regimen with improvements in hyperinsulinemia and insulin resistance but it is unclear if this may be as a result of energy restriction or time-restricted eating [39]. This is contrary to a review reporting isocaloric time-restricted feeding had greater benefit in reducing fasting insulin levels and insulin resistance than ad libitum time-restricted feeding [37]. This study was limited in that it was a non-randomised uncontrolled

intervention study with small patient numbers. The short duration of the intervention was also a limitation of this study [39], as well as the studies on Ramadan fasting [111, 113].

3.5 Implications for future research

The results of this review illustrate the large research gap that exists on this topic. Along with the narrative review in Chapter 2 there is a compelling case for the design of a study investigating the effect of time-restricted eating on important metabolic and clinical parameters in women with PCOS. Specifically, a prospective randomised cohort study with crossover design with the primary outcome assessment of short and long-term effects on insulin levels with secondary outcome assessment of effect on androgens and fertility would provide valuable data on the potential effects of such an intervention.

3.6 Conclusion

Data are limited on the effect of time-restricted eating in PCOS. The results of this study led to the design of a prospective interventional study on the effects of time-restricted eating on metabolic and clinical indices in women with PCOS, described in Chapters 4 and 5.

Author, year Study details Country	n	Age	Mean Age	Outcome Measured	Significance (p)	95% CI	Effect size (d) ^a
Chunxhu Li 2021 8 Hour Time-Restricted Eating Duration: 6 weeks Non-Randomised Uncontrolled Trial China	25 (15 completed and analysed)	18-31	-	Body weight	<0.001*	Not reported	Not reported
				Body mass index	<0.001*	Not reported	Not reported
				Waist-to-hip ratio	0.050*	Not reported	Not reported
				Skeletal muscle mass	0.062	Not reported	Not reported
				Body fat mass	<0.001*	Not reported	Not reported
				Body fat percentage	0.012*	Not reported	Not reported
				Visceral fat area	0.015*	Not reported	Not reported
				Fasting glucose	0.614	Not reported	Not reported
				Fasting Insulin	0.017*	Not reported	Not reported
				HOMA-IR	0.025*	Not reported	Not reported
				Total testosterone	0.048*	Not reported	Not reported
				Sex hormone-binding globulin	<0.001*	Not reported	Not reported
				Free androgen index	0.001*	Not reported	Not reported
				Luteinizing hormone	0.176	Not reported	Not reported
				Follicle-stimulating hormone	0.252	Not reported	Not reported
				LH/FSH ration	0.515	Not reported	Not reported
				IGF-1	0.006*	Not reported	Not reported
Asemi 2015 Ramadan Fasting Duration: 4 weeks Non-Randomised Uncontrolled Trial Iran	27	18-40	27.5	Body mass index	0.646	Not reported	Not reported
				Fasting plasma glucose	0.183	Not reported	Not reported
				Insulin	0.474	Not reported	Not reported
				HOMA-IR	0.364	Not reported	Not reported
				HOMA-B	0.736	Not reported	Not reported
				QUICKI	0.308	Not reported	Not reported

Table 3.2: Reported study characteristics and outcome statistics of relevant studies which were excluded from the review

^aEffect sizes classified as follows: small effect: $d=0.2$, medium effect: $d=0.5$, large effect: $d=0$

*Parameters which had a significant (positive) change during the study intervention

Abbreviations: HOMA-IR Homeostatic Model Assessment Insulin Resistance, IGF-1 Insulin-like Growth Factor-, HOMA-B Homeostatic Model Assessment Quantify Beta-Cell Function, QUICKI Quantitative Insulin-Sensitivity Check Index, CI Confidence Interval

Chapter 4:

Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (TimeMAP): A feasibility study of real-world clinical advice

4.1 Introduction and Research Rationale

Chapter 1-3 discussed the background of PCOS, in particular its association with insulin resistance and other metabolic abnormalities. One in three women with PCOS having impaired glucose tolerance and one in ten have type 2 diabetes, with the majority (60%) being overweight or obese [2]. Many dietary interventions have been trialled in PCOS with large variation in results with many diets unsustainable and ineffective [27-36]. Current dietary recommendations for patients with PCOS include regular eating, low glycaemic index foods with calcium/vitamin D supplementation [4]. Time-restricted eating in PCOS could offer more sustainable weight loss, cardiometabolic changes and may be more acceptable as a permanent lifestyle change [38]. Fasting-induced fuel switching has the potential to overcome the issues seen in patients with PCOS, as seen in other populations with underlying insulin resistance, including metabolic syndrome, obesity and type 2 diabetes [58-60]. After identifying a research gap for a well-designed randomised study of time-restricted eating in patients with PCOS, the study protocol, NCT05126199, was designed (Appendix 4).

The main aims of this study ‘Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (the TimeMAP study)’ are described in the next section. Primarily, this was designed as a feasibility study to assess if this dietary intervention was a safe and feasible treatment option for women with PCOS. The feasibility design was imperative as this was the first such trial on this intervention in women with PCOS. In addition to the feasibility aspect, the study was designed as a randomised cross-over trial

in which clinical (secondary) outcomes were measured (Section 4.3.3). The ‘real-world clinical advice’ aspect of the study refers to the aim for flexibility as is required in clinical practice. Specifically, women were advised to choose their own eating windows and did not give any dietary instructions on ‘what to eat’ but rather ‘when to eat’. That said, a strict inclusion and exclusion criteria was adhered to in order to avoid any confounding factors that may have also affected insulin sensitivity, such as pharmacological therapies. The feasibility study is described in Chapter 4, and the (secondary) clinical outcomes are described in Chapter 5.

4.2 Study Aims

Aims and Objectives

The primary aim this study was:

To determine the feasibility of time-restricted eating in polycystic ovarian syndrome

The secondary aims of this study were:

To investigate the effect of time-restricted compared to ad libitum eating on

- a. Insulin levels and insulin resistance
- b. Anthropometric data
- c. Androgen profiles
- d. Lipid profiles
- e. Nutritional intake
- f. Subjective and objective hunger and satiety measures

4.3 Methods

4.3.1 Participant Recruitment

Women with PCOS were recruited from the endocrinology clinic in the Department of Endocrinology Tallaght University Hospital and St. James's Hospital, Dublin, between May and October 2022. Patients were identified by screening new referrals with PCOS or identifying patients with PCOS currently attending these specialist reproductive endocrinology clinics. Patients were contacted either by phone or face-to-face in clinic to discuss the study. If interested, the study information leaflet was emailed and patients were given time to consider participation. Data collection for the purposes of this thesis was completed by March 2023. However, study data collection continued until May 2023. These additional data collected after March 2023 are not part of this thesis as the additional data was not available at the time of data analysis and thesis write up.

4.3.2 Inclusion and Exclusion Criteria

The inclusion criteria were:

- 18 to 42 year old women
- Diagnosis of PCOS according to the Rotterdam Criteria [4] (presence of two of three of the following criteria: oligo-anovulation, hyperandrogenism and polycystic ovaries (≥ 12 follicles measuring 2-9 mm in diameter and/or an ovarian volume > 10 mL in at least one ovary))
- Able to give informed consent
- Able to participate in follow up for a period of 28 weeks

The exclusion criteria were:

- Type 1 diabetes; medication-controlled type 2 diabetes
- Pregnancy
- Currently participating in weight loss programme, or reported weight change in the three months preceding participation (>5% of current body weight)
- History of eating disorder
- Ovulation medication or undergoing active fertility treatment
- Weight loss medication affecting weight or appetite or insulin sensitivity including weight loss medications, antipsychotic drugs or other medications as determined by the physician (eg. Semaglutide, liraglutide, orlistat, amphetamines, Qsymia (phentermine-topiramate), bupropion-naltrexone (Contrave))
- Known liver, renal or thyroid dysfunction*

**Whilst the original study design specified the exclusion of patients with non-alcoholic fatty liver disease and hypothyroidism on treatment or subclinical hypothyroidism, this was determined to be prohibitive for recruitment as a high proportion of patients have these concurrent conditions. Therefore, these were allowed.*

- Unable to participate in follow-up for at least 28 weeks
- Unable or unwilling to provide explicit, informed consent

4.3.3 Study Design

The protocol for this study was pre-registered under the identifier NCT05126199 at clinicaltrials.gov (Appendix 4). The study protocol was approved by the Tallaght University Hospital/St. James's Hospital Joint Research Ethics Committee. Informed consent was obtained from each participant prior to initiating the study. The study was

designed as a randomised crossover study where participants were randomised to start with either 12 weeks of time-restricted eating followed by a four week window period prior to crossing over to 12 weeks of ad libitum eating without dietary restriction allowing participants to return to their normal eating pattern, or vice versa. Envelopes were selected as the allocation concealment method. A randomised sequence was created and brown envelopes were labelled with sequential numbers and stored securely. The envelope was opened once the participant was recruited and consented to participation in the study at the first study visit. The crossover design for this study is illustrated in Figure 4.1. As such, this was not a randomised controlled trial, but rather, participants completed both the time-restricted eating intervention and the 'ad libitum' arm. Participants were met at baseline, midway and conclusion of each regimen with a total of six study visits over the 28 week study period. Informed consent was complete after full education on all aspects of the trial by a pre-assessment/eligibility telephone call, email including study documents and face-to-face assessment. Participants were given a copy of an instruction leaflet on how to use Nutritics/Libro software, a simplified one-page step by step guide on the study and a copy of the consent form and patient information leaflet (Appendices 5,6 and 7, respectively).

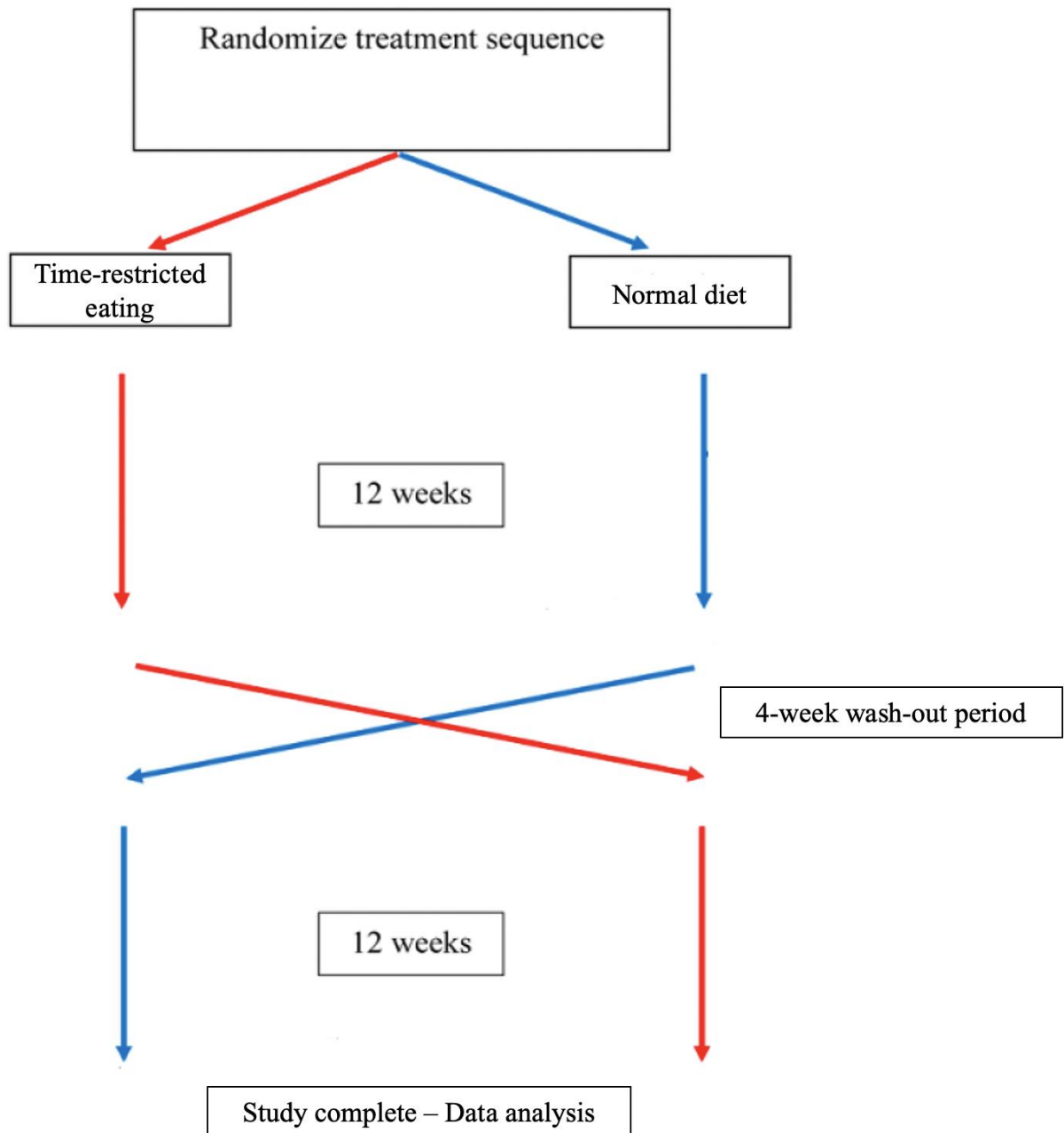


Figure 4.1: Randomised crossover design where participants were randomised to start with either 12 weeks of time-restricted eating followed by a four week window period prior to crossing over to 12 week ad libitum eating without dietary restriction allowing participants to return to their normal eating pattern, or vice versa

4.3.4 Time-restricted eating

At the initial study visit, participants underwent education on time-restricted eating and were given written instructions to follow. During fasting, still or sparkling plain water was allowed and encouraged. Black coffee and black tea without flavours/additives were allowed. Calorie free, sugar free sweetened beverages were not allowed, nor were herbal teas, alcohol or chewing gum. As a pragmatic study, participants were allowed to choose their own 6-hour eating window according to lifestyle and preference. However, an eating window between 8am to 2pm and 12 noon to 6pm or any time in between was suggested while ideally, avoiding a late-night eating window to avoid fasting side effects during the day and for optimal results.

4.3.5 Data Collection

Participants completed a full comprehensive medical history and assessment of clinical symptoms of PCOS, a visual analogue scale on appetite, and anthropometric measurements (including weight, height, hip, and waist circumference) at the initial visit. Baseline PCOS symptoms assessment was carried out and measured using Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (Table 4.1). Exercise engagement was clarified in line with that defined in the Nutritics food intake analysis software. Exercise activity level was characterised by the Nutritics software, based on the 2011 Compendium of Physical Activities, as sedentary if there was little or no regular exercise engagement, light exercise activities such as walking 1-3 days/week, moderate if strenuous activity exercise 3 days per week or light exercise activity 5 days per week, very active if strenuous activity 6 days per week and ultra-active if training twice daily [116]. All baseline assessments were repeated at each of the six study visits with the addition of a questionnaire about compliance and side effects with the time-restricted eating regimen. Assessment of compliance was

self-reported by patients who were asked the number of days that they did not adhere strictly to the six hour eating window. Participants were asked specifically about potential side effects of fasting at each visit, based on those reported in other studies of time-restricted eating [42, 117, 118]. These included headaches; dizziness; pre-syncope; syncope; constipation; diarrhoea; bloating; nausea; vomiting; gastro-oesophageal reflux; dehydration; irritability; anxiety; concentration impairment; fatigue; low energy; bad breath; insomnia/unable to fall asleep; tremor; alopecia. As with baseline PCOS symptoms, these were again assessed using the CTCAE v5.0.

Anthropometric measurements including height and weight were measured with a SECA 769 electronic column scales from which BMI was calculated (Kg/M^2). Body weight was recorded to the nearest 0.1kg. Waist and hip measurements were measured using a SECA lass-o-tape from which waist-to-hip ratio was calculated. Height, waist and hip circumference were measured to the nearest 0.5cm. Participants were instructed to wear similar clothing to each appointment such as ‘string top or light top and shorts or light pants or leggings’ and removed shoes and any heavy jewellery or accessories for measurements. These measurements were blinded from the participants and participants were advised not to take any home measurements during the study as not to impact dietary behaviours. Measurements were taken by the study lead only at every appointment to prevent inter-operator variability.

A 9-question 1-minute questionnaire, based on the Simplified Nutritional Appetite Questionnaire (SNAQ) and the Council of Nutrition Appetite Questionnaire (CNAQ), was completed by participants at each study visit to assess subjective appetite and satiety both while undergoing time-restricted eating and ad libitum diet (Appendix 3).

4.3.6 Blood draw and sample preparation

Blood samples were taken from an antecubital vein to assess insulin, glucose, HbA1c, testosterone, free testosterone, DHEA-S, androstenedione, SHBG, 17-hydroxyprogesterone (17-OHP), lipid profile, lipoprotein lipid A, apolipoprotein A1 and apolipoprotein B (five 3ml serum tubes, one 3ml vacutainer lithium heparin tube, one 3ml EDTA vacutainer tube and one 3ml fluoride oxalate plasma vacutainer tube). Without delay, these were transferred to the laboratory on ice and immediately separated and processed. All blood samples were collected in the morning while fasting. Insulin, glucose, HbA1c, testosterone, lipid profile, lipoprotein lipid A, apolipoprotein A1 and apolipoprotein B were processed at the laboratory at Tallaght University Hospital. Free testosterone, DHEA-S, androstenedione, SHBG and 17-OHP were prepared and transferred to external laboratories to be processed. Free testosterone was processed at Eurofins Biomnis and the remaining reproductive hormones were processed at St. James's Hospital laboratory.

Insulin samples were prepared and frozen on arrival to the laboratory as these are ran three times per week only. These were processed on Roche Cobas 801. Glucose, lipid profile, lipoprotein lipid A, apolipoprotein A1 and apolipoprotein B samples were all processed on arrival to the laboratory on Roche Cobas 702. HbA1c was prepared on arrival to the laboratory and processed daily on Arkray Ha8190. Testosterone levels were also processed daily on Roche Cobas 801. As mentioned, free testosterone, DHEA-S, androstenedione, SHBG, 17-OHP were processed at Eurofins Biomnis and St James's Hospital laboratories. They were transported on the day of samples being taken and results were returned electronically.

In order to assess for GLP-1, PYY and oxyntomodulin, a further 8ml EDTA sample was collected and immediately centrifuged for 10 minutes at 3000rpm. The supernatant was then removed via pipette and transferred into 2 Eppendorf tubes and immediately transferred on ice and stored in a -80degC freezer. These samples were prepared by the study lead only at every appointment to ensure timely processing and avoid degradation of these fragile gut hormones. All of these samples are currently stored until the end of the study when all samples will be transferred to the Conway Institute of Biomolecular and Biomedical Research laboratory at University College Dublin to be processed. However, due to the external laboratory's protocol requirement to receive all study samples together and to batch process, the results of these are not available for the current data analysis.

4.3.7 Dietary data collection and analysis

To assess changes in nutritional intake, all participants recorded a 3-day food diary. Participants were educated on completion of a 3-day food diary using the nutritional software package Nutritics (Nutritics Limited, version 5.7, Ireland) and the associated Libro phone application available on Android and Apple smartphone devices. Participants were asked to record all oral intake over a three day period including one weekend day and two weekdays. The Nutritics/Libro application was used to record daily food intake and portion sizes which automatically calculated the calories and nutrition content. Participants were also asked to time stamp all entries. A fact sheet was also provided on how to download, use the application and advice on recording entries (Appendix 5). Food records were analysed using the Nutritics software at the conclusion of the study for each participant to determine dietary intake, specifically, calorie (energy) intake as well as a macronutrient intake (carbohydrate, sugar, protein, fat, saturated fat) and micronutrient intake (calcium and vitamin D). Nutritics software compiled this data for each of these

variables. Averages of the 3-day food diaries were carried out for the participants while following the time-restricted eating regimen and ad libitum.

4.3.8 Determining feasibility

Adverse events and overall changes were assessed by questioning each participant regarding a list of side effects, commonly reported in other studies of time-restricted eating [42, 117, 118]. These were recorded at each study visit during both the time-restricted eating and ad libitum regimens. Participants were advised to contact a specific study email if they experienced any adverse events. The severity of side effects or symptoms reported were assessed by the CTCAE) version 5.0 as described in section 4.3.5.

Compliance and adherence were calculated based on the (self-reported) total number of days during which participants maintained an eating window of less than 6 hours. Compliance was calculated by dividing the number of days where participants consumed any caloric item (as specified in the instruction sheet Appendix 6) *outside* this 6 hour window by the total number of days in the 12 week regimen (84 days). Participants were expected to adhere to the regimen 85% of the time at a minimum, but were strongly advised to have full compliance.

A dropout figure of <20% was expected based on a similar study of time-restricted eating in PCOS discussed in Chapter 3, where they observed a dropout rate of 16.7% [39].

4.3.9 Statistical analysis

For the secondary, clinical outcomes, data were inspected for normality by examining QQ plots and histograms. Due to small subjects numbers, non-parametric testing was employed for the majority of the parameters. Normally distributed data were presented with means

and standard deviations, while medians and interquartile ranges were used for non-normally distributed/non-parametric data. If data were not linear and were non-normally distributed, they were natural log transformed (ln) for certain variables to enable analysis using linear modelling.

Statistical analysis of the non-parametric parameters (including insulin-related, anthropometric, reproductive hormones and lipid parameters) was conducted using the Wilcoxon Matched-Pairs Test. The energy (calorie) intake parameter of the nutritional data was normally distributed and was analysed using a t-test, while a Wilcoxon Rank Sum Test was used to analyse other non-normally distributed macronutrient and micronutrient parameters. This calculated a t-value or z-value with a corresponding p-value. The t-value illustrates the difference between the sample mean and the given number to the standard error of the mean, whereas the z-value illustrates the difference between the sample median and the given number to the interquartile range. The larger magnitude of the t-value or z-value signifies a smaller standard error of the mean giving the small p-value and strong evidence of a significant difference.

Certain parameters were missing data due to sample haemolysis, missing specimens, or incorrect samples. For responses with missing values, the values were not included in the analyses.

Based on the crossover design, patients were randomised to start either the time-restricted eating intervention or to continue with ad libitum eating for 12 weeks. Following a 4-week wash-out period, patients then crossed over to the alternative intervention. Each participant was therefore analysed 'twice': from baseline to the end of the respective 12 week

interventions (Figure 4.1). The differences between the ‘time-restricted eating’ and ad libitum results were compared to assess the change from baseline to both the six week interval and the final 12 week analysis using multilevel linear regression. The multilevel linear regression models were used to analyse data changes over the various data collection points of the study. This method of analysis was used given that the number of participants were low, but there was multiple test points for each participant, increasing the power of the overall results and allowing trends to be identified. The advantage of this statistical analysis is its consideration of greater than one factor of independent variables that subsequently influence the variability of dependent variables. This can lead to more accurate and realistic conclusions which are more reflective of the real-life condition.

For each group of variables, multi-level linear regression was used with a *TRE X Weeks* interaction term as the independent (predictor) variable of interest. A fixed-effect covariate was incorporated for condition assignment order (time-restricted eating first vs ab libitum first). Random effects estimates for participant were incorporated into the model to take into account the multiple measures per participant over the six visits. Results are presented as beta coefficients and corresponding 95% confidence intervals for the interaction term pertaining to impact (per week) of time-restricted eating on the dependent variable of interest (in comparison to the ab libitum condition).

In order to assess the effect of the order of assignment on each parameter, multivariate analysis was also used. The beta co-efficient was calculated for each parameter to assess if allocation order had a significant effect on the outcomes. In other words, if the crossover design and starting on either ad libitum diet or time-restricted eating, had any effect on the results for each outcome tested.

All data analysis was carried out on Stata Statistics Software Version 17. GraphPad Prism (version 9.5.1 GraphPad Software) was used for data analysis and graph representation of data. $P < 0.05$ was considered significant for all tests.

4.3.10 Sample size and power

A sample size and power calculation was not deemed appropriate as the primary aim of the study was to determine the feasibility and safety of the intervention. Whilst clinical outcomes were analysed as secondary outcomes (Chapter 5), this was intended to guide the setting of parameters, methodology, and outcomes for a future substantive study. At the outset of the study, it was planned to recruit $n=50$ patients to participate in the crossover study design. After starting the study, given the recruitment difficulties, this aim was revised downwards to $n=20$ patients.

4.3.11 Study outcomes

Primary outcome measures (Chapter 4):

1. Recruitment to the study
2. Adverse outcomes during the intervention
3. Compliance with the intervention

Secondary Outcomes Measures (Described in Chapter 5):

1. Serum insulin and glucose and ratios (HOMA-IR and QUICKI)
2. HbA1c
3. Anthropometric data (weight, height, BMI, waist circumference, hip circumference, waist-hip ratio)

4. Lipid profile (lipoprotein lipid A, Apolipoprotein A1, Apolipoprotein B)
5. Hormonal measures (testosterone, free testosterone, DHEAS, 17-OHP, androstenedione, SHBG)
6. Dietary intake of macro- and micronutrients during the intervention
7. Appetite and cravings during the intervention, with analogue scale
8. Satiety and hunger (GLP-1, PYY and oxyntomodulin)

The secondary outcome measures were broadly categories in to five groups for presentation of results: 1) Insulin-related, 2) Anthropometry, 3) Lipids, 4) Reproductive hormones and 5) Dietary intake. As mentioned, the appetite and gut hormone data was not available for analysis at the time of data analysis for this thesis.

4.3.12 Clinical applicability and statistical integrity

Acknowledgement must be made to the fact that low subject numbers mean that inferential statistics are unsound and will not provide meaningful or generally applicable clinical conclusions. Nevertheless, further statistics were analysed in the context of this feasibility study with the objective of aiding the design and planning of future substantive studies, and in particular the objectives, methodology and outcomes.

4.4 Results

4.4.1 Participant Characteristics

Table 4.1 depicts the baseline demographics, characteristics and PCOS symptomatology for the 9 participants analysed as part of the study. Due to small study numbers, the mean and interquartile ranges were used for presenting age, BMI, years with PCOS, length of menstrual cycle and length of period. The remaining characteristics and symptoms are presented as the number of participants.

Baseline Patient Characteristics		Number
Age (years)		28 (26-32)
BMI (Kg/m ²)		35.5 (30.5-43.3)
Years with PCOS Diagnosis		3 (3-8)
Meets Rotterdam Criteria, n		9
COCP, insulin sensitisers or weight loss medication, n		0
Parity, n	Primiparous	7
	Multiparous	2
Smoking status, n	Current smokers	4
	Non-smoker	2
	Ex-smoker	3
Exercise engagement, n	Sedentary	4
	Lightly active	2
	Moderately active	2
	Very active	1
Length of cycle (days)		60 (45-102)
Length of period (days)		7 (6-7)
Dysmenorrhea (Grade 1-2 [#]), n		6
Amenorrhoeic, n		4
Oligomenorrhea, n		9
Polymenorrhea, n		0

Menorrhagia (Grade 1-2 [#]), n	5
Hirsutism (Grade 1-2 [#]), n	6
Acne (Grade 1-2 [#]), n	9
Infertility (self-reported, TTC >12 months), n	5 ^a

Table 4.1: Details of n=9 study participant characteristics at baseline

Data presented as median (interquartile range) or number (n) of participants

[#]Symptoms measured using Common Terminology Criteria for Adverse Events (CTCAE) version 5.0

^a Unknown fertility status in n=4 patients

Abbreviations: IQR inter quartile range, Kg kilograms, M metre, COCP combined oral contraceptive pill, TTC trying to conceive

4.4.2 Feasibility, recruitment, drop-outs and adverse effects

4.4.2.1 Recruitment and drop-outs

Although a sample size calculation was not required due to the nature of the study (feasibility), the initial aim was to recruit n=50 participants. The charts of n=134 potential participants were screened, and n=66 were immediately excluded for not meeting the inclusion/exclusion criteria, giving a group of n=68 eligible subjects. Contact was attempted with the eligible subjects to invite them to participate via an initial screening call or consultation to discuss the study. Of these patients, n=24 patients were uncontactable due to incorrect details or not answering telephone calls. The remaining n=44 who were interested were given time to decide, and study information and requirements were sent by email, along with a patient information leaflet, from a study dedicated email account. Of the n=44 patients invited to participate, n=28 declined to participate for a variety of reasons as follows:

- Did not want to fast due to personal/lifestyle/work reasons n=8
- No reply to email with study details n=5
- Planning pregnancy/fertility treatments n=2
- Denied having PCOS condition n=1
- Unable to attend due to disability n=1
- In Direct Provision (programme for those applying for asylum in Ireland) n=1
- Distance to appointments n=1
- Out of country n=1
- Planning medical management of weight loss n=1
- Incorrect date of birth and outside age eligibility n=1
- No reason given n=6

At this stage of the recruitment process, the likelihood of achieving this planned sample size (n=50) was diminished. Therefore, the sample size was revised downwards to n=20, reasoning that the crossover design would add additional power given all patients would carry out both the time-restricted eating and ad libitum regimen, rather than a traditional randomised controlled trial. This change was recorded in the amended study protocol NCT05126199 (Appendix 4). Of the n=16 patients that agreed to participate, n=3 did not attend their first appointment and did not respond to further contact via email/telephone. Therefore, n=13 patients were randomised to a treatment sequence (Figure 4.2). Following recruitment, n=4 patients dropped out of the study, giving a dropout rate of 31% (Table 4.2). The n=4 participants who dropped out, did so due to reasons including inability to attend appointments or other unrelated medical issues. No patients reported dropping out due to difficulties with the intervention. N=9 subjects concluded the study with full data from six visits available for analysis. Their characteristics and baseline data is displayed in Table 4.1.

No.	Randomised	Completed study	Point of dropout	Reason for dropout
1	Started with ad libitum	Yes	-	-
2	Started with TRE	Yes	-	-
3	Started with TRE	Yes	-	-
4	Started with TRE	Yes	-	-
5	Started with ad libitum	Yes	-	-
6	Started with ad libitum	Yes	-	-
7	Started with TRE	Yes	-	-
8	Started with TRE	Yes	-	-
9	Started with ad libitum	No	After 3 visits	Uncontactable by email or phone.
10	Started with TRE	No	After 3 visits	Non-compliant with TRE due to unrelated medical condition (70% compliance). Not keen to proceed with remainder of the study.
11	Started with ad libitum	Yes	-	-
12	Started with ab libitum	No	After 4 visits	Uncontactable by email or phone.
13	Started with ab libitum	No	After 1 visit	Initially uncontactable by email or phone. Study appointments not possible due to work commitments.

Table 4.2: List of participants including randomised arm and detailing dropouts, point of dropout and reasons for dropout

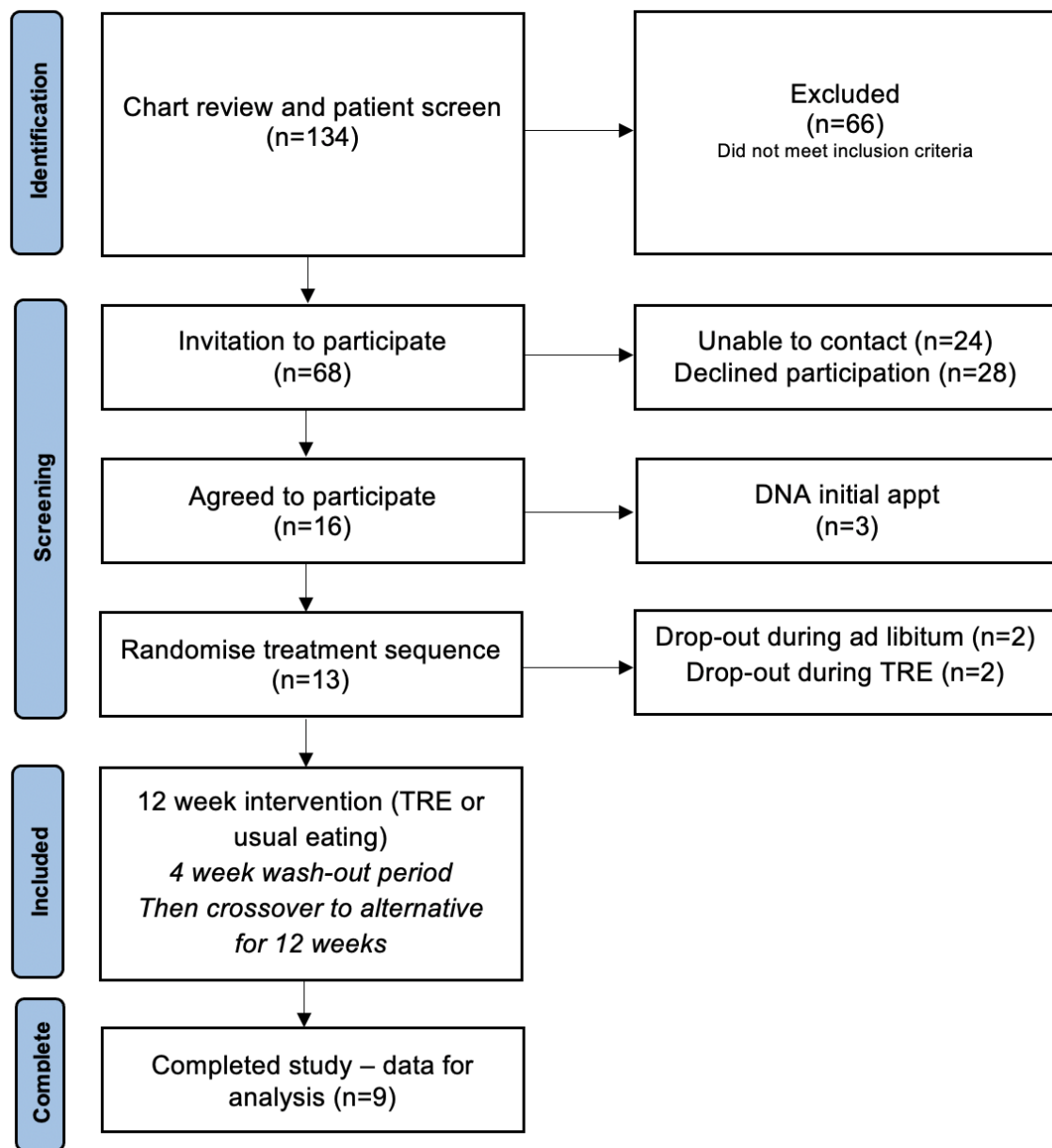


Figure 4.2: Flow sheet representing recruitment and dropouts at various stages of the study with numbers for final analysis. N=9 subjects participated in the full study, completing both the time-restricted eating and ad libitum arms. Therefore, there are n=9 subjects for analysis in each arm.

Abbreviations: DNA did not attend, TRE time-restricted eating

4.4.2.2 Compliance and eating patterns adopted

Of the n=9 women that completed the study, there was near total compliance with time-restricted eating with a mean (SD) compliance rate of 94 (4.6)%. Compliance was calculated as described earlier in the methods section 4.3.8. As part of the feasibility aspect of the study, participants chose their own fasting window, with instructions to preferably avoid a later fasting window as there may be higher insulin resistance in late evening. The most commonly adopted eating window was 12:00-18:00 (n=4). The remainder adopted the following eating windows: 13:00-19:00 (n=3), 10:00-16:00 (n=1), and 12:30-18:30 (n=1). The reported reason for choosing each eating window was to allow the eating window to fit in with work/college routines. One participant was unemployed and choose her fasting window of 12:00-18:00 to balance feeling hunger in the late evenings. Two participants were in full time education and 6/9 of the participants were in full time employment. These n=8 participants chose their fasting windows in order to fit around their allocated lunch hour and timing to coincide eating an evening meal with their family. The participant that chose the 10:00-16:00 window was the only participant to eat an early morning meal and not delay eating until the afternoon. This participant was one of those in full time education and was keen to have a meal prior to attending university classes and then had an earlier evening meal.

4.4.2.3 Assessment of adverse events

There were no major adverse events reported in either group, as assessed by CTCAE v5.0. Reported symptoms are reported below in Table 4.3 and Figure 4.3. Overall, the side effect profile of time-restricted eating involved solely Grade 1 or mild symptoms/side effects while fasting.

Of note, while assessing for side effects during the study, there were some positive benefits recorded by participants. Two-thirds of the women in the study reported less bloating while in the time-restricted eating group. Two-thirds had an improvement in energy levels and almost half of patients had a reduction in fatigue levels with one-third noticing an improvement in concentration. These positive benefits are reported in Figure 4.4.

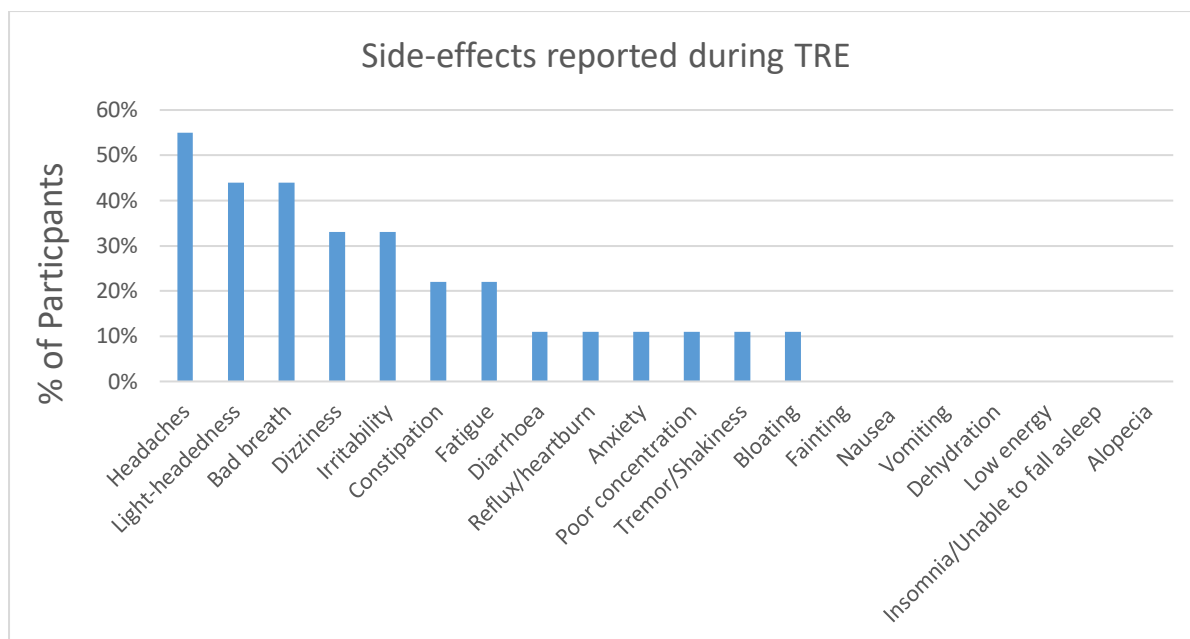


Figure 4.3: Side effects of time-restricted eating reported during this aspect of the study

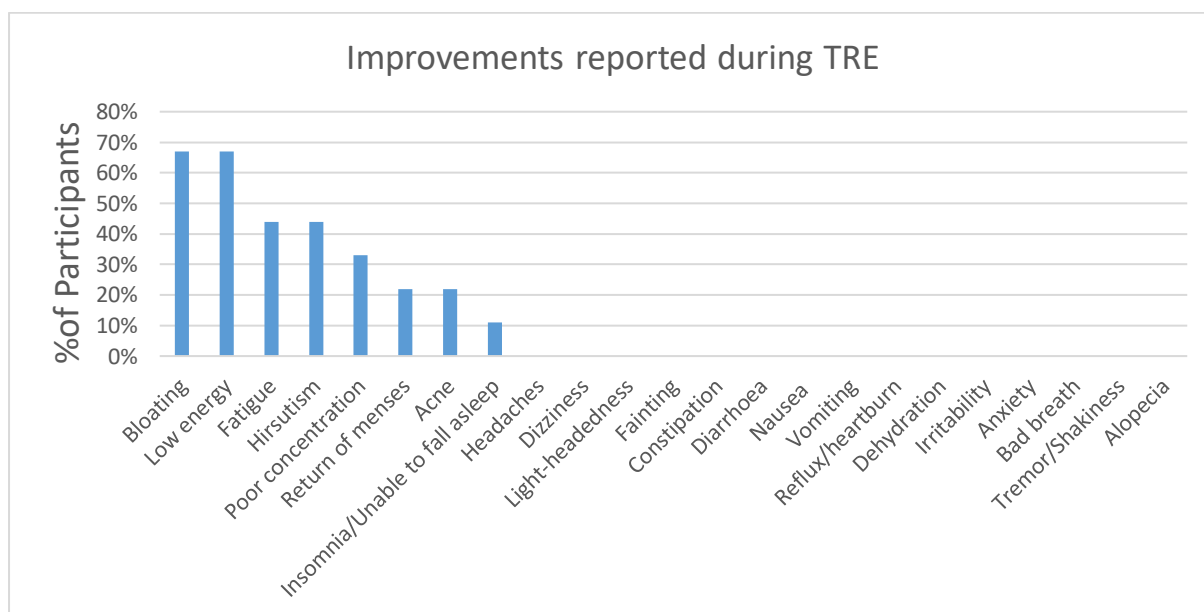


Figure 4.4 Improvements/benefits noted during the time-restricted eating regime

Participant No.	Eating window	Compliance	Side-effects TRE compared to ad libitum	Benefits TRE compared to ad libitum
1	12:30-18:30	94%	Increased light-headedness Increased irritability Increased halitosis No change to menstrual cycle	Reduced bloating Reduced fatigue Improved energy Improved concentration Improved sleep Reduced hirsutism
2	12:00-18:00	95.2%	Increased dizziness Increased light-headedness No change to menstrual cycle	Reduced bloating Improved energy Reduced hirsutism
3	12:00-18:00	96.4%	Increased headache Increased hirsutism No change to menstrual cycle	Improved energy Reduced acne
4	13:00-19:00	88.1%	Increased dizziness Increased light-headedness Increased tremor No change to menstrual cycle	Reduced bloating Improved energy No change to clinical hyperandrogenism
5	12:00-18:00	94%		Reduced bloating Reduced fatigue Improved energy Improved sleep Return of spontaneous menses No change to clinical hyperandrogenism
6	13:00-19:00	100%	Increase in constipation Increase in reflux symptoms Increase in fatigue	No change to clinical hyperandrogenism

			No change to menstrual cycle	
7	13:00-19:00	98.8%	Increased headaches Increased light-headedness Increased halitosis	Increased water intake Increased concentration Increased energy levels Return of spontaneous menses Reduced hirsutism Reduced acne
8	12:00-18:00	85.7%	Increased headaches Increased dizziness Increased diarrhoea Increased irritability Reduced concentration Increased fatigue Increased halitosis No change to menstrual cycle No change to clinical hyperandrogenism	
11	10:00-16:00	95.2%	Increased headaches Increased halitosis No change to menstrual cycle	Increased water intake Reduced hirsutism

Table 4.3: Side effect profile of each individual participant while following a time-restricted eating protocol (6 hours eating window per day). Severity of adverse effects classified using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5. All side effects reported as detailed above were Grade 1 only with none requiring medical intervention.

Abbreviations: TRE Time-restricted eating

4.4.2.4 Willingness to continue with the intervention

Of the 9 women completing the study, 89% (n=8) reported being keen to continue with time-restricted eating, or to try again in the future.

4.4.2.5 Resource usage

Each single appointment were scheduled for 1 hour. This included face-to-face time, time to process blood samples taken and transport directly to the laboratory. Each participant had six visits over 28 weeks for a total time per participant of six hours. Therefore, the study as designed was quite time-consuming for both the participant and researcher.

The food diaries were completed by patients on the Libro app. A simple guide sheet was given to patients on how and when to complete these diaries which included links to explanatory videos (Appendix 5). However, participants needed regular education regarding this, and reminders to complete these diaries. Participants found it difficult to use, time-consuming in comparison to other food and calorie tracking applications and reported difficulty finding certain foods. This difficulty in recorded may have led to the underreporting.

Analysis of GLP-1, PYY and oxyntomodulin were included in the study design as (secondary) clinical outcomes. Sample preparation for these blood measures was labour intensive, requiring immediate centrifuging at the bedside, transfer to Eppendorf tubes, and immediate transfer to the lab freezers for storage at -80 degrees Celsius. They also require specific assays and processing in an external lab (described in section 4.3.6), which also required significant co-ordination. Consequently, these results were not processed in time for the results of this thesis.

4.5 Discussion

4.5.1 Summary of findings

Of a cohort of $n=134$ patients, only $n=68$ (50.7%) fulfilled the strict inclusion and exclusion criteria. Of the $n=68$ eligible patients, only $n=44$ were contactable, of which a third ($n=16$, 36.4%) agreed to participate, an objectively poor participation rate. Reasons for not participating were varied, but only $n=8$ patients did not want to follow the dietary regimen (for personal, lifestyle or work reasons). Patients with PCOS are, by definition, young women of child-bearing and working age who, therefore, have family and employment commitments that may preclude their participation in a study which requires six clinic attendances over 28 weeks. There were also drop-outs at pre-participation and post-recruitment stage. This must be borne in mind when designing a well-powered substantive study. Of those that did participate, there was near total compliance, suggesting that the intervention itself was well-received and feasible.

Assessment of safety was a core aspect of this study, mainly due to the possible societal perceptions that fasting may be unsafe. There were no major adverse events reported in either time-restricted eating or ad libitum groups. The side-effects that were reported all fell under the mild/grade 1 category. Notably, there were some positive side-effects reported by those undergoing the time-restricted eating intervention, including less bloating, an improvement in energy and a reduction in fatigue, an improvement in concentration, reduced hirsutism, reduced acne and a return of spontaneous menses. The vast majority (8/9 women) who completed the study reported wished to continue with the intervention, illustrating that it was overwhelmingly positively received.

Overall, the intervention was deemed to be safe, acceptable and feasible. However, for the design of a larger study, practical, comprehensive strategies for improving recruitment and study retention are needed. The results of the feasibility study will be explored in further detail the context of other studies in Chapter 6.

In Chapter 5, the secondary outcomes of this study are presented.

Chapter 5:

Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (TimeMAP): Analysis of (secondary) clinical outcomes

5.1 Introduction

Chapter 4 described the assessment of feasibility and safety for time-restricted eating as an intervention in patients with PCOS – the TimeMap Study. Chapter 5 will describe the results of the (secondary) clinical outcomes. The secondary outcomes may be broadly categorised as follows: 1) insulin-related parameters, 2) anthropometry, 3) reproductive hormones, 4) lipid profiles and 5) dietary intake.

As discussed in Chapter 4, subjective and objective data on appetite and satiety by means of visual analogue scales and serum biomarkers were also collected. The results of this data was not available at the time of writing this thesis, as these samples will all be processed together and analysed in one assay at completion of the entire study. The purpose of this is to reduce inter-assay co-efficient of variation and allow for easier comparison of results.

5.2 Methods

The study design and statistical analysis were described in Sections 4.3 and are presented here again in brief. On entry into the study, patients were randomised to either time-restricted eating or ad libitum eating for a 12 week period. Following this, each patient completed a 4-week wash-out period, before crossing over to the alternative intervention for a further 12 weeks. Therefore, the entire study duration was 28 weeks, and patients presented for six clinic appointments during this time (three for each arm of the study). The 9 participants that are included in the analysis completed *both* the time-restricted eating and ad libitum arms of the study, and are compared. Therefore each arm had a sample size of $n=9$.

For each of the five outcomes categories, the change in each intervention group at beginning and end of each regimen was analysed. In addition, analysis of the crossover study's effect on each parameter was performed to identify if randomised assignment (to either start with time-restricted eating or ad libitum diet) had any significant effect.

5.3 Results

5.3.1 The effect of time-restricted eating on plasma HbA1c, insulin, and insulin resistance indices compared to ad libitum eating

The median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the baseline assessment and the 12 week point are detailed in Table 5.1. There was no statistically significant difference for any of the insulin-related parameters including insulin, glucose, HOMA-IR, QUICK-I or HbA1c when the medians and interquartile ranges from baseline assessment to the 12 week assessment for both the time-restricted eating and the ad libitum study arms were examined individually. Of note, there was missing insulin values in the data sets due to haemolysis of 6 out of 54 (11%) insulin samples analysed.

Outcome	Study Arm	Baseline	12 Weeks	Statistic
Insulin (mU/L)	Ad libitum	13.6 (9.3-32.7)	13.4 (7.9-21.7)	$z = -0.87, p = 0.50$
	Time-restricted eating	19.4 (8.4-31.2)	8.15 (5.7-36.05)	$z = 1.26, p = 0.21$
Glucose (mmol/L)	Ad libitum	4.9 (4.8-5.2)	4.8 (4.7-5)	$z = 0.42, p = 0.68$
	Time-restricted eating	5 (4.9-5.1)	4.9 (4.5-5.1)	$z = 0.54, p = 0.59$
HOMA-IR	Ad libitum	2.9 (2.1-7.6)	3.2 (1.7-4.6)	$z = -0.67, p = 0.50$
	Time-restricted eating	4.2 (1.8-7.1)	1.78 (1.2-8.1)	$z = 1.12, p = 0.26$
QUICK-I	Ad libitum	0.33 (0.29-0.34)	0.31 (0.27-0.32)	$z = 1.21, p = 0.22$
	Time-restricted eating	0.32 (0.29-0.35)	0.35 (0.27-0.37)	$z = -1.12, p = 0.26$
HbA1c (mmol/mol)	Ad libitum	36 (35-36)	36 (36-39)	$z = -1.34, p = 0.18$
	Time-restricted eating	37.5 (37-38.5)	37 (36-37)	$z = 1.07, p = 0.39$
HbA1c (% DCCT)	Ad libitum	5.4 (5.4-5.4)	5.4 (5.4-5.7)	$z = -0.63, p = 0.53$
	Time-restricted eating	5.6 (5.5-5.7)	5.5 (5.4-5.5)	$z = 0.85, p = 0.40$

Table 5.1: Median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the 0 week and 12 week timepoints are presented.

The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p value for all insulin related parameters.

Abbreviations: HOMA-IR Homeostatic Model Assessment for Insulin Resistance, QUICK-I Quantitative Insulin-Sensitivity Check Index, HbA1c Haemoglobin A1c, DCCT Diabetes Control and Complications Trial) unit of percentage (%), CI confidence interval

Following the assessment of the change from 0 to 12 weeks for both the ad libitum and time-restricted eating group for each individual parameter, the change observed for each parameter in both groups was examined and comparison between these changes was carried out to assess if these changes were statistically significant. There was no statistically significant difference in the changes between both groups for any of the parameters including insulin, glucose, HOMA-IR, QUICK-I or HbA1c as portrayed below in Table 5.2. The difference in changes for insulin, HOMA-IR and QUICK-I were calculated as $p=0.07$, which neared the arbitrary significant cut-off of $p<0.05$, but did not reach significance.

Difference 0-12 Weeks	Ad Libitum	Time-restricted Eating	Statistic
Insulin (mU/L)	1.3 (-4.9, 6.3)	-3.3 (-12.6, 0.7)	$z = 1.83, p = 0.07$
Glucose (mmol/L)	-0.3 (-0.3, 0.3)	0 (-0.3, 0.2)	$z = 0.12, p = 0.91$
HOMA-IR	0.12 (-1.25, 1.88)	-0.88 (-2.57, 0.24)	$z = 1.83, p = 0.07$
QUICK-I	-0.36 (-0.04, 0.02)	0.01 (-0.01, 0.05)	$z = -1.83, p = 0.07$
HbA1c (mmol/mol)	1 (0, 1)	-1 (-1.5, 0.5)	$z = 1.48, p = 0.16$
HbA1c (DCCT %)	0 (0, 0.1)	-0.1 (-0.2, 0.1)	$z = 1.35, p = 0.18$

Table 5.2: Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased).

The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p -value for change across 12-weeks for insulin related parameters.

Abbreviations: HOMA-IR Homeostatic Model Assessment for Insulin Resistance, QUICK-I Quantitative Insulin-Sensitivity Check Index, HbA1c Haemoglobin A1c, DCCT Diabetes Control and Complications Trial) unit of percentage (%), CI confidence interval

5.3.2 Change per week for plasma HbA1c, insulin and insulin-resistance

The data were subsequently examined with a multi-level linear regression analysis which allowed analysis of the change in each parameter over the 12 week period and compare if this change seen in the time-restricted eating group and ad libitum group showed statistical significance. This analysis was carried out as a proof of concept and further assessment of feasibility. This was carried out to assess if going forward with larger numbers in a larger scale study of the intervention, if these higher order statistical tests would be of value for the dataset. The results of the positive or negative change seen in the time-restricted eating 12-week regimen across the three study points was compared with the positive or negative change seen in the ad libitum eating 12-week regimen across the three study points. As this was a crossover design, this comparison was of same participants who completed both arms.

The insulin and HOMA-IR dataset was log transformed to reduce the skewness of these variables and normalise the dataset and stabilise the variation between the groups (due to small study numbers). There was no statistical difference in the log transformed insulin and HOMA-IR parameters when comparing the change over the 12-week period between the time-restricted eating and ad libitum group of the same participants. There was a significant improvement seen in the natural log transformed QUICK-Index measure of insulin sensitivity (95% CI: 0.00, 0.02, $p = 0.016$). This benefit can be interpreted as a statistically significant increased insulin sensitivity using the QUICK-I ratio calculated as a 0.01 increase per week while time-restricted eating in comparison to ad libitum eating. Similarly, there was a statistically significant reduction in HbA1c per week of -0.15 mmol/mol (95% CI: -0.28, -0.02, $p=0.02$).

Outcome	β Coefficient (95% CI)	p-value
$\ln$ Insulin (mU/L)	-0.43 (-0.09, 0.00)	0.06
Glucose (mmol/L)	-0.00 (-0.04, 0.03)	0.84
$\ln$ HOMA-IR	-0.05 (-0.10, 0.00)	0.07
$\ln$ QUICK-I	0.01 (0.00, 0.02)	0.016*
HbA1c in mmol/mol	-0.15 (-0.28, -0.02)	0.02*
HbA1c in DCCT (%)	-0.01 (-0.02, 0.00)	0.05*

*Table 5.3: Results of multi-level linear regression of insulin resistance data showing beta coefficients and p values for each parameter. The β Coefficient gives a change in parameter over time (per week in this case) and the p value indicates whether or not this change is significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*

Abbreviations: HOMA-IR Homeostatic Model Assessment for Insulin Resistance, QUICK-I Quantitative Insulin-Sensitivity Check Index, HbA1c Haemoglobin A1c, DCCT Diabetes Control and Complications Trial) unit of percentage (%), CI confidence interval

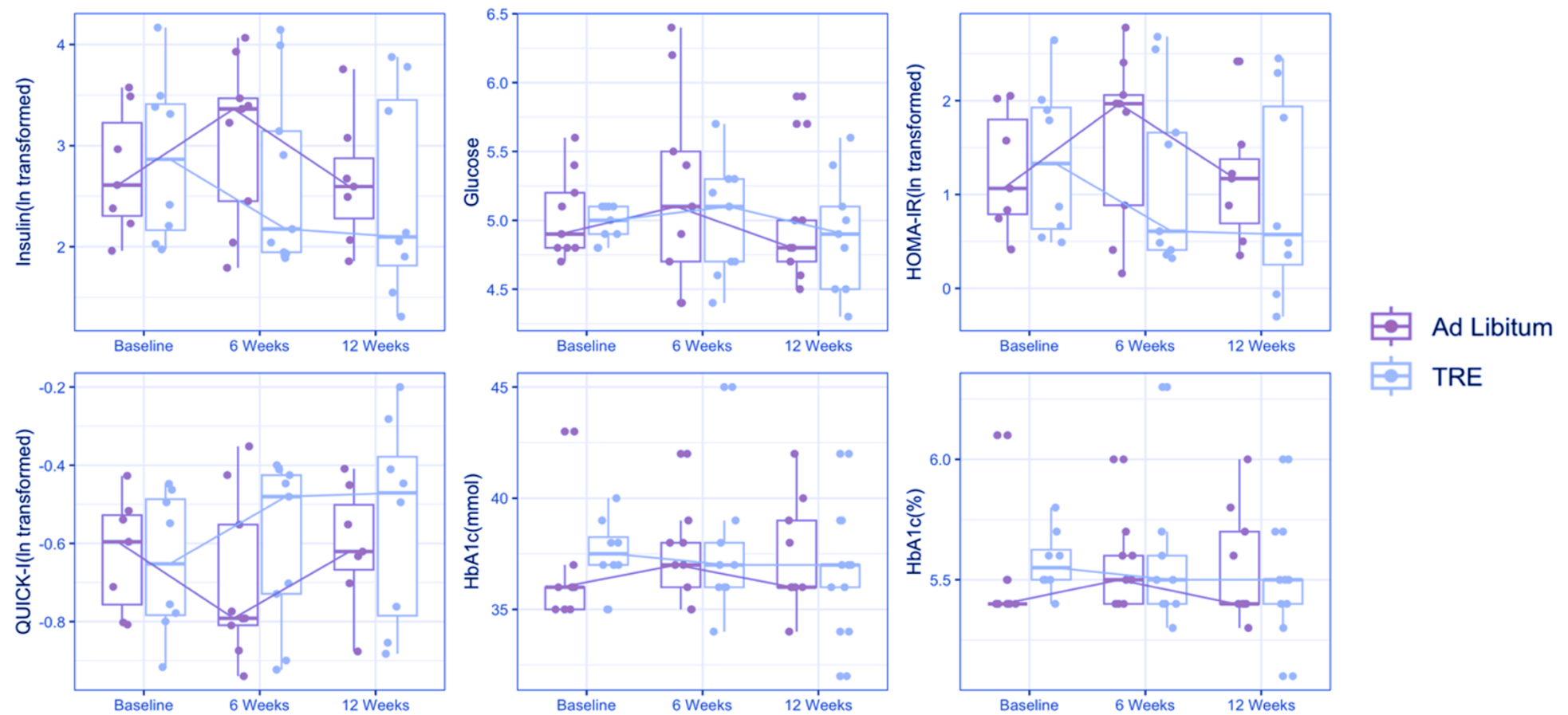


Figure 5.1: Graphical display of the medians and interquartile ranges of the insulin sensitivity parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks.

5.3.3 Time-restricted eating effect on anthropometric parameters compared to ad libitum eating

The median and interquartile ranges for anthropometric parameters (weight, BMI, waist circumference, hip circumference, and waist:hip ratio) for participants for both the ad libitum and time-restricted eating groups at baseline assessment and 12-week point are detailed in Table 5.4.

When assessing the medians and IQRs from baseline assessment to the 12 week assessment for both the time-restricted eating and the ad libitum study arms examined individually, there was a statistically significant increase in weight in the ad libitum group ($z = -2.07$, $p = 0.04$), and a statistically significant decrease in weight in the time-restricted eating group ($z = 2.13$, $p = 0.03$). There was a corresponding increase and decrease in BMI in the ad libitum and time-restricted eating groups, respectively (Table 5.4)

In the ad libitum group there was no significant change in participant's median waist circumference, hip circumference or waist:hip ratio from start to finish of this 12-week regimen. There was a significant decrease in waist circumference ($z = 2.02$, $p = 0.04$), hip circumference ($z = 2.69$, $p = 0.004$), but not waist:hip ratio in the time-restricted eating group.

Outcome	Study Arm	Baseline	12 Weeks	Statistic
Weight (kg)	Ad libitum	95.3 (88.80-110)	97.2 (94-109.3)	$z = -2.07, p = 0.04^*$
	Time-restricted eating	97.7 (90.4-110)	95.0 (86.7-110.3)	$z = 2.13, p = 0.03^*$
BMI (kg/m ²)	Ad libitum	34.9 (10.7-42.8)	37.0 (32.5-43.0)	$z = -2.07, p = 0.04^*$
	Time-restricted eating	37.1 (31.3-43.3)	36.2 (30-43.3)	$z = 2.19, p = 0.03^*$
Waist circumference (cm)	Ad libitum	97 (94-119)	105 (97-116)	$z = -0.18, p = 0.86$
	Time-restricted eating	103 (96-116)	96 (94-116)	$z = 2.02, p = 0.04^*$
Hip circumference (cm)	Ad libitum	120 (115-136)	121 (119-136)	$z = -0.96, p = 0.34$
	Time-restricted eating	124 (119-135)	119 (117-133)	$z = 2.69, p = 0.004^{**}$
Waist:Hip Ratio	Ad libitum	0.86 (0.80-0.89)	0.85 (0.80-0.89)	$z = 0.48, p = 0.64$
	Time-restricted eating	0.86 (0.82-0.87)	0.87 (0.79-0.88)	$z = 0.30, p = 0.82$

Table 5.4: Median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the 0 week and 12 week timepoints are presented.

The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p value for all anthropometric parameters.

** $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*

Abbreviations: Kg kilogram, kg/m² kilograms per metre squared, cm centimetres, BMI body mass index

Similar to the analysis of the insulin parameters, following the assessment of the change from 0 to 12 weeks for both the ad libitum and time-restricted eating group independently for each individual parameter, the changes observed for each parameter in both groups were compared to assess if the difference in these changes were statistically significant.

There was a median 2.65kg weight gain in the ad libitum group and a 1.5kg weight loss in the TRE group, of which the difference reached statistical significance ($z = 2.43$, $p = 0.01$). Most (78%, $n=7$) of participants in the time-restricted eating group lost weight compared to the ad libitum group where 78% ($n=7$) of participants gained weight. This corresponded with a statistically significant difference in BMI change between the groups ($z = 2.43$, $p = 0.02$). There was also a statistically significant difference in the changes in hip circumference between both groups with a median increase of 1cm in the ad libitum group and a median decrease of 2cm in the TRE group ($z = 2.62$, $p = 0.01$). A similar trend was seen with 100% ($n=9$) of participants having a decrease in hip circumference in the TRE group compared to 56% ($n=5$) of participants having an increase in hip circumference in the ad libitum group. There was no statistically significant difference in the change in waist circumference or waist:hip ratio. 67% ($n=6$) of participants in the time-restricted eating group had a decrease in waist circumference compared to the ad libitum group where 33% ($n=3$) of participants had an increase in waist circumference.

Difference 0-12 Weeks	Ad Libitum	Time-restricted Eating	Statistic
Weight (kg)	2.65 (0.3, 3.5)	-1.5 (-2.7, -0.1)	$z = 2.43, p = 0.01^*$
BMI (kg/m ²)	0.97 (0.13, 1.37)	-0.52 (-0.99, -0.04)	$z = 2.43, p = 0.02^*$
Waist Circumference (cm)	0 (-1, 2.5)	-3 (-6, 0)	$z = 1.54, p = 0.14$
Hip Circumference (cm)	1 (0, 1)	-2 (-4, -2)	$z = 2.62, p = 0.01^*$
Waist:Hip Ratio	-0.00 (-0.01, 0.01)	0.01 (-0.02, 0.01)	$z = 0.77, p = 0.44$

Table 5.5: Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased).

The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p -value for change across 12-weeks for anthropometric parameters. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Abbreviations: Kg kilogram, kg/m² kilograms per metre squared, cm centimetres, BMI body mass index

5.3.4 Change per week for anthropometric parameters

As with the data assessing for insulin resistance, for each anthropometric parameter, a multi-level linear regression was used to compare the change in each parameter seen in the time-restricted eating group compared to the ad libitum group. By using the data recorded at the 0, 6 and 12 week time points and using the time-restricted eating each week interaction term as the dependent variable, the β -coefficient was generated to show the median change per week for each variable. As before, these higher level statistics were carried out as part of the overall feasibility to demonstrate the potential for regression analysis and generation of the β coefficient, to be performed on a more highly powered study with a larger sample size.

The β -coefficient calculated by the multilevel regression analysis demonstrated that for each week of time-restricted eating, the participants following time-restricted eating lost significantly more weight than when following the ad libitum regimen. Participants had a weight loss of 0.37 kg per week when comparing time-restricted eating to the ad libitum regimen (95% CI: -0.54, -0.20, $p < 0.001$). This also contributed to a statistically reduction in BMI of 0.13 kg/m² per week (95% CI: -0.20, -0.07, $p < 0.001$). Waist circumference reduced by an average of 0.34cm per week (95% CI: -0.58, -0.10, $p = 0.005$) and hip circumference reduced by an average of 0.31cm per week (95% CI: -0.44, -0.17, $p < 0.001$), all reaching statistics significance.

An additional multi-level regression analysis was carried out to assess if the weight gain observed from baseline to 12 weeks for the ad libitum group, was statistically significant. The multi-level regression analysis produced a quantifiable average weight gain per week in kg by generating a β -coefficient. When the weight gain during the ad libitum regimen over 12 week is examined statistically with the impact of time on weight, for each week of

ad libitum eating, there was a statistically significant weight increase of 0.19 kg per week (0.10-0.29kg; $p<0.001$).

Outcome	β Coefficient (95% CI)	p-value
Weight (kg)	-0.37 (-0.54, -0.20)	<0.001***
BMI (kg/m ²)	-0.13 (-0.20, -0.07)	<0.001***
Waist Circumference (cm)	-0.34 (-0.58, -0.10)	0.01**
Hip Circumference (cm)	-0.31 (-0.44, -0.17)	<0.001***
Waist:Hip Ratio	-0.00 (-0.00, 0.00)	0.38

*Table 5.6: Results of multi-level linear regression of anthropometric data showing beta coefficients and p values for each parameter * $p<0.05$, ** $p<0.01$, *** $p<0.001$*

Abbreviations: Kg kilogram, kg/m² kilograms per metre squared, cm centimetres, BMI body mass index, CI confidence interval

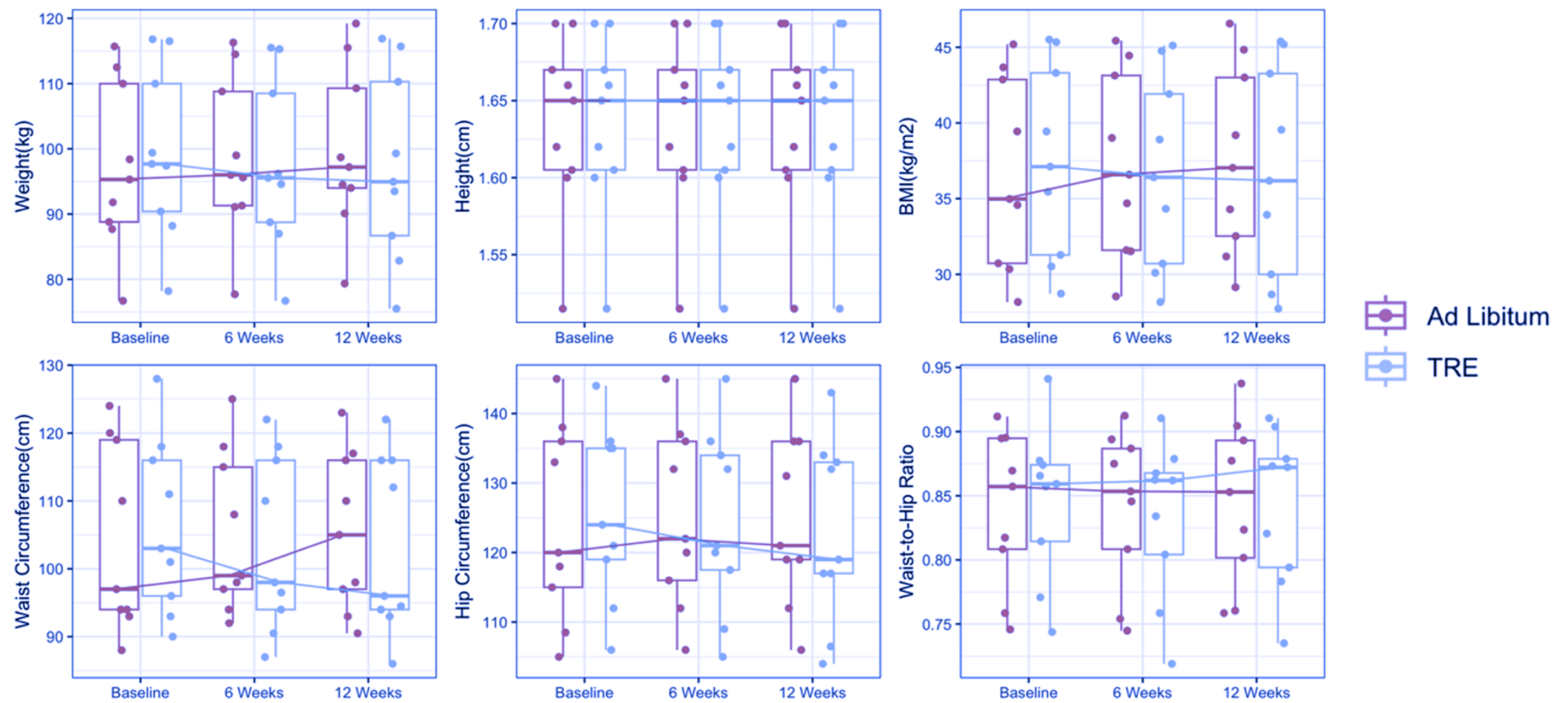


Figure 5.2: Graphical display of the medians and interquartile ranges of the anthropometric parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks.

5.3.5 The effect of time-restricted eating on reproductive hormones compared to ad libitum eating

To assess changes in reproductive hormones, all participants had the following included in an androgen profile measured at each visit: testosterone, free testosterone, androstenedione, DHEAS, SHBG and 17-OHP. The median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at baseline assessment and 12-week point are described in Table 5.7. There was no statistically significant difference for reproductive hormone parameters including testosterone, free testosterone, androstenedione, DHEAS or 17-OHP when the medians and interquartile ranges from baseline assessment to the 12-week assessment for both the time-restricted eating and the ad libitum study arms were examined individually. There was a significant increase in median SHBG from 20.85 at baseline to 28.8 following the 12-week time-restricted eating regimen ($z = -2.10$, $p = 0.04$).

Outcome	Study Arm	Baseline	12 Weeks	Statistic
Testosterone (nmol/L)	Ad libitum	1.5 (1.3-2)	1.45 (0.65-2.2)	$z = 0.70, p = 0.99$
	Time-restricted eating	1.8 (0.9-2)	1.7 (1.3-2.2)	$z = -0.71, p = 0.48$
Free Testosterone (nmol/L)	Ad libitum	2.6 (2.1-2.8)	2.5 (2.1-2.8)	$z = -0.30, p = 0.77$
	Time-restricted eating	2.4 (1.4-3.7)	2.8 (2-3.7)	$z = 0.14, p = 0.89$
Androstenedione (nmol/L)	Ad libitum	7.06 (5.25-8.42)	6.6 (4.52-9.72)	$z = 0.18, p = 0.86$
	Time-restricted eating	7.9 (5.05-11.5)	8.46 (4.5-9.66)	$z = 0.31, p = 0.85$
DHEAS (μ mol/L)	Ad libitum	5.46 (3.28-6.46)	4.15 (3.25-4.89)	$z = 0.39, p = 0.77$
	Time-restricted eating	6.12 (3.7-8.35)	4.98 (3.22-7.06)	$z = -0.52, p = 0.60$
SHBG (nmol/L)	Ad libitum	23.1 (15.8-33.7)	23.9 (15.3-31.8)	$z = 1.60, p = 0.11$
	Time-restricted eating	20.85 (15.6-34.15)	28.8 (15-32)	$z = -2.10, p = 0.04^*$
17-OHP (nmol/L)	Ad libitum	2 (1.7-3.5)	1.9 (1.8-3.5)	$z = -0.53, p = 0.60$
	Time-restricted eating	1.9 (1.4-3.3)	1.85 (0.95-3.35)	$z = 0.32, p = 0.75$

Table 5.7 Median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the 0 week and 12 week timepoints are presented.

The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p value for all reproductive hormone parameters. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Abbreviations: DHEAS dehydroepiandrosterone sulphate, SHBG sex hormone binding globulin, 17-OHP 17-hydroxyprogesterone

As per previous analyses, following the assessment of the change from 0 to 12 weeks for both the ad libitum and time-restricted eating group for each individual parameter, the change observed for each parameter in both groups was examined and comparison between these changes was carried out to assess if these changes were statistically significant. There was no statistically significant difference in the changes between both groups for the testosterone, free testosterone, androstenedione, DHEAS or 17-OHP (Table 5.8). A statistically significant difference was observed between the changes in SHBG in the ad libitum and time-restricted eating groups, with a median reduction of -1.1 (-1.9, -0.3) in the ad libitum group compared to an increase of 2.4 (0.25, 6.2) in the TRE group ($z = -2.10$, $p = 0.04$).

Difference 0-12 Weeks	Ad Libitum	TRE	Statistic
Testosterone (nmol/L)	-0.1 (-0.4, 0.5)	0.1 (-0.1, 0.3)	$z = 0.35$, $p = 0.73$
Free Testosterone (nmol/L)	0.1 (-0.4, 0.2)	-0.1 (-0.3, 0.6)	$z = -0.68$, $p = 0.50$
Androstenedione (nmol/L)	-0.13 (-0.73, 1.1)	-0.16 (-2.32, 1.31)	$z = -1.15$, $p = 0.25$
DHEAS ($\mu\text{mol/L}$)	-0.51 (-0.69, 0.8)	0.12 (-0.23, 0.34)	$z = 0.31$, $p = 0.75$
SHBG (nmol/L)	-1.1 (-1.9, -0.3)	2.4 (0.25, 6.2)	$z = -2.10$, $p = 0.04^*$
17-OHP (nmol/L)	0.2 (-0.1, 0.4)	-0.1 (-0.7, 0.1)	$z = 0.31$, $p = 0.75$

Table 5.8 Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased). The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p -value for change across 12-weeks for the reproductive hormone parameters. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Abbreviations: DHEAS dehydroepiandrosterone sulphate, SHBG sex hormone binding globulin, 17-OHP 17-hydroxyprogesterone

5.3.6 Change per week for reproductive hormones

As before, a multi-level linear regression was used with a time-restricted eating each week interaction term as the dependent variable. This was carried out for feasibility of these statistics with this small dataset. This allowed assessment of the change in each parameter over the 12 week period and compare if this change seen in the time-restricted eating group and ad libitum group showed statistical significance. There were no significant effects of time-restricted eating on participant's androgen profiles, for any of the parameters (Table 5.9). The β -coefficient measures the degree of change in each outcome variable for every 1-unit of change in the predictor variable. It remained close to zero for all parameters above. As with the insulin variables, log transformation was carried out for the DHEAS, SHBG and 17-OHP variables to normalise the datasets. These changes with time-restricted eating over time of these natural log transformed parameters did not reach statistical significance.

Outcome	β Coefficient (95% CI)	p-value
Testosterone (nmol/L)	0.01 (-0.02, 0.04)	0.58
Free Testosterone (nmol/L)	-0.02 (-0.08, 0.05)	0.60
Androstenedione (nmol/L)	0.02 (-0.15, 0.19)	0.82
$\ln$ DHEAS (μ mol/L)	0.01 (-0.02, 0.04)	0.52
$\ln$ SHBG (nmol/L)	0.01 (-0.00, 0.03)	0.08
$\ln$ 17-OHP (nmol/L)	0.01 (-0.07, 0.06)	0.88

Table 5.9: Results of multi-level linear regression of androgen profile data showing β -coefficients and p values for each parameter * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Abbreviations: DHEAS dehydroepiandrosterone sulphate, SHBG sex hormone binding globulin, 17-OHP 17-hydroxyprogesterone

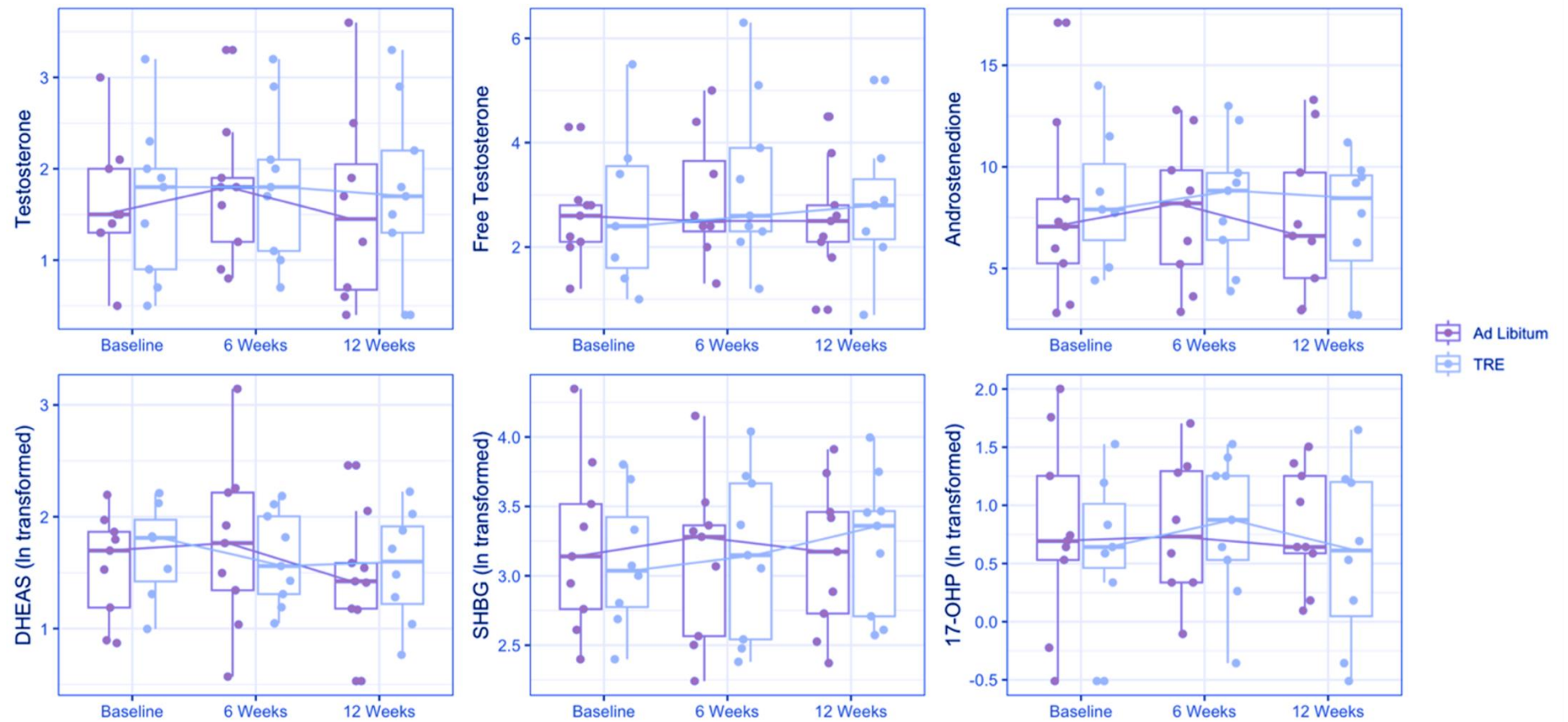


Figure 5.3: Graphical display of the medians and interquartile ranges of the reproductive hormone parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks

5.3.7 The effect of time-restricted eating on lipids compared to ad libitum eating

To assess changes in lipid profiles, all participants had the following included in an extended lipid profile measured at each visit: total cholesterol, triglycerides, HDL, LDL, Non-HDL, lipoprotein lipid A, apolipoprotein A1 and apolipoprotein B. The median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at baseline assessment and 12-week point are detailed in Table 5.10. There was no statistically significant difference for parameters including total cholesterol, HDL, LDL, Non-HDL, lipoprotein lipid A, apolipoprotein A1 and apolipoprotein B when the medians and interquartile ranges from baseline assessment to the 12-week assessment for both the time-restricted eating and the ad libitum study arms were examined individually. There was a significant decrease in median triglycerides from 1.82 mmol/L (1.61-3.31mmol/L) at baseline to 1.6mmol/L (1.23-2.91mmol/L) following the 12-week time-restricted eating regimen ($z = 2.02$, $p = 0.04$).

Outcome	Study Arm	0 Weeks	12 Weeks	Statistic
Total Cholesterol (mmol/L)	Ad libitum	5.5 (4.9-6.1)	6 (5.3-6.5)	$z = -1.72, p = 0.09$
	Time-restricted eating	6.1 (5.1-6.6)	6.1 (5-6.8)	$z = 0.30, p = 0.77$
Triglycerides (mmol/L)	Ad libitum	1.58 (1.19-2.34)	1.56 (1.14-2.95)	$z = -0.89, p = 0.37$
	Time-restricted eating	1.82 (1.61-3.31)	1.6 (1.23-2.91)	$z = 2.02, p = 0.04^*$
HDL (mmol/L)	Ad libitum	1 (0.94-1.24)	1.09 (1.05-1.22)	$z = -1.60, p = 0.11$
	Time-restricted eating	1.1 (1.03-1.26)	1.11 (1.03-1.27)	$z = -0.83, p = 0.41$
LDL (mmol/L)	Ad libitum	3.6 (2.9-4)	3.8 (3.5-4.3)	$z = -1.36, p = 0.17$
	Time-restricted eating	4.05 (3.05-4.25)	3.8 (3.2-4.3)	$z = 0.35, p = 0.73$
Non-HDL (mmol/L)	Ad libitum	4.5 (3.7-4.8)	5.2 (4.1-5.4)	$z = -1.48, p = 0.16$
	Time-restricted eating	5 (3.9-5.8)	4.7 (3.8-5.5)	$z = 0.36, p = 0.72$
Lipoprotein Lipid A (nmol/L)	Ad libitum	82.6 (31-188.5)	78.95 (23.75-123.2)	$z = 1.49, p = 0.14$
	Time-restricted eating	65.45 (25.1-223.2)	82.3 (39-176.5)	$z = -1.35, p = 0.18$
Apolipoprotein A1 (g/L)	Ad libitum	1.26 (1.11-1.31)	1.38 (1.16-1.41)	$z = -1.68, p = 0.10$
	Time-restricted eating	1.29 (1.26-1.31)	1.23 (1.13-1.46)	$z = 0.59, p = 0.61$
Apolipoprotein B (g/L)	Ad libitum	1.16 (0.84-1.33)	1.11 (0.97-1.28)	$z = 0.28, p = 0.78$
	Time-restricted eating	1.25 (0.97-1.36)	1.1 (0.94-1.3)	$z = 0.43, p = 0.43$

Table 5.10 Median and interquartile ranges for participants for both the ad libitum and time-restricted eating groups at the 0 week and 12 week timepoints are presented.

The statistic column shows the result of a Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p value for all lipid parameters.

** $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*

Abbreviations: HDL high density lipoprotein, LDL low density lipoprotein

Following the assessment of the change from 0 to 12 weeks for both the ad libitum and time-restricted eating group for each individual parameter, the change observed for each parameter in both groups was examined and comparison between these changes was carried out to assess if these changes were statistically significant. There was no statistically significant difference in the changes between both groups for the lipid profile parameters (Table 5.11), except for Apolipoprotein A1, which increased by 0.12 in the ad libitum group and decreased by -0.02 in the TRE group ($z = 2.52$, $p = 0.01$).

Difference 0-12 Weeks	Ad Libitum	TRE	Statistic
Total Cholesterol (mmol/L)	0.5 (-0.1, 0.9)	-0.1 (-0.4, 0.1)	$z = 0.89$, $p = 0.37$
Triglycerides (mmol/L)	0.1 (-0.07, 0.42)	-0.39 (-0.53, -0.11)	$z = 1.66$, $p = 0.10$
HDL (mmol/L)	0.1 (-0.01, 0.2)	0.2 (-0.01, 0.07)	$z = 1.24$, $p = 0.22$
LDL (mmol/L)	0.3 (-0.1, 0.6)	-0.05 (-0.2, 0.1)	$z = 1.12$, $p = 0.26$
Non-HDL (mmol/L)	0.5 (-0.2, 0.8)	-0.2 (-0.3, 0.3)	$z = 0.98$, $p = 0.33$
Lipoprotein Lipid A (nmol/L)	-4.3 (-21.4, 0)	6.1 (0, 12.3)	$z = -2.15$, $p = 0.06$
Apolipoprotein A1 (g/L)	0.12 (0.05, 0.21)	-0.02 (-0.06, 0)	$z = 2.52$, $p = 0.01^*$
Apolipoprotein B (g/L)	-0.04 (-0.13, 0.13)	-0.06 (-0.17, 0.05)	$z = 0.42$, $p = 0.74$

Table 5.11 Table showing the subtracted baseline from 12 weeks median with IQR values to get a change (positive value indicates that increased by 12 weeks and negative value that it decreased).

The statistic column shows results of the Wilcoxon Matched-Pairs Test (non-parametric) where z is test statistic and corresponding p -value for change across 12-weeks for the lipid parameters.

Abbreviations: HDL high density lipoprotein, LDL low density lipoprotein

5.3.8 Change per week for lipids

As with previous parameters, a multi-level linear regression was used with a time-restricted eating each week interaction term as the dependent variable. There were no significant effects of time-restricted eating on lipid profiles (Table 5.12). The β -coefficient was close to zero for all parameters when the change in each parameter over the 12 week period was compared between the time-restricted eating group and ad libitum group.

Outcome	β Coefficient (95% CI)	p-value
Total Cholesterol (mmol/L)	-0.03 (-0.08, 0.01)	0.17
Triglycerides (mmol/L)	-0.05 (-0.10, 0.00)	0.06
HDL (mmol/L)	-0.00 (-0.00, 0.01)	0.78
LDL (mmol/L)	-0.02 (-0.06, 0.02)	0.37
Non-HDL (mmol/L)	-0.03 (-0.07, 0.01)	0.13
lnLipoprotein lipid A (nmol/L)	0.01 (-0.00, 0.03)	0.15
lnApolipoprotein A1 (g/L)	-0.01 (-0.02, 0.00)	0.25
lnApolipoprotein B (g/L)	-0.00 (-0.00, 0.00)	0.99

Table 5.12: Results of multi-level linear regression of lipid profile data showing beta coefficients and p values for each parameter * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Abbreviations: HDL high density lipoprotein, LDL low density lipoprotein

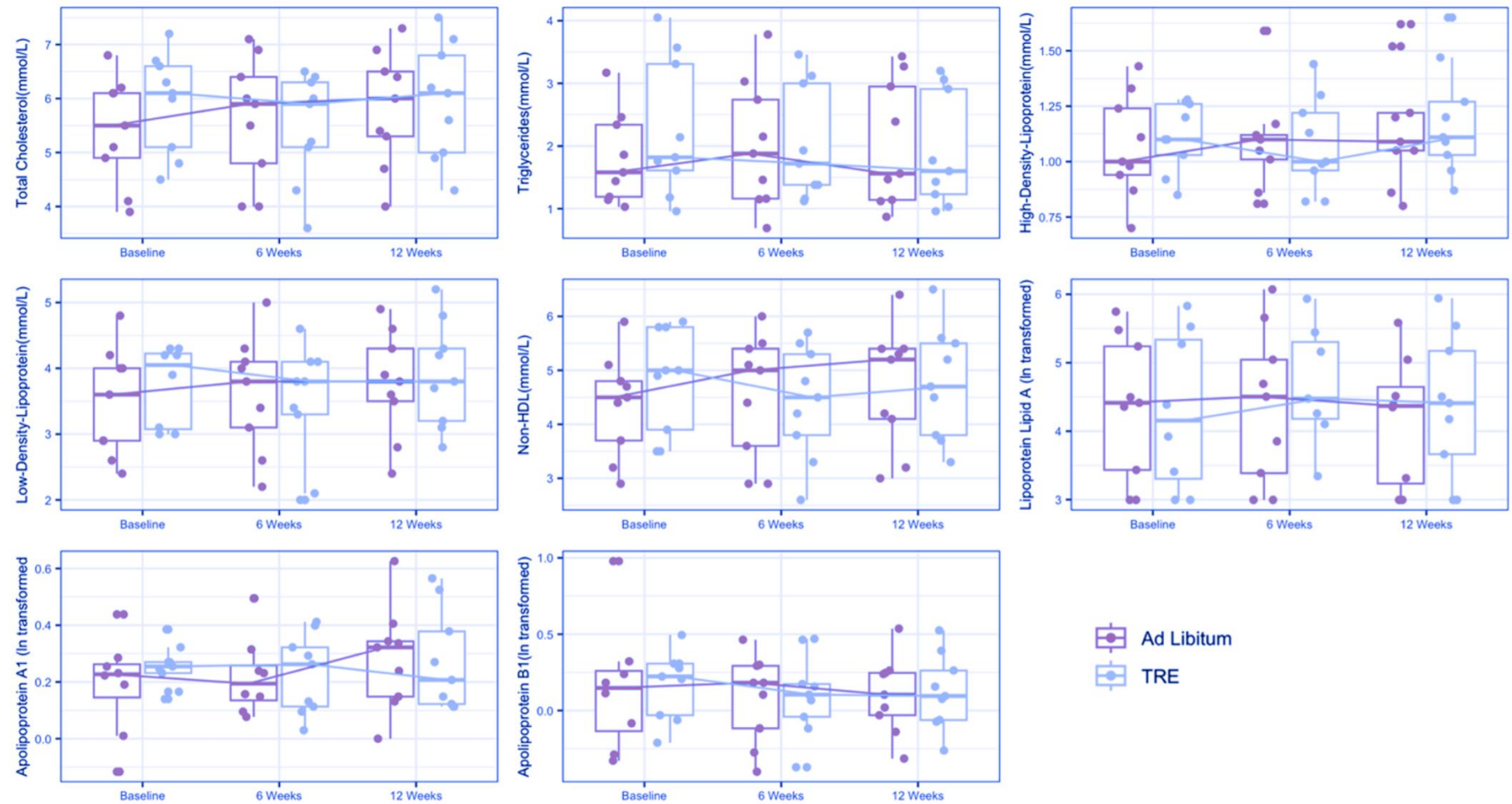


Figure 5.4: Graphical display of the medians and interquartile ranges of the lipid parameters between participants following ad libitum and time-restricted eating regimens from 0-12 weeks.

5.3.9 Coefficients For Assignment

Table 5.13 shows the β -coefficients for time-restricted eating over time ($TRE \times time$) and include controlling for random order of assignment to either arm, namely starting with time-restricted eating or ad libitum. As described in section 4.3.8, this is a multivariate analysis and so both are significant predictors independent of each other. This means that the intervention is working hence the significant time-restricted eating over time ($TRE \times time$) interaction, but allocation order is also still having a significant effect. Even when allocation was controlled for, intervention ($TRE \times time$) still has a significant effect; similarly even when controlling for intervention ($TRE \times time$), allocation order still has an effect. The non-significant p-values above signify that the results discussed are significant, independent of assignment, even though assignment is statistically significant for insulin parameters serum insulin, HOMA-IR, QUICKI-I and SHBG. 4/25 (16%) variables are significant. It is possible that the five patients randomised to time-restricted eating first as detailed in Table 4.2 (and crossed over to ad libitum eating) experienced benefits with regard to insulin sensitivity beyond the time-restricted eating period, and therefore had residual benefits during the ad libitum period.

Overall, the assignment of participants to either intervention to start had no effect on the outcomes.

Outcome	β Coefficient (95% CI)	p-value
lnInsulin (mU/L)	1.29 (0.85, 1.73)	<0.001***
Glucose (mmol/L)	-0.00 (-0.05, 0.04)	0.872
lnHOMA-IR	1.36 (0.92, 1.81)	<0.001***
lnQUICK-I	-0.31 (-0.41, -0.21)	<0.001***
HbA1c in mmol/mol	1.58 (-1.00, 4.16)	0.229
HbA1c in DCCT (%)	-0.01 (-0.04, 0.01)	0.297
Weight (kg)	6.81 (-8.82, 22.44)	0.393
BMI (kg/m ²)	1.29 (-6.62, 9.21)	0.748
Waist Circumference (cm)	4.09 (-11.25, 19.43)	0.601
Hip Circumference (cm)	4.42 (-11.59, 20.43)	0.588
Waist:Hip Ratio	0.00 (-0.08, 0.08)	0.914
Testosterone (nmol/L)	-0.00 (-1.03, 1.03)	0.999
Free Testosterone (nmol/L)	0.64 (-0.76, 2.05)	0.369
Androstenedione (nmol/L)	0.63 (-3.52, 4.79)	0.765
lnDHEAS (μ mol/L)	0.49 (-0.02, 0.99)	0.062
lnSHBG (nmol/L)	-0.13 (-0.00, 0.03)	0.011*
ln17-OHP (nmol/L)	0.13 (-0.40, 0.64)	0.636
Total Cholesterol (mmol/L)	-0.03 (-0.14, 0.08)	0.546
Triglycerides (mmol/L)	-0.05 (-0.14, 0.05)	0.320
High-Density Lipoprotein (mmol/L)	-0.00 (-0.03, 0.02)	0.896
Low-Density Lipoprotein (mmol/L)	-0.13 (-0.14, 0.77)	0.794
Non-HDL (mmol/L)	0.17 (-1.05, 1.39)	0.784
lnLipoprotein lipid A (nmol/L)	0.01 (-0.00, 0.03)	0.151
lnApolipoproteinA1(g/L)	-0.03 (-0.19, 0.12)	0.675
lnApolipoproteinB (g/L)	-0.03 (-0.35, 0.29)	0.867

Table 5.13: Results of beta coefficients and p values for all parameters for assignment to assess if assignment had an effect on outcomes

Abbreviations: HOMA-IR Homeostatic Model Assessment for Insulin Resistance, QUICK-I Quantitative Insulin-Sensitivity Check Index, HbA1c Haemoglobin A1c, DCCT Diabetes Control and Complications Trial unit of percentage (%), CI confidence interval, Kg/(m²)

kilograms (per metre squared), cm centimetres, BMI body mass index, DHEAS dehydroepiandrosterone sulphate, SHBG sex hormone binding globulin, 17-OHP 17-hydroxyprogesterone, HDL high density lipoprotein, LDL low density lipoprotein

5.3.10 The effect of time-restricted eating on nutritional intake compared to ad libitum eating

Caloric intake between both groups was found to be normally distributed, after examining QQ plots and histograms, so a t-test was used for this data. As described in section 4.3.7 of the Methods, averages of the 3-day food diaries collected at baseline, 6 week and 12 week mark for both the time-restricted and ad libitum regimens, were carried out for the participants while following the time-restricted eating regimen and as well as ad libitum. Therefore, the nutrients consumed represent average consumption over the 12 week intervention period for each intervention, respectively. Overall mean calorie intake in the time-restricted eating group (1256.4 kcal +/- 404.3 kcal) and the ad libitum group (1,650.6 kcal +/- 512.9 kcal) differed significantly ($t = 3.09$, $p = 0.002$). The t-statistic illustrates the difference between the sample mean and the given number to the standard error of the mean. The larger magnitude of the t-value signifies a smaller standard error of the mean giving the small p-value and evidence that the difference was significant.

The other macronutrient and mineral parameters were non normally distributed. The Wilcoxon Rank Sum Test was used for this reason at all timepoints and overall for each parameter. Those following the time-restricted eating intervention consumed less carbohydrate with a median intake of 165.1g (IQR 149.3g-201g) in the ad libitum group compared to 134.4g (IQR 95.6g-171g) in the time-restricted eating group ($z = 2.25$, $p = 0.03^*$). Those in the time-restricted eating group also consumed less saturated fat (median fat intake 20.9g (IQR 15.3g-28.5g) in the ad libitum group compared to 15.2g (IQR 12.8g-22.2g) in the time-restricted eating group, $z = 2.271$, $p = 0.03$). Calcium intakes also differed significantly between the groups with those in the ad libitum group consuming a

median of 515mg (IQR 312mg-717mg) compared to 332mg (IQR 213mg-441mg) in the time-restricted eating group.

There was no difference in protein intake between the ad libitum group who consumed a median 55g (IQR 51.4g-87.2g) compared with the time-restricted eating group who consumed a median of 55.5g (IQR 50.4g-75g) ($z = 1.44$, $p = 0.15$). There was also no difference in vitamin D intake between the ad libitum group who consumed a median 1.9ug (IQR 1.1ug-2.7ug) compared with the time-restricted eating group who consumed a median of 1.4ug (IQR 0.7ug-2.3ug) ($z = 1.11$, $p = 0.27$).

Overall, participants following time-restricted eating consumed significantly fewer calories, and less carbohydrate, saturated fat and calcium (Table 5.14).

Measurement	Ad libitum	TRE	Statistical Test
Calories (kcal)			
0 Weeks	1,585 (592.7)	1,258.7 (300.1)	t = 1.48, p = 0.07
6 Weeks	1,676.1 (599.6)	1,163.3 (507.8)	t = 1.95, p = 0.03
12 Weeks	1,701.7 (306.9)	1,347.3 (405.2)	t = 1.92, p = 0.04
Overall	1,650.6 (512.9)	1256.4 (404.3)	t = 3.09, p = 0.002**
Carbohydrate (g)			
0 Weeks	152.3 (129.6-209.6)	133.7 (113.5-168.7)	z = 1.28, p = 0.20
6 Weeks	165.1 (149.3-177.6)	97.6 (82-153.6)	z = 1.37, p = 0.17
12 Weeks	178.5 (154.2-196.7)	160.6 (122.6-134.4)	z = 1.01, p = 0.32
Overall	165.1 (149.3-201)	134.4 (95.6-171)	z = 2.25, p = 0.03*
Sugar (g)			
0 Weeks	42.9 (40.7-76.4)	53.9 (35.3-65.4)	z = 0.13, p = 0.93
6 Weeks	57.2 (46.9-76.5)	30.5 (24.9-47.4)	z = 1.72, p = 0.08
12 Weeks	52.1 (43.8-72)	45.4 (31-48.2)	z = 1.43, p = 0.18
Overall	52.1 (40.8-76.4)	45.4 (26.9-60.1)	z = 1.84, p = 0.08
Fat (g)			
0 Weeks	57.3 (42.3-70.8)	45.6 (40.1-54.3)	z = 0.93, p = 0.38
6 Weeks	47.5 (36.7-87.2)	47.2 (35.4-49.7)	z = 0.40, p = 0.69
12 Weeks	78.9 (56.9-82.8)	50 (41.4-72.1)	z = 1.64, p = 0.11
Overall	57.8 (42.3-82.8)	47.2 (36.3-69.2)	z = 1.75, p = 0.08
Saturated Fat (g)			
0 Weeks	19 (15.1-22.6)	15.2 (13.2-15.8)	z = 0.93, p = 0.36
6 Weeks	20.6 (15.1-32.6)	14 (12.8-19.7)	z = 1.19, p = 0.26
12 Weeks	26.7 (20.9-28.5)	17.9 (10.7-23.6)	z = 2.06, p = 0.04
Overall	20.9 (15.3-28.5)	15.2 (12.8-22.2)	z = 2.271, p = 0.03*
Protein (g)			
0 Weeks	62.5 (58-80.1)	57 (52.7-78.5)	z = 0.44, p = 0.66
6 Weeks	66 (51.2-88.4)	50.4 (41.9-58.2)	z = 1.63, p = 0.11
12 Weeks	81 (51.4-88.8)	55.5 (54-63.4)	z = 0.69, p = 0.49
Overall	55 (51.4-87.2)	55.5 (50.4-75)	z = 1.44, p = 0.15
Calcium (mg)			
0 Weeks	336 (312-529)	279 (169-341)	z = 1.72, p = 0.09
6 Weeks	515 (246-808)	304 (213-411)	z = 1.37, p = 0.17
12 Weeks	571 (452-720)	393 (281-547)	z = 0.90, p = 0.37
Overall	515 (312-717)	332 (213-441)	z = 2.24, p = 0.02*
Vitamin D (ug)			
0 Weeks	1.9 (1.1-2.4)	1.5 (1.3-2.4)	z = -0.13, p = 0.89
6 Weeks	1.9 (0.69-3.5)	0.90 (0.52-2.3)	z = 0.40, p = 0.69
12 Weeks	2.1 (1.1-2.7)	0.82 (0.69-2.1)	z = 1.5, p = 0.12
Overall	1.9 (1.1-2.7)	1.4 (0.7-2.3)	z = 1.11, p = 0.27

Table 5.14: Results of the Wilcoxon Rank Sum Tests for nutritional intake data showing means/medians and standard deviation/interquartile ranges as well as p values for each parameter.

*Overall is the average of each nutritional measurement, across the three visits while undertaking the ad libitum or time-restricted eating arm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*

Abbreviations: TRE time-restricted eating, g gram, mg milligram, ug microgram, kcal calorie

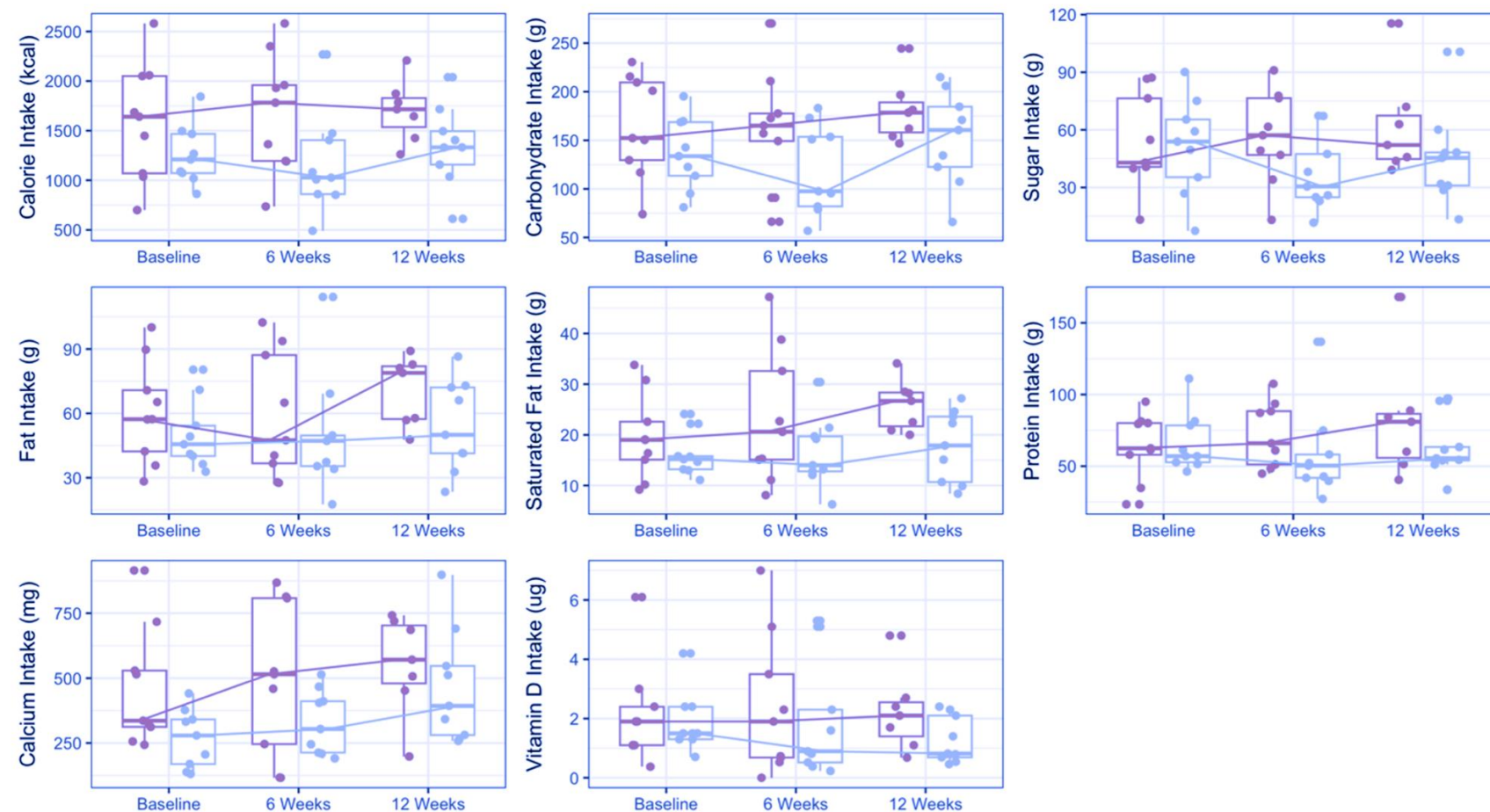


Figure 5.5: Graphical display of the differences in the means and standard deviation of calorie intake (normally distributed) and medians and interquartile ranges of the macronutrient and mineral intake averages between participants following ad libitum and time-restricted eating regimens from 0-12 weeks

5.4 Discussion

5.4.1 Summary of findings

As stated in Section 5.1, to categorise the results the secondary outcomes were grouped as follows: 1) insulin-related, 2) anthropometry, 3) reproductive hormones, 4) lipid profiles and 5) dietary intake. For each of the first four groups, the *change from baseline to 12 weeks* for the various parameters was analysed separately for the two dietary groups, the *two dietary groups were compared* regarding the change in the various parameters over 12 weeks, and the change *per week* was analysed and compared for the two dietary groups. For the fifth group, the averages of the 3-day food diaries collected at baseline, 6 week and 12 week mark for both the time-restricted and ad libitum regimens were directly compared. There was no difference between time-restricted eating and ad libitum eating regarding insulin-related parameters, with the exception of a modest increase in insulin sensitivity (measured by QUICKI) per week with time-restricted eating, and a similar improvement in HbA1c.

Anthropometric analyses showed a greater change. When looking at the change in each group separately, the time-restricted eating group exhibited a *decrease* in weight, BMI, waist circumference and hip circumference (but not waist:hip ratio). The ad libitum group showed an *increase* in weight and BMI (but not waist circumference, hip circumference and waist:hip ratio). When analysing the change in these parameters over 12 weeks and comparing the two diet groups, the time-restricted eating group had a weekly decrease in weight of 0.37Kg compared to the ad libitum group's weekly increase in weight of 0.19Kg. Like insulin-related parameters, there was no appreciable difference in any of the reproductive hormones measured, with the exception of SHBG, which increased in the time-restricted eating group and decreased in the ad libitum group, representing an improvement in insulin resistance.

Similarly, there were no changes in lipid profiles from baseline to 12 weeks in either group, with the exception of a reduction in triglycerides in the time-restricted eating group.

Those who followed the time-restricted eating intervention ate less than those who were assigned to the ad libitum arm. Overall, intake appeared to be low in both group allocations which likely represents underreporting of food intake in both groups. This was true for calories, carbohydrate, and saturated fat (but not total fat). Those in the time-restricted eating arm also consumed less calcium, although calcium intakes were remarkably low in both groups.

Similarly, the intake of vitamin D for both diet arms was worryingly low (median intake was at most 2.1 ug / day), and did not differ between the groups. The dietary vitamin D requirement to maintain serum 25(OH)D ≥ 30 nmol/L in healthy individuals aged 12–65 years in Ireland is 15 µg/day according to the Food Safety Authority of Ireland Scientific Committee Report [119].

5.4.2 Conclusions

Based on this small, underpowered, sample which was analysed a part of a feasibility study, time-restricted eating appeared to confer benefits in terms of weight loss, with smaller benefits in insulin-related parameters and triglycerides. Participants following this intervention consumed fewer calories overall, as well as less dietary calcium. To reiterate prior assertions, these findings do not provide evidence of time-restricted eating's superiority over ad libitum eating regarding the measured indices, rather, they bolster the case for a large, well-designed study to investigate the hypothesised benefits of this novel intervention. By definition, feasibility studies may be used to determine whether an intervention is appropriate for further testing, and these findings provide a strong and valid reason for pursuing further study. It should be noted that while the numbers were too low

for meaningful conclusions, the significant and near-significant changes in parameters seen in each instance were in favour of time-restricted eating, and not in favour of ad libitum eating.

In the final chapter (Chapter 6), the results of the study will be discussed in the context for the prior literature, clinical implications will be explored, the limitations and strengths will be described, and a roadmap for a future substantive study will be presented.

Chapter 6

Discussion, Conclusion & Future Directions

6.1 Brief Chapter Summary

Chapter 1 and 2 of this thesis provided a general background to PCOS, insulin resistance and the clinical consequences of this condition. The potential of time-restricted eating as a putative therapeutic intervention for PCOS, particularly for the reduction of insulin levels, was described in detail.

In chapter 3, the results of the first systematic review of the effect of time-restricted eating on various metabolic parameters in women with PCOS were presented. The review retrieved only one study that fulfilled the criteria.

The studies described in Chapters 4 and 5 represent the first feasibility study of time-restricted eating in PCOS, as well as the first randomised cross-over study exploring the effects of time-restricted eating on various critical outcomes in this condition. In terms of feasibility, the study struggled to attract large participant numbers for various reasons. Those who participated were adherent to the intervention, had high compliance and demonstrated its safety and feasibility with minimal side effects. While a high dropout rate was observed, those not completing the study reported being unable to do so due to practical and lifestyle reasons rather than for reasons related to time-restricted eating. The difficulties with recruitment will help to design a more effective future study.

In the analysis of clinical parameters (the secondary outcomes of the study, Chapter 5), notwithstanding the clear limitations of the small sample size, following 12 weeks of time-restricted eating there was a significant improvement in body weight, BMI, quantitative insulin sensitivity check index and HbA1c levels. There was no marked effect on reproductive hormones or lipid profiles. In the time-restricted eating arm, participants consumed significantly fewer calories, and less carbohydrate, saturated fat and, notably,

calcium. The analysis of clinical parameters contributed to the development of a proposed design for a larger substantive study to determine the efficacy of time-restricted eating in PCOS, described later.

6.2 Key Findings of the Thesis

6.2.1 A lack of research on this topic

There is minimal evidence for the effect of time-restricted eating in PCOS [112]. Zengeneh et al and Asemi et al showed no effect of Ramadan fasting on insulin levels [111, 113] whereas Li et al showed significant improvements in fasting insulin levels, HOMA-IR and SHBG after a 6-week time-restricted eating regimen with a 16-hour daily fasting period [112]. Another recent retrospective cohort study which examined a 6-week time-restricted eating regimen with a 16-hour daily fast, reported significant improvements in fasting insulin, glucose, HbA1c and HOMA-IR in women with PCOS [120]. A high quality randomised controlled trial showed improvements in the HOMA-IR levels of healthy, non-obese, female volunteers [121]. However, results of its effects on insulin parameters in other populations are mixed [122-124] Considering these studies in combination with the results of this study, there is still insufficient quality evidence to conclude that time-restricted eating can improve insulin levels and associated parameters specifically in women with PCOS. However, this is a growing area of research with many registered studies of time-restricted eating in PCOS on clinicaltrials.gov including NCT04452968, NCT04580433, NCT03792282 and NCT05629858.

6.2.2 Time-restricted eating is an acceptable, safe, and feasible intervention for PCOS

The feasibility study showed that time-restricted eating in PCOS was a feasible therapeutic intervention for women with PCOS. However, there were considerable difficulties in

recruiting patients to the study, and a large number of dropouts. These appeared to relate mainly to the time and commitment burden of the study (along with the difficulty in contacting some patients) rather than a problem with the intervention itself. In fact, majority of patients relayed that they were keen to continue with the intervention, showing broad acceptability. Crucially, time-restricted eating was found to be a safe intervention in this cohort, with minimal adverse events recorded during the study and good compliance over the twelve weeks of time-restricted eating. While this is the first feasibility study of time-restricted eating in the PCOS population, these findings mirror results in other populations (including those with obesity and type-2 diabetes mellitus) with no major adverse events and overall being a safe dietary intervention [122, 125].

The recruitment rate as a measure of feasibility was very low, a factor that needs to be addressed as a priority ahead of undertaking a larger substantive study. Recruitment was undoubtedly affected by the strict inclusion criteria. To obtain a clean sample of participants with minimal confounding factors, this strict criterion was deemed to be essential, excluding many potential participants. As participants were recruited from secondary care, patients had long standing PCOS and were established on therapies including insulin sensitising medications including mainly metformin, oral contraceptive pill, and weight loss medications such as GLP-1 agonists. To isolate the potential insulin sensitising effects of time-restricted eating, this was required. The reasoning behind the low recruitment rate is not unique to this study with other feasibility studies in time-restricted eating quoting strict inclusion/exclusion as a reason for this [125].

The most common side effect reported was headache, followed by pre-syncope episodes, halitosis, dizziness, and irritability. Other feasibility studies for time-restricted eating in other populations had minimal detailed recording of adverse events or side effects

experienced by participants [122, 125]. These side effects of prolonged fasting have been previously reported in other studies of fasting regimens [45, 121, 126].

Notably, there were important positive effects of the time-restricted eating intervention, including a subjective improvement in bloating, energy, fatigue, and concentration levels. The overall improvement in well-being and motivation of participants has obvious potential benefits which may improve other aspects of PCOS management such as exercise engagement. Similar positive effects of time-restricted eating were also reported in other studies [45, 126].

6.2.3 Time-restricted eating did not affect insulin-related parameters

There were no changes in insulin-related parameters in either the ad libitum and time-restricted eating groups in this study, nor was there a change across the 12-week regimen in either the ad libitum or time-restricted eating groups. A total of 11% of insulin samples were unable to be processed due to haemolysis. It is likely that these missing samples may have affected the results and had a subsequent downstream effect for the insulin sensitivity ratios including QUICK-I and HOMA-IR which require the insulin values to be calculated. The statistical evaluation of the dataset with multi-level regression analysis did show a significant beneficial change in QUICK-I ratio and HbA1c values. This regression analysis takes into account the six-week interval data set which may have improved the power of the statistical test. These results should be interpreted with some caution given the small number in the final analysis with this type of statistical analysis, nevertheless may provide a framework for the setting of outcomes in a larger study.

Glucose clamp studies are the gold standard for the measurement of insulin sensitivity but they are inefficient and costly. QUICK-I was an appropriate test for this study as it correlates well and is a useful tool clinically for the measurement of insulin resistance, with

lower numbers reflecting greater insulin resistance [73]. QUICKI ranges from 0.45 in healthy individuals to 0.30, which is the value associated with diabetes. A score below 0.339 indicates insulin resistance [127]. 78% (n=7) of the participants in this study had a baseline QUICKI ratio showing insulin resistance with a median value of 0.319 (IQR 0.287-0.338). HOMA-IR is also a useful simple formula to calculate insulin resistance with reasonable correlation between HOMA-IR and clamp gold standard studies; a result <1.0 meaning optimal insulin sensitivity, > 1.9 indicating early insulin resistance and >2.9 demonstrating significant insulin resistance. Other sources use >2.0 as signifying a clinically significant insulin resistance. In the present study, 78% of participants had insulin resistance at baseline when assessed with HOMA-IR, with a median value of 3.6 (IQR 1.9-7.5).

6.2.4 Time-restricted eating conferred significant improvements in weight and other anthropometric indices

All of the participants (n=9) in this study were overweight or obese, and obesity is one of the major symptoms and contributors to the worsening of the cardiometabolic issues seen in women with PCOS. A high BMI is associated with greater prevalence of irregular menstrual cycles and oligomenorrhea, as well as biochemical hyperandrogenism and its various clinical manifestations [39]. Dietary intervention and lifestyle modifications are at the foundation of PCOS management with many dietary interventions trialled and tested in this patient cohort [112]. Weight loss is one of the key components of management of all cardiometabolic and reproductive aspects of the condition and therefore anthropometric indices related to weight were secondary outcomes in this work [37].

A statistically significant improvement in anthropometric data including weight, BMI as well as waist and hip measurements was observed in the change in each parameter from

baseline assessment to the end of the 12-week regimen of TRE. When the differences in the change from baseline to end of each of the TRE and ad libitum group was examined and compared against each other, a statistically significant difference was noted in weight, BMI and hip circumference also. It is difficult to separate this confounding factor from the improvements in other variables of insulin resistance and assess if the reduction in insulin resistance occurs independent of weight loss and hypocaloric diet. A statistically significant reduction in waist circumference has significant health benefits, with larger waist circumference levels being associated with a higher mortality risk, even with adjustment for BMI [128]. Waist circumference has a strong correlation with visceral fat and is an additional descriptive variable, helpful in allowing consideration of body composition, not reflected in BMI [128]. Statistically significant improvements in all anthropometric parameters with time-restricted eating, despite the small number of participants in this study (n=9), demonstrates that time-restricted eating could be a powerful weight loss tool in this patient cohort, with 78% of participants in the time-restricted eating group losing weight. An unexpected finding in the study was the significant weight gain observed in participants while in the ad libitum arm of the study with 78% of participants gaining weight. This was independent of whether participants were randomised to start with time-restricted eating or ad libitum eating and crossover.

Accumulating evidence in various cohorts shows significant weight loss is achievable with time-restricted eating in various patient cohorts, particularly obese patients. Time-restricted eating has been shown in one meta-analysis to significantly improve weight compared with other weight loss regimens [44]. It was also highlighted that data regarding the long-term effect and benefits of time-restricted eating, remain unstudied [44]. More well-designed randomised trials are needed to definitively recommend time-restricted eating in various patient cohorts as a weight loss tool.

6.2.5 Time-restricted eating was associated with improved triglyceride levels but did not affect other lipid indices

Dyslipidaemia is a feature in PCOS, independent of obesity. Patterns of increased triglycerides, LDL-cholesterol and low HDL cholesterol are seen in up to 70% of this patient cohort [129, 130]. Lipoprotein lipid A, apolipoprotein A1 and apolipoprotein B were also analysed as secondary outcomes in this study. These are markers of assessment of overall cardiovascular risk of coronary artery disease particularly in combination with high LDL levels [131]. Elevated lipoprotein A levels have been reported in women with PCOS, independent of obesity status [131]. High levels of Apolipoprotein A1 are cardio-protective. Reduced levels have been observed in women with PCOS [132]. A statistically significant difference in Apolipoprotein A1 levels were noted between the change from baseline to 12 weeks of time-restricted eating and ad libitum. However, there was a decrease in the time-restricted eating group and increase in the ad libitum group, the potential clinical significance of which is unclear.

The general consensus in the literature for the management of dyslipidaemia associated with PCOS is lifestyle modification of diet and exercise as first line with the addition of statins if these measures prove ineffective [133]. Statins had several other proven benefits in PCOS including improvements in other parameters including testosterone and insulin resistance [14]. Dietary interventions for women with PCOS and dyslipidaemia should centre on a balanced diet with controlled energy intake, low glycaemic index, and enrichment with long chain polyunsaturated fatty acids such as Omega-3 [133]. Overall, a 12-week regimen of time-restricted eating was ineffective in bringing about a statistically significant improvement in lipid profiles including total cholesterol, HDLs, LDLs, non-HDLs as well as lipoprotein lipid A and apolipoproteins A1 and B in this small cohort of

women with PCOS. There was a statistically significant improvement in triglyceride levels seen from baseline to the end of the 12-week time-restricted eating in isolation. There was no significant difference when this change was compared with the ad libitum change over the 12 weeks. There was a reduction in triglycerides of -0.05mmol/L with time-restricted eating over time in the multilevel regression analysis also but this did not reach statistical significance. Small sample size most likely affected the ability to achieve a statistically significant change in the other aspects of the lipid profile (if a change existed). These findings do not correlate with published beneficial effect of time-restricted eating in dyslipidaemia in other populations [134, 135].

6.2.6 Time-restricted eating improved SHBG but did not affect other reproductive hormones

There was no significant differences in reproductive hormones in this study's small cohort, with the exception of SHBG. SHBG is a good reflection of insulin resistance and significantly improved in the participants in the time-restricted eating arm, when examined in isolation. There was also a statistically significant difference seen when the changes observed in SHBG between the ad libitum and time-restricted eating groups were compared. SHBG is a glycoprotein which attaches to circulating androgens and oestrogens, limiting their downstream effect on organs. These increases in SHBG seen in the time-restricted eating group have the potential to translate into a reduction in circulating androgens and subsequently clinical hyperandrogenism. Low SHBG levels seen in PCOS, are associated with insulin resistance and hyperandrogenism creating the classical phenotype seen in PCOS of hirsutism, acne as well as menstrual dysregulation.

It is feasible that time-restricted eating may offer benefit to those seeking fertility in that it can lead to a modest weight decrease and potential improvement in insulin resistance.

About one-third of women with PCOS have impaired glucose tolerance and one-tenth have type 2 diabetes, with the majority (60%) being overweight or obese [2]. Fertility issues are estimated to affect up to 80% of women with PCOS [136]. Pregnant women with PCOS have higher rates of pregnancy loss in the first trimester, higher rates of gestational diabetes, and subsequent macrosomia, increased risk of congenital anomalies, higher rates of pre term birth and neonatal intensive care unit admissions and higher rates of hypertensive disorders of pregnancy including pregnancy-induced hypertension and pre-eclampsia [137, 138]. These are thought to be potentially related to hyperandrogenism [136]. Given the higher risk of pregnancy complications there is a higher rate of Caesarean delivery in women with PCOS with its independent associated morbidities. With the close relationship of PCOS and obesity, there is an additional risk for further pregnancy complications and poorer maternal and neonatal outcomes [139-141].

Weight loss is associated with reduced incidence of complications during pregnancy and the neonatal period. Lifestyle change remains the first line intervention for weight loss prior to fertility treatments or pregnancy, particularly considering that medications to reduce weight can be associated with significant side effects and may be contraindicated in those actively trying to conceive [136]. Bariatric surgery is also associated with preterm and small for gestational age births as well as significant morbidity in pregnancy and the requirement to avoid pregnancy until up to two years following surgery [142].

A large spectrum of dietary interventions in PCOS have been investigated which are varied with many being unsustainable and ineffective [27-36]. While ‘lifestyle changes’ are considered the first line for management prior to pregnancy, there are minimal data on specific recommendations outside of regular eating with low glycaemic index foods and avoidance of refined sugars [4]. It is suggested that 5-10% weight loss in a six month period

can improve central obesity, hyperandrogenism and ovulation rate [136]. Calorie restriction and exercise can improve insulin sensitivity in obese women with PCOS but sustaining a calorie-restricted diet over time is challenging [38]. Time-restricted eating may potentially confer a more permanent lifestyle change and allow more sustainable and sustained weight-related and cardiometabolic benefits [38]. Time-restricted eating may deplete liver glycogen stores and induce a fuel switch to fatty acids and ketones, which can improve cellular performance and potentially overcome the insulin resistance seen in PCOS, similar to its effects in metabolic syndrome, obesity and type 2 diabetes [58-60]. Time-restricted eating offers a novel dietary solution which may be beneficial for women embarking on fertility treatment or prior to trying to conceive.

There is some evidence to suggest that time-restricted eating decreases testosterone while increasing SHBG levels in women with obesity and without altering oestrogen, gonadotropins or prolactin levels [143]. These results are reflective of the reduction in SHBG seen in the present study but without any significant change in androgens such as testosterone. There have been very few studies on this topic to comprehensively assess the effect of this dietary intervention on reproductive hormones, making it difficult to draw conclusions on its effect. It may be a valuable tool in women seeking fertility treatments to aid weight loss, particularly in women where ovulation induction is contraindicated due to a significantly raised BMI and risks associated with obesity and pregnancy. It may also be beneficial to treat hyperandrogenism, regulate menstruation and potentially aid ovulation [144]. More research is certainly necessary in this area. Overall, this study did not show statistically significant change in androgen results, although it was underpowered to do so.

The effect of time-restricted eating on oocyte quantity and quality has not been assessed. There is limited but mixed evidence in animal studies. One study showed in female mice

that time-restricted feeding impairs the developmental competence of oocytes after fertilisation [145]. This study attributed this finding to reduced serum cholesterol levels with the reduction of LDL, a vital component of the cellular membrane, causing cell dysfunction including ovarian and oocyte incompetence [145]. This reduction of LDL cholesterol was not observed in this study's analysis, as discussed above in section 6.1.5. They also discussed the increased intracellular reactive oxygen species level in oocytes causing a pro-inflammatory environment and decreasing oocyte developmental competence [145]. Other studies have found that time-restricted feeding ameliorates ovarian follicle loss, which is seen in obesity, by induction of liver fibroblast growth factor 21 (FGF21) secretion similar to other dietary interventions such as the ketogenic diet [146]. Increased circulating levels of FGF21 as seen in animal studies has an important role in the regulation of hypothalamic release of gonadotropin releasing hormone (GnRH) and subsequent action on the release of gonadotropins from the anterior pituitary (LH and FSH) which improves the reproductive function by stimulation of gametogenesis and sex hormone production in response [146, 147]. The full mechanism behind the effects of (animal) time-restricted feeding on oocyte health requires further investigation as well as research into its translation into human studies of time-restricted eating.

6.2.7 Those following time-restricted eating intervention ate fewer calories.

Patients in the time-restricted eating groups ate fewer calories, and less carbohydrate, saturated fat, and calcium than those who ate ad libitum. There was no statistically significant difference in protein and vitamin D intake between the time-restricted eating and ad libitum groups.

The recommended energy intake for a women in Ireland is 1,800-2,000 calories. Participants ate a mean of 1256.4 calories while intermittent fasting. There was most likely

an underreporting of caloric intake with both the mean intake in both the time-restricted eating and ad libitum groups well below the daily recommended amount for adult females. The hypocaloric dietary intake recorded by participants undergoing the time-restricted eating regimen, makes it difficult to ascertain whether putative benefits were derived from the time-restricted eating, or if these benefits were due to a low caloric and low carbohydrate intake. As previously mentioned, published studies that hail the therapeutic benefits of fasting regimens in various patient cohorts concurrently report weight loss, making it difficult to separate the effect of fasting and weight loss on cardiometabolic improvements [87-90]. As demonstrated in this study's findings, time-restricted eating results in caloric restriction with limited eating windows and resultant inevitable weight loss. However, time-restricted eating has benefits over hypocaloric diets in that it has the potential to be a more sustainable weight loss intervention. The long term outcomes of time-restricted eating in the literature is limited. If time-restricted eating results in a sustainable, long term reduction in energy intake due to its effect on appetite or satiety then this would be of clear value for a myriad of patient types. While 89% of participants in this study said that they would try time-restricted eating again or continue with the intervention after the study, there was no long term follow up incorporated into this study's design to assess its effectiveness over a longer term. One recent RCT studying the effect of time-restricted eating in adults at risk of type-2 diabetes found that at 18 month follow up there was no difference in weight or body composition between the three groups of time-restricted eating, calorie restriction and standard care (provision of weight loss booklet) [148]. In this specific RCT, the time-restricted eating regimen involved consumption of 30% of their energy requirements between 08:00 and 12:00 hours, followed by a 20 hour fasting period on three non-consecutive days per week and ad libitum eating on other days

and the calorie restriction group consumed 70% of their calorie requirements daily, without giving a time prescription [148].

Time-restricted eating did not have a significant effect on protein intake. The median protein intakes in both groups were above the recommended protein intake of 50g per day for an average adult female. Protein is an important macronutrient to ensure fullness and satiety which is fundamental in time-restricted eating in avoiding hunger and cravings in prolonged fasting windows.

As previously discussed, current dietary recommendations for patients with PCOS recommend calcium and vitamin D supplementation [4]. Both calcium and vitamin D supplementation have been suggested to be beneficial for improving insulin resistance, dyslipidaemia, cardiovascular complications, androgen profiles, menstrual regularity, and ovulation [149-151]. Of concern, there was a statistically significant difference in calcium intake between the groups, with those in the time-restricted eating group consuming significantly less calcium than the ad libitum group. In fact, both groups reported a markedly suboptimal calcium intake when compared to the recommended daily average for calcium (800mg/d) [152]. There was no difference in vitamin D consumption between the two groups. However, both the ad libitum and time-restricted eating groups dietary intake consumed much less vitamin D than the required amount, with participants consuming a median of 1.9ug and 1.4ug respectively with no participants consuming the recommended daily allowance of Vitamin D of 15ug [119]. Vitamin D plays an important role in PCOS and is recommended as a dietary supplement for women with the condition. Vitamin D deficiency has been linked with metabolic and hormonal disorders in women with PCOS. They are more likely to have dysglycaemia measured by HOMA-IR than those without vitamin D deficiency [153-155]. Dietary quality is imperative whether patients

follow a time-restricted eating pattern or consume food ad libitum, however, investigating whether time-restricted eating results in a lower consumption of critical micronutrients should be an integral component of a larger study.

6.3 Limitations and Strengths

These studies had several important limitations and strengths.

1) Whilst n=9 patients is an acceptable number for a feasibility study, it is insufficient for the analysis of clinical data and cannot provide meaningful conclusions. Nevertheless, analysis of the clinical data provides crucial direction for the development and design of a larger study.

2) The strict inclusion and exclusion criteria were both a limitation and strength. The majority of women with a diagnosis of PCOS are counselled on the lifestyle and dietary interventions as first line management of their condition. In addition, the vast majority attending an endocrinology clinic will be on at least one insulin sensitising agent to improve metabolic parameters with many also, more recently, on weight loss medications such as GLP-1 agonists. Patients included were not on medications that may have altered insulin sensitivity in order to achieve reliable results from the study sample without confounding factors and assess the effect of time-restricted eating alone on insulin resistance. This study was very carefully designed with careful consideration of the confounding factors, and therefore this aim for a very clean sample of participants may have been at the expense of concluding the study with small numbers.

3) This study assessed one specific time-restricted eating regimen of an 18 hour fasting window. While there are many other regimens, it was postulated that this regimen with a

prolonged daily fasting window would be most beneficial in PCOS to overcome the marked insulin resistance seen in this population. In addition to this, participants' self-reporting of compliance rates were relied upon which may have introduced a margin of error to the results. However, a time-restricted eating regimen with a daily fast between 14-18 hours is the most popular and widely used in the literature [156, 157]. Using this regimen was possibly the best balance between being acceptable and effective in testing feasibility in this patient cohort.

4) Participants in this study were not given any dietary advice outside of the time-restriction aspect. Participants were not given any specific instructions on what to eat in the 6 hour eating window. Ideally, an isocaloric diet with set calorie meals would allow assessment of a time-restricted eating regimen alone on the various metabolic parameters and avoid the confounding factor of hypocaloric intake as demonstrated in this study. On the other hand, imposing a measured 'metabolic kitchen' style regimen would remove the pragmatic, real-world aspect of the study. Arguably, this is one of the study's strengths. In clinical practice, as in this study, patients are advised to follow a particular eating plan or regimen, and must therefore incorporate it into their lives. In addition, by allowing patients to set their own eating patterns, a better understanding is gained on how this intervention affects appetite, behaviour, dietary intake and adverse events.

5) The study was designed as a randomised cross-over study. Cross-over studies are useful in that they can be conducted with fewer study participants, they avoid any difference in baseline participant characteristics, and removes potential confounding factors introduced by participants, as they act as their own study control. While the study design likely affected recruitment due to the study length being 28 weeks in total, the study results were

significant for multiple parameters, particularly anthropometrics, despite small numbers. Crossover studies have the obvious disadvantage of being significantly longer than other interventional studies. They are frequently used in dietary intervention studies [88, 158-160].

6.4 A roadmap for a substantive future study to determine the effectiveness of time-restricted eating in patients with PCOS

The results of this thesis support the design and development of a large, substantive study to investigate the effect of time-restricted eating on metabolic parameters in women with PCOS. The feasibility study has provided invaluable insight into how such a study should be designed. Based broadly on the PICO model, below are recommendations arising from the experiences of designing and running this feasibility study and the wider findings of the thesis:

6.4.1 Patients / Population

Future studies with a larger sample size could consider including patients on other medications including metformin, oral contraceptive pills and weight loss medications such as GLP-1 agonists in combination and potentially conduct a sub-group analysis for these groups to assess if time-restricted eating is a useful dietary intervention in combination with the medical management and if it has an additional effect in improving insulin resistance. PCOS frequently co-exists with insulin resistance and obesity which are complex conditions, most likely requiring a multimodal approach to management.

To guide future larger studies and RCTs, a sample size calculation was carried out based on reported mean serum insulin levels in obese women with PCOS (22.98 ± 18.44) [161]. There would be an expectation of a decrease in serum insulin of 40% with time-restricted

eating as seen in other dietary intervention studies in PCOS aiming to improve insulin resistance [31]. The majority of medical literature uses a beta cut-off of 20% (type II error rate of 0.2) indicating a 20% chance that a significant difference is missed and an alpha cut-off of 5% (type I error rate of 0.005) indicating a 5% chance of a significant difference is random rather than a true difference [162]. These figures would give a required sample size of $n=64$, with $n=32$ in each phase. In order, to allow for attrition (of around 30%) and subgroup analysis, a sample size of $n=100$ with $n=50$ in each phase, would be recommended for an RCT design. Recruitment success in a future larger study would be measured on the ability to reach the desired sample size based from this calculation.

We recruited from secondary care where patients are often established on guideline based medical therapies or attending secondary care for specialised, time-sensitive management such as fertility treatment. This undoubtedly affected the large number of patients excluded. Recruitment from primary care may be beneficial in finding women with PCOS who are newly diagnosed or not established on therapy. Expanding the trial to multiple centres may also help in boosting recruitment numbers. Multiple means of contacting patients were utilised to screen for eligibility and recruit participants including email, telephone and face-to-face clinic appointments. Improvement in study marketing with posters, email reminders or alternative advertisements may have also helped to increase recruitment numbers.

6.4.2 Intervention

As previously discussed, only one specific time-restricted eating regimen, of a daily 18-hour fasting window, was assessed in this trial. This type of prolonged daily fast has the benefit of aiming to overcome the marked insulin resistance seen in the PCOS population and is most popular and widely used in the literature [156, 157]. While 89% of participants said they would consider continuing time-restricted eating in the future, the question of

sustainability of this fasting regimen remains unanswered. Potentially, an alternate day fasting (as described in Table 1.1) may be more amenable to patient's lifestyle or varying routines by allowing alternative days of *ad libitum* eating which could allow later dining, snacking or evening alcohol intake which may be more acceptable, sustainable and allow variations across seasons. Within the context of an 18:6 regimen (a six hour eating window), patients could be counselled that they may change their window on a day-to-day basis (dependant on their requirements) and that they do not need to commit to one 'eating window' for the duration of the study. This may help with compliance and may make the intervention more practical and conducive to normal living.

6.4.3 Control / study design / analysis

The crossover design had pros and cons. It was on one hand an attraction to participants in that all participants had the opportunity to try out the time-restricted eating regimen and also to assess how they felt while doing both time-restricted eating and *ad libitum* diet in a study setting. This is more attractive than a randomised controlled trial for patients as they may be randomised to a control group without the opportunity to try out the intervention of interest. This is balanced against the obvious disadvantage of the crossover trial in that it doubles the time commitment to the study and subsequently potentially led to an increased dropout rate as participants were unable to dedicate 28 weeks to the study and had the potential to lose momentum. A carefully designed randomised controlled trial design would be the most beneficial design in conclusively assessing the intervention's benefit in PCOS. The randomised controlled trial study design described in the currently recruiting trial protocol NCT05629858 has three study arms: 6-hour time-restricted eating, calorie restriction (25% energy restriction daily), or control group (*ad libitum* intake with no meal timing restriction) in PCOS.

The data was assessed with a broad range of statistics which will be a beneficial basis and reference for any larger studies. The progression from simple comparative statistics to the multi-level regression analysis allowed assessment of the feasibility of more complex statistics on this data, despite the smaller numbers. Missing data certainly affected the results with small participant numbers. Great care should be taken when processing insulin samples in particular including avoiding butterfly needles, warming venepuncture site to increase blood flow, adequate bottle filling and immediate transfer to the laboratory to be centrifuged and processed.

The intervention length was appropriate. While other studies discussed in the systematic review in Chapter 3 used shorter study regimen length, the 12-week regimen was important to assess the sustainability of time-restricted eating and allow adaption to the lifestyle change. While this was balanced against a longer overall study length, assessment of sustainability of the intervention is vital, particular in the setting of a feasibility study. Assessment at baseline, 6 weeks and 12 weeks was also appropriate. The 6 week interim study visit allowed discussion of progress with time-restricted eating and adverse events which was important to assess in a setting with minimal data in the literature in the PCOS population. The interim visit increased the power of the results and allowed additional data points in order to be able to trial multi-level regression analysis statistics.

6.4.4 Outcomes

This study assessed a significant number of outcomes as part of the feasibility assessment of time-restricted eating in PCOS. The outcomes assessed were valuable in the overall assessment and if conducting a similar study or progressing to a larger scale study, should be included. Other studies on time-restricted eating included markers of inflammation,

stress response, continuous glucose monitoring, sleep quality and quality of life assessments which may also be beneficial to consider in the study design.

This feasibility study showed the safety of this intervention in women with PCOS. While a larger study sample would be required to assess for rarer occurring events, in this small cohort, time-restricted eating in PCOS was safe. While the initial planned sample size aim was optimistic for this study, with a larger research time and incorporation of the suggestions mentioned above for recruitment, a sample size of n=50 in each group of an RCT design, may be achievable.

6.4.5 Other considerations

The financial implication of the study included a subscription to the Nutritics database, appropriate stationary, printing of study documents, equipment for measuring anthropometric data, blood samples equipment, storage of blood samples in -80 degree Celsius freezer, processing of blood samples by the hospital laboratory and transfer to external laboratories as well as ordering of specific assays for processing samples.

I conducted all the research visits and data collection. Ideally, this study would have a dedicated phlebotomist for blood draw, and portering services to the laboratory. A recruitment team including doctors and a research nurse would ideally be required to screen patients charts, conduct phone calls and assess eligibility. The addition of a research dietitian would be of great benefit in supporting the nutritional analysis and patient education regarding this. These would particularly be necessary with large study numbers. Despite small study numbers, approximately 150 hours of face-to-face time was spent with patients for research visits and approximately 100 hours on telephone calls and emails

screening and contacting potential participants. Undoubtedly, with larger numbers – a large time commitment and availability would be required.

While the idea of virtual appointments using Microsoft teams or Zoom was explored, participants were required to physically attend to allow blood sample collection and accurate anthropometric data collection. Other technologies used in the study were google forms for appetite questionnaire and food diary logging using the Libro app available on handheld smartphone devices.

6.5 Conclusions

In conclusion, time-restricted eating represents a novel solution to aid in the control of insulin resistance in patients with PCOS. While caution should be taken when interpreting these results and drawing clinical conclusions, this feasibility study will aid in the design of a larger, well-designed randomised controlled trial, to investigate the hypothesised benefits of this novel intervention.

Appendices and References

Appendix 1: Search Strategies

EMBASE (1909 Results)

1. 'ovary polycystic disease'/exp
2. ('stein leventhal syndrome' OR 'cystic ovar*' OR 'micropolycystic ovar*' OR 'multiple follicle cyst*' OR 'ovar* polycystic disease' OR 'ovar* polycystic syndrome' OR 'polycystic ovarian disease' OR 'polycystic ovar*' OR 'polycystic ovar* disease' OR 'polycystic ovar* syndrome' OR 'stein cohen leventhal syndrome' OR 'stein leventhal disease' OR 'syndrome stein leventhal'):ti,ab
3. #1 OR #2
4. 'insulin'/exp OR 'hyperinsulinism'/exp OR 'insulin response'/exp
5. insulin:ti,ab
6. ('hyperinsulinism' OR 'hyperinsulism' OR 'hyperinsulinaemia' OR 'hyperinsulinema' OR 'hyperinsulinemia' OR 'insulinaemia' OR 'insulinemia' OR 'insulin hypoglycaemia' OR 'insulin hypoglycemia'):ti,ab
7. #4 OR #5 OR #6
8. 'fasting'/exp
9. (Time NEAR/2 restrict* NEAR/2 (Eating OR diet* OR feeding OR fast*)):ti,ab
10. (('Feeding time*' OR diet OR food OR fast*) NEAR/3 restrict*):ti,ab
11. (Fasting OR 'whole day fast*' OR 'food tim*'):ti,ab
12. (fast* NEAR/3 diet*):ti,ab
13. ((intermittent* OR alternat* OR modified) NEAR/3 fast*):ti,ab
14. (food NEAR/1 (abstinence or fast*)):ti,ab
15. (Ramadan or Ramadhan):ti,ab
16. ('16 8 method' OR '5 2 diet'):ti,ab
17. #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16
18. #3 AND #7 AND #17
19. 'conference abstract':it OR 'conference report':it OR letter:it OR editorial:it
20. #18 NOT #19

Medline (1737)

1. exp Polycystic Ovary Syndrome/
2. (stein leventhal syndrome OR cystic ovar* OR micropolycystic ovar* OR multiple follicle cyst* OR ovar* polycystic disease OR ovar* polycystic syndrome OR polycystic ovarian disease OR polycystic ovar* OR polycystic ovar* disease OR polycystic ovar* syndrome OR stein cohen leventhal syndrome OR stein leventhal disease OR syndrome stein leventhal).ti,ab.
3. or/1-2
4. exp Insulin/ OR exp Hyperinsulinism/
5. (insulin OR hyperinsulinism OR hyperinsulism OR hyperinsulinaemia OR hyperinsulinema OR hyperinsulinemia OR insulinaemia OR insulinemia OR insulin hypoglycaemia OR insulin hypoglycemia).ti,ab.
6. or/4-5
7. fasting/
8. (Time adj2 restrict* adj2 (Eating OR diet* OR feeding OR fast*)):ti,ab.
9. ((Feeding time* OR diet OR food OR fast*) adj3 restrict*).ti,ab.
10. (Fasting OR whole day fast* OR food tim*).ti,ab.
11. (fast* adj3 diet*).ti,ab.
12. ((intermittent* OR alternat* OR modified) adj3 fast*).ti,ab.
13. (food adj1 (abstinence or fast*)):ti,ab.
14. (Ramadan or Ramadhan).ti,ab.
15. (16 8 method OR 5 2 diet).ti,ab.
16. or/7-15
17. 3 AND 6 AND 16

CINAHL (339)

1. (MH "Polycystic Ovary Syndrome")
2. TI ("stein leventhal syndrome" OR "cystic ovar*" OR "micropolycystic ovar*" OR "multiple follicle cyst*" OR "ovar* polycystic disease" OR "ovar* polycystic syndrome" OR "polycystic ovarian disease" OR "polycystic ovar*" OR "polycystic ovar* disease" OR "polycystic ovar* syndrome" OR "stein cohen leventhal syndrome" OR "stein leventhal disease" OR "syndrome stein leventhal") OR AB ("stein leventhal syndrome" OR "cystic ovar*" OR "micropolycystic ovar*" OR "multiple follicle cyst*" OR "ovar* polycystic disease" OR "ovar* polycystic syndrome" OR "polycystic ovarian disease" OR "polycystic ovar*" OR "polycystic ovar* disease" OR "polycystic ovar* syndrome" OR "stein cohen leventhal syndrome" OR "stein leventhal disease" OR "syndrome stein leventhal")
3. S1 OR S2
4. (MH "Insulin+") OR (MH "Insulin Sensitivity") OR (MH "Hyperinsulinism+")
5. TI (insulin OR "hyperinsulinism" OR "hyperinsulism" OR "hyperinsulinaemia" OR "hyperinsulinema" OR "hyperinsulinemia" OR "insulinaemia" OR "insulinemia" OR "insulin hypoglycaemia" OR "insulin hypoglycemia") OR AB (insulin OR "hyperinsulinism" OR "hyperinsulism" OR "hyperinsulinaemia" OR "hyperinsulinema" OR "hyperinsulinemia" OR "insulinaemia" OR "insulinemia" OR "insulin hypoglycaemia" OR "insulin hypoglycemia")
6. S4 OR S5
7. (MH "Fasting")
8. TI (Time N2 restrict* N2 (Eating OR diet* OR feeding OR fast*)) OR AB (Time N2 restrict* N2 (Eating OR diet* OR feeding OR fast*))
9. TI (("Feeding time*" OR diet OR food OR fast*) N3 restrict*) OR AB (("Feeding time*" OR diet OR food OR fast*) N3 restrict*)
10. TI (Fasting OR "whole day fast*" OR "food tim*") OR AB (Fasting OR "whole day fast*" OR "food tim*")
11. TI (fast* N3 diet*) OR AB (fast* N3 diet*)
12. TI ((intermittent* OR alternat* OR modified) N3 fast*) OR AB ((intermittent* OR alternat* OR modified) N3 fast*)
13. TI (food N1 (abstinence or fast*)) OR AB (food N1 (abstinence or fast*))
14. TI (Ramadan or Ramadhan) OR AB (Ramadan or Ramadhan)
15. TI ("16 8 method" OR "5 2 diet") OR AB ("16 8 method" OR "5 2 diet")
16. S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15
17. S3 AND S6 AND S16

Cochrane Library (268)

1. (mh "Polycystic Ovary Syndrome")
2. ("stein leventhal syndrome" OR "cystic ovar*" OR "micropolycystic ovar*" OR "multiple follicle cyst*" OR "ovar* polycystic disease" OR "ovar* polycystic syndrome" OR "polycystic ovarian disease" OR "polycystic ovar*" OR "polycystic ovar* disease" OR "polycystic ovar* syndrome" OR "stein cohen leventhal syndrome" OR "stein leventhal disease" OR "syndrome stein leventhal"):ti,ab,kw
3. #1 OR #2
4. (mh "Insulin") OR (mh "Hyperinsulinism")
5. (Insulin OR hyperinsulinism OR hyperinsulism OR hyperinsulinaemia OR hyperinsulinema OR hyperinsulinemia OR insulinaemia OR insulinemia OR "insulin hypoglycaemia" OR "insulin hypoglycemia"):ti,ab,kw
6. #4 OR #5
7. (mh "fasting")
8. (Time NEAR/2 restrict* NEAR/2 (Eating OR diet* OR feeding OR fast*)):ti,ab,kw
9. (("Feeding time*" OR diet OR food OR fast*) NEAR/3 restrict*):ti,ab,kw
10. (Fasting OR "whole day fast*" OR "food tim*"):ti,ab,kw
11. (fast* NEAR/3 diet*):ti,ab,kw
12. ((intermittent* OR alternat* OR modified) NEAR/3 fast*):ti,ab,kw
13. (food NEAR/1 (abstinence or fast*)):ti,ab,kw
14. (Ramadan or Ramadhan):ti,ab,kw

15. ("16 8 method" OR "5 2 diet"):ti,ab,kw
16. #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15
17. #3 AND #6 AND #16

Web of Science (2086)

1. TS =("ovary polycystic disease" OR "stein leventhal syndrome" OR "cystic ovar*" OR "micropolycystic ovar*" OR "multiple follicle cyst*" OR "ovar* polycystic disease" OR "ovar* polycystic syndrome" OR "polycystic ovar*" OR "polycystic ovar* disease" OR "polycystic ovar* syndrome" OR "stein cohen leventhal syndrome" OR "stein leventhal disease" OR "syndrome stein leventhal")
2. TS =(Insulin OR "hyperinsulinism" OR "hyperinsulism" OR "hyperinsulinaemia" OR "hyperinsulinema" OR "hyperinsulinemia" OR "insulinaemia" OR "insulinemia" OR "insulin hypoglycaemia" OR "insulin hypoglycemia")
3. TS =((Time NEAR/1 restrict* NEAR/1 (Eating OR diet* OR feeding OR fast*)) OR (("Feeding time*" OR diet OR food OR fast*) NEAR/3 restrict*) OR Fasting OR "whole day fast*" OR "food tim*" OR (fast* NEAR/3 diet*) OR ((intermittent* OR alternat* OR modified) NEAR/3 fast*) OR (food NEAR/1 (abstinence or fast*)) OR Ramadan or Ramadhan OR "16 8 method" OR "5 2 diet")
4. #1 AND #2 AND #3

Appendix 2: Excluded Trials

Study ID number (Covidence) and Author	Reason for exclusion
1. #2560 Agowska 2021	Wrong intervention
2. #169 Altieri 2013	Wrong study design
3. #2263 Anderson 1995	Wrong intervention
4. #582 Armutcu 2019	Wrong study design
5. #2348 Asemi 2014	Wrong intervention
6. #419 Asemi 2015	Non randomised and without control group
7. #2213 Chiofalo 2017	Wrong study design
8. #1889 El-Bandrawy 2016	Wrong intervention
9. #2019 Farshchi 2007	Wrong study design
10. #2085 Fransk 1991	Wrong study design
11. #2652 Frary 2016	Wrong study design
12. #2097 Hartmann 2019	Wrong study design
13. #1369 Jyotsna 2018	Wrong study design
14. #651 Kiddy 1992	Wrong intervention
15. #2634 Kite 2019	Wrong study design
16. #2132 Lass 2011	Wrong intervention
17. #2181 Li 2021	Non randomised and without control group
18. #113 Marsh 2015	Wrong study design
19. #1920 Micić 2003	Wrong intervention
20. #2573 Moran 2006	Wrong study design
21. #1230 Moran 2017	Wrong study design
22. #432 Moran 2011	Wrong study design
23. #2345 Moran 2019	Wrong study design
24. #321 Moran 2006	Wrong intervention
25. #1066 NCT 2019	Study ongoing
26. #1065 NCT 2018	Study ongoing
27. #708 Papakonstantinou 2016	Wrong intervention
28. #742 Pasquali 2011	Wrong study design
29. #1284 Pundir 2019	Wrong study design
30. #1788 Shang 2020	Wrong study design
31. #1331 Song 2020	Wrong intervention
32. #943 Stamets 2004	Wrong intervention
33. #2543 vanDammen 2018	Wrong intervention
34. #462 Varady 2016	Wrong study design
35. #146 Wang 2016	Wrong intervention
36. #2489 Wong 2016	Wrong intervention

Appendix 3: Appetite Questionnaire

The Effect of Time-restricted Eating on Insulin Levels and Other Metabolic Abnormalities in Polycystic Ovarian Syndrome: A Randomised Feasibility Study of Real-world Clinical Advice - APPETITE ASSESSMENT SCALE

This is a questionnaire regarding your appetite with visual analog scales about hunger, satiety, prospective food consumption, cravings and nausea in general recently. You should fill it out to the best of your ability. It should only take you about 1 minute and has 9 questions.

It should be filled in on the morning of having your bloods taken and should be answered with the last 3 days in mind rather than just your feelings at the time of completing.

pcosresearch22@gmail.com [Switch accounts](#)

*Required

Email *

Your email address

1. How would you rate how hungry you are?

1 2 3 4 5 6 7 8 9 10

I am not hungry at all ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Extremely hungry

2. How would you rate how full you feel?

1 2 3 4 5 6 7 8 9 10

I do not feel full or satisfied at all ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ I feel full and satisfied

3. How much food do you think you could eat right now?

1 2 3 4 5 6 7 8 9 10

Nothing at all ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ A large amount

4. I have a desire to eat something fatty

1 2 3 4 5 6 7 8 9 10

No craving ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Very strong craving

5. I have a desire to eat something salty

1 2 3 4 5 6 7 8 9 10

No craving ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Very strong craving

6. I have a desire to eat something sweet

1 2 3 4 5 6 7 8 9 10

No craving ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Very strong craving

7. I have a desire to eat something savoury

1 2 3 4 5 6 7 8 9 10

No craving ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Very strong craving

8. Do you usually find your food/meals tasty and enjoyable when you eat?

1 2 3 4 5 6 7 8 9 10

Not tasty or not enjoyable at all ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Very tasty

9. How nauseous have you felt overall?

1 2 3 4 5 6 7 8 9 10

No nausea ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Extremely nauseous

Appendix 4: NCT05126199 Protocol

ClinicalTrials.gov PRS
Protocol Registration and Results System

ClinicalTrials.gov Protocol Registration and Results System (PRS) Receipt
Release Date: September 15, 2022

ClinicalTrials.gov ID: NCT05126199

Study Identification

Unique Protocol ID: TimeMAP

Brief Title: Time-restricted Eating to Improve Metabolic Abnormalities in Polycystic Ovarian Syndrome (TimeMAP)

Official Title: The Effect of Time-restricted Eating on Insulin Levels and Other Metabolic Abnormalities in Polycystic Ovarian Syndrome: A Randomised Feasibility Study of Real-world Clinical Advice

Secondary IDs:

Study Status

Record Verification: September 2022

Overall Status: Recruiting

Study Start: May 5, 2021 [Actual]

Primary Completion: December 31, 2022 [Anticipated]

Study Completion: January 31, 2023 [Anticipated]

Sponsor/Collaborators

Sponsor: Ruairi Floyd

Responsible Party: Sponsor-Investigator

Investigator: Ruairi Floyd [rfloyd]

Official Title: Study Co-ordinator and Researcher

Affiliation: Tallaght University Hospital

Collaborators: Tallaght University Hospital

Oversight

U.S. FDA-regulated Drug: No

U.S. FDA-regulated Device: No

U.S. FDA IND/IDE: No

Human Subjects Review: Board Status: Approved

Approval Number: Project ID: 0482

Board Name: St. James' Hospital (SJH) / Tallaght University Hospital (TUH)

Joint Research Ethics Committee (REC)

Board Affiliation: Tallaght University Hospital/St. James's Hospital

Phone:

Email: ResearchEthics@tuh.ie

Address:

TUH/SJH Joint Research Ethics Committee
Tallaght University Hospital
Dublin
Ireland

Data Monitoring: No

FDA Regulated Intervention: No

Study Description

Brief Summary: Polycystic ovarian syndrome (PCOS) is associated with metabolic symptoms such as hyperinsulinemia. Time-restricted eating may reduce serum insulin and improve insulin resistance in patients with PCOS. Currently, there are few studies investigating time-restricted eating in patients with PCOS. The investigators plan to test the feasibility of time-restricted eating in the management of PCOS by means of a real-world clinical intervention. The investigators will determine if an 18:6 eating protocol reduces insulin levels by means of a randomised controlled crossover trial.

Detailed Description: Background: Polycystic ovarian syndrome (PCOS) is the most common reproductive endocrinopathy in women of reproductive age with many associated metabolic symptoms, in particular hyperinsulinemia, insulin resistance and a high lifetime risk of type 2 diabetes mellitus. The effects of time-restricted eating on metabolic profiles have been investigated in many endocrinopathies, but there are minimal data in PCOS.

Methods: This study will investigate the feasibility of time-restricted eating in the management of PCOS, and its effects on insulin levels and other metabolic parameters.

To achieve this, the investigators will recruit 20 patients with PCOS (normal weight, overweight, obese).

In a randomised cross-over design, participants will be observed for two consecutive 12 week periods (with a 4 weeks washout period in between) following either 'time-restricted eating' or 'usual eating', detailed below.

1. 18:6 protocol: 18 hours of fasting and a 6-hours eating window, with no other specific dietary advice. Participants choose their own 6-hour period according to their lifestyle and preference.
2. Usual eating: follow usual eating patterns, no time restriction, no other dietary advice

When fasting, participants are permitted to consume plain water, unflavoured/unsweetened sparkling water, black breakfast tea and black coffee.

Dietary intake will be determined at baseline, at midpoint of each study arm, and at the end of the study using Nutritics software. Participants will self-record dietary intake using the Nutritics 'app'.

The primary endpoints will be serum insulin and feasibility of the intervention as well as safety, acceptability, and compliance with time-restricted eating.

Secondary endpoints will be insulin resistance (Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)), androgens (testosterone, free testosterone, dehydroepiandrosterone sulfate (DHEA-S), androstenedione, 17-Hydroxyprogesterone (17-OHP) and sex hormone binding globulin (SHBG)), appetite (10-point visual analogue scale), hunger/satiety (glucagon-like peptide 1 (GLP-1), ghrelin, PYY and oxyntomodulin, fasting glucose, HbA1c, lipid profile, lipoprotein lipid A, apolipoprotein A1, apolipoprotein B, anthropometrics

(weight, body mass index, hip and waist circumference), dietary intake (calorie and macronutrient intake; micronutrient intake including iron, calcium; dietary pattern including timing).

Results: Safety and acceptability will be measured by adverse event reporting and measurement of adherence. Paired t-test will be used to assess between baseline and post intervention measurements. Results considered statistically significant if $p < 0.05$.

Discussion: Time-restricted eating has potential to aid in improvement of insulin resistance in patients with PCOS based on studies in other populations. There is no substantial literature on this subject to date in the PCOS patient cohort, with this being the first randomised study to date. The investigators will discuss the effects of time-restricted eating on insulin levels in the specific population of women with PCOS based on the results.

Conditions

Conditions: PCOS
Hyperinsulinemia
Insulin Resistance

Keywords: PCOS
Intermittent Fasting
Fasting
Hyperinsulinemia
Insulin Resistance

Study Design

Study Type: Interventional
Primary Purpose: Treatment
Study Phase: N/A
Interventional Study Model: Crossover Assignment
Number of Arms: 2
Masking: Single (Outcomes Assessor)
Allocation: Randomized
Enrollment: 20 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Time restricted eating Time-restricted eating using 18:6 protocol (12 weeks) Washout Period (4 weeks) Crossover to Normal ad libitum diet (12 weeks)	Time restricted eating Following a 3 day baseline dietary assessment using the Nutritics 'app', patients will immediately commence time-restricted eating on a 18:6 basis (18 hours fasting, 6 hours eating window) for 12 weeks. Participants will consume all their meals within a daily 6-hour period of their choosing, and this may change according to patient's lifestyle and preference to reflect a real-world situation. Participants may eat ad libitum / according to appetite during the eating period. Participants will fast for 18 hours per day, consuming only plain water, unflavoured/unsweetened sparkling

Arms	Assigned Interventions
	water, black breakfast tea or black coffee. Alcohol must not be consumed during fasting periods Dietary intake will again be measured using the Nutritics 'app' midpoint through the 12-week period (week 6 +/- 1 week) and in the last week of the intervention (week 11/12).
Active Comparator: Normal ad libitum diet Normal ad libitum dietary patterns without defined eating window, fasting or restrictions on types of food or drink consumed (12 weeks) Washout Period (4 weeks) Crossover to time-restricted eating using 18:6 protocol (12 weeks)	Normal ad libitum diet Following a 3-day baseline dietary assessment using the Nutritics 'app', participants will be directed to continue with their usual dietary intake without any time-related restrictions for 12 weeks. There will be no defined eating window and fasting or restrictions regarding types of food or drink consumed. Dietary intake will again be measured using the Nutritics 'app' midpoint through the 12-week period (week 6 +/- 1 week) and in the last week of the intervention (week 11/12).

Outcome Measures

Primary Outcome Measure:

- Drop-out rate
Assessing intervention feasibility
[Time Frame: 6 weeks]
- Drop-out rate
Assessing intervention feasibility
[Time Frame: 12 weeks]
- Adverse outcomes as assessed by CTCAE v4.0
Assessing intervention feasibility
[Time Frame: 6 weeks]
- Adverse outcomes as assessed by CTCAE v4.0
Assessing intervention feasibility
[Time Frame: 12 weeks]
- Change in serum insulin
Measured with serum insulin levels to assess effects
[Time Frame: 6 weeks]
- Change in serum insulin
Measured with serum insulin levels to assess effects
[Time Frame: 12 weeks]
- Change in food diaries
Assessment of change of eating behaviours
[Time Frame: 6 weeks]
- Change in food diaries
Assessment of change of eating behaviours
[Time Frame: 12 weeks]

Secondary Outcome Measure:

- Change in insulin resistance

- Assessed by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and other ratio calculations measuring insulin resistance
- [Time Frame: 6 weeks]
10. Change in insulin resistance
Assessed by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and other ratio calculations measuring insulin resistance
 - [Time Frame: 12 weeks]
 11. Change in testosterone levels
Assessed by plasma testosterone
 - [Time Frame: 6 weeks]
 12. Change in testosterone levels
Assessed by plasma testosterone
 - [Time Frame: 12 weeks]
 13. Change in free testosterone levels
Assessed by plasma free testosterone
 - [Time Frame: 6 weeks]
 14. Change in free testosterone levels
Assessed by plasma free testosterone
 - [Time Frame: 12 weeks]
 15. Change in dehydroepiandrosterone sulfate (DHEA-S) levels
Assessed by plasma dehydroepiandrosterone sulfate (DHEA-S)
 - [Time Frame: 6 weeks]
 16. Change in dehydroepiandrosterone sulfate (DHEA-S) levels
Assessed by plasma dehydroepiandrosterone sulfate (DHEA-S)
 - [Time Frame: 12 weeks]
 17. Change in androstenedione levels
Assessed by plasma androstenedione
 - [Time Frame: 6 weeks]
 18. Change in androstenedione levels
Assessed by plasma androstenedione
 - [Time Frame: 12 weeks]
 19. Change in sex hormone binding globulin (SHBG) levels
Assessed by plasma sex hormone binding globulin (SHBG)
 - [Time Frame: 6 weeks]
 20. Change in sex hormone binding globulin (SHBG) levels
Assessed by plasma sex hormone binding globulin (SHBG))
 - [Time Frame: 12 weeks]
 21. Change in 17-Hydroxyprogesterone (17-OHP) levels
Assessed by 17-Hydroxyprogesterone (17-OHP)
 - [Time Frame: 6 weeks]
 22. Change in 17-Hydroxyprogesterone (17-OHP) levels
Assessed by 17-Hydroxyprogesterone (17-OHP)
 - [Time Frame: 12 weeks]
 23. Change in appetite

Measured by a validated 10-point visual analogue scale on a scale of 1-10, 1 being not hungry at all and 10 being very hungry

[Time Frame: 6 weeks]

24. Change in appetite

Measured by a validated 10-point visual analogue scale on a scale of 1-10, 1 being not hungry at all and 10 being very hungry

[Time Frame: 12 weeks]

25. Change in markers of satiety

Assess by plasma GLP-1

[Time Frame: 6 weeks]

26. Change in markers of satiety

Assess by plasma GLP-1

[Time Frame: 12 weeks]

27. Change in markers of satiety

Assess by plasma PYY

[Time Frame: 6 weeks]

28. Change in markers of satiety

Assess by plasma PYY

[Time Frame: 12 weeks]

29. Change in markers of satiety

Assess by plasma oxyntomodulin

[Time Frame: 6 weeks]

30. Change in markers of satiety

Assess by plasma oxyntomodulin

[Time Frame: 12 weeks]

31. Change in markers of hunger

Assess by plasma ghrelin

[Time Frame: 6 weeks]

32. Change in markers of hunger

Assess by plasma ghrelin

[Time Frame: 12 weeks]

33. Change in fasting glucose

Assessed in serum glucose measurements

[Time Frame: 6 weeks]

34. Change in fasting glucose

Assessed in serum glucose measurements

[Time Frame: 12 weeks]

35. Change in HbA1c

Assessed in serum HbA1c measurements

[Time Frame: 6 weeks]

36. Change in HbA1c

Assessed in serum HbA1c measurements

[Time Frame: 12 weeks]

37. Change in lipids

Assessed by Lipid profile

- [Time Frame: 6 weeks]
38. Change in lipids
Assessed by Lipid profile
[Time Frame: 12 weeks]
39. Change in lipids
Assessed by Lipoprotein lipid A levels
[Time Frame: 6 weeks]
40. Change in lipids
Assessed by Lipoprotein lipid A levels
[Time Frame: 12 weeks]
41. Change in lipids
Assessed by Apolipoprotein A1 levels
[Time Frame: 6 weeks]
42. Change in lipids
Assessed by Apolipoprotein A1 levels
[Time Frame: 12 weeks]
43. Change in lipids
Assessed by Apolipoprotein B levels
[Time Frame: 6 weeks]
44. Change in lipids
Assessed by Apolipoprotein B levels
[Time Frame: 12 weeks]
45. Change in body weight
Body weight (kg)
[Time Frame: 6 weeks]
46. Change in body weight
Body weight (kg)
[Time Frame: 12 weeks]
47. Change in body mass index
BMI (kg/m²)
[Time Frame: 6 weeks]
48. Change in body mass index
BMI (kg/m²)
[Time Frame: 12 weeks]
49. Change in anthropometric measurements (waist circumference)
Waist circumference (cm)
[Time Frame: 6 weeks]
50. Change in anthropometric measurements (waist circumference)
Waist circumference (cm)
[Time Frame: 12 weeks]
51. Change in anthropometric measurements (waist-hip ratio)
Waist-hip ratio
[Time Frame: 6 weeks]
52. Change in anthropometric measurements (waist-hip ratio)

Waist-hip ratio

[Time Frame: 12 weeks]

53. Change in dietary intake

Assessed using interval dietary assessments with Nutritics 'app'

[Time Frame: 6 weeks]

54. Change in dietary intake

Assessed using interval dietary assessments with Nutritics 'app'

[Time Frame: 12 weeks]

Eligibility

Minimum Age: 18 Years

Maximum Age: 42 Years

Sex: Female

Gender Based:

Accepts Healthy Volunteers: Yes

Criteria: Inclusion Criteria:

- Adult women of reproductive age with confirmed diagnosis of PCOS (Rotterdam Criteria, including at least 2 of 3 characteristics: oligomenorrhea, clinical and/or biochemical hyperandrogenism and ultrasound criteria)
- No BMI restriction
- Able and willing to provide explicit, informed consent

Exclusion Criteria:

- Type 1 diabetes, medication-controlled type 2 diabetes
- Pregnancy
- Currently participating in weight loss programme, or reported weight change in last 3 months (>5% of current body weight)
- Documented history of eating disorder
- Ovulation medication, such as clomiphene citrate
- Weight loss medication affecting weight or appetite in last 6 months, including weight loss medications, antipsychotic drugs or other medications as determined by the physician (eg. Semaglutide, liraglutide, orlistat, amphetamines, Qsymia (phentermine-topiramate), bupropion-naltrexone (Contrave))
- Known liver, renal or thyroid dysfunction (not including non-alcoholic fatty liver disease with hypothyroidism on treatment or subclinical hypothyroidism seen in a large proportion of patients with PCOS)
- Unable to participate in follow-up for at least 24 weeks
- Unable or unwilling to provide explicit, informed consent

Contacts/Locations

Central Contact Person: Ruairi Floyd, BSc BMBS
Telephone: (01) 414 2000
Email: floyd@tcd.ie

Central Contact Backup:

Study Officials: Lucy-Ann Behan, MD
Study Principal Investigator

Robert Graves Institute of Endocrinology, Tallaght University Hospital

Sinead Duggan, R.D. PhD
Study Director
University of Dublin, Trinity College

Locations: Ireland

Robert Graves Institute of Endocrinology, Tallaght University Hospital
[Recruiting]

Dublin, Leinster, Ireland, D24 NR0A

Contact: Ruairi Floyd, BSc BMBS floyd@tcd.ie

IPDSharing

Plan to Share IPD: No

References

- Citations: Asemi Z, Samimi M, Taghizadeh M, Esmailzadeh A. Effects of Ramadan Fasting on Glucose Homeostasis, Lipid Profiles, Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome in Kashan, Iran. *Arch Iran Med*. 2015 Dec;18(12):806-10. PubMed 26621011
- Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Pittonen T, Norman RJ; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril*. 2018 Aug;110(3):364-379. doi: 10.1016/j.fertnstert.2018.05.004. Epub 2018 Jul 19. PubMed 30033227
- Anton SD, Moehl K, Donahoo WT, Marosi K, Lee SA, Mainous AG 3rd, Leeuwenburgh C, Mattson MP. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting. *Obesity (Silver Spring)*. 2018 Feb;26(2):254-268. doi: 10.1002/oby.22065. Epub 2017 Oct 31. PubMed 29086496
- Li C, Xing C, Zhang J, Zhao H, Shi W, He B. Eight-hour time-restricted feeding improves endocrine and metabolic profiles in women with anovulatory polycystic ovary syndrome. *J Transl Med*. 2021 Apr 13;19(1):148. doi: 10.1186/s12967-021-02817-2. PubMed 33849562
- de Cabo R, Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. *N Engl J Med*. 2019 Dec 26;381(26):2541-2551. doi: 10.1056/NEJMr1905136. No abstract available. Erratum In: *N Engl J Med*. 2020 Jan 16;382(3):298. *N Engl J Med*. 2020 Mar 5;382(10):978. PubMed 31881139
- Albosta M, Bakke J. Intermittent fasting: is there a role in the treatment of diabetes? A review of the literature and guide for primary care physicians. *Clin Diabetes Endocrinol*. 2021 Feb 3;7(1):3. doi: 10.1186/s40842-020-00116-1. PubMed 33531076
- Zangeneh F, Salman Yazdi R, Naghizadeh MM, Abedinia N. Effect of Ramadan Fasting on Stress Neurohormones in Women with Polycystic Ovary Syndrome. *J Family Reprod Health*. 2015 Jun;9(2):51-7. PubMed 26175759

Pellegrini M, Cioffi I, Evangelista A, Ponzo V, Goitre I, Ciccone G, Ghigo E, Bo S. Effects of time-restricted feeding on body weight and metabolism. A systematic review and meta-analysis. *Rev Endocr Metab Disord*. 2020 Mar;21(1):17-33. doi: 10.1007/s11154-019-09524-w. Erratum In: *Rev Endocr Metab Disord*. 2020 Feb 18;; PubMed 31808043

Links: URL: <https://www.indi.ie/fact-sheets/fact-sheets-on-women-s-health/542-polycystic-ovarian-syndrome-pc...>

Description The Irish Nutrition and Dietetic Institute - Polycystic Ovary Syndrome (PCOS)

URL: <https://www.bda.uk.com/resource/polycystic-ovary-syndrome-pcos-diet.html>

Description BDA The Association of UK Dieticians - Polycystic Ovary Syndrome (PCOS) Food Fact Sheet

Available IPD/Information:

U.S. National Library of Medicine | U.S. National Institutes of Health | U.S. Department of Health & Human Services

Appendix 5: PIL for Nutritics App



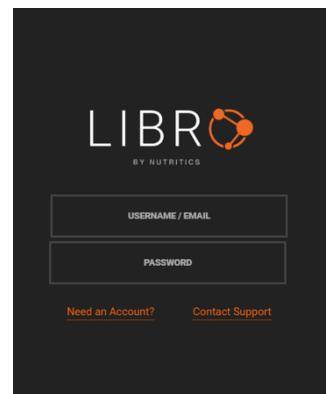
Participant Information Leaflet: Food and Drink Record

Libro App is the way you will track your food and drink intake during the study.

You will be sent an email with a link to invite you to download the Libro App. Please note that you must have an iPhone or Android Device to download the application.

Logging Into Libro for the First Time

- When you open Libro for the first time you will be presented with the screen below.
- You will need to enter a username and password to log in. The username and password you choose will be your credentials any time you log in.
- Once you have entered the username and password of your choice, click the tick in the bottom of the screen to log in.



When should I record my intake?

- Record your intake for three consecutive days.
 - One of these days must be a weekend, e.g. Thursday, Friday & Saturday or Sunday, Monday, & Tuesday.
- Record *all food and drink* you consume *at the time* you have it. This will help the study to be as accurate as possible.
 - Drink includes water, coffee, tea, diet drinks, as well as soft drinks (including Diet/Zero varieties), juices, smoothies etc.

How do I log my food and drink intake?

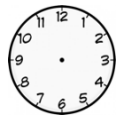
Please read and watch the following *before* starting to track your intake. This will help you to understand how to log your portion sizes.

1. https://www.nutritics.com/p/userguide&c=623_libro
2. https://www.nutritics.com/p/userguide&c=504_libro

It is *not necessary to weigh* everything you eat and drink but try to be as accurate as possible.

Please note that information about your energy, protein, and vitamin intake etc. will *not* be available to you but the researcher can see this information.

Appendix 6: Step-by-step instructions for participants



The TIMEMAP Study

Time-restricted eating to improve metabolic abnormalities in poly-cystic ovarian syndrome:

A randomised feasibility study of real-world clinical advice

Step-by-step instructions for participants

1. Please **fast for 18 hours per day** with a 6-hours eating window. There is no other specific dietary advice.
2. During fasting periods, you must consume only plain water, unflavoured/unsweetened sparkling water, black breakfast tea or black coffee.
3. Choose the fasting period according to your lifestyle and preference. You can **choose your own 6-hour eating window** – we suggest having your eating window between 8am to 2pm and 12 noon to 6pm or any times in between. Ideally, avoid having a late eating window to avoid fasting side effects during the day and for optimal results.
4. Complete a **3-day food diary** as instructed and using Libro factsheet provided. Please also **record the times** you eat foods on the app.
5. Please attend the appointments we give you to allow us to fully assess how you are doing while fasting – there will be **at least 6 appointments**, approximately every 6 weeks for the duration of the trial (total trial duration is approx. 28 weeks or 7 months).



The TIMEMAP Study

Time-restricted eating to improve metabolic abnormalities in poly-cystic ovarian syndrome:

A randomised feasibility study of real-world clinical advice

Participant Information Leaflet/Consent Form

Principle Investigator:

Dr Lucy-Ann Behan, M.D., Consultant Endocrinologist, Robert Graves
Institute of Endocrinology, Tallaght University Hospital

Co-investigators:

Dr Ruairí Floyd, BSc. BMBS, Trinity College Dublin
Dr Sinead N Duggan, R.D. PhD, Trinity College Dublin
Professor James Gibney, M.D., Tallaght University Hospital & Trinity College Dublin
Dr Ana Racovac-Tisdall, M.D., Tallaght University Hospital
Professor Carel W le Roux, M.D. PhD, University College Dublin

Funding:

The Meath Foundation of Tallaght University Hospital

INTRODUCTION

You are being invited to take part in a research study in Tallaght University Hospital (TUH) related to your diagnosis of poly-cystic ovarian syndrome (PCOS). If you wish to be included in the study, we would like to explain the study in detail. This information leaflet contains important information on the study. Please read the leaflet carefully and feel free to discuss the information with your us or GP before you make a decision. If you have any queries regarding the study, you can contact us at 01 4144786 or on email pcosresearch22@gmail.com.

PART 1: The Study

Why is this study being done?

We are investigating if a certain way of eating (where the time / hours of eating are restricted every day) has positive effects on patients with PCOS. Specifically, we are investigating if limiting when someone eats to a 6 hour window in a day reduces a hormone called insulin and how your body responds to insulin. We also will see if this way of eating reduces weight and/or waist circumference (measurement around the belly), which can be associated with health benefits.

For those with PCOS, insulin levels in the blood are often too high, and this leads to other problems including overweight and obesity, as well as problems with reproductive hormones like testosterone, and for some, a difficulty with ovulating and conceiving a child.

We find that many women with PCOS struggle to lose weight, and the typical ‘diets’ of calorie reduction and exercise do little to help.

We believe that a period of ‘fasting’ on a daily basis may reduce insulin, and may in turn help the other symptoms of PCOS.

This study is a feasibility study of a randomised controlled trial (RCT). An RCT means that we allocate participants to one of two groups randomly. The feasibility aspect means that we are conducting a preliminary study to see if a larger study is safe, tolerated and possible. If we find that it is feasible, we will then expand the study to bigger numbers of participants over a longer period of time.

The study name ‘TimeMAP’ is an acronym of the longer title.

Why am I being asked to take part?

We are looking for participants with a diagnosis of PCOS. Participants must be available to attend the clinic for measurements at the beginning of the study, 6 weeks after the start of the study and at 12 weeks after the start of the study. If you fit these criteria you may be asked to take part.

There are certain criteria that may mean you are not eligible to take part, such as type 1 diabetes, or if you are taking certain medications, and this will be explained to you in detail during a screening.

Do I have to take part? What happens if I say no? Can I withdraw after agreeing to take part?

Your participation is voluntary and you can refuse to participate in this study. If you decide to take part in the study, you may quit the study at any time. Whatever your decision, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your current or future treatment or your rights. If you wish to opt out, please contact the study lead Dr Ruairi Floyd and he will be able to organise it for you. You don't need to give any reason for opting out.

How will the study be done?

You will be invited to participate during your routine PCOS clinic appointment, complete a consent form, and screened for eligibility. If you are eligible and wish to take part, the baseline measures will be taken on day one.

You will be randomly assigned to group 1 or group 2 (group 1 starts the time-restricted eating right away, while group 2 starts after 16 weeks).

In any case, you will receive full instruction regarding measuring your dietary intake with 3-day food diaries, and recording eating hours via a smart-phone app.

The interim measures are done in week 6 of the study, and the final measures are done in week 12.

What will happen to me if I agree to take part?

As above, you will be screened for eligibility, complete informed consent form, randomised and given education and instruction.

Are there any benefits to me if I take part?

We believe that this way of eating will reduce your insulin levels and may have other benefits regarding weight. This is our study hypothesis.

Are there any risks if I decide to take part?

While there is a perception that ‘fasting’ for 18 hours per day may result in feeling faint, weakness, light-headedness, this is rarely if ever the case (as long as the fast is a true fast, which will prevent insulin being stimulated).

You may feel hungry at the beginning, but this should not be distressing or overwhelming, and you may ease into the hours if this suits you better (e.g. 12 hours, 14 hours, 16 hours then 18 hours over a few days or weeks).

If at any time you do experience the symptoms above, you should eat and just restart the next day, recording your experience as directed.

What happens if something goes wrong while I am taking part in the study?

You will have access to the study lead Dr Ruairí Floyd to report any adverse symptoms and the Principal Investigator Dr Lucy-Ann Behan will be available for an appointment to determine if any symptoms are study related.

What other treatments are available to me?

If you choose not to take part in the study, you will attend the PCOS clinic as normal, and your usual care won’t be affected in any way.

Will I be told of the outcome of the study?

Your own results will be communicated to you.

PART 2: Data Protection

What information about me (personal data) will be used as part of the study? Will my medical records be accessed?

To learn about how time-restricted eating affects insulin, other hormones, weight, appetite and dietary intake, we will take measurement at baseline, 6 weeks (half way through) and at the end of the study (12 weeks). We don't anticipate needing to access your medical notes for any other information.

What will happen to my personal data?

We will file the data collection form in locked filing cabinets in locked offices, in a secure research building, and only the data lead Dr Ruairí Floyd and other members of the Research Team (PI Lucy-Ann Behan, Endocrinologist Dr James Gibney, and co-investigator Dr Sinead Duggan) will have access to the files. We will create a database for the study which will be on password protected desktop computers in password-protected files.

Who will access and use my personal data as part of this study?

As above.

Will my personal data be kept confidential? How will my data be kept safe?

As above. All data is entirely confidential. Your name will never be identified or identifiable in any future presentation or publication which arise from this work. Your data will not leave the country at any time.

What is the lawful basis to use my person data?

It is lawful to use personal data if specific consent is taken. This is in accordance with article 6 and article 9 of GDPR of data protection,

According to the Article 6 of GDPR

“1. Processing shall be lawful only if and to the extent that at least one of the following applies:

*(a) the data subject has given **consent** to the processing of his or her personal data for one or more specific purposes;”*

And in Article 9 of GDPR

*“1. Processing of personal data revealing racial or ethnic origin, political opinions, religious or philosophical beliefs, or trade union membership, and the processing of genetic data, biometric data for the purpose of uniquely identifying a natural person, data concerning health or data concerning a natural person's sex life or sexual orientation **shall be prohibited**.*

2. Paragraph 1 shall not apply if one of the following applies:

*(a) the data subject has given **explicit consent** to the processing of those personal data for one or more specified purposes, except where Union or Member State law provide that the prohibition referred to in paragraph 1 may not be lifted by the data subject;”*

In your case, we will obtain explicit, informed consent for this study. This means that you will be fully aware of the nature of the study and the data collection. We will only use your data for the purposes of this specific study.

The investigators have completed, or will have completed by the start of the study, the relevant GDPR courses.

What are my rights?

If you wish to, you will have the

- Right to access data held
- Right to restrict the use of the data held
- Right to correct inaccuracies
- Right to have information deleted
- Right to data portability
- Right to object to profiling

PART 3: Costs, funding and approval

Will it cost me anything if I agree to take part?

There is no added cost for you to participate in the study. Your doctors / nurses / dietitians are covered by standard malpractice insurance. Nothing in this document restricts or curtails your rights

Who is funding the study?

We successfully applied for a grant from the Meath Foundation of Tallaght Hospital (A Tallaght Hospital Charity). The grant is called the John Barragry Award and was for the value of 75,000 Euro.

Has the study been approved by a Research Ethics Committee?

We have applied for ethical approval from the Joint Research Ethics Committee of Tallaght Hospital and St. James Hospital (JREC AMNCH/SJH).

PART 4: Future Research

Will my personal data and/or biological material be used in future studies?

No, we don't plan to use your data or blood samples for any future research. If any research must be done, new data will be obtained.

PART 5: Further Information

Where can I get more information?

If you would like further information, please contact the study lead (yet to be appointed).

What happens if I want to make a complaint?

If you wish to make a complaint, please contact the *Patient Advocacy Department* of Tallaght University Hospital:

Tel: (01) 414 4709

Email: patient.advocacy@tuh.ie

Letter: Patient Advocacy Department, Tallaght University Hospital, Tallaght, D24

Will I be contacted again?

If members of your team learn of important new information that might affect your desire to remain in the study, he or she will tell you.

St James's Hospital / Tallaght University Hospital Research Ethics Committee

CONSENT FORM

Time-Restricted Eating to Improve Metabolic Abnormalities in Poly-Cystic Ovarian Syndrome: A *randomised feasibility study of real-world clinical advice* (The TimeMAP Study)

To be completed by the **PARTICIPANT**:

I have read and understood the information leaflet.	YES ☐	NO ☐
I have had the opportunity to discuss the study, ask questions about the study and I have received satisfactory answers to all my questions.	YES ☐	NO ☐
I have received enough information about this study.	YES ☐	NO ☐
I understand that I am free to withdraw from the study at any time without giving a reason and this will not affect my future medical care.	YES ☐	NO ☐
I agree to allow the researchers use my information (personal data) as part of this study as outlined in the information leaflet.	YES ☐	NO ☐
I agree to allow the researchers access my medical records as part of this study	YES ☐	NO ☐
I agree to be contacted by researchers as part of this study	YES ☐	NO ☐
I agree to give blood samples as outlined as part of this study.	YES ☐	NO ☐
I consent to take part in this research study having been fully informed of the risks, benefits and purpose of the study	YES ☐	NO ☐
I give my explicit consent to have my data processed as part of this research study'	YES ☐	NO ☐
I understand that my data or blood samples will not be used for any further studies not described in the participant information leaflet.	YES ☐	NO ☐

Participant's Name (Block Capitals):	
Participant's Signature:	
Date:	

To be completed by the **RESEARCHER**:

I have fully explained the purpose and nature (including benefits and risks) of this study to the participant in a way that he/she could understand. I have invited him/her to ask questions on any aspect of the study.	YES ☐	NO ☐
I confirm that I have given a copy of the information leaflet and consent form to the participant.	YES ☐	NO ☐

Researcher's Name (Block Capitals):	
Researcher's Title & Qualifications:	
Researcher's Signature:	
Date:	

References

1. Keating, K., *INTERMITTENT FASTING FOR PCOS*, in *PCOS Living*. 2018: Access at: <https://www.pcosliving.com/pcos-living-blog/intermittent-fasting-for-pcos>.
2. Pirotta, S., et al., *Obesity and the Risk of Infertility, Gestational Diabetes, and Type 2 Diabetes in Polycystic Ovary Syndrome*. *Semin Reprod Med*, 2021.
3. Ndefo, U.A., A. Eaton, and M.R. Green, *Polycystic Ovary Syndrome: A Review of Treatment Options With a Focus on Pharmacological Approaches* *Pharmacy & Therapeutics*, 2013. **38(6)**: p. 336-338, 348, 355.
4. Teede, H.J., et al., *Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome*. *Human Reproduction*, 2018. **33(9)**: p. 1602–1618.
5. Lord, J.M., I.H. Flight, and R.J. Norman, *Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome*. *Cochrane Database of Systematic Reviews*, 2003(3): p. CD003053.
6. Harmanci, A., et al., *Oral contraceptive plus antiandrogen therapy and cardiometabolic risk in polycystic ovary syndrome*. *Clinical Endocrinology*, 2013. **78(1)**: p. 120-125.
7. Rabe, T., et al., *Hirsutism - Medicinal treatment. Joint statement of the German Society of Gynaecological Endocrinology and Reproductive Medicine and the Professional Association of Gynaecologists*. *Journal fur Reproduktionsmedizin und Endokrinologie*, 2015. **12(3)**: p. 102-148.
8. Brown, J., et al., *Clomiphene and anti-oestrogens for ovulation induction in PCOS*. *Cochrane Database of Systematic Reviews* 2009.
9. Niafar, M., et al., *A systematic review of GLP-1 agonists on the metabolic syndrome in women with polycystic ovaries*. *Archives of Gynecology and Obstetrics*, 2016. **293(3)**: p. 509-515.
10. Zhong, W.Y., et al., *Effect of thiazolidinedione amide on insulin resistance, reactive protein and endothelial function in young women with polycystic ovary*

- syndrome*. Tropical Journal of Pharmaceutical Research, 2015. **14**(12): p. 2287-2292.
11. Du, Q., et al., *Effects of thiazolidinediones on polycystic ovary syndrome: A meta-analysis of randomized placebo-controlled trials*. Advances in Therapy, 2012. **29**(9): p. 763-774.
 12. Fulghesu, A.M., et al., *N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome*. Fertility and Sterility, 2002. **77**(6): p. 1128-1135.
 13. Nehra, J., et al., *A comparative study of myo inositol versus metformin on biochemical profile in polycystic ovarian syndrome in women*. International Journal of Pharmaceutical Sciences and Research, 2017. **8**(4): p. 1664-1670.
 14. Raval, A.D., et al., *Statins for women with polycystic ovary syndrome not actively trying to conceive*. Cochrane Database of Systematic Reviews, 2011(10).
 15. de Medeiros, S.F., *Risks, benefits size and clinical implications of combined oral contraceptive use in women with polycystic ovary syndrome*. Reproductive biology and endocrinology : RB&E, 2017. **15**(1): p. 93.
 16. Costello, M., et al., *Insulin-sensitising drugs versus the combined oral contraceptive pill for hirsutism, acne and risk of diabetes, cardiovascular disease, and endometrial cancer in polycystic ovary syndrome*. Cochrane Database of Systematic Reviews, 2007(1).
 17. Katsikis, I., et al., *Phenotypic expression, body mass index and insulin resistance in relation to LH levels in women with polycystic ovary syndrome*. European Journal of Obstetrics and Gynecology and Reproductive Biology, 2011. **156**(2): p. 181-185.
 18. Shamdeen, M.Y. and M.A. Saber, *Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome*. Middle East Fertility Society Journal, 2005. **10**(3): p. 223-230.
 19. Marsden, P.J., A.P. Murdoch, and R. Taylor, *Tissue insulin sensitivity and body weight in polycystic ovary syndrome*. Clinical Endocrinology, 2001. **55**(2): p. 191-199.
 20. Bennett, C.M., M. Guo, and S.C. Dharmage, *HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review*. Diabet Med, 2007. **24**(4): p. 333-43.

21. Velling Magnussen, L., et al., *Hemoglobin A1c as a tool for the diagnosis of type 2 diabetes in 208 premenopausal women with polycystic ovary syndrome*. Fertility and Sterility, 2011. **96**(5): p. 1275-1280.
22. Tarkun, I., et al., *Endothelial dysfunction in young women with polycystic ovary syndrome: Relationship with insulin resistance and low-grade chronic inflammation*. Journal of Clinical Endocrinology and Metabolism, 2004. **89**(11): p. 5592-5596.
23. Amato, M.C., et al., *Hyperinsulinism and polycystic ovary syndrome (PCOS): role of insulin clearance*. J Endocrinol Invest, 2015. **38**(12): p. 1319-26.
24. Farshchi, H., et al., *Diet and nutrition in polycystic ovary syndrome (PCOS): Pointers for nutritional management*. Journal of Obstetrics and Gynaecology, 2007. **27**(8): p. 762-773.
25. Hirschberg, A.L., et al., *Impaired cholecystokinin secretion and disturbed appetite regulation in women with polycystic ovary syndrome*. Gynecological Endocrinology, 2004. **19**(2): p. 79-87.
26. Lim, S.S., et al., *The effect of obesity on polycystic ovary syndrome: A systematic review and meta-analysis*. Obesity Reviews, 2013. **14**(2): p. 95-109.
27. Van Dam, E.W.C.M., et al., *Increase in daily LH secretion in response to short-term calorie restriction in obese women with PCOS*. American Journal of Physiology Endocrinology and Metabolism, 2002. **282**(4): p. 865-872.
28. Legro, R.S., *Obesity and PCOS: Implications for Diagnosis and Treatment*. Semin Reprod Med., 2012. **30**: p. 496–506.
29. Barrea, L., et al., *Adherence to the Mediterranean Diet, Dietary Patterns and Body Composition in Women with Polycystic Ovary Syndrome (PCOS)*. Nutrients, 2019. **11**(10).
30. Porchia, L.M., et al., *Diets with lower carbohydrate concentrations improve insulin sensitivity in women with polycystic ovary syndrome: A meta-analysis*. Eur J Obstet Gynecol Reprod Biol, 2020. **248**: p. 110-117.
31. Shang, Y., et al., *Effect of Diet on Insulin Resistance in Polycystic Ovary Syndrome*. J Clin Endocrinol Metab, 2020. **105**(10).
32. Kazemi, M., et al., *A Comparison of a Pulse-Based Diet and the Therapeutic Lifestyle Changes Diet in Combination with Exercise and Health Counselling on the Cardio-Metabolic Risk Profile in Women with Polycystic Ovary Syndrome: A Randomized Controlled Trial*. Nutrients, 2018. **10**.

33. Phy, J.L., et al., *Low Starch/Low Dairy Diet Results in Successful Treatment of Obesity and Co-Morbidities Linked to Polycystic Ovary Syndrome (PCOS)*. Journal of Obesity & Weight Loss Therapy, 2015. 5(2).
34. Pohlmeier, A.M., et al., *Effect of a low-starch/low-dairy diet on fat oxidation in overweight and obese women with polycystic ovary syndrome*. Applied physiology, nutrition, and metabolism = Physiologie appliquée, nutrition et métabolisme, 2014. 39(11): p. 1237-1244.
35. Mavropoulos, J.C., et al., *The effects of a low-carbohydrate, ketogenic diet on the polycystic ovary syndrome: A pilot study*. Nutrition and Metabolism, 2005. 2.
36. Shishehgar, F., et al., *Does a restricted energy low glycemic index diet have a different effect on overweight women with or without polycystic ovary syndrome?* BMC Endocrine Disorders, 2019. 19(1).
37. Fauser, B.C., et al., *Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group*. Fertil Steril, 2012. 97(1): p. 28-38.e25.
38. Anton, S.D., et al., *Flipping the Metabolic Switch: Understanding and Applying Health Benefits of Fasting*. Obesity, 2017. 26(2): p. 254–268.
39. Li, C., et al., *Eight-hour time-restricted feeding improves endocrine and metabolic profiles in women with anovulatory polycystic ovary syndrome*. J Transl Med, 2021. 19.
40. de Cabo, R. and M.P. Mattson, *Effects of Intermittent Fasting on Health, Aging, and Disease*. N Engl J Med 2019. 381: p. 2541-2551.
41. Armutcu, F., *Fasting may be an alternative treatment method recommended by physicians*. Electronic Journal of General Medicine, 2019. 16(3).
42. Allaf, M., et al., *Intermittent fasting for the prevention of cardiovascular disease*. Cochrane Database of Systematic Reviews, 2021.
43. Zarrinpar, A., et al., *Diet and feeding pattern affect the diurnal dynamics of the gut microbiome*. Cell Metab, 2014. 20(6): p. 1006-17.
44. Pellegrini, M., et al., *Effects of time-restricted feeding on body weight and metabolism. A systematic review and meta-analysis*. Rev Endocr Metab Disord, 2020. 21(1): p. 17-33.
45. Wilhelmi de Toledo, F., et al., *Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects*. PLOS One, 2019. 14 (1).

46. Tey, S.L., et al., *Effects of aspartame-, monk fruit-, stevia- and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake.* *International Journal of Obesity* volume, 2017. **41**(pages 450–457).
47. Anton, S.D., et al., *Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels.* *Appetite*, 2010. **55**(1): p. 37-43.
48. Nettleton, J.E., R.A. Reimer, and J. Shearer, *Reshaping the gut microbiota: Impact of low calorie sweeteners and the link to insulin resistance?* *Physiology & Behavior*, 2016. **164**: p. 488-493.
49. Mathur, K., et al., *Effect of artificial sweeteners on insulin resistance among type-2 diabetes mellitus patients.* *J Family Med Prim Care*, 2020. **9**(1): p. 69-71.
50. Leahy, M., et al., *Consumption of Low-Calorie Sweetened Beverages Compared to Water Is Associated with Reduced Intake of Carbohydrates and Sugar, with No Adverse Relationships to Glycemic Responses: Results from the 2001-2012 National Health and Nutrition Examination Surveys.* *Nutrients*, 2017. **9**(9).
51. Simundic, A.M., et al., *Standardization of collection requirements for fasting samples: for the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).* *Clin Chim Acta*, 2014. **432**: p. 33-7.
52. Horne, B.D., *Considerations for the Optimal Timing, Duration, Frequency, and Length of an Intermittent Fasting Regimen for Health Improvement.* *Nutrients*, 2020. **12**(9): p. 2567.
53. Zangeneh, F., et al., *The Effect of Ramadan Fasting on Hypothalamic Pituitary Ovarian (HPO) Axis in Women with Polycystic Ovary Syndrome.* *Women's Health Bulletin*, 2014. **1**(1): p. 1-6.
54. Diamanti-Kandarakis, E. and A.G. Papavassiliou, *Molecular mechanisms of insulin resistance in polycystic ovary syndrome.* *Trends Mol Med*, 2006. **12**(7): p. 324-32.
55. Malin, S.K., et al., *Pancreatic β -cell dysfunction in polycystic ovary syndrome: Role of hyperglycemia-induced nuclear factor- κ B activation and systemic inflammation.* *American Journal of Physiology - Endocrinology and Metabolism*, 2015. **308**(9): p. E770-E777.

56. Prelevic, G.M., M.I. Wurzbarger, and L.A. Peric, *Pancreatic beta cell function in polycystic ovary syndrome: its relationship to body weight, serum testosterone and serum prolactin levels*. Experimental & Clinical Endocrinology, 1987. **90**(1): p. 76-82.
57. Dunaif, A. and D.T. Finegood, *Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome*. J Clin Endocrinol Metab, 1996. **81**(3): p. 942-7.
58. Brede, S., et al., *Clinical Scenario of the Metabolic Syndrome*. Visceral Medicine, 2016. **32**(5): p. 336-341.
59. López-Jaramillo, P., et al., *The role of leptin/adiponectin ratio in metabolic syndrome and diabetes*. Horm Mol Biol Clin Investig, 2014. **18**(1): p. 37-45.
60. Albosta, M. and J. Bakke, *Intermittent fasting: is there a role in the treatment of diabetes? A review of the literature and guide for primary care physicians*. Clin Diabetes Endocrinol, 2021. **7**(1): p. 3.
61. Koleva, D.I., et al., *Adipokines and soluble cell adhesion molecules in insulin resistant and non-insulin resistant women with polycystic ovary syndrome*. Archives of Physiology and Biochemistry, 2016. **122**(4): p. 223-227.
62. Gözükcük, M., et al., *Adiponectin and leptin levels in normal weight women with polycystic ovary syndrome*. Hormone Molecular Biology and Clinical Investigation, 2020. **41**(4).
63. Rosenfield, R.L. and D.A. Ehrmann, *The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited*. Endocrine Reviews, 2016. **37**(5): p. 467–520. .
64. Banaszewska, B., L. Pawelczyk, and R. Spaczynski, *Current and future aspects of several adjunctive treatment strategies in polycystic ovary syndrome*. Reproductive biology, 2019. **19**(4): p. 309-315.
65. Legro, R.S., V.D. Castracane, and R.P. Kauffman, *Detecting Insulin Resistance in Polycystic Ovary Syndrome: Purposes and Pitfalls*. Obstetrical and Gynecological Survey, 2004. **59**(2): p. 141-154.
66. Moran, L.J., R.J. Norman, and H.J. Teede, *Metabolic risk in PCOS: phenotype and adiposity impact*. Trends Endocrinol Metab, 2015. **26**(3): p. 136-43.
67. Majid, H., Q. Masood, and A.H. Khan, *Homeostatic model assessment for insulin resistance (HOMA-IR): A better marker for evaluating insulin resistance than*

- fasting insulin in women with polycystic ovarian syndrome*. Journal of the College of Physicians and Surgeons Pakistan, 2017. **27**(3): p. 123-126.
68. Koleva, D.I., et al., *Indexes of insulin sensitivity/resistance in women with metabolic syndrome and women with polycystic ovary syndrome*. Endokrinologiya, 2016. **21**(2): p. 93-102.
 69. Azziz, R., *Evaluation for Insulin Resistance and Comorbidities Related to Insulin Resistance in Polycystic Ovary Syndrome*, in *Insulin Resistance and Polycystic Ovarian Syndrome: Pathogenesis, Evaluation, and Treatment*, E. DiamantiKandarakis, et al., Editors. 2007. p. 145-157.
 70. Lankarani, M., et al., *Evaluation of insulin resistance and metabolic syndrome in patients with polycystic ovary syndrome*. Gynecological Endocrinology, 2009. **25**(8): p. 504-507.
 71. Gurbuz, T. and O. Gokmen, *A Novel index to overcome the assessment limitations of insulin resistance in polycystic ovary syndrome: Homeostatic model assessment index to red blood cell distribution width ratio*. Journal of Reproductive Medicine, 2021. **66**(1-2): p. 9-14.
 72. Fulghesu, A.M., et al., *Failure of the homeostatic model assessment calculation score for detecting metabolic deterioration in young patients with polycystic ovary syndrome*. Fertility and Sterility, 2006. **86**(2): p. 398-404.
 73. Wilma, D.S.C., N. Kavya, and S. Kulkarni, *Evaluation of insulin sensitivity status in polycystic ovarian syndrome*. Asian Pacific Journal of Tropical Disease, 2011. **1**(1): p. 67-70.
 74. Hajder, M. and E. Hajder, *Insulin sensitivity and free androgen index in women with polycystic ovarian syndrome*. Healthmed, 2009. **3**(4): p. 513-519.
 75. Xu, X., et al., *Adiponectin/(FBG×FIns) as a predictor of insulin sensitivity and metabolic syndrome in patients with polycystic ovary syndrome*. Medicine (United States), 2016. **95**(49): p. e5524.
 76. Yildiz, Y., et al., *Serum adiponectin level and clinical, metabolic, and hormonal markers in patients with polycystic ovary syndrome*. International Journal of Fertility and Sterility, 2014. **7**(4): p. 331-336.
 77. Akin, F., et al., *SHBG levels correlate with insulin resistance in postmenopausal women*. European Journal of Internal Medicine, 2009. **20**(2): p. 162-167.
 78. Jayagopal, V., et al., *The biological variation of testosterone and sex hormone-binding globulin (SHBG) in polycystic ovarian syndrome: Implications for SHBG*

- as a surrogate marker of insulin resistance. *Journal of Clinical Endocrinology & Metabolism*, 2003. **88**(4): p. 1528-1533.
79. Dahan, M.H. and J. Goldstein, *Serum sex hormone-binding globulin levels show too much variability to be used effectively as a screening marker for insulin resistance in women with polycystic ovary syndrome*. *Fertility and Sterility*, 2006. **86**(4): p. 934-941.
 80. DeUgarte, C.M., Y.I. Chen, and R. Azziz, *What's the best way to diagnose insulin resistance and hyperinsulinemia?* *Contemporary OB/GYN*, 2005. **50**(3): p. 66-70.
 81. Goodman, N.F., et al., *American association of clinical endocrinologists, American college of endocrinology, and androgen excess and pcos society disease state clinical review: Guide to the best practices in the evaluation and treatment of polycystic ovary syndrome - Part 2*. *Endocrine Practice*, 2015. **21**(12): p. 1415-1426.
 82. Matsuda, M., *Measuring and estimating insulin resistance in clinical and research settings*. *Nutrition, Metabolism and Cardiovascular Diseases*, 2010. **20**(2): p. 79-86.
 83. Shahin, L., et al., *Insulin Sensitivity Indices in Patients with Polycystic Ovary Syndrome with Different Body Mass Index Categories*. *Current Diabetes Reviews*, 2020. **16**: p. 483-489.
 84. Gutch, M., et al., *Assessment of insulin sensitivity/resistance*. *Indian J Endocrinol Metab.* , 2015. **19**(1): p. 160–164.
 85. Kant, A.K. and B.I. Graubard, *40-year trends in meal and snack eating behaviors of American adults*. *J Acad Nutr Diet*, 2015. **115**(1): p. 50-63.
 86. Ritskes-Hoitinga, M., et al., *Chapter 4.3 - Nutrition of the Laboratory Mouse*, in *The Laboratory Mouse (Second Edition)*, H.J. Hedrich, Editor. 2012, Academic Press: Boston. p. 567-599.
 87. Trepanowski, J.F., et al., *Effect of Alternate-Day Fasting on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically Healthy Obese Adults: A Randomized Clinical Trial*. *JAMA Intern Med*, 2017. **177**(7): p. 930-938.
 88. Soeters, M.R., et al., *Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism*. *Am J Clin Nutr*, 2009. **90**(5): p. 1244-51.
 89. Harvie, M.N., et al., *The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women*. *Int J Obes (Lond)*, 2011. **35**(5): p. 714-27.

90. Halberg, N., et al., *Effect of intermittent fasting and refeeding on insulin action in healthy men*. J Appl Physiol (1985), 2005. **99**(6): p. 2128-36.
91. Farey, J.E., et al., *Effect of Laparoscopic Sleeve Gastrectomy on Fasting Gastrointestinal, Pancreatic, and Adipose-Derived Hormones and on Non-Esterified Fatty Acids*. Obesity Surgery, 2017. **27**(2): p. 399-407.
92. Machado Júnior, A.S., et al., *The Effect of Sleeve Gastrectomy on the Hormonal Profile of Patients with Polycystic Ovary Syndrome*. Obesity Surgery, 2019. **29**(8): p. 2415-2419.
93. Sutton, E.F., et al., *Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even Without Weight Loss in Men with Prediabetes*. Cell Metab, 2018. **27**(6): p. 1212–1221.e3. .
94. Hatori, M., et al., *Time restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high fat diet*. Cell Metab. , 2012 **15**(6): p. 848–860.
95. Belkacemi, L., et al., *Intermittent fasting modulation of the diabetic syndrome in streptozotocin-injected rats*. Int J Endocrinol, 2012. **2012**: p. 962012.
96. Olsen, M.K., et al., *Time-restricted feeding on weekdays restricts weight gain: A study using rat models of high-fat diet-induced obesity*. Physiol Behav, 2017. **173**: p. 298-304.
97. Sherman, H., et al., *Timed high-fat diet resets circadian metabolism and prevents obesity*. Faseb j, 2012. **26**(8): p. 3493-502.
98. Woodie, L.N., et al., *Restricted feeding for 9h in the active period partially abrogates the detrimental metabolic effects of a Western diet with liquid sugar consumption in mice*. Metabolism, 2018. **82**: p. 1-13.
99. Wu, T., et al., *Differential roles of breakfast and supper in rats of a daily three-meal schedule upon circadian regulation and physiology*. Chronobiol Int, 2011. **28**(10): p. 890-903.
100. Joham, A.E., et al., *Prevalence of infertility and use of fertility treatment in women with polycystic ovary syndrome: data from a large community-based cohort study*. J Womens Health (Larchmt), 2015. **24**(4): p. 299-307.
101. Homburg, R., *Pregnancy complications in PCOS*. Best Practice & Research Clinical Endocrinology & Metabolism, 2006. **20**(2): p. 281-292.

102. Van Dam, E.W., et al., *Increase in daily LH secretion in response to short-term calorie restriction in obese women with PCOS*. Am J Physiol Endocrinol Metab, 2002. **282**(4): p. E865-72.
103. Llewellyn-Waters, K. and M.M. Abdullah, *Intermittent fasting - a potential approach to modulate the gut microbiota in humans? A systematic review*. Nutrition and Healthy Aging, **2021**. **Pre-press**: p. 1-8.
104. Hermes, G.D.A., et al., *Individual and cohort-specific gut microbiota patterns associated with tissue-specific insulin sensitivity in overweight and obese males*. Scientific Reports, Nature Research, 2020. **10**.
105. Gurung, M., et al., *Role of gut microbiota in type 2 diabetes pathophysiology*. EBioMedicine, 2020. **51**: p. 102590.
106. Carvalho, B.M. and M.J. Abdalla Saad, *Influence of Gut Microbiota on Subclinical Inflammation and Insulin Resistance*. Mediators of Inflammation, 2013. **2013**.
107. Tabrizi, R., et al., *The Effects of Probiotic Supplementation on Clinical Symptom, Weight Loss, Glycemic Control, Lipid and Hormonal Profiles, Biomarkers of Inflammation, and Oxidative Stress in Women with Polycystic Ovary Syndrome: a Systematic Review and Meta-analysis of Randomized Controlled Trials*. Probiotics and Antimicrobial Proteins, 2019.
108. Heshmati, J., et al., *The Effects of Probiotics or Synbiotics Supplementation in Women with Polycystic Ovarian Syndrome: a Systematic Review and Meta-Analysis of Randomized Clinical Trials*. Probiotics and Antimicrobial Proteins, 2019. **11**(4): p. 1236-1247.
109. Cozzolino, M., et al., *Therapy with probiotics and synbiotics for polycystic ovarian syndrome: a systematic review and meta-analysis*. European Journal of Nutrition, 2020. **59**(7): p. 2841-2856.
110. Liberati, A., et al., *Research Methods & Reporting The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration*. BMJ, 2009. **339**.
111. Zangeneh, F., et al., *Effect of Ramadan Fasting on Stress Neurohormones in Women with Polycystic Ovary Syndrome*. Journal of Family & Reproductive Health, 2015. **9**(2): p. 51-7.

112. Floyd, R., et al., *The Effect of Time-Restricted Eating on Insulin Levels and Insulin Sensitivity in Patients with Polycystic Ovarian Syndrome: A Systematic Review*. Int J Endocrinol, 2022. **2022**: p. 2830545.
113. Asemi, Z., et al., *Effects of Ramadan Fasting on Glucose Homostasis, Lipid Profiles, Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome in Kashan, Iran*. Archives of Iranian Medicine, 2015. **18**(12): p. 806-810.
114. Yeoh, E.C., et al., *Fasting during Ramadan and Associated Changes in Glycaemia, Caloric Intake and Body Composition with Gender Differences in Singapore*. Ann Acad Med Singap, 2015. **44**(6): p. 202-6.
115. Kiyani, M.M., et al., *Study of Human Biochemical Parameters During and After Ramadan*. J Relig Health, 2017. **56**(1): p. 55-62.
116. Ainsworth, B.E., et al., *2011 Compendium of Physical Activities: a second update of codes and MET values*. Med Sci Sports Exerc, 2011. **43**(8): p. 1575-81.
117. Soliman, G.A., *Intermittent fasting and time-restricted eating role in dietary interventions and precision nutrition*. Frontiers in Public Health, 2022. **Volume 10**.
118. Varady, K.A., et al., *Clinical application of intermittent fasting for weight loss: progress and future directions*. Nat Rev Endocrinol, 2022. **18**(5): p. 309-321.
119. Food_Safety_Authority_Ireland, *Scientific Committee Report - Vitamin D: Scientific Recommendations for 5 to 65 Year Olds Living in Ireland*. 2023.
120. Feyzioglu, B.S., C.M. Güven, and Z. Avul, *Eight-Hour Time-Restricted Feeding: A Strong Candidate Diet Protocol for First-Line Therapy in Polycystic Ovary Syndrome*. Nutrients, 2023. **15 (10)**: p. 2260.
121. Xie, Z., et al., *Randomized controlled trial for time-restricted eating in healthy volunteers without obesity*. Nat Commun, 2022. **13**
122. Chow, L.S., et al., *Time-Restricted Eating Effects on Body Composition and Metabolic Measures in Humans who are Overweight: A Feasibility Study*. Obesity, 2020. **28**: p. 860-869.
123. Lowe, D.A., et al., *Effects of Time-Restricted Eating on Weight Loss and Other Metabolic Parameters in Women and Men With Overweight and Obesity : The TREAT Randomized Clinical Trial* . JAMA Intern Med., 2020. **180**(11): p. 1491–1499.

124. Moon, S., et al., *Beneficial Effects of Time-Restricted Eating on Metabolic Diseases: A Systemic Review and Meta-Analysis*. *Nutrients*, 2020. **12(5)**: p. 1267.
125. Parr, E.B., et al., *Time-Restricted Eating as a Nutrition Strategy for Individuals with Type 2 Diabetes: A Feasibility Study*. *Nutrients*, 2020. **12(11)**: p. 3228.
126. Tinsley, G.M., et al., *Time-restricted feeding plus resistance training in active females: a randomized trial*. *The American Journal of Clinical Nutrition*, 2019. **110(3)**: p. 628–640.
127. Hřebíček, J.i., et al., *Detection of Insulin Resistance by Simple Quantitative Insulin Sensitivity Check Index QUICKI for Epidemiological Assessment and Prevention*. *The Journal of Clinical Endocrinology & Metabolism*, 2002 **87(1)**: p. 144.
128. Koster, A., et al., *Waist Circumference and Mortality*. *American Journal of Epidemiology*, 2008. **167(12)**: p. 1465–1475.
129. Rocha, M.P., et al., *Dyslipidemia in women with polycystic ovary syndrome: Incidence, pattern and predictors*. *Gynecological Endocrinology*, 2011. **27(10)**: p. 814-819.
130. Kim, J.J. and Y.M. Choi, *Dyslipidemia in women with polycystic ovary syndrome*. *Obstetrics & Gynecology Science*, 2013. **56(3)**: p. 137-142.
131. Nordestgaard, B.G., et al., *Lipoprotein(a) as a cardiovascular risk factor: current status*. *European Heart Journal*, 2010. **31(23)**: p. 2853.
132. Lewandowski, K.C., et al., *Prevalence of Dyslipidaemia and Pre-Diabetes among Women with Polycystic Ovary Syndrome (PCOS): Do We Overestimate Cardiovascular Risk?* *Hormone and Metabolic Research*, 2019. **51(8)**: p. 539-545.
133. Newman, C.B., et al., *Lipid management in patients with endocrine disorders: An endocrine society clinical practice guideline*. *Journal of Clinical Endocrinology and Metabolism*, 2020. **105(12)**.
134. Meng, H., et al., *Effects of intermittent fasting and energy-restricted diets on lipid profile: A systematic review and meta-analysis*. *Nutrition*, 2020. **77**.
135. Santos, H.O. and R.C.O. Macedo, *Impact of intermittent fasting on the lipid profile: Assessment associated with diet and weight loss*. *Clinical Nutrition ESPEN*, 2018. **24**: p. 14-21
136. Melo, A.S., R.A. Ferriani, and P.A. Navarro, *Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice*. *Clinics (Sao Paulo)*, 2015. **70(11)**: p. 765-9.

137. Boomsma, C.M., B.C. Fauser, and N.S. Macklon, *Pregnancy complications in women with polycystic ovary syndrome*. Semin Reprod Med, 2008. **26**(1): p. 72-84.
138. Palomba, S., et al., *Pregnancy complications in women with polycystic ovary syndrome*. Hum Reprod Update, 2015. **21**(5): p. 575-92.
139. Galtier-Dereure, F., C. Boegner, and J. Bringer, *Obesity and pregnancy: complications and cost*. The American Journal of Clinical Nutrition, 2000. **Volume 71**(Issue 5): p. Pages 1242S–1248S.
140. Poston, L., et al., *Obesity in Pregnancy: Implications for the Mother and Lifelong Health of the Child. A Consensus Statement*. Pediatr Res, 2011. **69**(2): p. 175-80.
141. Yu, C.K.H., T.G. Teoh, and S. Robinson, *Review article: Obesity in pregnancy*. BJOG: An International Journal of Obstetrics & Gynaecology, 2006. **113**: p. 1117-1125.
142. Heusschen, L., et al., *A Matter of Timing-Pregnancy After Bariatric Surgery*. Obes Surg, 2021. **31**(5): p. 2072-2079.
143. Cienfuegos, S., et al., *Effect of Intermittent Fasting on Reproductive Hormone Levels in Females and Males: A Review of Human Trials*. 2022.
144. Jurczewska, J. and D. Szostak-Węgierek, *The Influence of Diet on Ovulation Disorders in Women—A Narrative Review*. 2022.
145. Konishi, N., et al., *Effects of time-restricted feeding and type of food on fertility competence in female mice*. Sci Rep 12 , 7064, 2022.
146. Hua, L., et al., *Time-restricted feeding improves the reproductive function of female mice via liver fibroblast growth factor 21*. Clin Transl Med, 2020. **10**(6): p. e195.
147. Tezze, C., V. Romanello, and M. Sandri, *FGF21 as Modulator of Metabolism in Health and Disease*. Frontiers in Physiology, 2019.
148. Teong, X.T., et al., *Intermittent fasting plus early time-restricted eating versus calorie restriction and standard care in adults at risk of type 2 diabetes: a randomized controlled trial*. Nature Medicine, 2023. **29**: p. 963–972.
149. Pal, L., et al., *Therapeutic implications of vitamin D and calcium in overweight women with polycystic ovary syndrome*. Gynecol Endocrinol, 2012. **28**(12): p. 965-8.
150. Asemi, Z., et al., *Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient*

- women with polycystic ovary syndrome. *Clinical Nutrition*, 2015. **34**(4): p. 586-592.
151. Tehrani, H.G., F. Mostajeran, and S. Shahsavari, *The effect of calcium and vitamin D supplementation on menstrual cycle, body mass index and hyperandrogenism state of women with poly cystic ovarian syndrome*. *J Res Med Sci*, 2014. **19**(9): p. 875-80.
 152. FSAI, *Recommended dietary allowances for Ireland*. 1999, Food Safety Authority of Ireland.
 153. Kuliczowska-Plaksej, J., et al., *Serum Vitamin D Binding Protein Level Associated with Metabolic Cardiovascular Risk Factors in Women with the Polycystic Ovary Syndrome*. *Hormone and Metabolic Research*, 2019. **51**(1): p. 54-61.
 154. He, C., et al., *Serum vitamin d levels and polycystic ovary syndrome: A systematic review and meta-analysis*. *Nutrients*, 2015. **7**(6): p. 4555-4577.
 155. Gupta, T., et al., *Study of Effect of Vitamin D Supplementation on the Clinical, Hormonal and Metabolic Profile of the PCOS Women*. *Journal of Obstetrics and Gynecology of India*, 2017. **67**(5): p. 349-355.
 156. Lustig, E., J.H. Shubrook, and K.M. Pfothenauer, *Time-Restricted Feeding and Potential for Type 2 Diabetes Mellitus: A Narrative Review*. *Journal of Osteopathic Medicine*, 2020. **120**: p. 560-567.
 157. Lee, S.A., et al., *Determinants of Adherence in Time-Restricted Feeding in Older Adults: Lessons from a Pilot Study*. *Nutrients*, 2020. **12**: p. 874.
 158. Douglas, C.C., et al., *Role of diet in the treatment of polycystic ovary syndrome*. *Fertility and Sterility*, 2006. **85**(3): p. 679-688.
 159. Papakonstantinou, E., et al., *Effect of meal frequency on glucose and insulin levels in women with polycystic ovary syndrome: A randomised trial*. *European Journal of Clinical Nutrition*, 2016. **70**(5): p. 588-594.
 160. Gower, B.A., et al., *Favourable metabolic effects of a eucaloric lower-carbohydrate diet in women with PCOS*. *Clinical Endocrinology*, 2013. **79**(4): p. 550-557.
 161. Tahergorabi, Z., et al., *Association of serum levels of vascular endothelial growth factor and thrombospondin-1 to body mass index in polycystic ovary syndrome: a case-control study*. *Obstetrics & Gynecology Science*, 2019. **62**(6): p. 420-428.
 162. B., R., *Fundamentals of Biostatistics*. 7th Edition ed. 2011.

