

Study Protocol

Efficacy of Metformin in Pregnant Obese Women, a Randomised Controlled Trial.

EMPOWaR

Short Title	EMPOWaR
Aim	The aim of this study is to determine if metformin, administered to obese women during pregnancy, reduces the future life risk of obesity and metabolic syndrome in their babies.
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PROTOCOL REVISIONS

PROTOCOL VERSION	DATE	REASON FOR UPDATE	SUBSTANTIAL AMENDMENT NUMBER	SUMMARY OF CHANGES
1	06 th January 2010	Submitted to Ethics and MHRA	N/A	Initial Protocol
1	04 th March 2010	Additional information provided to the MHRA who considered this an amended request for CTA.	1	Additional information provided to MHRA after initial non acceptance.
2	20 th September 2010	Updated protocol and other supporting documents.	2	Protocol modified Version 2 Expanded details about the substudies PIL and consent forms amended to version 3 September 2010; to update due to the additional information added to the protocol Addition of new site and PI Sheffield Dr. Hany Lashen Additional documents: EMPOWaR Treatment Diary Version 1 16/06/2010 EMPOWaR Participant contact information Version 1 13/09/2010 EMPOWaR GP Letter Version 1 20/09/2010

PROTOCOL VERSION	DATE	REASON FOR UPDATE	SUBSTANTIAL AMENDMENT NUMBER	SUMMARY OF CHANGES
3	13 th April 2011	Change in site and addition of site. Changes to the protocol and supporting documents	3	PI contact address and site changed for Prof. Siobhan Quenby Additional site: Dr. George Bugg Nottingham. PIL and consent forms amended to version 4, April 2011 – all references to obesity removed Table of study assessments - errors corrected 1 hour GTT blood sample removed from visits Para 6.1 additional text added: "where a letter inviting women to participate may be issued" G.P. Letter, Advert for paper, Advert for waiting rooms (poster text) and Slip for notes (invitation letter) updated to version 2, April 2011 All reference to obesity removed.
3	19 th July 2011	Addition of new PI	4	Professor Tuffnell added as PI Bradford.
4	30 th September 2011	Change to reference range for entry liver function test Clarification re exclusion criteria for GDM	5	Change to reference range for entry liver function test Clarification re exclusion criteria for GDM - in future relate just to WHO and not SIGN guidelines Names of recruiting hospitals deleted from general protocol. Delete reference to Matsuda index

CONTENT

PROTOCOL APPROVAL	4
LIST OF ABBREVIATIONS	5
FLOWCHART	6
1. INTRODUCTION	7
1.1 BACKGROUND	9
1.2 RATIONALE FOR STUDY	10
2. STUDY OBJECTIVES	13
2.1 OBJECTIVES	13
2.2 ENDPOINTS	14
3. STUDY DESIGN	15
4. STUDY POPULATION	15
5. NUMBER OF PARTICIPANTS	16
5.1 INCLUSION CRITERIA	16
5.2 EXCLUSION CRITERIA	16
6. PARTICIPANT SELECTION AND ENROLMENT	17
6.1 IDENTIFYING PARTICIPANTS	17
6.2 CONSENTING PARTICIPANTS	18
6.3 SCREENING FOR ELIGIBILITY	18
6.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS	18
7. RANDOMISATION	18
7.1 TREATMENT ALLOCATION	19
7.2 EMERGENCY UNBLINDING PROCEDURES	19
7.3 PREMATURE WITHDRAWAL	19
8. INVESTIGATIONAL MEDICINAL PRODUCT AND PLACEBO	20
8.1 STUDY TREATMENTS	20
8.2 STUDY DRUG MANUFACTURER	20
8.3 MARKETING AUTHORISATION HOLDER	21
8.4 MARKETING AUTHORISATION NUMBER(S)	21
8.5 LABELLING AND PACKAGING	21
8.6 STORAGE	21
8.7 SUMMARY PRODUCT CHARACTERISTICS	21
8.8 DOSING REGIME	22
DOSING REGIME TABLE:	22
8.9 DOSE CHANGES	23
8.10 PARTICIPANT COMPLIANCE	23
8.11 OVERDOSE	23
8.12 OTHER MEDICATIONS	23
9. STUDY ASSESSMENTS	24
9.1 SAFETY ASSESSMENTS	24
9.2 STUDY VISIT 1	24
STUDY ASSESSMENTS TABLE	25
9.3 STUDY VISIT 2	27
9.4 STUDY VISIT 3	28
9.5 STUDY VISIT 4	29
9.6 STUDY VISIT 5	29
9.7 STUDY VISIT 6	30
9.8 STUDY VISIT 7	30
9.9 STUDY VISIT 8	31
9.10 STUDY VISIT 9	32
9.11 SUB-STUDIES	32
10. DATA COLLECTION	34
11. STATISTICS AND DATA ANALYSIS	34
11.1 SAMPLE SIZE CALCULATION	34
11.2 PROPOSED ANALYSES	35
12. ADVERSE EVENTS	35
12.1 DEFINITIONS	36
12.2 DETECTING AEs AND SAEs	36
12.3 RECORDING AEs AND SAEs	37
12.4 EVALUATION OF AEs AND SAEs	38
12.5 REPORTING OF SAEs/SARs/SUSARs	40

12.6	REGULATORY REPORTING REQUIREMENTS	40
12.7	FOLLOW UP PROCEDURES.....	41
12.8	OUT OF HOURS COVER.....	41
13.	TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS.....	42
13.1	TRIAL MANAGEMENT GROUP	42
13.2	CENTRAL TRIAL OFFICE	42
13.3	TRIAL STEERING COMMITTEE.....	43
13.4	DATA MONITORING COMMITTEE.....	43
13.5	INSPECTION OF RECORDS	43
13.6	STUDY MONITORING.....	43
13.7	RISK ASSESSMENT	43
14.	GOOD CLINICAL PRACTICE	44
14.1	ETHICAL CONDUCT	44
14.2	REGULATORY COMPLIANCE.....	44
14.3	INVESTIGATOR RESPONSIBILITIES	44
15.	STUDY CONDUCT RESPONSIBILITIES.....	47
15.1	PROTOCOL AMENDMENTS	47
15.2	PROTOCOL VIOLATIONS AND DEVIATIONS.....	47
15.3	STUDY RECORD RETENTION.....	48
15.4	SERIOUS BREACH REQUIREMENTS	48
15.5	END OF STUDY.....	48
15.6	CONTINUATION OF DRUG FOLLOWING THE END OF STUDY	49
15.7	INSURANCE AND INDEMNITY.....	49
16.	REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS.....	50
16.1	AUTHORSHIP POLICY.....	50
16.2	PUBLICATION	50
17.	PEER REVIEW.....	50
18.	REFERENCES	50
	APPENDIX 1: SUMMARY OF PRODUCT CHARACTERISTICS.....	53
	APPENDIX 2: TRIAL STEERING COMMITTEE	54
	APPENDIX 3: DATA MONITORING COMMITTEE.....	55
	APPENDIX 4: WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI.....	56
	APPENDIX 5: TIMELINES FOR NOTIFICATION	57
	APPENDIX 6: DRUG LABEL, VERSION 3 20/09/2010	58
	APPENDIX 7: VASCULAR STUDIES	59
	APPENDIX 8: HYPERINSULINAEMIC EUGLYCAEMIC CLAMP FOR THE EMPOWAR STUDY.....	62
	APPENDIX 9: MRI SCANNING.....	66

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EudraCT number 2009-017134-47

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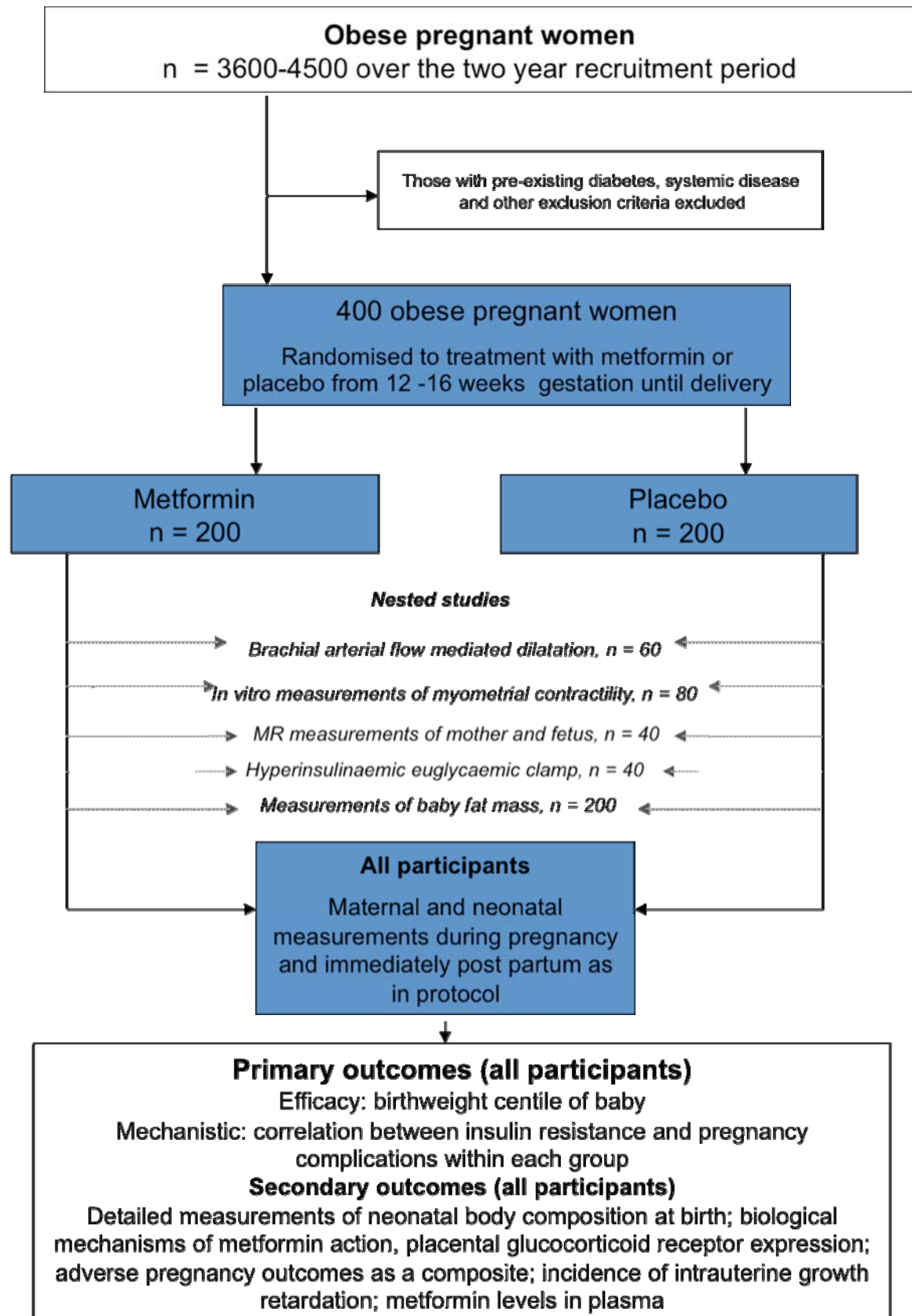
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LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research and Development
AE	Adverse Event
AR	Adverse Reaction
BMI	Body Mass Index
DNA	Deoxyribonucleic acid
eCRF	Electronic Case Report Form
ECTU	Edinburgh Clinical Trials Unit
GCP	Good Clinical Practice
IMP	Investigational Medicinal Product
IR	Insulin Resistance
ISF	Investigator Site File
NICE	National Institute for Health and Clinical Excellence
PCOS	Polycystic Ovary Syndrome
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UAR	Unexpected Adverse Reaction

FLOWCHART



1. INTRODUCTION

Obesity amongst adults and children is emerging as a major public health issue in the UK as in other developed nations. Increasing evidence, suggests that adult obesity has its origins at or prior to birth, with positive correlations between high birthweight and adult obesity in men and women in both large epidemiological studies (Curhan, Chertow et al. 1996; Curhan, Willett et al. 1996) and in a systematic review (Parsons, Power et al. 1999). Although the relationship between birthweight and adult obesity is J shaped, with low birthweight also predicting adult obesity, the rapid rise in the incidence of high birthweight (Surkan, Hsieh et al. 2004) and in maternal obesity means that the links between maternal obesity, high birthweight and later life obesity are a major concern.

The prevalence of obesity amongst pregnant women is rising rapidly and is now over 15% in many UK hospitals. Women who are obese during pregnancy have a significantly increased risk of adverse outcomes including maternal death, gestational diabetes, pre-eclampsia and caesarean section. Their babies are more likely to be stillborn and/ or larger than average when compared with lean pregnant women. In addition to these immediate problems, there is evidence that the bad effects of maternal obesity during pregnancy persist into the baby's adult life.

The higher than average baby birthweight leads to increased risk of the baby having obesity when he/she becomes an adult. If unchecked, this will lead to a further rise in the rates of obesity in the UK and other developed countries, with all the attendant medical problems (including diabetes, heart disease and premature death). We do not know how obesity in pregnant women causes these problems. We do know that obese pregnant women are more "insulin resistant" than lean pregnant women – i.e. they need to produce higher levels of the hormone insulin to keep their blood glucose levels at the same level. A modest degree of insulin resistance (IR) is normal in pregnancy so that there is a good supply of glucose and other food substrates for the baby to grow. However, excessive insulin resistance means that the food supply to the baby is potentially too great, leading to a birthweight that is too high. This link between high insulin resistance and high birthweight has already been demonstrated. Additionally, women with higher levels of blood glucose tend to have bigger babies and a greater incidence of pregnancy problems. Reducing their blood glucose seems to reduce the amount of pregnancy problems.

We believe that giving metformin to obese pregnant women will reduce their insulin resistance and therefore reduce the incidence of high birthweight. Importantly, the small studies that have been done so far suggest that metformin does not increase the risk of excessively low birthweight. We hope to confirm this in our study. By looking at changes in the blood of obese pregnant women and their babies given metformin or placebo, we will further be able to understand how obesity causes pregnancy problems. We will also look at women given metformin and those given placebo (likely to be those with normal and high insulin resistance) and relate this to the pregnancy outcomes they have. This should give us important information on whether reducing insulin resistance improves other pregnancy problems for obese pregnant women and their babies.

We will evaluate an intervention with the drug metformin (an insulin sensitizing agent) using a comparator placebo to measure the outcome birthweight centile, a surrogate marker of future life risk of obesity and metabolic syndrome. Additionally, we will use metformin administration as a tool to explore the mechanism by which obesity causes adverse pregnancy outcomes, with a particular focus on insulin resistance (IR). Lastly, we will explore mechanisms of action of metformin, and explore the efficacy of metformin in reducing other adverse pregnancy events.

Metformin reduces insulin resistance and is widely used to treat type II diabetes. Metformin has been shown to be safe during pregnancy and the National Institute for Health and Clinical Excellence (NICE) in the UK now recommends metformin as an alternative to insulin in women with established diabetes in pregnancy.

We believe that birthweight centile is an appropriate surrogate marker for the lifetime risk of obesity and metabolic syndrome. Increasing evidence suggests that adult obesity has its origins at or prior to birth, and that intrauterine events leading to high birthweight cause later life obesity.

A second major aim is to use this intervention study to try and determine how obesity causes adverse pregnancy outcomes. Previous studies and our other ongoing studies focus on correlation analyses but gives only restricted information on cause and effect. In this study, by reducing IR, we will be able to determine whether abnormalities in IR are responsible for some or all of the adverse effects of obesity on pregnancy outcome.

1.1 BACKGROUND

The WHO defines as obesity as "a state of excess fatness which increases the risk of ill health". A more pragmatic definition of obesity is a body mass index (BMI) of ≥ 30 kg/m². Rates of obesity in adults and children are rising exponentially in the UK as in other developed nations, and are major causes for concern. Around 24% of adult males, 27% of adult females, and 20% of children aged 11-12 now are obese (Scottish Health Survey 2003). There has been a significant increase in both mean birthweight and in the incidence of being born large for gestational age over the last few decades (Surkan, Hsieh et al. 2004) which will fuel the increase in subsequent child/ adult obesity. The increase in average UK BMI during pregnancy in over the last twenty years has been well documented (Kanagalingam, Forouhi et al. 2005) and rates of maternal obesity (BMI >30 kg/m²) in women booking for antenatal care are over 15% in both Edinburgh and Liverpool. The secular rise in maternal weight at delivery appears to be the factor most strongly correlated with the increase in birthweight (Surkan, Hsieh et al. 2004; Catalano and Ehrenberg 2006). Thus the increase in rates of maternal obesity are setting up a vicious cycle, leading to increased birthweight and increased rates of child and adult obesity, contributing to an epidemic of obesity which has become one of the most significant contributors to global ill health (Organisation 2000; Catalano 2003).

Although, lifestyle (diet and exercise) interventions might be seen as the logical approach to the problem of obesity in pregnancy, studies hitherto have shown no evidence of benefit (Dodd, Crowther et al. 2008). Others in the UK and around the world are trialling the effects of an intensive lifestyle intervention in pregnancy (e.g. Poston, NIHR funded [UK with whom we have close collaborative links] and Dodd [Australia]). In this study, we will apply current standards of dietary advice and exercise promotion to all women in this study. However, we believe that metformin, a pharmacological intervention, not only increases options for women, but it is likely more readily translatable into clinical practice, and by rapidly reducing IR will likely have beneficial therapeutic effects over and above lifestyle interventions.

The link between maternal obesity, high birthweight of the baby and later life obesity of the baby has been shown in many studies. As more women enter pregnancy with a body mass which would define them as "obese" an intervention is urgently required to stop this health risk being passed on to the next generation. Unfortunately many drugs that might be effective are unsuitable for use in pregnancy. In contrast,

metformin appears safe during pregnancy. In this study, we will (for the first time) test its efficacy in reducing high birthweight in the children of obese pregnant women.

1.2 RATIONALE FOR STUDY

This study is timely because of emerging evidence about the safety and efficacy of metformin, a licensed first line therapy for the treatment of type II diabetes, in pregnancy (Rowan, Hague et al. 2008) and preliminary data in pregnant women with polycystic ovary syndrome (PCOS) (Vanky, Salvesen et al. 2004). The use of metformin in gestational diabetes is now endorsed by NICE. To our knowledge, there have been no studies in obese pregnant women to determine the efficacy of metformin in reducing birthweight and adverse pregnancy outcomes.

The problem of maternal obesity, leading to programming of future life obesity risk in the offspring, and manifest by excess birthweight, is reaching epidemic proportions. We believe that metformin will likely be an effective therapy in interrupting this cycle, and improving pregnancy outcome for both baby and mother. Additionally, intervention with metformin will allow us to explore the mechanism by which obesity causes adverse pregnancy outcome. This study sets out a multidisciplinary programme for testing this hypothesis.

Positive correlations have been shown between birthweight and both maternal pregravid weight (Eastman and Jackson 1968; Sewell, Huston-Presley et al. 2006; Hull, Dinger et al. 2008) and weight gain in pregnancy (Humphreys 1954), with odd ratios of high birthweight some 2.4 times greater in morbidly obese compared with lean women (Heslehurst, Simpson et al. 2008). Thus high maternal weight and weight gain “programmes” obesity in the baby, perpetuating a vicious cycle. An effective intervention applied in pregnancy could make a major impact in interrupting this cycle, and reversing upward secular trend in obesity rates.

A systematic review showed a positive correlation between birthweight and obesity during adulthood (Parsons, Power et al. 1999) and the link between high birthweight and later life obesity is also supported by two large epidemiological studies. For example, in the Health Professionals Follow Up Study which surveyed over 22,000 men aged between 40 and 75, the age-adjusted odds ratio of being in the highest versus the lowest quintile of adult body mass index for men with birth weight greater or equal to 4.55 Kg (10.0 lbs) (compared to the referent group) was 2.08 (95% CI, 1.73 to 2.50)(Curhan, Willett et al. 1996). In the Nurses Health Studies I and II

(consisting of over 160,000 women aged 25 -55) those who weighed >10 lb at birth had an age-adjusted odds ratio of 1.62 (95% CI, 1.38 to 1.90) of being in the highest (>29.2 kg/m²) versus the lowest (<21.9 kg/m²) quintile of body mass index in midlife (Curhan, Chertow et al. 1996). For both studies, the authors concluded that “These findings support the hypothesis that early life exposures, for which [high and low] birth weight is a marker, are associated withchronic diseases in adulthood”. These two studies found obesity but not metabolic syndrome to be a long term correlate of high birthweight. However, a subsequent prospective study found that children who were large for gestational age at birth and exposed to an intrauterine environment of maternal obesity are also at increased risk of developing metabolic syndrome (hazard ratios of developing metabolic syndrome of 2.19 [1.25 – 3.82] and 1.81 [1.03 –3.19] in association with being large for gestational age and maternal obesity respectively) (Boney, Verma et al. 2005).

Other systematic reviews have highlighted the relationship between maternal obesity and other pregnancy complications such as pre-eclampsia (with the risk of pre-eclampsia doubling for each 5–7 kg/m² increase in prepregnancy body mass index) [30] caesarean section (OR 2.00, [95% CI 1.87-2.15]), longer hospital stay (OR 2.84 [2.77 – 2.91]) and maternal haemorrhage (OR 1.24 [95% CI 1.20 – 1.28]); and baby complications such as longer neonatal intensive care unit stay (OR 1.35 [1.22-1.49]) and stillbirth in addition to high birthweight (Heslehurst, Simpson et al. 2008).

The increased risk of maternal death amongst obese pregnant women was highlighted in the most recent publication from CEMACH (the Confidential Enquiry on Maternal and Child Health - www.cemach.org.uk). Importantly, there is a dose response effect between interpregnancy weight gain and adverse pregnancy outcomes such as pre-eclampsia, caesarean section, stillbirth and birth of a large for gestational weight offspring, even in women who remain in the “normal” pre-pregnancy BMI range (Villamor and Cnattingius 2006). The mechanism by which obesity increases pregnancy and peripartum complications is unclear, but there are likely several candidate mechanisms. IR and endothelial dysfunction are associated both with obesity (in pregnant and non pregnant individuals) and pre-eclampsia, and are highly likely contender pathways by which obesity increases pre-eclampsia risk (Walsh 2007). Increased birthweight (probably driven in part by maternal IR as reviewed above) likely contributes to the greater risk of caesarean section and maternal haemorrhage in obese women. Additionally, myometrial contractility is poorer in obese pregnant women (Zhang, Bricker et al. 2007) with our own

unpublished data suggesting that this relates to IR: further increasing the risk of caesarean section and maternal haemorrhage.

If our hypotheses about the causes of high birthweight and selected other adverse pregnancy outcomes in obese pregnant women are correct, then metformin might be the ideal therapy. Metformin's principal mechanism of action includes improvement of IR in liver and smooth muscle, which also improves endothelial function [33] increased peripheral uptake of glucose, improved lipid profile, redistribution of fat from visceral to subcutaneous deposits and antioxidant effects – all of which are likely to contribute to its clinical efficacy in reducing adverse outcomes in obese pregnant women (Scarpello and Howlett 2008). The MIG study recently compared the effect of metformin versus placebo in women with gestational diabetes (Rowan, Hague et al. 2008). Metformin reduced the proportion of women requiring insulin by 50% with no adverse pregnancy outcomes. Weight gain in pregnancy (a known additional driver of birthweight) was lower in the metformin group (difference of 1.6kg, $p < 0.001$). A small randomised study of pregnant women with a history of PCOS (polycystic ovarian syndrome - a condition often accompanied by IR) showed that metformin reduced pre-defined pregnancy complications from 32% in the placebo group to 0% in the metformin group ($p=0.01$) [16]. Importantly, if metformin is to be effective in reducing high birthweight in obese women, it should not increase the proportion of babies with low birthweight - two small studies on women with a mean BMI $< 30\text{kg/m}^2$ are reassuring on this point (Vanky, Salvesen et al. 2004; Nawaz, Khalid et al. 2008).

Metformin, an insulin sensitizing agent, might be ideal for this purpose. Considerable evidence implicates IR (the inability of a defined concentration of insulin to effect a predictable biologic response of nutrient metabolism at the level of target tissue) and / or hyperglycaemia as the mechanism by which maternal obesity causes excessive neonatal birthweight as follows:

- A. Whilst modest IR is physiological in pregnancy and generates maternal glucose, free fatty acids and amino acids as substrates for fetal growth; obese pregnant women are significantly more insulin resistant than their lean counterparts (Catalano and Ehrenberg 2006) leading to a further amplification of nutrient availability with consequent excessive fetal growth.
- B. There is a strong correlation between IR in late gestation and both birthweight and fat free mass at birth (Catalano, Thomas et al. 2003).

- C. The HAPO trial (Metzger, Lowe et al. 2008) confirms that there is a linear relationship between hyperglycaemia and birthweight, even at glucose levels not normal considered abnormal during pregnancy
- D. The ACHOIS study (Crowther, Hiller et al. 2005) confirms that treating hyperglycaemia has a highly significant effect in reducing both the incidence of large for gestational age babies and other perinatal complications.

Thus metformin, by reducing IR, could have a major impact in reducing excess birthweight in obese pregnant women. Importantly metformin is unique amongst insulin sensitizing agents in being weight neutral and not promoting weight gain. This appears true in pregnancy also: in the MIG study, pregnancy weight gain in the metformin group was significantly lower than in the placebo arm (Rowan, Hague et al. 2008). Given the additional role of weight gain during pregnancy in promoting high birthweight (Humphreys 1954) metformin's actions in reducing IR and in minimizing weight gain are likely to act synergistically to reduce birthweight.

2. STUDY OBJECTIVES

The aim of this study is to determine if metformin, administered to obese women during pregnancy, reduces the future life risk of obesity and metabolic syndrome in their babies. We will use birthweight centile as a surrogate marker for future life events as its validity has been shown in large epidemiological studies.

Secondly, we will use metformin administration as a tool to explore the mechanism by which obesity causes adverse pregnancy outcomes, with a particular focus on insulin resistance (IR). We seek to evaluate the efficacy of metformin (in comparison with placebo) in reducing future life risk of obesity and metabolic syndrome in the babies of obese pregnant women. We will use birthweight centile as a surrogate marker for future life risk of obesity and metabolic syndrome.

2.1 OBJECTIVES

2.1.1 Primary Objective

1. What is the efficacy of metformin (up to 2500mg daily), given to obese pregnant women from 12 - 16 weeks gestation until delivery in reducing birthweight centile of the baby?
2. What is the pattern of association between insulin resistance and adverse pregnancy outcome in obese pregnant women?

2.1.2 Secondary Objectives

Our secondary objectives aim to determine whether abnormalities in IR are responsible for some or all of the adverse effects of obesity on pregnancy outcome, to explore detailed effects of metformin on the baby's body composition, the mechanistic effects of metformin, and metformin's efficacy in reducing clinical complications of mother and baby. Thus our specific secondary questions are, in relation to administration in obese pregnant women:

- i) What is the effect of metformin on detailed measurements of the baby's body composition?
- ii) What is the effect of metformin on maternal IR at 28 and 36 weeks gestation and on hepatic and skeletal muscle insulin sensitivity at 36 weeks gestation?
- iii) What is the effect of metformin on maternal inflammatory variables (measured at 28 and 36 weeks gestation) and on neonatal inflammatory variables (measured in cord blood at birth)?
- iv) What is the effect of metformin on maternal endothelial dependent flow mediated dilatation at 36 weeks gestation?
- v) What is the effect of metformin on myometrial contractility increased peripheral uptake of glucose, measured in vitro in myometrial biopsies removed at the time of caesarean section?
- vi) What is the effect of metformin on maternal and fetal visceral and subcutaneous adipose tissue depth and fetal hepatic volume?
- vii) What is the efficacy and effect size of metformin on composite adverse maternal and baby clinical outcomes including incidence of pregnancy induced hypertension and/or pre-eclampsia, caesarean section, primary post partum haemorrhage, maternal weight gain during pregnancy, and incidence of the baby's admission to the neonatal unit?
- viii) Can we confirm that metformin does not increase the rate of babies with a low birthweight centile?
- ix) What is the efficacy (as opposed to effectiveness) of metformin when analysis is restricted to those with pharmacological circulating levels of drug?

2.2 ENDPOINTS

2.2.1 Primary Endpoint

1. Efficacy outcome – z-score corresponding to the gestational age and sex adjusted birthweight centiles of the baby

2. Mechanistic outcome - maternal insulin resistance (IR) at 36 weeks gestation which will be correlated with adverse pregnancy outcomes.

2.2.2 Secondary Endpoints

- (i) More detailed measurements of neonatal body composition at birth including ponderal index; skinfold thickness; and (Edinburgh babies only) neonatal fat mass measured using air displacement plethysmography.
- (ii) Biological mechanisms of metformin action including
 - a. change in whole body IR (longitudinal studies at 28 and 36 weeks); hepatic and skeletal insulin sensitivity (36 weeks)
 - b. maternal and neonatal inflammatory and lipid and fatty acid indices including CRP, IL-6, leptin, full lipid profile, non-esterified fatty acids, polyunsaturated fatty acids and PAI1/PAI2 ratio
 - c. placental glucocorticoid receptor expression
 - d. maternal brachial arterial endothelial dependent flow mediated dilatation (FMD)
 - e. in vitro measurements of maternal myometrial contractility
- (iii) Maternal, fetal and neonatal anthropometry.
- (iv) Adverse pregnancy outcomes as a composite; incidence of low birthweight centile.
- (v) Gas chromatography mass spectrometry measurements of metformin in maternal plasma to determine compliance.

3. STUDY DESIGN

The design is a double blind randomised placebo controlled trial, with embedded sub-studies to explore mechanisms of action of metformin, in a population of 400 obese pregnant women.

4. STUDY POPULATION

We will invite obese pregnant women to participate in a double blind randomised study where they are given either metformin or placebo (dummy) tablets. We will track them during pregnancy and look at the birthweight of the baby. Additionally we will collect information on other pregnancy complications for mother and baby. We will look at the effect of metformin on biochemical factors in the mother and baby's blood, and in the placenta.

We plan to focus recruitment in the following: Edinburgh Royal Infirmary, Birmingham Heartlands NHS Foundation Trust, Liverpool Women's Maternity Hospital and the Jessop Hospital for Women, Sheffield. These centres care for a combined population of over 20,000 women per year, of whom 10 - 15% fulfil the inclusion criteria for this project. Additionally, these hospitals are amongst the very few in the UK to have set up specialist multidisciplinary antenatal clinics for obese pregnant women. However, other centres with the enthusiasm, infrastructure and the resource to recruit participants may be included in due course.

5. NUMBER OF PARTICIPANTS

We aim to recruit 400 women attending the antenatal clinics specialising in the care of obese pregnant women in maternity hospitals in the UK. Women will be recruited over a period of 2 years at an anticipated rate of 1-2 participants per week in each centre. Women will be between 12 and 16 weeks gestation and must be willing to be randomised to either active (metformin) or placebo treatment.

5.1 INCLUSION CRITERIA

- Caucasian obese (BMI ≥ 30 kg/m²) pregnant women between $\geq 12^{\text{w}}$ and $\leq 16^{\text{w}}$ weeks gestation
- Women age ≥ 16 years
- Signed informed consent form

5.2 EXCLUSION CRITERIA

- Non Caucasian women
- Women with a BMI ≤ 29 kg/m²
- Gestation $>$ than 16 weeks
- Women with pre-existing diabetes
- Women with gestational diabetes in a previous pregnancy
- Women with systemic disease at the time of trial entry, with the disease either requiring regular medication, or having required treatment with systemic steroids within the last 3 years
- Gestational diabetes in index pregnancy (diagnosed with 75g OGTT (WHO criteria – see page 27) prior to randomisation
- Previous delivery of a baby $<$ 3rd centile or previous pregnancy with pre-eclampsia prompting delivery before 32 weeks gestation

- A known hypersensitivity to metformin hydrochloride or to any of the excipients
- Known liver failure or dysfunction at the time of trial entry (tested prior to randomisation)
- Known renal failure or dysfunction at the time of trial entry (tested prior to randomisation)
- Acute conditions at the time of trial entry with the potential to alter renal function such as:
 - Dehydration sufficient to require intravenous infusion
 - severe infection
 - shock
 - intravascular administration of iodinated contrast agents
 - acute or chronic diseases which may cause tissue hypoxia such as:
 - cardiac or respiratory failure,
 - recent myocardial infarction,
 - hepatic insufficiency, acute alcohol intoxication, alcoholism,
- Lactation
- Multiple pregnancy

6. PARTICIPANT SELECTION AND ENROLMENT

6.1 IDENTIFYING PARTICIPANTS

We will recruit pregnant women who are booked for their delivery at one of the hospitals participating in the study and who meet the study eligibility criteria. Case notes of pregnant women will be reviewed for the patient's potential eligibility from women attending the antenatal clinics into the trial; where a letter inviting women to participate may be issued. Additionally, we will ask midwives to identify eligible participants when the woman books for antenatal care. Eligible patients will be informed of the study at routine antenatal appointments and will be asked for permission for their details to be passed to the Principal Investigator (or their team) at each site

No personal identifiers will be recorded at pre-screening although the reason for an eligible patient being excluded will be recorded where possible for input into trial metrics as per the CONSORT statement (<http://www.consort-statement.org>).

6.2 CONSENTING PARTICIPANTS

Women will be approached by a member of the clinical staff, normally an obstetrician or midwife. Women will be given information about the study and they will be allowed adequate time (at least 24 hours) to read the information and consider the invitation to participation in the study.

6.3 SCREENING FOR ELIGIBILITY

Participants who fulfil all the potential eligibility criteria and who express an interest in the study will have a blood test for renal and liver function and a formal glucose tolerance tests. These tests will be performed after consent for the study is signed but before randomisation. Recruitment targets are based on randomised patients.

6.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

No further information will be collected on women who are ineligible solely because of abnormalities in glucose handling, liver or renal function, others than the number of such women for inclusion in trial metrics.

7. RANDOMISATION

A central randomisation facility based at the ECTU will be available by telephone, or, randomisation can be carried out online via the web portal. Users will be assigned a personal identifier number. Randomisation will be stratified by treatment centre and BMI 30-39 kg/m² versus ≥ 40 kg/m².

Following completion of the consenting procedures patients will be randomly assigned to active treatment with metformin or an identical looking placebo.

The patient's paper case records and/or computer file will be noted to show that they are participating in this trial.

The study will be double blind, so neither the patient nor the Investigator will know which treatment has been allocated.

7.1 TREATMENT ALLOCATION

Following randomisation to either metformin or matching placebo, patients will be given a study prescription to take to the site Pharmacy where they will receive their allocated blinded treatment. The prescription will provide details of number of tablets with instructions for tablets to be taken with food.

The PI should advise the local pharmacy when a patient finishes treatment as scheduled or stops taking treatment.

7.2 EMERGENCY UNBLINDING PROCEDURES

The study will be performed double blinded so neither the patient nor the Investigator will know which treatment has been allocated. Breaking of the study blind should only be performed where knowledge of the treatment is absolutely necessary for further management of the patient.

There will be no central unblinding facility but the site pharmacies are provided with the key which links drug pack number to treatment. Thus unblinding (emergency or otherwise) can be carried out by a pharmacist if requested by a senior clinician (normally a consultant). The name of the clinician requesting the unblinding, the reasons for it and notification of any unblinding will be sent to the Chief Investigator via e-mail. Reasons for unblinding will be collected via the e-CRF. Unless there is a clinical requirement, the blind will not be broken until after data entry is complete, the validity of the data is checked, all queries resolved and the patient populations agreed.

7.3 PREMATURE WITHDRAWAL

Participation in the study is voluntary. A patient has the right to discontinue the drug or completely withdraw from the study at any time for any reason. The Investigator has the right to discontinue a patient taking the drug at any time if it is deemed to be in the patient's best interest.

If the participant is withdrawn due to a serious adverse event, the Principal Investigator (PI) will arrange for follow-up visits or telephone calls until the event has resolved or stabilised. However as the participants are pregnant women the data will

be collected to outcome (i.e. Delivery) and data will be used in the analysis unless the consent to collect the outcome is specifically refused by the participant.

Women may also temporarily halt the treatment and restart if it considered appropriate by the local PI. The reason, duration and circumstances for premature discontinuation will be documented in the eCRF.

8. INVESTIGATIONAL MEDICINAL PRODUCT AND PLACEBO

8.1 STUDY TREATMENTS

Active drug: Metformin (Glucophage) 500mg film-coated tablet

Placebo: Placebo tablets will be identical to the active tablets except for the active ingredient.

Both placebo and active drugs will be supplied by Merck Sante.

8.2 STUDY DRUG MANUFACTURER

Active drug:

Merck Serono
Merck Santé s.a.s.
Centre de Production
2, rue du Pressoir Vert
45400 SEMOY
FRANCE

Supplied by:

Merck Santé S.A.S
Merck Santé s.a.s.
10 avenue de Lattre de Tassigny
69330 MEYZIEU
France

Placebo:

DELPHARM SA
rue du Petit Paris
F - 91731
Bretigny Sur Orge Cedex

8.3 MARKETING AUTHORISATION HOLDER

Active drug:

Lipha Pharmaceuticals Limited
Bedfont Cross
Stanwell Road
Feltham
Middlesex
TW14 8NX
UK

Placebo drug:

Not applicable

8.4 MARKETING AUTHORISATION NUMBER(S)

Active drug: PL 03759/0012-0013

Placebo: Not Applicable

8.5 LABELLING AND PACKAGING

Both the active and placebo treatments will be labelled and packaged by the supplier (Merck Santé) in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004. Drugs will be supplied in bottles each containing 90, 500mg tablets. Six bottles will normally be dispensed to a participant at any one time.

8.6 STORAGE

This IMP does not require any special storage conditions.

8.7 SUMMARY PRODUCT CHARACTERISTICS

The Summary of Product Characteristics (SmPC) is given in Appendix 1.

Licensed indications are for the treatment of type 2 diabetes mellitus in adults, particularly in overweight patients, when dietary management and exercise alone

does not result in adequate glycaemic control. Metformin will not be used within its licensed indications for this study.

Contraindications are noted as the exclusion criteria for this study.

8.8 DOSING REGIME

Women will be prescribed metformin tablets up to 2500mg from 12-16 weeks gestation and will stop once they have delivered their baby.

Women will be asked to start with 500mg metformin (1 tablet, Once Daily) taken with food, increasing in week 2 by an increment of 500mg per day (in other words to 1 tablet, twice daily). Week 3: a further increment of 500mg per day (in other words to 1 tablet, three times daily). In the fourth week the women will increase the evening dose of metformin by a further 500mg and in the fifth week the morning dose will also be increased by a further 500mg.

If side effects (largely anticipated to be gastro-intestinal) are experienced, the woman should drop to the previous week's dose or 500mg metformin (whichever is the greater) and wait for a week before increasing the dosage again. The maximum recommended dose is 2500mg daily, taken as three divided doses.

The usual starting dose is one tablet 2 or 3 times daily given during or after meals.

DOSING REGIME TABLE:

WEEK	Total Daily Dose	Route
1	500mg	Orally one tablet daily
2	1000mg	Orally one tablet, twice daily (BID)
3	1500mg	Orally one tablet, three times daily (TID)
4	2000mg	Orally, three times daily Morning: 500mg Midday: 500mg Evening: 1000mg

WEEK	Total Daily Dose	Route
5	2500mg	Orally, three times daily Morning: 1000mg Midday: 500mg Evening: 1000mg
6 to delivery	2500mg MAX NB: The dose may be adjusted down by the PI	Orally, three times daily Morning: 1000mg Midday: 500mg Evening: 1000mg

8.9 DOSE CHANGES

The local investigator may alter the above treatment regimen at his/her discretion, so long as the maximum daily dose does not exceed 2500mg in three divided doses. Changes to treatment dose will be recorded on the patient's eCRF as soon as practicable.

8.10 PARTICIPANT COMPLIANCE

Participants will be asked to keep a diary to record drug intake and to bring all medication to each study visit so that the midwife can record how much study drug has been taken. Additionally, metformin levels will be analysed in a blood sample taken in the third trimester by gas chromatography mass spectrometry.

8.11 OVERDOSE

Hypoglycaemia has not been seen with metformin hydrochloride doses of up to 85g, although lactic acidosis has occurred in such circumstances. High overdose of metformin hydrochloride or concomitant risks may lead to lactic acidosis. Lactic acidosis is a medical emergency and must be treated in hospital. The most effective method to remove lactate and metformin hydrochloride is haemodialysis.

8.12 OTHER MEDICATIONS

Prohibited Medications

- Alcohol and alcohol containing medicinal products since there is increased risk of lactic acidosis in acute alcohol intoxication, particularly in case of fasting, malnutrition, or hepatic insufficiency. Pregnant women should avoid alcohol in any case, so this is not likely to be a significant issue in practice.
- Iodinated contrast agents should also be avoided since intravascular administration of iodinated contrast agents may lead to renal failure, resulting in metformin hydrochloride accumulation and an increased risk of lactic

acidosis. Thus any woman in the study who needs such an agent will be advised to discontinue metformin hydrochloride must be discontinued prior to, or at the time of the test and not restart metformin until 48 hours afterwards, and only after renal function has been re-evaluated and found to be normal.

- Glucocorticoids (systemic and local routes), beta-2-agonists, and diuretics may attenuate metformin's potential hypoglycaemic effect and ACE inhibitors may amplify it. Use of these agents is not prohibited but they should be used with care in women participating in this study and after discussion with the local principal investigator.

9. STUDY ASSESSMENTS

9.1 SAFETY ASSESSMENTS

Safety will be assessed at each routine clinic visit and documented as noted in the section 12. Additionally, interim un-blinded outcome data will be made available to the Data Monitoring Committee (DMC).

9.2 STUDY VISIT 1

Information Visit - Conducted between Week 10⁰ and 16⁺⁰ weeks gestation

Women attending the antenatal clinics who meet the eligibility criteria will be provided with a copy of the Patient Information Leaflet at or prior to the routine visit and given the opportunity to discuss the study and to ask any questions. The inclusion / exclusion checklist will be used as a guide to eligibility at this stage, and may be completed in part if wished.

After the woman has had the opportunity to consider whether she would like to participate (24 hours is recommended) she will be invited to attend the randomisation visit.

Additionally at the Edinburgh site women will also be invited to donate tissue, blood and other relevant samples to the ethically approved pregnancy bio-bank, which has a separate information sheet and consent form.

STUDY ASSESSMENTS TABLE

Responsibility, Site PI and Local teams									
Visit Number	1	2	3	4	5	6	7	8	9
Pregnancy gestation	10-16 weeks	12-16 weeks	12-16 weeks	18-20 weeks	28 Weeks	36 Weeks	Term	Labour/Delivery/Neonatal (discharge/ 28 days)	3 months postnatally
	Screening	Consent	Randomisation (baseline)	Study visit (could be by telephone)	Study visit	Study Visit	Study visit (could be by telephone)	Study visit	Study visit
Inc/Exc Criteria	X								
Patient Information Leaflet	X								
Consent Form		X							
Demographics		X							
Medical History		X							
Height and Weight		X							
Maternal anthropometry		x				x			x
Bloods for baseline assessments incl. LFT/renal function		X							
Fasted GTT (sampling at baseline and 2 hrs)		X			X	X			
Stored sample for inflammatory markers etc		x			x	x			
Vital Signs		X							
Study Drug dispensed			X		X				
Randomisation			X						
Unused Study Drug /packaging returned								x	
Review SAE's				X	X	X	X	X	
Complete side effects questionnaire				X	X	X			
Review and record pregnancy complications					X	X	X	X	

Responsibility, Site PI and Local teams									
Visit Number	1	2	3	4	5	6	7	8	9
Pregnancy gestation	10-16 weeks	12-16 weeks	12-16 weeks	18-20 weeks	28 Weeks	36 Weeks	Term	Labour/Delivery/Neonatal (discharge/ 28 days)	3 months postnatally
	Screening	Consent	Randomisation (baseline)	Study visit (could be by telephone)	Study visit	Study Visit	Study visit (could be by telephone)	Study visit	Study visit
Saliva samples for cortisol measurements (N = 50 each group or greater if possible)			x		x	x			
Bodpod measurements (Edinburgh participants only)		X or visit 3	X (or visit 2)			x			X
Hyperglycaemic euglycaemic clamp (Edinburgh subgroup N=40+)						X			X
Endothelial FMD (Edinburgh subgroup N=60+)						X			
MR scanning (Edinburgh subgroup n = 40+)					24-28w	x			
Labour/Delivery Information								x	
Cord blood & placenta (including samples for RNA/DNA)								X	
Myometrial, rectus sheath and adipose samples (if delivered by C/S)								X	
Baby's weight and anthropometry								X	X
Peapod assessment (Edinburgh babies only)								x	X
Child assessment									X

9.3 STUDY VISIT 2

Conducted between 12⁻⁰ and 16⁺⁰ weeks gestation - Consent and further eligibility screening.

Women who wish to participate will be asked to sign a consent form (including the bio-bank consent form for women at the Edinburgh site only). Consent for the mechanistic studies (where relevant) will be obtained either at Visit 2 or prior to randomisation at Visit 3. The original consent form(s) will be stored in the Investigator Site File (ISF) file, a copy is given to the woman, a copy added to the medical notes and a copy faxed to the Trial Office 0131 242 2686

Following completion of the consenting procedures the following baseline assessments will be performed:

- Demographics (patient and baby's putative father)
- Medical History including previous pregnancies
- Height and Weight
- Vital signs
- Baseline Blood and 75g oral glucose tolerance test (OGTT) with samples at baseline and 2 hrs.
- Blood sampling to test for liver and renal function
- Fasting blood sampling so that a sample can be stored for subsequent analysis of maternal inflammatory and metabolic indices (including but not limited to DNA CRP, IL-6, leptin, PAI1/PAI2 ratio, cortisol, cortisol binding globulin, [fasting] lipids (total cholesterol, HDL, calculated LDL) and triglycerides (serum gel sample); non esterified fatty acids (NEFAs)(serum sample); fatty acids in red cell membranes (red cell portion of spun EDTA sample).
- Other anthropometry measurement e.g. waist, hip, mid-arm and mid-thigh circumference, skinfolds triceps, biceps and subscapular, and bioimpedance (subset of participants only)
- Detailed measurement of body fat using BodPod machine (Edinburgh participants only, to be performed at visit 2 or visit 3)
- A sample pot will be given for the patient to supply a morning and evening salivary samples, which will be posted in to the laboratory.

9.4 STUDY VISIT 3

Conducted between 12-0 and 16+0 weeks gestation - Randomisation

Once renal and liver function and glucose tolerance have been confirmed to be normal women may be randomised to the study.

The Bodpod measurements may be performed at this visit if not done at the previous visit (Edinburgh participants only).

RENAL FUNCTION RANGE

A normal serum creatinine at baseline will be considered to be evidence of normal renal function. Reference ranges for renal function variables for the purposes of the study are as follows:

Urea	2.5 – 6.6 mmol/l
Creatinine	≤ 85 µmol/l (Girling, Dow et al. 1997; Girling 2000)
Sodium	135 - 145 mmol/l
Potassium	3.6 – 5.0 mmol/l

(It is accepted that these may differ slightly from the reference range in each hospital. The ranges provided were derived from the literature and planned to be “conservative” to avoid recruitment of women with renal or liver dysfunction).

LIVER FUNCTION RANGE

Reference ranges for liver function variables are as follows:

Bilirubin	3- 16 µmol/l
ALT	10 - 60 iu/l

(**Note**, if ALT is greater than 43 iu/l, please confirm that blood lactate levels are within the local laboratory reference range before randomisation. Women with an ALT of 43 or less do not need measurement of blood lactate).

OGTT TEST RANGE

Women with gestational diabetes diagnosed using the WHO guidelines will be excluded from the study. The guideline requires a fasting 8-10 hour sample and a 75 g OGTT: WHO criteria: fasting glucose ≥ 7.0 and 2 hour ≥ 7.8 mmol/l. If all tests are within or below the ‘normal ranges’ women will then be randomised and assigned to metformin or placebo.

For women participating in the vascular studies (flow mediated dilatation, augmentation index and pulsed wave velocity measurements), baseline measurements will be made either at visit 3 or at a subsequent additional visit prior to 16 weeks gestation AND prior to initiation of study medication.

A prescription will be issued for dispensing of the IMP by the local hospital pharmacy department. Medication will be dispensed in boxes containing 90 tablets. Six boxes will be dispensed at the first visit, sufficient for 16 weeks of treatment. A date for starting treatment should be agreed with the participant, and this should be no later than 16+0 weeks gestation.

9.5 STUDY VISIT 4

Conducted at 18 – 20weeks gestation.

Approximately 1 month after the commencement of treatment, information on any side effects, adverse events and pregnancy complications will be collected. Patients will be asked if they are complying with their study medication. This could be a virtual visit conducted by telephone.

ADDITIONAL STUDY VISIT

Week 24 – 28

Participants in the MR sub-study will have their first MR scan at a visit between 24 and 28 weeks gestation (**see Appendix 9**).

9.6 STUDY VISIT 5

Week 28 (+/- 7 days)

Information on pregnancy complications will be collected. Adverse events (AE) will be recorded in the patient notes, serious adverse events (SAEs) will be recorded on the SAE form. Patients will be asked if they are complying with their study medication. A repeat 75g OGTT (baseline and 2 hr samples) will be performed and fasting blood again taken and stored for inflammatory and metabolic indices (including but not limited to CRP, IL-6, leptin, PAI1/PAI2 ratio cortisol, cortisol binding globulin, and [fasting] full lipid profile (total cholesterol, HDL, calculated LDL) and triglycerides (serum gel sample); non esterified fatty acids (NEFAs)(serum sample); fatty acids in red cell membranes (red cell portion of spun EDTA sample). Women will be given a further sample pot for bed time and early morning salivary collection to be posted.

The results of the glucose tolerance test will be revealed to the woman and her caregivers. If a participant develops gestational diabetes during the study, she will be asked to continue with the IMP but she will be referred to the obstetric diabetic team for further advice and treatment which may include diet and/or insulin. The details of any treatments will be recorded on the database (eCRF). It is not anticipated that the development of gestational diabetes will be an indication for unblinding.

A further drug supply (up to 6 bottles, sufficient for up to 14 weeks of treatment) will be dispensed at this study visit.

9.7 STUDY VISIT 6

Week 36 (+/- 7 days)

Information on pregnancy complications will be collected. Adverse events (AE) will be recorded in the patient notes, serious adverse events (SAEs) will be recorded on the SAE form. Patients will be asked if they are complying with their study medication. A

75g OGTT (baseline and 2 hr samples) will be performed. A fasting blood sample will be stored for inflammatory and metabolic indices (including CRP, IL-6, leptin, PAI1/PAI2 ratio, cortisol, [fasting] lipids (total cholesterol, HDL, calculated LDL) and triglycerides (serum gel sample); non esterified fatty acids (NEFAs)(serum sample); fatty acids in red cell membranes (red cell portion of spun EDTA sample). Repeat anthropometry measurements e.g. waist, hip, mid-arm and mid-thigh circumference will be obtained. Repeat detailed measurements of body fat will be made using the BodPod machine (subset of participants in Edinburgh only). A further set of bottles for bed time and early morning salivary samples will be provided.

Vascular measurements, an MR scan and a hyperglycaemic euglycaemic clamp will be performed on women participating in these mechanistic studies either at study visit 6 or an alternative visit around this time if more convenient for the participant.

Again, the 36 week glucose tolerance test will be revealed to the participant and her caregivers. Levels above the reference range should prompt regular glucose monitoring and specialist advice.

9.8 STUDY VISIT 7

Week 40 (+/- 7 days)

Information on pregnancy complications will be collected. Adverse events (AE) will be recorded in the patient notes, serious adverse events (SAEs) will be recorded on the SAE form. This could be a telephone visit.

9.9 STUDY VISIT 8

Labour/Delivery/ Neonatal Assessments

Admission for delivery will not be a formal study visit and as it is anticipated it will not be recorded as an SAE. Information on pregnancy complications will be collected. Adverse events (AE) will be recorded in the patient notes, serious adverse events (SAEs) will be recorded on the SAE form. Information will be obtained on the maternal outcomes of delivery, including method of delivery, indication for delivery method, date and gestation of delivery, and blood loss. Adverse events and pregnancy complications will be reviewed where possible at this visit and unused study medication returned.

At delivery, a sample of cord blood will be taken from the placenta at delivery and stored for future analysis including DNA, fetal metabolic and inflammatory variables including CRP, IL-6, leptin, PAI1/PAI2 ratio, cortisol, cortisol binding globulin, lipids (total cholesterol, HDL, calculated LDL) and triglycerides (serum gel sample); non esterified fatty acids (NEFAs)(serum sample); fatty acids in red cell membranes (red cell portion of spun EDTA sample) and C peptide.

Where facilities exist (eg Edinburgh), a sample of placenta will be taken at delivery, flash frozen and stored for future analysis.

A sample of myometrium and rectus sheath muscle will be taken in women being delivered by caesarean section. One portion of the myometrial sample will be stored in normal saline and the other flash frozen and stored at -20 ° C until analysis. Samples will be couriered to S Wray Laboratory for measurement of muscle glycogen storage.

The baby outcomes of delivery including baby weight, gender, and birth outcome (e.g. live birth, stillbirth or death in the delivery room) will be collected and recorded by local investigators mainly from hospital notes, and the data will be entered on to the eCRF. Z score of birthweight centile will be calculated using www.gestation.net. Anthropometry of the babies – length, head circumference and skin fold thicknesses will also be recorded within 72 hours of birth. Babies of women delivered in Edinburgh and selected other hospitals will have body composition measured by the air displacement plethysmography (PeaPod <http://www.lifemeasurement.com/>) as soon as possible after birth, and certainly within 72 hours..

SAE's will be recorded and be followed up to 28 days after birth.

9.10 STUDY VISIT 9

Three months after delivery.

Babies will be reviewed at three months of age. Weight, length, head circumference and skin fold thicknesses will be measured. In Edinburgh, body composition will additionally be measured in the baby using the PEAPOD and in the mother using the BodPod. We will ask women for permission to view their own and their baby's record in the future, and also to invite them for some future studies. Such studies are outside the scope of this project and will likely be the subject of other funding applications.

9.11 SUB-STUDIES

In parallel with the main trial and study measurements, in which all participants will be involved, we will conduct several sub-studies in which more detailed measurements of metformin action will be carried out. Women will be approached to participate in these studies prior to randomisation. More detailed protocols for these studies are included in Appendices 7- 9 these sub-studies are as follows:

Vascular function:

Endothelial dependent flow mediated dilatation (FMD), augmentation index (AI) and pulsed wave velocity (PWV) will be measured in a subset (n = 30 each group) of women at baseline (12 – 16 weeks) and again at 36 weeks gestation using a non invasive, validated, and reproducible method suitable for use in pregnancy (Appendix 7)

Detailed measurements of insulin resistance function: in a subgroup of women (n = 20 in each group) insulin resistance will be measured by the hyperinsulinaemic euglycaemic clamp at around 36 weeks gestation, to characterise the relative effects of metformin on hepatic and peripheral skeletal muscle insulin sensitivity (Appendix 8)

Magnetic resonance imaging (MR): Twenty women in each of the active and placebo groups (40 altogether) will be scanned using The Verio 3 Tesla MRI system (Siemens Medical Systems, Germany) in the Clinical Research Imaging Centre (CRIC), Queen's Medical Research Centre, University of Edinburgh firstly at around

24 - 28 weeks gestation and then again at around 36 weeks gestation. MRI does not involve the use of ionising radiation and when used in accordance with National Radiological Protection Board guidelines may be safely performed in pregnancy after the first trimester. We have ethical approval and have developed protocols involving the MRI scanning of pregnant women at 3T.

Maternal and fetal abdominal and visceral fat depth and fetal liver volume will be measured. Additionally, proton MR spectroscopy (^1H MRS) of maternal and fetal liver and of maternal thigh muscle (quadriceps) will be performed. (Appendix 9). We will also perform MR imaging of the maternal and fetal liver in order to quantitate fat in the whole liver using a technique that creates a 3D map over the liver. Commercial software packages will be used to analyse the data as well as in-house analytical techniques.

Hypothalamo pituitary adrenal axis: During pregnancy there is an increase in activity of the hypothalamic-pituitary-adrenal axis with a significant rise in circulating maternal cortisol levels. Over-exposure of the developing fetus to cortisol impacts on birthsize and health in adult life. We plan to measure diurnal cortisol levels (bed-time and waking samples) in saliva as well as morning serum cortisol levels at 3 time points during pregnancy, baseline, 28 weeks and 36 weeks. Saliva samples will be collected into a salivette (Sarsted) container at bed-time and upon waking. Time of sample collection will be recorded. The subject will be asked to avoid eating and smoking and not to clean their teeth for half an hour prior to collection. Samples are stable in a refrigerator at 40C for up to 1 week. Samples will be posted or delivered back to the research clinic and then stored at -20C. Morning venous blood samples will be collected from an antecubital fossa vein after an overnight fast. Serum will be separated and stored at -80C.

Cortisol will be measured in saliva by ELISA (Salimetrics kit) and in serum by RIA. Levels of corticosteroid binding globulin (CBG) will also be measured by RIA allowing calculation of free cortisol levels.

Myometrial contractility:

Will be measured in biopsies of lower segment myometrium obtained during Caesarean section as we have previously described ⁴. The myometrial biopsies will be divided, with one portion placed in physiological saline for contractility analysis within 24 hours, and the other snap frozen for glycogen storage measurements. In order to measure contractility myometrial strips will be mounted on a tension transducer, stretched to 1.5× their slack length and perfused with physiological saline

at 5ml/min at pH 7.4 and 35°C. The strips will be allowed to contract spontaneously and once a regular, consistent pattern of contraction is established the peak amplitude, duration of contraction, integral of force (area under the curve) and frequency of contractions will be measured over a 60 minute period and the mean value for each variable, for each patient calculated. We will measure both force of contraction and simultaneous force and Ca²⁺ recordings (using the fluorescent calcium- indicator Indo-1 and excited using 350nm wavelength light). Glycogen storage will be measured by conversion to glucose and enzymatic assay using the hexokinase/glucose-6-phosphate (G-6-P) dehydrogenase reaction. The NADPH produced will be read on a spectrophotometer at 340 nm .

An optional extra sample of rectus sheath (skeletal muscle) and adipose tissue (visceral and subcutaneous) will also be taken (Edinburgh participants only) to measure inflammatory variables and elements of the insulin signalling cascade and to determine the effect of metformin on these variables.

10. DATA COLLECTION

The patient's clinical record will be considered to be source data. Information will be abstracted from the clinical record into the electronic case report form (eCRF). Data will be collected as it becomes available – i.e. at or shortly after each patient visit.

11. STATISTICS AND DATA ANALYSIS

11.1 SAMPLE SIZE CALCULATION

11.1.1 BIRTHWEIGHT CENTILE

In a previous study, the mean (SD) birthweight in a cohort of obese women (mean BMI 34) was 4.0kg (0.6kg)[9]. We hypothesise that metformin will reduce mean birthweight by 200g, corresponding to a reduction in birthweight centile of 0.33 SD. We believe that this reduction in birthweight centile is clinically relevant, but is a relatively conservative estimate of likely reduction in birthweight centile induced by metformin, given that mean birthweight in the study described above in a parallel non obese cohort was 3.4kg. A sample size of 143 in each group will have 80% power to detect a difference in means birthweight centile of 0.33 SD (the difference between a placebo mean of 4.0kg and a metformin mean of 3.8kg) at the 5% significance level (2-sided) using a two group t-test; a sample size of 163 in each group will give the study 85% power to detect these differences. In practice we will recruit 200 women to

each group to allow loss to follow up (anticipated < 5% by extrapolation from our previous studies) and suboptimal compliance.

11.1.2 INSULIN RESISTANCE (IR)

In our previous study of obese women with PCOS, fasting insulin was lowered by 25% after 6 months treatment with metformin 1500mg daily [10], consistent with meta-analyses [11]. Based on published levels of fasting insulin in obese pregnant women 26.9 IU/L [12], and deriving the standard deviation as 15.7

IU/L from the published standard error of 3.5 IU/L with n=20, if a reduction in mean fasting insulin of 22% (5.4 IU/L) is achieved by metformin in pregnancy, a sample size of 306 will be required for the study to have 85% power to demonstrate differences between the placebo and metformin group at the 5% significance level.

11.2 PROPOSED ANALYSES

Mean birthweight centile will be compared between the groups using essentially a two-sample t test, but with the analysis stratified for the same factors as the randomization. Correlations within the metformin and placebo groups will be used to determine association between IR and adverse pregnancy outcomes

A detailed statistical analysis plan will be written specifying the methods of analysis, plans for handling missing data, non compliance and withdrawals. At this stage, no formal interim analysis is planned (other than those requested by the DMEC). The primary analysis will be by intention to treat.

12. ADVERSE EVENTS

The Investigator is responsible for the detection and documentation of events meeting the criteria and definitions detailed below.

Full details of contraindications and side effects that have been reported following administration of the trial drug can be found in the relevant Summary of Product Characteristics (SmPC) in Appendix 1.

Participants should be instructed to contact their Investigator at any time after consenting to join the trial if any symptoms develop. All adverse events (AEs) that occur after joining the trial must be reported in detail in the participant's medical notes. In the case of an AE, the Investigator should initiate the appropriate treatment

according to their medical judgment. Participants with AEs present at the last visit must be followed up until resolution of the event.

12.1 DEFINITIONS

An **adverse event** (AE) is any untoward medical occurrence in a clinical trial subject who is administered a medicinal product, which does not necessarily have a causal relationship with the treatment.

An **adverse reaction** (AR) is any untoward or unintended response to an investigational medicinal product related to any dose administered.

An **unexpected adverse reaction** (UAR) is an adverse reaction that is not consistent with the product information in the SoPC.

A **serious adverse event** (SAE), **serious adverse reaction** (SAR) or **suspected unexpected serious adverse reaction** (SUSAR) is any AE, AR or UAR that at any dose:

- results in death;
- is life threatening (i.e. the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- requires hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect.

12.2 DETECTING AEs AND SAEs

All AEs and SAEs must be recorded from the time a participant is randomised until after the last baby is born and discharged from hospital or the end of the postnatal period (28 days after the birth), which ever is sooner.

The Investigator should ask about the occurrence of AEs/SAEs at every visit during the study. Open-ended and non-leading verbal questioning of the participant should be used to enquire about AE/SAE occurrence. Participants should also be asked if they have been admitted to hospital, had any accidents, used any new medicines or changed concomitant medication regimens. If there is any doubt as to whether a clinical observation is an AE, the event should be recorded.

Information to be collected includes type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

12.3 RECORDING AEs AND SAEs

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator should then record all relevant information in the patient notes. If the AE meets the criteria of serious it should also be recorded on the SAE form. For example, a post partum haemorrhage (PPH) loss >500ml (>750ml following a caesarean section) may be medically judged to be an AE. However, if the PPH was considered life threatening at the time of the event or if it resulted in prolonging the women's existing hospitalisation it would qualify an SAE and should be recorded on the SAE form and reported as described below.

Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

All adverse medical events reported by the patient should be noted in the patient's hospital notes, together with a note of the date of starting, the duration, and any medical treatment received.

The clinician will assess **ALL** reported SAEs. Some adverse events are expected and will not therefore be reported as SAEs **but** will be recorded in the eCRF and presented to the DMC, as part of the ongoing safety review.

For this study the following events are **NOT** considered SAEs:

- Pregnancy is not considered an AE or SAE, as it is part of the inclusion criteria
- Hospitalisations for treatment planned prior to randomisation and hospitalisation for elective treatment of a pre-existing condition will not be considered as an SAE. This includes pregnancy. However, ***complications occurring during such hospitalisation will be AE/SAEs.***
- Miscarriage

- Preterm labour
- Preterm delivery in maternal interest
- Preterm delivery in fetal interest
- Hospitalisation for pregnancy induced hypertension
- Hospitalisation for “maternal discomfort”
- Hospitalisation for “rest”.
- Hospitalisation for “observation” or “monitoring” for which the women is admitted for a period of less than 12 hours
- Delivery complications such as caesarean section or post partum haemorrhage
- Admission of the baby to the neonatal unit for a period of up to 14 days.

12.4 EVALUATION OF AEs AND SAEs

Seriousness, causality, severity and expectedness should be evaluated as though the participant is taking active drug. Cases that are considered serious, possibly, probably or definitely related to drug and unexpected are suspected unexpected serious adverse reactions (i.e. SUSARs). Women should only be unblinded if the information is needed to inform the care team on a course of treatment. However the women should be treated as though they were on the active treatment.

12.4.1 Assessment of Seriousness

The Investigator should make an assessment of seriousness as defined in Section 12.1.

12.4.2 Assessment of Causality

The Investigator must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions. All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably, definitely) to the study drug will be considered as ARs/SARs. If concomitant or rescue/escape drugs are given, the Investigator must also make an assessment of whether the AE/SAE is likely to be related to an interaction between the study drug and concomitant or rescue/escape drugs or whether the AE/SAE might be linked to either the study drug or concomitant or rescue/escape drugs but cannot be attributed

to only one of these drugs. All AEs/SAEs judged as being related (e.g. possibly, probably, definitely) to an interaction between the study drug and concomitant or rescue/escape drugs, or any AE/SAE that cannot be attributed to only the study drug or the concomitant or rescue/escape drugs will also be considered to be ARs/SARs .

Unrelated: where an event is not considered to be related to the study drug.

Possibly: although a relationship to the study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably: the temporal relationship and absence of a more likely explanation suggest the event could be related to the study drug.

Definitely: The known effects of the study drug or its therapeutic class, or based on challenge testing, suggest that study drug is the most likely cause.

Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated. The blind should not be broken for the purpose of making this assessment.

12.4.3 Assessment of Severity

The Investigator should make an assessment of severity for each AE/SAE and record this on the eCRF according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

Note: the term 'severe', used to describe the intensity, should not be confused with 'serious' which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

12.4.4 Assessment of Expectedness

If an event is judged to be an AR/SAR, the evaluation of expectedness should be made based on knowledge of the reaction and the relevant product information documented in the SmPC.

12.5 REPORTING OF SAEs/SARs/SUSARs

Once an Investigator becomes aware that an SAE has occurred in a study participant, they must report the information to the ACCORD Research Governance & QA Office within 24 hours.

The SAE form must be completed as thoroughly as possible with all available details of the event, signed by the Investigator or designee. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before completing the SAE form. The form can be updated when the additional information is received.

The SAE report must provide an assessment of causality and expectedness at the time of the initial report to the ACCORD Research Governance & QA Office according to Sections 10.4.2, Assessment of Causality and 10.4.4, Assessment of Expectedness.

The SAE form should be transmitted by fax to the ACCORD Research Governance & QA Office on 0131 242 9447 or may be transmitted by hand to the office.

12.6 REGULATORY REPORTING REQUIREMENTS

The ACCORD Research Governance & QA Office is responsible for Pharmacovigilance reporting on behalf of the Co-Sponsors (Edinburgh University and Lothian Health Board).

The ACCORD Research Governance & QA Office has a legal responsibility to notify the regulatory competent authority and the relevant ethics committee (main Research Ethics Committee (REC) that approved the trial). Fatal or life threatening SUSARs will be reported no later than 7 calendar days and all other SUSARs will be reported no later than 15 calendar days after the ACCORD Research Governance & QA Office is first aware of the reaction.

An Annual Safety Report will be submitted to the regulatory competent authority and the main REC listing all SARs and SUSARs. Additional reports will be made to Merck as requested in the contract between Merck and Edinburgh.

12.7 FOLLOW UP PROCEDURES

After initially recording an AE or recording and reporting an SAE, the Investigator is required to follow each participant until resolution. Follow up information on an SAE should be reported to the ACCORD Governance & QA Office.

AEs still present in participants at the last study visit should be monitored until resolution of the event or until no longer medically indicated.

12.8 OUT OF HOURS COVER

The Trial Management Group, Trial Steering Committee, Trial managers and Co-sponsors do not provide out of hours advice for study participants. The protocol will be available on the labour ward of each participating institutions, and the study team will attempt to ensure that all senior obstetricians within participating units are aware of the study. Out of hours emergency unblinding will be available via the local pharmacy on request by a senior clinician.

There is experience of using the study medication within pregnancy and each participating site is covered by local senior obstetric 'on-call' provision at all times.

The rationale for the out of hours arrangements is informed by a number of considerations:

- The medication being used in the study is recommended by NICE for pregnancy, although not for the specific indication under trial.
- Treatment unblinding is available 24 hours per day.
- Use of these drugs during pregnancy is consistent with the off-label use of these products.
- The treatment is being used at prophylactic doses and is terminated on delivery of the baby.

13. TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

13.1 TRIAL MANAGEMENT GROUP

The trial will be coordinated by a Trial Management Group (TMG), consisting of the grant holders (Chief Investigator and Principal Investigator in Edinburgh), a Trial Manager, a representative from the ECTU (Data Centre) and a Pharmacist.

Members of the Trial Management Group (or a delegated deputy) will be invited to attend the Trial Steering Committee meetings.

The Trial Manager will oversee the study and will be accountable to the Chief Investigator. The ECTU will be responsible for checking the eCRFs for completeness, plausibility and consistency. Any queries will be resolved by the site Principle Investigator or delegated member of the trial team.

A Delegation Log will be prepared for each site, detailing the responsibilities of each member of staff working on the trial.

13.1.1 Division of Responsibilities of the Trial Management Group (TMG):

The responsibilities of the investigators are as follows:

- Chief Investigator, Norman: overall responsibility for the design, conduct, analyses and reporting of the trial; assisted by the trial management group.
- Murray for trials unit support including statistics and management of ECTU.
- The remaining members include the trial clinicians and scientists listed as participating centres will have responsibilities for the conduct of the trial in their participating centres.

13.2 CENTRAL TRIAL OFFICE

The Trial manager will co-ordinate the study based at the Simpson's Centre for Reproductive Health, Edinburgh. The eCRF and statistics will be developed and supported by the Edinburgh Clinical Trials Unit (ECTU). The trial manager and ECTU will provide support to the participating sites. The ECTU will be responsible for randomisation, collection of data (eCRF) in collaboration with the Trial Manager, data processing and analysis.

13.3 TRIAL STEERING COMMITTEE

A Trial Steering Committee (TSC) will be established to oversee the conduct and progress of the trial. The terms of reference of the Trial Steering Committee will be developed separately. The names and contact details of the TSC are detailed in Appendix 2.

13.4 DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established to oversee the safety of subjects in the trial. The terms of reference of the Data Monitoring Committee will be developed separately. The contact details of the DMC are detailed in Appendix 3.

13.5 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit trial related monitoring, audits, REC review, and regulatory inspection(s). In the event of an audit, the Investigator agrees to allow the Sponsor, representatives of the Sponsor or regulatory authorities direct access to all study records and source documentation.

13.6 STUDY MONITORING

The trial will be monitored by the ACCORD Clinical Trials Monitor and/or the Trial Manager on behalf of the Co-Sponsors. A study start-up visit will be completed prior to recruitment, ongoing monitoring will be performed during recruitment to verify eligibility, consent and trial data. A closure visit will be completed after recruitment has finished.

13.7 RISK ASSESSMENT

An independent risk assessment will be carried out by the ACCORD Clinical Trials Monitor.

13.7.1 Potential Risks

The risks of metformin outside pregnancy are minimal, and largely relate to the (small) risk of lactic acidosis in subjects with renal and/or liver impairment. Safety data on metformin in pregnancy is provided by the MIG trials [13] in which there were no serious adverse events in the 363 women assigned to metformin. A meta-analysis suggests that the incidence of major fetal malformation is actually

reduced in babies of women exposed to metformin in the first trimester (odds ratio of 0.50 [95% confidence interval of 0.15 to 1.60])[15].

Regarding other neonatal adverse events, the risk of neonatal hypoglycaemia was lower in the metformin +/- insulin group compared with the insulin group alone in the MIG trial (relative risk 0.41 [95% CI 0.21–0.78]) [13]. Rates of the remaining neonatal outcomes were similar in the two groups, with the sole exception of preterm birth, which was modestly elevated in the metformin group. Two studies have (to our knowledge) evaluated the use of metformin in pregnant women without diabetes [16 17]. Although their combined sample size is small (< 150) they are again reassuring on the safety of metformin in non diabetic pregnancy, with outcomes being better in the metformin compared with the control group. Metformin does not provoke hyperinsulinaemia, and thus is not associated with hypoglycaemia, either in women with PCOS or in normoglycaemic individuals at high risk for diabetes [11 18].

13.7.2 Minimising Risk

Risk is minimised in this study by ensuring normal renal and liver function in all women prior to randomisation.

14. GOOD CLINICAL PRACTICE

14.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of Good Clinical Practice (GCP). A favourable ethical opinion will be obtained from the appropriate REC and local R&D approvals will be obtained prior to commencement of the study.

14.2 REGULATORY COMPLIANCE

A Clinical Trial Authorisation (CTA) has been obtained from the appropriate Regulatory Authority (ref no 01384/0217/001-0001). The protocol and study conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004, and any relevant amendments.

14.3 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of GCP, the following areas listed in this section are also the responsibility of the Investigator.

Responsibilities may be delegated to an appropriate member of study site staff. Delegated tasks must be documented on a Delegation Log and signed by all those named on the list.

14.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant should be performed by the Investigator or designated person, and must cover all the elements specified in the Participant Information Sheet/Informed Consent Form(s).

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant should be informed and agree to their medical records being inspected by regulatory authorities but understand that their name will not be disclosed outside the hospital.

The Investigator or delegated member of the trial team and the participant should sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant should receive a copy of this document and a copy filed in the Investigator Site File (ISF).

14.3.2 Study Site Staff

The Investigator must be familiar with the IMP, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the IMP, protocol and their trial related duties.

14.3.3 Data Recording

The Investigator is responsible for the quality of the data recorded in the eCRF.

14.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the ACCORD Governance & QA Office, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents);
- Curriculum vitae (CV) signed and dated by the Investigator indicating that it is accurate and current.
- A Valid GCP certificate

The ACCORD Research Governance & QA Office will ensure all other documents required by GCP are retained in a Trial Master File (TMF) and that appropriate documentation is available in local ISFs.

14.3.5 GCP Training

All study staff must hold evidence of appropriate GCP training or undergo GCP training. The Co-sponsors require that GCP is updated every two years throughout the trial.

14.3.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the REC. The Investigator and study site staff involved with this study may not

disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

14.3.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants.

Computers used to collate the data will have limited access measures via user names and passwords. Published results will not contain any personal data that could allow identification of individual participants.

15. STUDY CONDUCT RESPONSIBILITIES

15.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant, must be reviewed and approved by the Chief Investigator.

Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to participants being enrolled into an amended protocol.

15.2 PROTOCOL VIOLATIONS AND DEVIATIONS

Investigators should not implement any deviation from the protocol without agreement from the Chief Investigator and appropriate REC, Regulatory Authority and R&D approval except where necessary to eliminate an immediate hazard to trial participants.

In the event that an Investigator needs to deviate from the protocol, the nature of and reasons for the deviation should be recorded in the eCRF. If this necessitates a

subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate.

15.3 STUDY RECORD RETENTION

This is a study involving pregnant women and research records should be retained according to NHS Guidelines for the retention of documentation involving pregnant women. All medical records will be retained for at least 25 years after publication of the final study report. Guidelines on retention of other research related documents are continually under review. We plan to retain all documents for 5 years and then review according to current guidance at that time.

15.4 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

- (a) the safety or physical or mental integrity of the subjects of the trial (this should be relevant to trial subjects in the UK); or
- b) the scientific value of the trial.

If a potential serious breach is identified by the Chief Investigator, Principal Investigator or delegates, the Co-sponsors must be notified within 24 hours. It is the responsibility of the Co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and take the appropriate action.

Not every deviation from the protocol needs to be reported to the regulatory authority as a serious breach. If the Co-sponsors deem the incident to be a minor deviation from the protocol when identified, corrective and preventative actions will be taken where appropriate and they will be recorded in file notes, held within the TMF or ISF.

15.5 END OF STUDY

The end of study declaration will be submitted to the relevant authorities after the last baby is born and discharged from hospital or the end of the postnatal period (28 days after the last birth), which ever is sooner. The end of the study will be reported to the REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants and ensure that the appropriate follow up is arranged for all involved.

A summary report of the study will be provided to the REC and Regulatory Authority within 1 year of the end of the study.

15.6 CONTINUATION OF DRUG FOLLOWING THE END OF STUDY

Participants in the study will be given study drug for the relevant duration of the index pregnancy. The study drug will not be provided for subsequent pregnancies and the decision to prescribe metformin for a subsequent pregnancy will be up to the participant's caregivers at that time.

15.7 INSURANCE AND INDEMNITY

The Co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the Co-Sponsors' responsibilities:

- The protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the Sites concerned. The Co-Sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's Nation Health Service will have the benefit of NHS Indemnity.
- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.
- The manufacturer supplying IMP has limited liability related to the manufacturing and packaging of the study treatments and to the losses, damages, claims or liabilities incurred by study participants based on known or unknown Adverse Events which arise out of the manufacturing and packaging

of the study drug, but not where there is any modification to the study drug (including without limitation re-packaging and blinding).

16. REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

16.1 AUTHORSHIP POLICY

Ownership of the data arising from this is set out in the collaborators' agreement and an authorship policy will be developed. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared in accordance with GCP guidelines.

16.2 PUBLICATION

The clinical study report will be used for publication and presentation at scientific meetings. The results of the study and any protocol deviations will be published in writing by a team headed by the Chief Investigator, which will report to the Trial Management Committee. Individual investigators may be able to produce oral reports with the permission of the Trial Management Committee

Summaries of results will also be made available to Investigators for dissemination within their clinics (where appropriate and according to their discretion).

17. PEER REVIEW

The study was extensively peer reviewed as part of the process of gaining EME grant funding.

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APPENDIX 1: SUMMARY OF PRODUCT CHARACTERISTICS

The manufacturer may change the SmPC, for this study as new information becomes available. The study will therefore adopt the manufacturer's current SmPC. The study team will monitor and review changes to the SmPC and consider the impact on the study and revise documents if required.

The SmPC is published on the electronic Medicines Compendium (eMC) showing the date it was published and the reasons for change.

At the time of writing this protocol the SmPC was last updated on the eMC website on 03/11/2008, and is available at

<http://emc.medicines.org.uk/medicine/1043/SPC/Glucophage+500+mg+and+850+mg+film+coated+tablets/>

APPENDIX 2: TRIAL STEERING COMMITTEE

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Lay representation tba

APPENDIX 3: DATA MONITORING COMMITTEE

Chair

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APPENDIX 4: WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)

55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)

59th WMA General Assembly, Seoul, October 2008

<http://www.wma.net/e/policy/pdf/17c.pdf>

APPENDIX 5: TIMELINES FOR NOTIFICATION

The table below identifies the predictable report types arising from the study, the dates such reports are required, the necessary recipients, the persons responsible for preparation, collation, dispatch, transmission and confirmation of receipt.

Report Type	Necessary Recipients	Person responsible for collation	Person Responsible for dispatch	Method of transmission	Person responsible for confirmation of receipt	First report due:
Six monthly Report to EME NIHR Funding Body (as indicated in their letter of 10 th February 2010)	TMG	CI	CI/ Trial Office (Edinburgh)	RECORDED MAIL	CI/ Trial Office (Edinburgh)	NOVEMBER 2011
	TSC					
	Co-Sponsors					
	MRC					
Annual Safety Report	Co-Sponsors	ECTU	CI/ Trial Office (Edinburgh)	RECORDED MAIL	CI/ Trial Office (Edinburgh)	MARCH 2011
	NRES					
	MHRA					
	DMC					
	TSC					
SAR/ SUSAR report	Co-Sponsors	Site Investigator/member of steering committee/CI/Co-Sponsors	Co-Sponsors	RECORDED MAIL	Co-Sponsors	7 days (for fatal/life threatening) or 15 days from receipt by member of steering committee
	PI					
	NRES					
	MHRA					
	TSC					
DMC						
Ethics Annual Progress Report	NRES	CI	CI/ Opptimum Trial Office (Edinburgh)	RECORDED MAIL	CI/ Trial Office (Edinburgh)	MARCH 2011
	Co-Sponsors					
Site termination Notifications	PI	CI/ Opptimum Trial Office (Edinburgh)	CI/ Trial Office (Edinburgh)	RECORDED MAIL	CI/ Trial Office (Edinburgh)	WITHIN 30 DAYS OF SITE CLOSE OUT VISIT
	CI					
	NRES					
Study termination notification	CO-SPONSORS	CI/ Trial Office (Edinburgh)	CI/ Trial Office (Edinburgh)	RECORDED MAIL	CI/ Trial Office (Edinburgh)	WITHIN 90 DAYS OF STUDY TERMINATION

APPENDIX 6: DRUG LABEL, VERSION 3 20/09/2010

Study: EMPOWaR Treatment Number : XXXXXX

EudraCT: 2009-017134-47 Batch number: XXX

Expiry date: MM/YYYY

FOR CLINICAL TRIAL USE ONLY - KEEP OUT OF REACH AND SIGHT OF
CHILDREN

This bottle contains 90 Metformin 500mg film-coated tablets or Placebo

Take ONE or TWO tablets orally as directed with food.

AVOID ALCOHOL AND ALCOHOL-CONTAINING MEDICINES

This medicinal product does not require any special storage conditions

Co-sponsors: University of Edinburgh/NHS Lothian, The Queen's Medical Research

Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ

Telephone: 0131 242 9461

Investigator: _____
Hospital: _____

Patient number: _____
Visit number: _____

APPENDIX 7: VASCULAR STUDIES

Setting and preparation

Women will be studied in a quiet, temperature controlled room after an overnight fast. All vasoactive medications will be withheld for at least two days, if possible. In addition, subjects should not exercise, should not ingest substances that might affect FMD such as caffeine, alcohol, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.

Flow mediated dilatation (FMD) measurements

The subject will be positioned in the 30° left lateral position with the arm in a comfortable position for imaging the brachial and radial artery. The brachial artery will be imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall will be selected for continuous 2D grayscale imaging. During image acquisition, anatomic landmarks such as veins and fascial planes will be noted to help maintain the same image of the artery throughout the study. A stereotactic probe-holding device will be employed to aid a stationary position.

Endothelial-dependent FMD.

To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff will first be placed below the antecubital fossa. A baseline rest image will be acquired, and blood flow velocity will be estimated by the pulsed Doppler velocity signal obtained from an angle-corrected mid-luminal sample volume. Thereafter, arterial occlusion will be created by cuff inflation to suprasystolic pressure (at least 50 mm Hg above systolic pressure) to occlude arterial inflow for 5 min. The longitudinal image of the artery will be recorded continuously from 30 s before and 2 min after cuff deflation. A further mid-luminal pulsed Doppler signal will be obtained upon immediate cuff release and no later than 15 s after cuff deflation to assess the hyperaemic response. The maximal increase in diameter is expected to occur approximately 60 s after release of the occlusive cuff.

The diameter of the brachial artery will be measured from longitudinal images in which the lumen-intima interface is visualized on the near (anterior) and far (posterior) walls. These boundaries are best visualized when the angle of insonation is perpendicular. Thus, clear visualization of both the near and far wall lumen-intima boundaries will indicate that the imaging plane is bisecting the vessel in the longitudinal direction, and diameters measured from these images likely reflect the

true diameter. Once the image for analysis has been chosen, the boundaries for diameter measurements (the lumen-intima or the media-adventitia interfaces) will be identified manually with electronic calipers or automatically using edge detection software. Brachial artery diameter will be measured at a defined time point in the cardiac cycle, identified using ECG gating during image acquisition. The onset of the R-wave will be used to identify end diastole, and the peak of the T-wave to identify end systole. An average measurement of diameter derived from at least 5 diameter measurements determined along a segment of the vessel will be used to minimize variability. Flow-mediated vasodilation will be expressed as the change in post-stimulus diameter as a percentage of the baseline diameter.

Endothelial independent FMD

These studies will be performed after at least 10 min of rest following the previous test in order to reflect the reestablished baseline conditions. A single low dose (0.025 mg) of nitroglycerin (GTN) spray will be used to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilatation reflecting vascular smooth muscle function.

Pulse Wave Analysis

Applanation tonometry of the radial artery will be performed using a micromanometer and the SphygmoCor system according to the manufacturer's instructions. Briefly, the arterial waveform will be recorded at the radial artery. This should be identifiable 3 cm from the wrist and medial to the brachioradialis muscle. Recordings will be made over at least 10 arterial waveforms. Readings will be used to derive the aortic pulse pressure waveform via a mathematical transfer function. Thereafter, the augmentation index (defined as the difference between the second and first systolic peaks and expressed as a percentage of the pulse pressure) will be calculated, both at "real time" values and corrected for a heart rate of 75 bpm. The augmentation index is a measure of systemic arterial stiffness and wave reflection. Arterial blood pressure varies with respiration; thus, to cover a complete respiratory cycle, 2 independent analyses, incorporating 10 arterial waveforms each, will be obtained and averaged from each subject.

Pulse Wave Velocity.

Carotid-femoral PWV and carotid-radial PWV will be determined by sequential acquisition of pressure waveforms from the carotid, femoral, and radial arteries. ECG recordings will be made in parallel in order to compare timing of the waveforms with the R wave on a simultaneously recorded ECG, and the time delay calculated. Two

consecutive waveform recordings will be obtained from each subject and the average reported.

Reference

Corretti, M.C., T.J. Anderson, E.J. Benjamin, D. Celermajer, F. Charbonneau, M.A. Creager, J. Deanfield, H. Drexler, M. Gerhard-Herman, D. Herrington, P. Vallance, J. Vita, and R. Vogel, Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*, 2002. 39(2): p. 257-65.

APPENDIX 8: HYPERINSULINAEMIC EUGLYCAEMIC CLAMP FOR THE EMPOWAR STUDY

Stable isotope use

Studies will be done with stable isotope infusions (also termed tracers). An isotope is a member of a chemical-element family of atomic species that has two or more nuclides with the same number of protons but a different number of neutrons. Because the atomic mass is determined by the sum of the number of protons and neutrons contained in the nucleus, isotopes differ in mass. Since they contain the same number of protons and hence electrons, isotopes have the same chemical properties. Importantly, stable isotopes are safe and not radioactive and have been used in human studies including pregnancy over the last four decades with no adverse effects.

All infusion studies will commence at 07.30h. Prior to the visits, all subjects will be instructed to abstain from strenuous physical activity for 24 h and to perform a 10- to 12-h overnight fast before the examination days. All subjects will ensure their diet includes a minimum of 250 g of carbohydrate and no alcohol for at least 3 days before the studies. The studies will take place in the WTCRF.

Subjects will have their height and weight recorded in the WTCRF. Their Body surface area (BSA) will be calculated using the Mosteller formula: $\sqrt{(\text{Weight (kg)} \times \text{Height (cm)}) \div 3600}$

(see HEC studies below where insulin is infused according to BSA).

Infusion Studies: A short polyethylene catheter will be inserted into an antecubital vein for infusion of test substances. Another catheter will be placed into a contralateral forearm vein for blood sampling. This arm will be kept at ~70°C with a heating box or pad to arterialise venous blood.

The infusions will be divided into three phases. The first part will be the enriched tracer infusates alone (=90 minutes) to provide an estimate of EGP and basal lipolysis. The second part will be a low dose HEC (=120 minutes) and the third part will be the high dose HEC (=120 minutes). It is anticipated that these times will be sufficient to allow steady state to be achieved, although they may need to be altered depending on information arising from our previous pregnancy studies. During the clamp studies the infusion of the tracer will be continued. During each of the stages

indirect calorimetry will be done to estimate glucose and lipid oxidation and to calculate non oxidative glucose utilisation (NOGU).

Stage 1 Endogenous glucose production - time 0-90 mins: A primed 25umol/kg, continuous 22umol/kg/hour 6,6-2H2 glucose (Cambridge Isotope Laboratories, Andover, Mass) with a primed 1.6 umol/kg, continuous 6.6 umol/kg/hour 1,1,2,3,3-2H5 glycerol infusion will be commenced and continued for 90 minutes as we have previously described (Forbes, 2006). (Again, this dose may be adjusted, depending on results from previous studies). Plasma samples will be taken at 0 for background enrichment of glucose and glycerol. (Other tests scheduled for the same gestation – eg CRP, lipids etc) could also be taken if necessary at this stage). Blood will then be taken at times 60, 70, 80 and 90 minutes for enriched and unenriched glucose and glycerol concentrations, insulin, lactate (metabolite of glucose) β hydroxybutyrate (metabolite of fat) and NEFA concentrations.

Basal EGP will be estimated according to the steady state equation of Steele (Steele 1959).

(Note turnover also termed rate of appearance (Ra)): $Ra = F \times (100-1)/E$; $Ra = EGP + F$; So $EGP = Ra - F$. Note at Steady State $Ra=Rd$, where F constant infusion rate, E isotopic enrichment of plasma glucose, Rd = Rate of disappearance) . Glycerol production will be calculated in the same way. The glycerol Ra x 3 will be assumed to reflect whole body lipolysis.

Stage 2: low dose HEC (90-210 minutes): Fast acting insulin (Actrapid 100/ml Novo Nordisk) will then be infused intravenously at a rate of 20 mU/m²/min (or adjusted depending on previous studies) for 120 minutes to achieve a plasma insulin concentration of 50 uU/ml. Glucose concentrations will be maintained at 4.7 mmol/l by a variable infusion of 20% glucose. Glucose will be sampled at 90, 130, and 160 minutes and then every 5 minutes and analysed via a blood glucose monitor to ensure a glucose concentration of 4.7 mmol/l. At 180, 190, 200, 210 minutes the plasma will be collected for future measurement of glucose and glycerol, insulin, lactate, β hydroxybutyrate and NEFA concentrations. The amount of glucose infused will be averaged from 180-210 minutes. This value is used to estimate glucose disposal as glucose uptake in peripheral tissues under steady state conditions.

Stage 3: high dose HEC (210-330 mins): Fast acting insulin (Actrapid Novo Nordisk) will then be infused intravenously at a rate of 40 mU/m²/min for 120 minutes to achieve a plasma insulin concentration of 100 uU/ml. Glucose will again be sampled

at 210 minutes then at 250 and 270 minutes and then every 5 minutes and analysed via a blood glucose monitor to ensure a glucose concentration of 4.7 mmol/l. From 80 to 120 mins the plasma will be collected for glucose and glycerol, insulin, lactate β hydroxybutyrate and NEFA concentrations. The amount of glucose infused will be averaged from 290 – 330 minutes. This value is used to estimate glucose disposal as glucose uptake in peripheral tissues under steady state conditions.

Note: Insulin sensitivity will be estimated as the glucose infusion rate required to maintain plasma glucose constant at 4.7 mmol/l during the clamp studies. The primary purpose of the 2 dose insulin infusion clamp studies will be to determine the ratio of the change in glucose infusion rate to change in insulin concentration from low to high dose insulin infusions. This enables a comparison of the dose response curves during pregnancy. Quantitative estimates of insulin sensitivity from glucose clamps conducted at a single insulin concentration may underestimate the degree of insulin resistance, because there is a large increase in noninsulin requiring glucose disposal during pregnancy resulting from utilisation by the fetus and placenta.

A secondary purpose of these two clamp procedures is to determine whether lower doses of insulin result in less suppression of EGP in pregnancy.

Indirect calorimetry: respiratory gas exchange rates will be determined via a ventilated hood system (GEM, Nutrition Ltd, Cheshire). The analyser will be calibrated before and after each procedure using standard gases. All subjects will be in the semi-recumbent position on their left side during measurements. The urinary nitrogen level will be determined using a urinary samples collected prior to the study. The quantity of urinary urea nitrogen excreted during the study will be used as an index of protein oxidation assuming that 1g nitrogen equals 6.25g protein. Whole body glucose oxidation and lipid oxidation may then be derived according to the tables of Frayn (Frayn 1983). Indirect calorimetry will be done at the following points: 1. 70 – 90 minutes, 2. 190 – 210 minutes, 3. 310-330 minutes

NOGU may be calculated: $\text{NOGU} = \text{Rd glucose} - \text{glucose oxidation}$.

The rate of FFA oxidation provides an index of the rate of FFA re-esterification because recycling is ultimately the fate of all non-oxidised FFA. Therefore:

$\text{FFA reesterification} = \text{glycerol Ra} \times 3 - \text{FFA oxidation}$

Preparation of infusates: Solutions of 6,6-²H₂-glucose (99% H) and 1,1,2,3,3-²H₅-glycerol (99% H) (Tracer Technologies, Inc., Somerville, MA, USA) will be prepared in pyrogen-free 0.9% saline and passed through a Ministart 0.2 mm filter. These infusates are prepared by Dr Alistair Millar at the Royal Infirmary, Edinburgh.

Metabolites and analysis: Plasma for 6,6-²H₂-glucose enrichment will be deproteinised with ethanol and derivatised with n-butyl boronic acid in pyridine and acetic anhydride. The resultant metabolites will be analysed by gas chromatography: mass spectrometry in the department of Endocrinology, Wellcome Trust Core Research Facility. Insulin will be quantified by an ELISA method as previously described (Merckodia, Uppsala, Sweden). Lactate and hydroxybutyrate will be determined by enzymatic kits (Randox, Co. Antrim, UK) as will NEFA (Alpha, Eastleigh, UK) on an in-house centrifugal analyser (Cobaas Mira).

Reference

Forbes S, Robinson S, Dungu J, Anyaoku V, Bannister P, Forster D, et al. Sustained endogenous glucose production, diminished lipolysis and non-esterified fatty acid appearance and oxidation in non-obese women at high risk of type 2 diabetes. *European journal of endocrinology / European Federation of Endocrine Societies*. 2006 Sep;155(3):469-76

Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol*. 1983 Aug;55(2):628-34.

Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. *Annals of the New York Academy of Sciences*. 1959 Sep 25;82:420-30.

APPENDIX 9: MRI SCANNING

Twenty women in each of the active and placebo groups (40 altogether) will be scanned using the Verio 3 Tesla MRI system (Siemens Medical Systems, Germany) in the CRIC, Queen's Medical Research Centre.

Women will be scanned at around 24 - 28 weeks gestation and then again at around 36 weeks gestation. Subjects will be positioned in the magnet in a left lateral position to prevent aorto-caval compression.

1. Abdominal MR imaging of maternal and fetal body fat content.

The maternal abdomen will be scanned using T1 weighted acquisitions to measure maternal subcutaneous abdominal and visceral fat, and then fetal abdomen also scanned to measure fetal subcutaneous fat and visceral fat. Images will be acquired as single slices and each slice analysed using a commercially available software program (SliceOmatic) that employs knowledge-base image processing to label pixels as fat or non-fat components. The adipose tissue mass thus derived will be converted into fat mass using a mathematical model.

2. Fetal liver volume

Isotropic voxel acquisition images of the fetal abdomen will be selected, through a large enough volume to cover the fetal liver. This data will be used to generate a volume measurement of the fetal liver using established research techniques.

3. ¹H MRS of liver – measurement of maternal and fetal intrahepatocellular lipid.

Single voxel 3x3x3 cm³ spectra will be obtained from the maternal and fetal liver using a PRESS sequence least square fitting method operating in the time domain. Peak areas for all resonances will be obtained and lipid resonances quantified with reference to water after correcting for T1 and T2. Maternal and fetal liver fat content will be calculated and expressed in the standard way as a percentage.

4. MRI of the maternal and fetal liver. MRI of the maternal and fetal liver will be done.

Senior scientists in CRIC are in the process of developing in-house software analysis programmes which will allow a 3D image to be mapped over the liver such that the total hepatic fat content may be estimated. This may potentially be more representative of total hepatic fat content as it is not restricted to a small sampling voxel site

5. ¹H MRS of muscle – measurement of intramyocellular lipid.

Single voxel spectra will be acquired from the maternal thigh (quadriceps). Other muscle fibers may also be scanned. Using a PRESS sequence, from a $3 \times 3 \times 3 \text{ cm}^3$ voxel with TE/TR=135/1500 ms and 256 averages. Peak areas for each signal will be obtained and lipid resonances quantified with reference to Crtot after correcting for T1 and T2 as previously described.

For each parameter, the change from 24 to 36 weeks will be calculated and compared between the metformin and the placebo groups. Additionally, since the baseline scan will (for safety reasons) be performed after the study drug is commenced, a secondary analysis will compare results at 36 weeks gestation between the two treatment groups.

Single voxel spectra will be acquired from the maternal thigh, using a PRESS sequence, from a $2 \times 2 \times 2 \text{ cm}^3$ voxel with TE/TR=135/1500 ms and 256 averages. Peak areas for each signal will be obtained and lipid resonances quantified with reference to Crtot after correcting for T1 and T2 as previously described.

For each parameter, the change from 24 to 36 weeks will be calculated and compared between the metformin and the placebo groups. Additionally, since the baseline scan will (for safety reasons) be performed after the study drug is commenced, a secondary analysis will compare results at 36 weeks gestation between the two treatment groups.