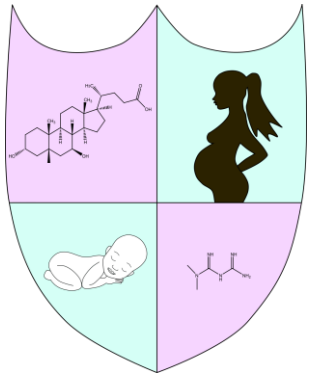
PROTOCOL FULL TITLE:

Randomised controlled trial of **G**estational treatment with **U**rsodeoxycholic **A**cid compared to Metformin to **R**educe effects of **D**iabetes mellitus

Protocol Short Title/Acronym:
GUARD



Trial Identifiers

EudraCT Number – 2019-002880-82
IRAS Number – 1003208
REC Number – 20/LO/0504
ISRCTN –
NIHR portfolio number – 44480
EDGE ID - 128219

Sponsor
King's College London
Representative: Helen Critchley
King's Health Partners Clinical Trial Office, 16th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
Telephone: 020 7188 5732
Fax: 020 7188 8330
helen.critchley@kcl.ac.uk

Co-sponsor
Guy's and St Thomas NHS Foundation Trust
Representative: Rachel Fay
Guy's and St Thomas NHS Foundation Trust R&D Department, 16th Floor Tower Wing, Great Maze Pond, London SE1 9RT
Telephone: 02071889811
R&D@gstt.nhs.uk

Chief Investigator

Professor Catherine Williamson
Professor of Women's Health
Division of Women and Children's Health, King's College London, London SE1 1UL
020 7848 6350 / 020 7848 6014
catherine.williamson@kcl.ac.uk

Name and address of Co-Investigators

Prof Helen Murphy
Professor of Medicine
Address: St Thomas' Hospital, London
helen.1.murphy@kcl.ac.uk

Dr Caroline Ovadia
Chadburn Lecturer in Obstetrics and Gynaecology
Address: King's College London, London
caroline.ovadia@kcl.ac.uk

Dr Alice Mitchell
Postdoctoral Research Associate
Address: King's College London, London
alice.mitchell@kcl.ac.uk

Dr Annette Briley
Consultant Midwife
Address: St Thomas' Hospital, London
annette.l.briley@kcl.ac.uk

Ms Claire Singh
Research Matron – Women's Services
Address: St Thomas' Hospital, London
claire.Singh@gstt.nhs.uk

Dr Peter Dixon
Research Fellow
Address: King's College London, London
peter.dixon@kcl.ac.uk

Prof Kennedy Cruickshank
Professor of Cardiovascular Medicine and Diabetes
Address: King's College London and Guy's and St Thomas NHS FT, London
Telephone: 02078484270
kennedy.cruickshank@kcl.ac.uk

Statistician

Mr Paul Seed
Address: King's College London, London
Email: paul.seed@kcl.ac.uk

Clinical Trial Manager

Ms Sonia Serrano
NIHR Biomedical Research Centre, Guy's and St Thomas' NHS Foundation Trust
Telephone: 020 7188 7188 ext 53369; Sonia.serrano@gstt.nhs.uk

1. Study Synopsis

Title of clinical trial	Randomised controlled trial of **G**estational treatment with **U**rsodeoxycholic **A**cid compared to Metformin to **R**educe effects of **D**iabetes mellitus
Protocol Short Title/Acronym	GUARD
Trial Phase if not mentioned in title	Pilot Phase 4 RCT
Sponsor name	King's College London & Guy's and St Thomas' NHS Foundation Trust
Chief Investigator	Professor Catherine Williamson
EudraCT number	2019-002880-82
REC number	31/03/2020
Medical condition or disease under investigation	Gestational Diabetes Mellitus (GDM)
Purpose of clinical trial	To compare the impact of treatment with ursodeoxycholic acid vs metformin on glycaemic control in women with GDM
Primary objective	To assess the efficacy of UDCA compared to metformin to improve glycaemic control in GDM.
Secondary objective (s)	- To evaluate the impact of the treatments on maternal and neonatal lipid metabolism. - To assess the acceptability of UDCA compared to metformin to women with GDM. - To establish whether continuous glucose monitoring gives more informative overall assessment of maternal glycaemic control in overweight or obese pregnant women. - To evaluate vascular responses in each arm. - To compare maternal and fetal outcomes that could relate to treatment with UDCA or metformin.
Trial Design	Two-armed, randomised, controlled, open label multicentre clinical trial with optional observational mechanistic study in a subgroup from each arm
PICO	P – Pregnant women of a BMI ≥25 with GDM requiring pharmacological treatment I – UDCA 500mg BD C – Metformin 1000mg BD O – Fasting glucose concentration at 36 weeks gestation
Endpoints	- Maternal fasting glucose concentration at 36 weeks gestation measured with a blood sample.

	- Quality of Life assessment at baseline and 36 weeks', and treatment satisfaction scores at 36 weeks gestation. - Biomedical and clinical maternal outcomes - Biomedical and clinical neonatal outcomes at birth
Sample Size	**Main study** 158 (79 per group) in the randomised controlled trial 40 additional participants in the mechanistic sub-study **Mechanistic sub-study (GUARD MEC)** 80 participants will be enrolled in total in the GUARD MEC. Of these, 40 will be GUARD participants: - 20 women randomised to metformin - 20 women randomised to UDCA Additionally, 40 controls will be enrolled into two additional arms of the mechanistic studies: - 20 women with GDM not requiring pharmacotherapy - 20 pregnant women without GDM
Summary of eligibility criteria	1. Women between 16 and 45 years of age with GDM diagnosed at 26^{+0} to 30^{+6} weeks' gestation in accordance with the NICE guidelines (one or more glucose concentration of ≥5.6 mmol/l fasting or ≥7.8 mmol/l 2 hours after a standard 75g OGTT, and requiring pharmacological treatment). 2. Overweight or obese (Booking BMI ≥25 kg/m2) 3. Planned antenatal, intrapartum and postpartum care at the participating centre (i.e. not planning to move before delivery).
IMP, dosage and route of administration	UDCA oral 500 mg BD
Active comparator product(s)	Metformin oral 1000 mg BD
Maximum duration of treatment of a participant	14 weeks + 3 month follow up
Version and date of protocol amendments	V 1.0 dated 11th March 2020

2. Glossary of Terms

AE	Adverse Event
AR	Adverse Reaction
BD	Twice a Day
BMI	Body Mass Index
BP	Blood Pressure
BRC	Biomedical Research Centre
CGM	Continuous Glucose Monitoring
CI	Chief Investigator
CRA	Clinical Research Associate
CS	Caesarean Section
CTIMP	Clinical Trial of an Investigational Medicinal Product
CTM	Clinical Trial Manager
CTO	Clinical Trials Office
eCRF / CRF	(Electronic) Case Report Form
FU	Follow Up
GCP	Good Clinical Practice
GDM	Gestational Diabetes Mellitus
GSTFT	Guy's and St Thomas' NHS Foundation Trust
HBA1C	Glycated hemoglobin
HDL	High Density Lipoprotein
HDPE	High-density polyethylene
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICP	Intrahepatic Cholestasis of Pregnancy
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ITT	Intention To Treat
QOLQ	Quality of Life Questionnair
KCP	King's Health Partners
KCL	King's College London
LCA	Lithocholic Acid
LGA	Large Gestational Age
LSCS	Lower Segment Caesarian Section
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NIMP	Non-Investigational Medicinal Product
OGTT	Oral Glucose Tolerance Test
PI	Principal Investigator
PIS	Patient Information Sheet
PMU	Pharmacy Manufacturing Unit
PPI	Patient and Public Involvement
PWV	Maternal Pulse Wave Velocity
RCT	Randomised Controlled Trial
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SMBG	Self-Monitoring of Blood Glucose
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Adverse Reaction
SVD	Spontaneous Vaginal Delivery
T2DM	Type 2 Diabetes Mellitus
TMF	Trial Master File
TSC	Trial Steering Committee
UDCA	Ursodeoxycholic Acid
U&E	Urea & Electrolytes

CONTENTS

3. Background & Rationale

Each year in the UK approximately 35,000 women develop diabetes during pregnancy, a condition called gestational diabetes mellitus (GDM), which increases the risk of adverse outcomes for both mother and child[1]. Complications for the mother include increased risk of hypertensive diseases of pregnancy, including preeclampsia[2], and higher rates of cardiovascular disease and type 2 diabetes mellitus (T2DM) in later life[3–5]. Aside from hyperglycaemia, GDM is further complicated by maternal dyslipidaemia. Specifically, triglyceride and free fatty acid concentrations are increased in maternal blood, whilst high density lipoprotein (HDL)-cholesterol is reduced[6,7]. Modern metabolomic studies show disturbances of lipid metabolism, particularly intermediary metabolites (e.g. acyl-carnitines, phospholipids)[7,8]. Early decline in plasma adiponectin, an indicator of poorer mitochondrial oxidation in overweight and obese women, is an almost universal finding in GDM pregnancy[9]. Thus, GDM is a potentially vasculotoxic condition, associated with abnormal lipid and glucose metabolism[10].

GDM is also associated with accelerated fetal growth and increased risk of being large for gestational age (LGA), defined as birth weight above the 90th percentile for sex and gestational age[1,11]. GDM is also complicated by higher rates of preterm birth, caesarean section and birth injuries, including shoulder dystocia, which is particularly increased with LGA[1,2,12]. Due to the complications of preterm delivery and LGA, GDM offspring are more likely to require admission to neonatal intensive care units for treatment of hypoglycaemia, jaundice and respiratory distress[11]. GDM causes fetal dyslipidaemia, with increased free fatty acids and triglycerides in the umbilical cord blood; this is also associated with increased risk of LGA[13–15]. The children of women with GDM have increased rates of obesity, childhood cardiovascular disease and T2DM in later life, likely related to exposure both to maternal hyperglycaemia and maternal hyperlipidaemia in utero[16,17].

3.1 Effectiveness of current treatments

In the UK, women with risk factors for GDM have a 75g oral glucose tolerance test (OGTT) at 24-28 weeks' gestation. Those that test positive (fasting glucose concentration ≥5.6 and/or 2-hr ≥7.8mmol/L) start self-monitoring of blood glucose (SMBG) and are given dietary and lifestyle advice. If unable to achieve the National Institute for Health and Care Excellence (NICE) recommended glucose control targets (fasting glucose <5.3, 1hr <7.8mmol/L and/or 2hr <6.7mmol/L), they are prescribed either pharmacological oral glucose lowering medications, e.g. metformin/glibenclamide, or subcutaneous insulin injections. Metformin is the most commonly used first line pharmacological treatment. However, there is increasing concern about its widespread use during pregnancy, both because of its limited efficacy and because of potential safety concerns. Metformin crosses the placenta, has growth inhibitory properties and suppresses mitochondrial respiration which could theoretically adversely affect the developing fetus[18,19]. The Metformin in Gestational Diabetes (MiG) trial demonstrated that mothers randomised to metformin, compared to insulin, had reduced maternal weight gain and gestational hypertension[20]. However, the rate of LGA was not affected and the offspring had more subcutaneous fat at 2 years of age[21]. A study of maternal metformin treatment for women

with polycystic ovary syndrome also did not show an impact on LGA, and the offspring were heavier at 1 year of age[22]. Thus, the current data have raised concerns that metformin, currently used by many women with GDM, does not adequately prevent adverse outcomes such as LGA, and may have negative long-term effects on the metabolic health of the children[23,24]. This may be, at least in part, because metformin has less effect on serum triglyceride concentrations than insulin[20]. It is noteworthy that in the MiG trial, metformin alone was inadequate for achieving glycaemic targets in approximately 50% of women, necessitating supplementary treatment with insulin[20]. Indeed, even insulin treatment (the "gold standard" pharmacological approach) was not shown to be of definitive benefit for GDM offspring in the most recent Cochrane review, and was thought to possibly increase the risk of raised blood pressure compared to oral treatments[25]. Glibenclamide is the other oral hypoglycaemic agent used to treat GDM. It has not been shown to be superior to insulin treatment used in randomised trials[26], or as an add-on therapy to metformin[27]. Therefore, there is an urgent unmet need for additional therapies that improve maternal-fetal glucose and lipid metabolism, and the longer-term health outcomes of GDM exposed offspring.

Ursodeoxycholic acid (UDCA) is currently not an established/licensed treatment for GDM. However, a meta-analysis that included data from 7 trials that reported the impact of UDCA on glycaemic markers showed that it improved fasting glucose, insulin and HbA1c concentrations[55]. Furthermore, our pilot data from studies of UDCA treatment of women with intrahepatic cholestasis of pregnancy have demonstrated improved insulin resistance, indicating that it has the potential to be an effective treatment to improve glycaemic control in GDM (see Appendix 2 for more details). UDCA is commonly used in pregnancy for the treatment of ICP, and a recent randomised, placebo-controlled trial did not show any increase in adverse events, including gastrointestinal symptoms, in women treated with UDCA compared to placebo[56]. Our pilot data also show that UDCA improves fetal serum lipid parameters (see Appendix 2), so it may be more effective than metformin at reduction of the frequency of large for gestational age infants in GDM.

3.2 Rationale

Our trial will compare the impact of treatment with ursodeoxycholic acid (UDCA) -a drug with pilot data to support its effectiveness to treat GDM- to metformin on glycaemic control (primary outcome) in women with GDM (see Appendix 1). We will evaluate maternal and fetal lipid and glucose metabolism, and maternal vascular outcomes, using biochemical and imaging assays and simple arterial measures. Neonatal health outcomes will also be studied, including the proportion of LGA offspring. Table 1 summarises the mechanisms by which UDCA and metformin influence glucose and lipid metabolism.

Table 1. Proposed mechanisms of action of UDCA and metformin in GDM

	UDCA	Metformin
Inhibition of the mitochondrial respiratory chain complex 1; leads to activation of hepatic AMPK, reducing SRBEP1c which controls glucose-stimulated genes associated with lipid, glucose and protein formation, and stimulates fatty acid oxidation and glucose uptake	X	✓

Activation of hepatic FXR, reducing SRBEP1c which controls glucose-stimulated genes associated with lipid, glucose and protein formation, and stimulates fatty acid oxidation and glucose uptake	✓	X
Brown adipose tissue activation of AMPK, breakdown of VLDL-TG, mitochondrial content	X	✓
Brown adipose tissue signalling via TGR5 to increase energy expenditure by increasing UCP1	✓	X
Increased skeletal muscle insulin sensitivity and insulin-mediated glucose uptake	X	✓
GLP-1 receptor increase and reduced GLP-1* breakdown	X	✓
GLP-1* release increase	✓	X
Reduction of endoplasmic reticulum stress in obese individuals, reducing insulin resistance	✓	X

* Glucagon-like peptide 1 (GLP-1) is thought responsible for 70% of the insulin release following meals, and levels are lower in GDM than unaffected pregnancies[28]

There is increasing evidence that the gut microbiota plays a role in maternal glucose and lipid metabolism. When faeces from pregnant women were transplanted into germ-free mice there were phenotypic differences in mice receiving faeces from women in the 3rd vs 1st trimester of pregnancy. The 3rd trimester faeces had a greater abundance of the pro-inflammatory Proteobacteria and Actinobacteria and the recipients had more weight gain and insulin resistance[29]. Women with GDM also have alterations in the gut microbiota. At the time of diagnosis, there is enrichment with a number of microbes, e.g. Desulfovibrio, the prevalence of which is also higher in T2DM[30]. Similar changes in the composition of the gut microbiota occur in women who are overweight in pregnancy[31]. Studies of the gut microbiota in T2DM have reported reductions in butyrate-producing bacteria in untreated compared to metformin-treated patients[32]. Butyrate is a metabolically-active short chain fatty acid (SCFA), increased levels of which are associated with improved glucose control[33].

We propose that UDCA is a new potential treatment for GDM. UDCA alters gut metabolites (via microbial modification of the drug). We hypothesise that this will result in increased release of gut hormones (GLP-1 and FGF19) that improve maternal/fetal blood concentrations of lipids, e.g. triglycerides, glycaemic control and reduce rates of obstetric and neonatal complications. We have pilot data to support this hypothesis from studies of women with intrahepatic cholestasis of pregnancy (ICP) treated with UDCA.

3.3 Pilot data

UDCA treatment improves HOMA-IR and GLP-1 secretion in the mother in women with ICP

Women with ICP have increased rates of GDM (odds ratio 2.81, 95% CI 2.32-3.41)[34]. Using continuous glucose monitoring, we demonstrated ICP-associated elevations in prandial glucose concentrations, abnormal glucose tolerance and reduced secretion of the gut hormone glucagon-like peptide-1 (GLP-1)[35], which acts to enhance glucose-mediated insulin secretion[36]. We have shown improvement in GLP-1 in women with ICP treated with

UDCA[35]. Of note, GLP-1 is thought responsible for 70% of the insulin release following meals, and levels are lower in GDM than unaffected pregnancies[28].

Research to support study of the gut microbiome

To understand the mechanism of action of UDCA treatment in ICP, we have performed a pilot study of faecal gut microbiota and bile acid profiles see Appendix 1.

UDCA treatment improves ICP-associated maternal and fetal dyslipidaemia

Women with ICP have dyslipidaemia in addition to increased susceptibility to ICP-associated GDM. This is characterised by elevated serum concentrations of total cholesterol and low density lipoprotein (LDL)-cholesterol. Our pilot data show improvement in maternal total cholesterol and LDL-cholesterol following UDCA treatment (see Appendix 2)

A recent study of UDCA treatment of 20 people with T2DM and hepatic impairment showed reduced weight and HbA1c (glycosylated haemoglobin) after treatment for 12 weeks (a similar duration to that proposed for this study)[38]. Furthermore, a recently published meta-analysis in people with non-alcoholic fatty liver disease (a disorder that is commonly associated with T2DM, previous GDM)[39,40] reported that UDCA treatment was associated with significant reduction in fasting glucose, HbA1c and plasma insulin concentration. UDCA is therefore a biologically plausible treatment but has not yet been evaluated in GDM. **We believe it is important and timely to evaluate the impact of UDCA on maternal and fetal outcomes in GDM.**

Dose rationale

The dose of UDCA taken by most women with ICP is 500mg BD, including those from whom the pilot data in Appendix 2 were obtained. Similarly, the recent PITCHES trial that compared UDCA to placebo treatment for ICP proposed a starting dose of 500mg BD that could be increased to a maximum of 2g daily[56]. There was no increase in adverse outcomes in women treated with UDCA compared to those that received placebo. The reason for increasing the dose in women with ICP is typically due to severity of the symptom of pruritus or worsening liver function tests. There is no evidence that an increase of UDCA dose will improve glycaemic control more than treatment with 500mg BD, and therefore we propose to only use a UDCA dose of 500mg BD for the GUARD Trial. This does not need to be increased as the lower dose was sufficient to have an impact on maternal insulin sensitivity and serum lipids.

3.4. Rationale for using continuous glucose monitoring (CGM), dietary assessment and vascular studies

CGM will be used in this trial alongside conventional capillary glucose monitoring to compare the impact of UDCA and metformin on maternal glycaemic control. CGM measures interstitial glucose concentration every five minutes through a sensor that is placed subcutaneously. With 288 glucose measurements/day, CGM provides detailed glucose information about overnight and post-prandial glucoses, providing direct insight into foetal exposure to maternal glycaemia[41]. A recent large international consensus paper

highlighted CGM as a robust research tool and emphasised the accuracy of contemporary sensors, the detailed information they provide and non-invasive nature compared to frequent capillary glucose monitoring[42].

There is evidence that the diet composition of pregnant women influences their susceptibility to GDM[57]. Therefore women will be asked to complete a 4 day dietary assessment at 36 weeks' gestation. This will enable evaluation of diet composition and whether this could influence treatment response. The diet will also affect the gut microbiota, so the 4 day diet questionnaire will be completed by all participants in the GUARD MEC study.

For vascular studies we will use a calibrated cuff-based blood pressure instrument, the Arteriograph, as recently used in a maternal hypertension trial[46]. We include vascular health as a secondary outcome of the proposed trial because arterial function measures are more powerful than, and independent of, standard blood pressure for later prognosis[47–49]. The arteriograph works by a minor supra-systolic inflation so that the cuff senses the waveform from each heartbeat for 4-6 beats, thereby providing both a BP measure and for arterial stiffening through sensing the waveforms. It has a British Hypertension Society (BHS) A/A grading for its BP measurement.

3.5 Future work

If we generate convincing evidence that UDCA improves maternal glycaemic control we intend to apply for funds to perform a large multicentre trial where we can personalise decisions about which women will respond to UDCA to improve maternal and baby outcomes.

Many women with GDM are susceptible to T2DM, a condition with a higher rate of the adverse outcomes associated with GDM and also increased risk of stillbirth. The underlying pathology is similar in many cases. At present not all women of reproductive age are aware that they have T2DM and some are only diagnosed in early pregnancy. If UDCA is effective in GDM, we will also apply to study UDCA as a potential therapy for treatment of women with established T2DM in pregnancy.

The children of women with GDM have increased rates of obesity, diabetes and cardiovascular disease in later life. If UDCA improves fetal metabolic parameters, e.g. umbilical cord blood LDL-cholesterol, triglycerides or free fatty acids, we also intend apply for future funds to evaluate offspring metabolic health as the drug may not only improve perinatal outcomes but also the future health of the children that were treated in utero.

4. Trial Objectives and Design

4.1. Trial Objectives

4.1.1 Primary objective

To assess the efficacy of UDCA compared to metformin to improve glycaemic control in GDM.

4.1.2 Secondary objectives (endpoints detailed below in 4.2.2)

- To evaluate the impact of the treatments on maternal and neonatal lipid metabolism.

- To assess the acceptability of UDCA compared to metformin to women with GDM.

- To establish whether continuous glucose monitoring gives more informative overall assessment of maternal glycaemic control in overweight or obese pregnant women,

- To evaluate vascular responses in each arm (optional element).

- To compare maternal and fetal outcomes that could relate to treatment with UDCA or metformin.

For objectives of the GUARD MEC sub-study please see section 19. Mechanistic Sub-study: GUARD MEC.

4.2 Trial endpoints

4.2.1 Primary endpoint

Maternal fasting glucose concentration at 36 weeks' gestation measured with a blood sample.

4.2.2 Secondary endpoints

- Quality of Life assessment (EQ-5D-5L) at baseline and 36 weeks, and treatment satisfaction scores at 36 weeks.

- Biomedical and clinical maternal outcomes:
    1. Glucose metabolism at baseline, Follow up 1 and 2 assessed by:
        a) Continuous glucose monitoring (CGM) to assess glycaemic control. This will determine the percentage time spent within target (glucose levels 3.5-7.8mmol/L), percentage time spent above target (>7.8mmol/l and ≥6.7mmol/l), time spent below target (≤3.5 and ≤3.0 mmol/l), measures of glucose variability including glucose standard variation (SD), co-efficient of variation (CV), frequency and duration of glycaemic excursions measured by the area under the curve (AUC) for the pre-specified glucose thresholds.
        b) Serum concentrations of 1,5-anhydroglucitol; a novel marker of short-term glycaemia [4,43]
        c) HbA1c concentration; a conventional marker of medium-term glycaemia (except at Follow up 1)
    2. Lipid metabolism at Follow up 2 assessed by blood triglyceride, total cholesterol, calculated LDL-cholesterol, HDL-cholesterol and free fatty acid concentrations

3. Biochemical analysis of maternal blood for liver function tests at Follow up 2 (ALT, bilirubin, ALP), bile acids, C reactive protein (including highly sensitive analyses)
4. Proportion of women requiring insulin treatment (time until treatment and total dose of insulin required)
5. Maternal gestational weight change at 36 weeks compared to weight at first trimester screening visit.
6. Measurement of vascular responses at Follow up 1 and 2, including: i) maternal pulse wave velocity (PWV), with systolic and diastolic blood pressure, ii) central arterial pressure (cP), and iii) augmentation index (AIx)
7. Estimated blood loss at time of delivery.

- Biomedical and clinical neonatal outcomes at birth:

1. Mode of birth (rates of caesarean section (CS), (elective & emergency), assisted vaginal birth and spontaneous vaginal delivery (SVD))
2. Gestational age at birth
3. Apgar scores @ 5 minutes post birth
4. Occurrence of shoulder dystocia
5. Cord blood C-peptide, triglyceride, total cholesterol, calculated LDL-cholesterol, HDL-cholesterol and free fatty acid concentrations
6. Infant birth weight (customised birth weight percentile[51], proportion of babies born large for gestational age (LGA), proportion of babies born small for gestational age (SGA)
7. Neonatal morbidity (treatment for neonatal hypoglycaemia, neonatal jaundice, respiratory distress or birth trauma)
8. Neonatal intensive care and special care unit admission (duration of hospital stay)
9. Stillbirth and neonatal death

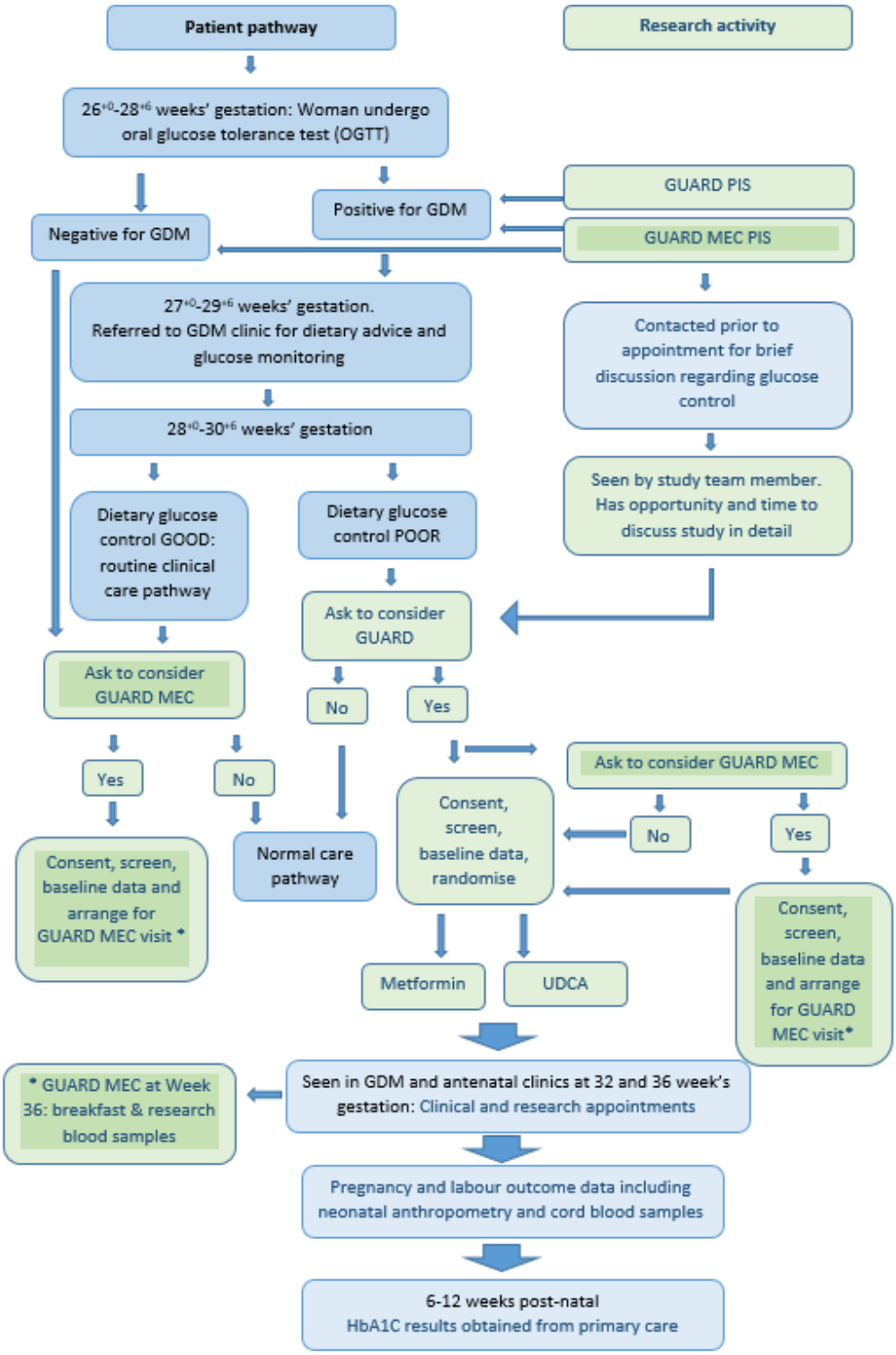
For endpoints of the GUARD MEC sub-study please see section 19. Mechanistic Sub-study: GUARD MEC.

4.3 Trial Design

GUARD is a pilot phase IV two-armed, open label, multi-centre randomised, controlled trial, designed to discover possible new uses for UDCA, a drug commonly used in pregnancy for other conditions.

158 overweight or obese women with a clinical diagnosis of GDM that requires management with pharmacological intervention will be recruited and randomised to one of two trial interventions in the UK.

4.4 Trial Flowchart



5. Trial Medication

5.1 Investigational Medicinal Product

The Pharmacy Manufacturing Unit, Guy's and St Thomas' NHS Foundation Trust (GSTFT PMU) Pharmaceuticals are licensed to support clinical trials under an MIA (IMP) licence granted by the MHRA license. They have a long standing history of servicing the clinical trials market, and are specialised in the manufacture, storage and distribution for trials. GSTFT PMU will supply, re-package, label and distribute both IMPs for this clinical trial. Analytical testing, Annex 13 compliant labelling, and temperature controlled and monitored storage and shipment will be implemented.

5.1.1 UDCA

Ursodeoxycholic acid 500mg film-coated tablets (Ursofalk®, Dr Falk) will be packed into packs of 28 tables (2 weeks' supply) in a high-density polyethylene (HDPE) container with child-resistant, tamper evident closure.

5.1.2 Metformin

Metformin 500 mg tablets (Medley) will be packed into packs of 56 tablets (2 weeks' supply) in an HDPE container with child-resistant, tamper-evident closure with integrated silica gel desiccant tablets.

The investigator should ensure that the participant has sufficient tables of the allocated treatment to last until the following scheduled appointment. Patients will return unused doses at the final visit.

5.2 Dosing Regimen

Starting treatment for UDCA is 500 mg twice a day (BD) orally with the morning and evening meals.

Metformin will be started following a dose escalation scheme to minimise side effects, until a dose of 1000 mg BD is reached:

- Days 1 & 2: 500 mg with evening meal
- Days 3 & 4: 500 mg with breakfast and 500 mg with evening meal
- Days 5 & 6: 500 mg with breakfast and 1000 mg with evening meal
- Day 7 and remaining: 1000 mg with breakfast and 1000 mg with evening meal

In both cohorts, participants will take the first dose within 2 days of the baseline visit, and will continue self-administration at home, while they undergo regular glucose control checks in line with current clinical practice.

The glucose control targets will follow NICE pregnancy guidelines (i.e. aiming to maintain all capillary glucose levels between 3.9-7.8mmol/l). The specific pre- and post-meal

SMBG targets are ≤5.3mmol/L before breakfast, ≤7.8mmol/L 1-hr post meal and ≤6.7mmol/L 2-hr post meal. All participants will be given education regarding diet and lifestyle as part of their standard clinical care pathway. Insulin may be added as a rescue medication if oral treatment does not control blood glucose levels, in accordance with standard antenatal clinical practice. Insulin will constitute a non-investigational medicinal product (nIMP). Also following clinical practice, doses could be reduced or temporarily discontinued if deemed appropriate by the patient's clinician.

Compliance will be checked with study participants at follow up visits by reviewing diary cards.

Last dose will be taken at the time of delivery, as per clinician instructions.

5.3 IMP Risks

For a list of up-to-date risks, latest Summary of Product Characteristics (SmPC) should be consulted. None of the two IMP are licensed for use in pregnancy, therefore this data is not based in pregnancy data.

Frequency of occurrence is defined as follows: very common: 1/10; common>1/100, <1/10; uncommon>1/1,000, <1/100; rare>1/10,000, <1/1,000; very rare<1/10,000, not known.

Metformin: The most common adverse reactions are nausea, vomiting, diarrhoea, abdominal pain, loss of appetite (very common) and taste disturbance (common), which usually resolve spontaneously. To prevent these a gradual dose increase is used. Very rare effects are skin reactions, lactic acidosis and liver function tests abnormalities or hepatitis.

UDCA: Pasty stools or diarrhoea are common adverse reactions. Nausea, vomiting and pruritus might be effects but the frequency is unknown. Very rare effects are: calcification of gallstones and urticaria.

Should the research team have concerns about any new symptoms when taking the IMP, they will act on it as per clinical standard, which might need consulting with the investigator.

5.4 Drug Accountability

The Co-Sponsors will arrange transfer of active IMP from GSTFT PMU to the participating sites' pharmacies. The Principal Investigator will then take responsibility for IMP accountability by ensuring that: the IMP is stored in a secure location, segregated from other medicines, used and returned medication is kept separate from unused medication, storage conditions are monitored and recorded, IMP is dispensed to participants in accordance with the trial protocol and any randomization list, and unused medication is returned to the study team or destroyed if requested by the sponsor. Full accountability records will be kept for all

aspects of IMP handling in pharmacy. IMP accountability records will be monitored by the Clinical Research Associate (CRA).

5.5 Storage of IMP

Both IMPs will be stored by pharmacy, kept at 15-25^{o}C and dispensed after each study visit. Temperatures will be monitored within the pharmacy departments but this data will not be collected.

5.6 Participant Compliance

Participant attendance and compliance will be recorded for all visits.

Women will receive a diary card to record taken/not taken doses. Drug accountability will be conducted at each study visit by asking patients if they missed any doses and reviewing the diary card. If discrepancies are identified, this should be discussed with the participant. Any reported missed doses will be recorded in the eCRF.

5.7 Concomitant Medication

A complete listing of all concomitant medication received from baseline to birth or study discontinuation must be recorded in the medical notes and eCRF, with the exception of standard medications given during labour.

Avoidance of the following concomitant medications is recommended:

- Metformin: Trimethoprim and vancomycin, which could theoretically cause acidosis
- Ursodeoxycholic acid should not be administered concomitantly with charcoal, colestyramine, colestipol or antacids containing aluminium hydroxide and/or smectite (aluminium oxide), because these preparations bind ursodeoxycholic acid in the intestine and thereby inhibit its absorption and efficacy

Each drug's SmPC should be reviewed for current information about management of concomitant medications.

6. Selection and Withdrawal of Participants

6.1 Inclusion Criteria

1. Women between 16 and 45 years of age with GDM diagnosed at 26^{+0} to 30^{+6} weeks' gestation in accordance with the NICE guidelines (one or more glucose concentrations of ≥5.6 mmol/l fasting or ≥7.8 mmol/l 2 hours after a standard 75g OGTT, and requiring pharmacological treatment).

2. Overweight or obese (Booking BMI ≥25 kg/m2)
3. Planned antenatal, intrapartum and postpartum care at the participating centre (i.e. not planning to move before delivery).

6.2 Exclusion Criteria

1. Unwilling/unable to give written informed consent and comply with the requirements of the study protocol
2. Multiple pregnancies (twins, triplets etc) in current pregnancy
3. Congenital anomaly on ultrasound requiring fetal medicine input
4. Previous diagnosis of diabetes outside pregnancy
5. HbA1c at booking >48 mmol/mol or ≥6.5% during current pregnancy (if available)
6. Significant pre-pregnancy comorbidities that increase risk in pregnancy, for example renal failure, severe liver disease, transplantation, cardiac failure, psychiatric conditions requiring in-patient admission (within previous year) in the opinion of the responsible clinician or the CI.
7. Significant co-morbidity in the current pregnancy, nephropathy (estimated GFR <60ml/min), other physical or psychological conditions likely to interfere with the conduct of the study and/or interpretation of the trial results in the opinion of the responsible clinician or the CI.
8. Not fluent in English and absence of interpreter or translation services (ie telephone translation services)
9. Participating in another intervention study where the results could influence GDM-related endpoints, in the opinion of the responsible clinician or the CI, or participation in a CTIMP during current pregnancy.
10. Known allergy/hypersensitivity/intolerance to the active substance or excipients, or patients taking any medications which are contraindicated as per IMP SmPC (as per Section 5.7).

The Inclusion/Exclusion for the sub-study GUARD MEC will be specified in section 19. Mechanistic Sub-study: GUARD MEC.

6.3 Selection of Participants

Women will be selected from the antenatal diabetes clinics at participating hospitals with specialist obstetric/diabetes multidisciplinary teams, expert in the management of GDM.

Women with a clinical diagnosis of GDM who require pharmacological intervention will be asked to provide written informed consent, recruited and randomised to one of the two trial interventions.

Women recruited into GUARD will subsequently be offered the opportunity to participate in the GUARD MEC sub study. Women with a clinical diagnosis of GDM who do not require medication to control the condition, and pregnant women without GDM, will be invited to participate in the GUARD MEC only.

6.4 Randomisation Procedure / Code Break

Allocation of treatment arm will be randomised by secure computerised web-based programme, provided by MedSciNet^{Ltd}. The groups will be minimised by;

* BMI category (25-29.9, 30-34.9, ≥35),
* Previous history of GDM,
* Disease severity (baseline fasting glucose <6.2 or ≥6.2),
* Centre

Regular checks during the recruitment phase will be carried out to confirm that the minimisation procedure has been applied correctly.

Once a participant has provided informed written consent, baseline details will be entered into the eCRF. As soon as eligibility has been confirmed, treatment allocation will be assigned via the database, and the study ID created. All parties will be aware of the participants' allocation. The pharmacy department will be informed so they can supply the appropriate IMP for the participant.

Emergency code breaking is not required as the treatment is open label.

6.5 Withdrawal of Subjects

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, AEs, SAE's, SUSAR's, protocol violations, cure, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible.

Should a patient withdraw from study drug only (and not from trial participation), efforts will be made to continue to obtain follow-up data, with the permission of the patient. Participants who withdraw from trial medication will be asked to confirm whether they are still willing to provide: i) trial specific data, including samples at delivery, ii) data collected as per routine clinical practice.

In case of withdrawn participants, they will be asked to return all unused IMP to the study team.

Patients can withdraw from GUARD MEC and still continue participating in GUARD study.

6.6 Expected Duration of Trial

It is expected that each participant will be in the treatment period for a maximum of approximately 14 weeks, with an additional data point collected 3 months post birth from the local GP (the result of the HbA1c sample only). Participants will be asked for permission to be contacted in the future for follow up of their offspring. Methods of, 'keep in touch' such as Christmas cards, newsletters etc, will be used to keep participants engaged after their participation is completed. Any follow up procedures will have all appropriate regulatory approvals in place.

The end of the trial will be defined as database lock, once all recruits have completed all the study related visits, and the data has been entered in the eCRF and cleaned.

7. Trial Procedures

Visit name & approximate pregnancy week	Participant identification	Baseline	Follow up 1	Follow up 2	Birth	Post-birth
	26^{+0}-30^{+6}	27^{+0}-31^{+6} [a]	$32^{+0} \pm 1$ [b]	$36^{+0} \pm 1$ [b]		3 month post birth[c]
Patient information *	X [h]					
Informed consent *		X				
Inclusion / exclusion criteria*		X				
Demographics *		X				
Medical and obstetric personal and family history *		X				
Adverse events*		X	X	X	X [n]	
Concomitant medication *		X	X	X	X [o]	
Weight *		X	X	X		
Blood pressure and pulse [d]		X	X	X		
Fasted glucose	X [u]			X		
HbA1c	X [i]	X [j]		X		X [t]
Liver function tests, bile acids and high sensitivity C-reactive protein, U&E		X		X		
Fasting lipid profile				X		
1,5-Anhydroglucitol and non-fasting metabolic hormones[e]		X	X			
1,5-Anhydroglucitol and fasting free fatty acids & metabolic hormones [e]				X		
Optional faecal sample [e] *				X [k]		
Optional GUARD MEC Blood samples after a breakfast [e] *				X [l]		
Cord blood samples [e]					X	
Meconium collection [e]					X	
Randomisation		X				
IMP dispensing		X	X	X		
IMP administration		X (continuously)				
Dispense diary card		X				
Drug diary review			X	X	X	
Continuous Glucose Monitoring [f] *		X	X	X		
Download CGM data *			X	X	X	
Optional vascular studies [g] *		X	X	X		
Quality of life questionnaires (EQ-5D-5L)		X		X		
Treatment satisfaction questionnaires (DTSQs)				X		
4-day food diary *			[m]	X [m]		
Labour and birth data *					X [p]	
Neonatal anthropometry *					X [q r]	
Neonatal data *					X [r s]	

* - assessments applicable to patients in GUARD MEC, to be done during the GUARD MEC study visit.
a - If participants are recruited earlier or later than expected, follow up visits will be adjusted according to clinical pathways and to ensure the participant has been receiving IMP for at least 2 weeks.
b - Women must fast for at least 3 hours
c - To occur at local GP practice approximately 3 months post-delivery (as per standard of care).
d - Blood pressure in triplicate and pulse only for woman who don't consent to the vascular studies. Use non-dominant arm.

e - Research samples, for storage.
f - CGM will be in place for 10 days after each study visit. Women to be trained to remove the device themselves.
g - Vascular studies: blood pressure pulse wave velocity, central arterial pressure, augmentation index.
h - PIS to be given after OGTT appointment.
i - Pre-enrolment HbA1c and glucose: samples analysed within 3 weeks before baseline can be used. If no results are available, an HbA1c sample must be collected at baseline.
j - Only collect if no previous results within 3 weeks are available
k - Faecal samples are optional and should be produced at approximately 36 weeks. If the woman is unable to give a faecal sample on the day, a stool sample collection kit may be provided and the sample collected by courier from the participant's home.
l – Optional GUARD MEC: a hospital standardised breakfast will be provided. Four blood samples will be taken at the following timepoints: before breakfast, 15 minutes, 1 hour and 2 hours postprandially.
m - Food diaries should be given to patients at FU 1 to be completed the 4 days prior to FU 2. This should occur prior to providing a faecal sample (if applicable).
n - Adverse event data will be collected at each visit from baseline to discharge from hospital of mother and infant.
o - Standard of care concomitant medication given at labour do not need to be recorded.
p - Labour and birth data: onset of labour, genital tract trauma, post-partum haemorrhage, mode of birth, gestational age at delivery, NICU admission, morbidity, feeding at discharge.
q - Neonatal anthropometry: birth weight and the following measurements in triplicate:

- With blank tapes (then checked measured these with a steel rule): head circumference, chest circumference, abdominal circumference, midarm circumference
- Skinfold thicknesses in triplicate: subscapular and triceps
- Crown rump length, crown foot length

r - Neonatal assessments will be performed on the day of delivery, or as soon as feasible.
s - Apgar scores: 5 min post-birth
t - HbA1c samples will be drawn at the local GP and samples requested where clinically available
u – Fasted and 2 hours post prandial glucose taken from the OGTT appointment

7.1 By Visit

7.1.1 Identification and Informed Consent

Women who test positive at their routine OGTT visit (usually at 26-28 weeks' gestation) will be provided with the main study and sub-study PIS/ICF by a member of the clinical research team, altogether with an explanation of the trial. At 28-30 weeks patients will be reviewed as per standard of care, and those formally diagnosed with GDM who require pharmacological treatment will be offered the opportunity to participate in the main study and the optional sub-study.

Patients without GDM, or those whose glucose is well controlled by diet and hence not requiring pharmacological treatment are not eligible to participate in the main study. These patients will be given the opportunity to participate in the sub-study GUARD MEC as controls (see more details in GUARD MEC section 19.5 Recruitment & assessments).

The trial will be explained in detail by trained and delegated clinical or research staff, as per local procedure. Should the patient agree to participate to any part of the research, a copy of the ICF will be signed by the patient and the investigator at that visit (or delegated member according to local standard practice), and a copy given to the patient. A copy will be uploaded into the woman's electronic maternity records or filed in the patient medical records. The investigator's original will be filed in the ISF.

7.1.2 Baseline

Following informed consent, screening procedures will begin to assess patient's eligibility. The following assessments will take place:

- Demographic (including post-code, which is not stored in the eCRF), family, medical and obstetric data will be collected (including data from OGTT appointment)
- Concomitant medication
- Adverse event data
- Inclusion/exclusion assessment
- Weight (height to be obtained from medical records)
- Blood sample for in-hospital analysis: LFT, total bile acids, U&E and high sensitivity C-reactive protein
- HbA1c (if none available within the last 3 weeks)
- Plasma sample to be stored for 1,5 anhydroglucitol and metabolic hormones
- Continuous Glucose Monitoring implementation and education
- *Optional* Vascular studies: blood pressure pulse wave velocity, central arterial pressure, augmentation index.
- Blood pressure and pulse (if not consented for the vascular studies)
- Quality of life questionnaire (EQ-5D-5L)
- Randomisation via the eCRF
- Dispense IMP and provide enough supply until following visit
- Dispense diary card for IMP

7.1.3 Follow up 1

Follow-up 1 will be scheduled to coincide with antenatal clinics/scans at approximately 32^{+0} weeks' gestation (±1 week). The following assessments will take place:

- Concomitant medications check
- Adverse event data
- Weight
- Blood sample to be collected, processed and stored for 1,5-anhydroglucitol and metabolic hormones analysis
- Download CGM data and new equipment supply
- **Optional** Vascular studies: blood pressure pulse wave velocity and augmentation index
- Blood pressure and pulse (if not consented for the vascular studies)
- Dispense IMP and provide enough supply until following visit
- Diary card checks
- 4 day food diary to be provided and training given so it can be completed in anticipation to follow up 2.

7.1.4 Follow up 2

Follow-up visit 2 will be scheduled to coincide with antenatal clinics/scans at approximately 36^{+0} weeks' gestation (±1 week). The following assessments will take place:

- Concomitant medications check
- Adverse event data
- Weight
- Blood samples collected for in-hospital analysis (minimum 3 hour fast): serum glucose, LFT, U&E, total bile acids, high-sensitivity C-reactive protein, HbA1c and fasting lipid profile.
- Blood sample to be collected, processed and stored for 1,5-anhydroglucitol, metabolic hormones and free fatty acid analysis
- Download CGM data and new equipment supply
- **Optional** Vascular studies: blood pressure pulse wave velocity and augmentation index
- Blood pressure and pulse (if not consented for the vascular studies)
- Dispense IMP and provide enough supply until following visit
- Diary card checks
- Quality of Life questionnaires (EQ-5D-5L) and treatment satisfaction (DTSQs)
- Collect 4-day food diary from participant
- **Optional** faecal sample (can be produced at the participant's home and shipped to hospital)
- **Optional** and separately consented mechanistic samples. See section 19. Mechanistic Sub-study: GUARD MEC for further information.

7.1.5 Birth and immediate postpartum period

Participants are expected to take the last dose of IMP on the day they give birth, where possible. The following assessments and data will take place on that visit and during the subsequent days, if at all possible:

- Adverse event data
- Concomitant medication (except any standard medication given when in labour)
- CGM data download and collection of device
- Collect remaining IMP and diary card check
- Cord blood samples (SST vacutainer; for storage and subsequent analysis of lipids and C-peptide)
- Meconium collection from nappy
- Labour and birth data: onset of labour, genital tract trauma, post-partum haemorrhage, mode of birth.
- Neonatal data: Apgar scores: 5 min post-birth, gestational age at birth, gender, NICU/SCBU admission, morbidity, feeding method at birth and discharge, inpatient night, shoulder dystocia and manoeuvres required for delivery.

- Neonatal anthropometry will be measured taken on day of delivery or as soon as feasible: birth weight and the following measurements in triplicate:

a) With blank tapes (then checked measured these with a steel rule): head circumference, chest circumference, abdominal circumference, midarm circumference

b) Skinfold thicknesses in triplicate: subscapular and triceps

c) Crown rump length, crown foot length

There may be instances where patients are unable to bring back material such as leftover IMP, diary card or CGM device. If women are not to return to hospital in the near future, a pre-paid postal package will be provided for them to send the material back. If substantial study data is missed from labour, the research midwife might call participants to enquire for missing information.

7.1.6 Post birth

As per standard care patients will have an HbA1c measurement at their local GP approximately 3 months post-delivery. This data will be obtained by the study team.

7.2 Laboratory Tests

At each visit we will require a different volume of blood, to a max of approximately 28 mL (FO, SST, EDTA vacutainers). Standard clinical measures for serum glucose, HbA1c, lipids (total, HDL- and LDL-cholesterol, triglycerides), liver function tests, U&E, total bile acids and high-sensitivity C-reactive protein tests will be performed at the local hospital laboratory. From the total volume, 6 mL from an EDTA vacutainer will be collected and processed at each visit, and the plasma stored for further analysis of metabolic hormones and 1,5 anhydroglucitol, a novel marker of short-term glycaemia. At Follow-Up 2, 5 mL from an SST vacutainer will be collected and processed, and the serum stored for further analysis of free fatty acids. The table below details the volume of blood and the vacutainer required for collection at each visit.

	Baseline	**Follow-Up 1**	**Follow-Up 2**
FO	N/A	N/A	4 mL
SST	7 mL	N/A	12 mL (5 mL storage)
EDTA	12 mL (6 mL storage)	6 mL (storage)	12 mL (6 mL storage)
Estimated total volume blood required:	19 mL	6 mL	28 mL

We will also evaluate gut microbes and metabolites in the maternal faeces to investigate the effect of UDCA/metformin on gut signals that can influence maternal metabolism and susceptibility to GDM and dyslipidaemia. Women will be asked to donate an optional faecal sample for the gut hormone studies at approximately week 36. This

sample will need to be frozen immediately. Gut microbiota will be determined in the faeces samples by 16S rRNA sequencing. Faecal bile acid profiles will be obtained using UPLC-MS/MS and SCFAs will be quantified by gas-liquid chromatography (GLC). Additional aliquots of faecal samples will be stored at -80^{o}C, as, if a change in maternal gut microbiota composition is observed, they will be used for future experiments.

Cord blood samples will be obtained for measurement of lipid and C-peptide levels (approximately 4 ml SST tube) and stored locally. Meconium will be collected from the nappy where possible and stored locally.

Stored samples will be shipped to KCL in batches and analysed centrally, either by courier or in person. Collection and shipping logs will be completed by the site staff.

Details about the collection, handling, shipment and analyses of research samples are described in the lab manual. Details about the sampling collection of the GUARD MEC will be described in section 19.6 Laboratory details and the lab manual. All details about sample processing will be described in the lab manual.

8. Assessment of Efficacy

8.1 Primary Efficacy Parameters

Glucose control at 36 weeks is the primary efficacy parameter. This will be measured by a fasted blood sample obtained via venepuncture at the follow up clinic.

8.2 Secondary Efficacy Parameters

1. Biomedical maternal
   Glucose metabolism
   Lipid metabolism
   Biochemical analysis of maternal blood
   Maternal gestational weight change (randomisation to 36 weeks)
   Blood pressure

2. Biomedical neonatal
   Gestational age at delivery, frequency of preterm birth
   Neonatal adjusted birth weight
   Cord blood analysis
   Apgar scores
   Neonatal morbidity
   Neonatal intensive care unit admission

8.3 Procedures for Assessing Efficacy Parameters

Maternal

- Venepuncture at follow up visits for the analyses of: glucose, HbA1C, lipids (triglycerids, total cholesterol, LDL-cholesterol, HDL-cholesterol), liver function tests, bile acids, C-reactive protein (including highly sensitive analyses), 1,5- anhydroglucitol, metabolic hormones and free fatty acids. When possible, these will be collected at the same time as any other required clinical samples.
- Continuous glucose monitoring (CGM) to assess glycaemic control.
- Quality of Life (at baseline and Follow up 2).
- Measurements of blood pressure with an arteriograph for the vascular studies.

Neonatal

Routinely collected clinical birth details will be collected for the trial, these will include;

- Birth data
  - Mode of birth (rates of primary & repeat CS, elective & emergency LSCS).
  - Gestational age at delivery, frequency of preterm birth.
  - Infant birth weight (customised birth weight percentile, proportion of large for gestational age infants (LGA), proportion of small for gestational age infants (SGA))
  - Apgar scores at 5 minutes.
  - Neonatal morbidity (treatment for neonatal hypoglycaemia, neonatal jaundice, respiratory distress or trauma).
  - Stillbirth
  - Neonatal special and intensive care unit admission (duration of hospital stay, highest level care).

- Cord blood for the analyses of C-peptide, triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol and free fatty acid concentrations.
- Infant feeding method at hospital discharge (breast, bottle, mixed).

9. Assessment of Safety

9.1 Specification, Timing and Recording of Safety Parameters

Adverse event data will be collected at each visit from baseline to discharge from hospital of mother and infant.

Blood pressure will be measured at each visit.

U&E will be performed at follow up 2 to monitor renal function. Liver function tests are also measured at this visit.

Other safety assessments will be performed as per standard of care down to the clinician's decision.

9.2 Procedures for Recording and Reporting Adverse Events

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

Adverse Event (AE): Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

Note 1: This definition includes events related to the investigational medical device or the comparator.

Note 2: This definition includes events related to the procedures involved.

Adverse Reaction (AR): Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Unexpected Adverse Reaction (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the summary of product characteristics (SmPC) for **metformin and UDCA**

Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR): Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

Results in death (including neonatal);
Is life-threatening;
Required hospitalisation or prolongation of existing hospitalisation;
Results in persistent or significant disability or incapacity;
Consists of a congenital anomaly or birth defect.

Important Medical Events (IME)

Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

9.2.1 Reporting Responsibilities

KCL & GSTFT have delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partners Clinical Trials Office (KHP-CTO).

All Adverse Events will be recorded in the medical notes.

All SAEs, SARs and SUSARs (excepting those specified in this protocol as not requiring reporting) will be reported immediately (and certainly no later than 24hrs) by the Investigator to the KHP-CTO and CI for review in accordance with the current Pharmacovigilance Policy, and subsequently recorded in the eCRF. All SAEs will be reported using MedDRA coding, in liaison with the study CRA.

The KHP-CTO will report SUSARs to the regulatory authorities (MHRA, competent authorities of other EEA (European Economic Area) states in which the trial is taking place. The Chief Investigator will report to the relevant Ethics Committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.

- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.

- The Chief Investigator and KHP-CTO (on behalf of the co-sponsors), will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

9.2.2 Definition of causality

The assignment of the causality should be made by the investigator responsible for the care of the participant as: definitely, likely, possibly, unlikely, not related or related to other study procedure.

An AE whose causal relationship to the study drug or study procedure is assessed by the investigator as “possibly”, “likely” or “definitely” is an Adverse Drug Reaction. All events judged by the investigator to be “possibly”, “likely” or “definitely” related to the therapy or study procedure, graded as serious and unexpected should be reported as a SUSAR.

9.2.3 Severity

Regardless of the classification of an AE as serious or not, its severity must be assessed according to medical criteria alone using the following categories:

Mild: does not interfere with routine activities

Moderate: interferes with routine activities

Severe: very difficult or impossible to perform routine activities

9.2.4 Expectedness

If there is at least a possible involvement of the trial medications, the investigator and sponsor must assess the expectedness of the event. An unexpected adverse reaction is one that is not reported in the current SPC, or one that is more frequently reported or more severe than previously reported. See SPC for a list of expected toxicities associated with the drugs being used in this trial. If a SAR is assessed as being unexpected, it becomes a SUSAR and it must be reported to the competent authority by the sponsor.

9.2.5 Adverse events that do not require reporting

All adverse events will be reported from baseline until discharge from hospital for mother and infant.

Events that are reported as outcomes in the eCRF, or those which are expected in this population or as result of routine care/treatment do not need to be reported as AEs or SAEs. This includes but is not limited to:

- Birth by C-Section
- Planned hospital admissions (including to give birth), including for treatment planned prior to trial entry, or for elective treatment of a pre-existing condition.
- Post-partum haemorrhage
- Genital tract trauma
- Antepartum haemorrhage (approx. > 100 ml)
- Postpartum haemorrhage (approx. > 500 ml)
- Neonatal admission to high level of neonatal care for less than 48 hours
- Shoulder dystocia
- Neonatal hypoglycaemia, neonatal jaundice, respiratory distress or birth trauma

Those events will only be reported to the sponsor if the investigator believes the event is a result of the GUARD intervention. All unexpected SAR will be reported.

Further information in the reporting of AE for participants of GUARD MEC are described in section 19.7.

9.3 Trial Stopping Rules

The trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Independent Data Monitoring Committee / Trial Steering Committee, regulatory authority or Ethics Committee concerned.

If the trial is prematurely discontinued, active participants will be informed and no further participant data will be collected. The Competent Authority and Research Ethics Committee will be informed within 15 days of the early termination of the trial.

10. Statistics

Appropriate summary statistics (means, medians, percentages and measures of dispersion such as the standard deviation and interquartile range) will be generated according to treatment assignment for important baseline covariates and for primary and secondary outcomes. At all follow-up visits, summary statistics for the observed values and for changes from baseline will be computed and tabulated for all primary, secondary and safety outcomes. In the event that a participant stops the intervention, they will be encouraged to continue to be part of the study. These participants will form part of the final analysis on an intention to treat basis.

10.1 Sample Size

A study size of 158 participants will provide sufficient statistical power whilst allowing for a 20% withdrawal rate. This gives 90% power to detect the primary outcome of a difference in maternal fasting glucose at 36 weeks of 6% (0.28mmol/L). This sample size calculation was performed using data obtained from a previous study that reported differences with UDCA treatment for non-alcoholic fatty liver disease (NAFLD) in a population of similar body mass index and age to our study group[44], and the difference in glucose level is thought clinically relevant as it is equivalent to the difference in glucose categories between which differences in LGA, primary caesarean section, cord blood serum C-peptide level >90th centile and clinical neonatal hypoglycaemia were evident in the HAPO study[43]. Using two-sided calculations with alpha 0.05, 63 women per arm would be required to determine this reduction with 90% power. Thus, allowing for approximately 20% dropouts, the numbers rise to 158 women in total.

10.2 Randomisation

Randomisation will be minimised in groups by four variables: BMI, by previous history of GDM, severity and by centre.

Due to the large pill size and different dosage, this will be an open label study.

10.3 Analysis

Interim analysis will be conducted as described in section 11.4.The stopping rule will be based on the Peto principle[53], that the trial should continue except in the face of overwhelming evidence (P<0.0001), sufficient to make a recommendation affecting all future obese or overweight pregnant women.

The main analysis will follow the intention to treat (ITT) principle, using all available data on randomised women, according to the intended treatment option (The ITT database). Should there be a large number of women (over 20%) not following the randomised treatment, a per protocol (PP) dataset limited to women following the intended treatment will also be established and a secondary PP analysis will be conducted.

All comparisons by treatment group will be adjusted for all variables used in the randomisation. Data derived from the CGM will be analysed at 36 weeks. The differences caused by the randomised treatment, adjusting for the baseline randomisation measurements by multiple regression. This method (also known as ANCOVA) will increase the power and accuracy of these comparisons.

UDCA will be declared non-inferior to metformin if metformin does not have a significant advantage, and the largest plausible advantage (by 95% Confidence Interval) is less than 0.28 mmol/L. If neither treatment shows a significant advantage, and the difference and CI are less than 0.28 mmol/L, the treatments will be regarded as equivalent[54].

10.4 Procedures for dealing with missing data, unused data and false data[52]

Missing data. We will follow a four-point framework for dealing with incomplete observations which will allow the correct method to be chosen and subsequently implemented [52].

1. Attempt to follow up all randomized participants, even if they withdraw from allocated treatment
2. Perform a main analysis of all observed data that is valid under a plausible assumption about the missing data. Specifically, we will assume data is missing at random (MAR). Under this assumption, imbalances between treatment groups due to dropout can be corrected by appropriate multiple regression models.
3. Perform a sensitivity analyses to explore the effect of departures from the assumption made in the main analysis. The MNAR (missing not at random) analysis will use the method of White et al. (2011)[52] as implemented in the Stata command rctmiss.
4. Account for all randomized participants, at least in the sensitivity analyses

This framework highlights the importance of using plausible assumptions with regards to the nature of the missing data. These assumptions will then be tested using appropriate sensitivity analyses on observed data using complete cases analysis. For the purpose of the main analysis we will make the assumption that missing data is missing at random and the effect of the intervention is the same in those with and without the observations. Furthermore, we will check whether there is an imbalance or is similar the percentage of missing data within each treatment allocation.

Unused data. The principal analysis will follow the intention to treat principle. All consenting women randomised for whom adequate data is collected will be included in the primary and main secondary endpoints. A secondary, per protocol analysis of the primary outcome will be limited to women who take the majority of their randomised medication.

False data. We will take all reasonable precautions to minimise the number of data errors. Everyone responsible for collecting data will be trained in the procedures to followed. All data entered will be checked by the study team as per the Data Monitoring Plan and Data Management Plan; and again by the statistician at the time of analysis; and corrections made wherever possible.

A detailed SAP, including dummy tables, will be prepared as a separate document.

11. Trial management and oversight

11.1 Overview of trial management

The conduct of the trial will be overseen by a Trial Steering Committee (TSC). The Trial Manager along with the Chief Investigator and Research Associate will be responsible on a day to day basis for overseeing and co-ordinating the work of the multi-disciplinary trial team.

The TMG will outsource services of an electronic data capture and randomisation services. The CRA and trial statistician will be responsible for monitoring data collection, processing data and conducting data validation.

The KHP-CTO acts on the behalf of the sponsor and provides quality assurance and trial monitoring.

The minutes from discussions of the study committees will be formally documented and a record kept in the TMF.

11.2 Trial Management Group

The Trial Management Group (TMG) will be chaired by the trial manager, and will include the Chief Investigator, CTM, selected co-investigators (or delegated individuals), a consultant midwife and a research matron, Statistician and CRA. For selected meetings, the TMG may additionally include the Trial Pharmacist, representatives from KHP-CTO and the Trial Sponsors as required. This group will have responsibility for the day to day operational management of the trial. Regular meetings of the TMG will be held to discuss and monitor trial progress and solve problems.

11.3 Trial Steering Committee

A TSC will be established prior to the start of the study, with a mix of independent and study team members. The TSC will be an executive committee, responsible for the overall supervision on behalf of the Sponsor and the Funder, and will ensure that the trial is conducted in accordance with the rigorous standards set out in the UK Policy Framework for Health and Social Care Research and Guidelines for Good Clinical Practice. The TSC will consist of an independent chair, the Chief Investigator, the statistician, independent members consisting of a group of experienced obstetricians and diabetologists, specialist nurses and midwives and PPI members. The TSC will discuss recommendations raised by the IDMC. A charter will be agreed by the members, listing the detailed Terms of Reference and frequency of meetings. The group will meet at least annually.

11.4 Independent Data Monitoring Committee

An IDMC will be appointed comprising two fully independent clinicians and an independent statistician. This group will be an advisory committee to the TSC. The IDMC will review outcomes after 40 (25%) of participants have given birth. Interim analysis will be performed and reported to the IDMC including rates of adverse pregnancy outcomes and to identify causes for participant withdrawal. The IDMC's responsibility is to safeguard the interests of the trial participants and advise the TSC to protect the validity and credibility of the trial. During the recruitment period, reports will be provided to the IDMC as per charter, which will include information on the AEs reported, recruitment, along with any other data that the committee may request. The IDMC will advise the TSC if, in their view, the randomised comparisons have provided both (i) 'proof beyond reasonable doubt' that for all, or some, the treatment is clearly indicated or clearly contra-indicated and (ii) evidence that might reasonably be expected to materially influence future patient management. Following

a report from the IDMC, the TSC will decide what actions, if any, are required. Frequency of meetings will be defined in a charter document.

11.5 Patient and Public Involvement

At least one, and preferably two, service users will be involved in the design and management of the trial (i.e. as a member of the TSC), developing participant information resources and contributing to the reporting of the research. This approach has been very successful in previous trials within the study team.

We performed an internet survey of mothers with GDM to establish whether they would be happy to take an alternative drug to metformin (we also asked if they would be happy to take both drugs, although this is not of relevance to current study design). The results were supportive of the study design. In brief, 30 women with previous GDM responded, 14 of whom were treated with metformin. Responses of relevance to this study were:

- 68% would prefer oral treatments rather than insulin
- 72% believe additional treatments are needed as well as diet and lifestyle change
- 68% would be happy to participate in a trial of a new drug that has good safety data in pregnancy but that has not been used to treat GDM
- 58% would be happy to take the new tablet instead of metformin or insulin

12. Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the UK Policy Framework for Health and Social Care Research and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents has been submitted for review to Health Research Authority (HRA), Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the Sponsor) and the REC within the timelines defined in the Regulations. The KHP-CTO or delegate will upload the final report to EudraCT on behalf of the Sponsor.

12.1 Ethical issues

There is a chance that UDCA treatment will not improve GDM control or could worsen maternal or fetal lipid profiles or outcomes. However, this is very unlikely given that our pilot data in humans and mice indicate that it will be beneficial. Furthermore, previous clinical trials by ourselves and others for ICP have not demonstrated adverse maternal or fetal outcomes associated with the drug. When UDCA was evaluated in a previous study to establish whether it was an acceptable drug for women with ICP (the PITCH pilot trial)[45], 13 (23%) adverse events took place among women randomised to UDCA compared with 10

(18%) among women randomised to placebo. No woman reported more than one adverse event. Most adverse events were mild, with the remainder classified as moderate (eight); none were classed as severe by the site principal investigators. Most adverse events related to gastrointestinal disturbances (nine in the UDCA arm versus five in the placebo arm). Importantly, the majority were thought not, or unlikely, to be caused by the trial drug (possible causality in four events in the UDCA arm versus two in placebo arm).

Given the glucose-lowering effects of UDCA, there is a theoretical potential for UDCA to lower glucose below safe levels (hypoglycaemia). However, there is no clinical or experimental evidence that UDCA treatment causes hypoglycaemia; in murine studies, treatment with UDCA lowers blood glucose levels for obese, but not healthy mice, to healthy levels[50]. In human studies, treatment of obese women with 6 weeks of UDCA did not lower their fasting glucose below normoglycaemia (2x SD below the mean)[44].

13. Quality Assurance

Trial committees (IDMC and TSC) will be appointed to oversee the conduct of the study. Monitoring to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained, by the KHP-CTO Quality Team.

13.1 Direct Access to Source Data and Documents

The Investigator will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsors, Regulators and REC direct access to source data and other documents (e.g. patients' case notes, blood test reports, scan reports etc).

13.2 Trial monitoring

Monitoring of this trial will be performed to ensure compliance with Good Clinical Practice, and scientific integrity will be managed and oversight retained, by the KHP-CTO Quality Team.

A study specific monitoring plan will be developed by the KHP-CTO on the basis of the risk assessment. The KHP-CTO will carry out on-site monitoring to undertake source data verification checks and confirm that records are being appropriately maintained by the PI and pharmacy teams. The site PI will be responsible for ensuring the findings are addressed appropriately. The CTM will ensure relevant findings are discussed with the CI and the report is filed in the TMF.

In addition to site monitoring, the CTM and CI will communicate regularly with sites via email, telephone and teleconferences, and will perform spot checks in the eCRF.

14. Data Handling

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Patient data will be pseudo-anonymised.
- All pseudo-anonymised data will be stored on a password protected computer system.
- Data entered onto the eCRF will be pseudo-anonymised and stored on a secure server.
- Full postcode will be collected and entered into the eCRF, but postcode will not be stored (the eCRF will match the postcode with the Census area (LSOA), which is the data that will be saved in the eCRF).
- All hard copies of source data worksheets and ISFs will be kept in a locked office within the trial site.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act 2018 and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Kings Health Partners Clinical Trials Office Archiving SOP, for at least 25 years.
- Patients will be asked to provide their email addresses if they wish to be informed of study results and to be contacted in the future. We will collect these in the ISF and a copy will be given to the sponsor at intervals or at the end of the trial.

15. Data Management

Electronic Case Report Forms (eCRFs) will used to capture the data of each subject considered in the study. No identifiable data will be collected for people who do not consent to participate in the trial, but reasons for not consenting will be collected. These forms will be standardised and protocol specific and create a time stamped electronic record of any amendments or additions to the document. eCRF pages will be monitored on an ongoing basis by the study monitor and the trial manager and queries raised to resolve discrepancies.

There will be some instances where data may be inputted into the eCRF directly. In those cases, the eCRF will become the source data. Those may be:

- Medical history & demographics
- Arteriograph results
- Time of blood samples
- Time and date of last meal
- Questions related to IMP compliance where a diary card has not been completed
- Reason for withdrawal
- Research laboratory samples processing information and barcode

This clinical trial will use the industry-standard secure database called MedSciNet^{Ltd}. All access to the MedSciNet^{Ltd} data system is controlled using a Username/Password login. Passwords are encrypted before storing to database, using SHA-1 hash (MS .NET SHA1CryptoServiceProvider). These are created and controlled by Administrative users of the system as identified by the Chief Investigator. The data is stored in the BRC eCRF servers and meet all MHRA requirements for CTIMP data storage. Only the server

administrator has access to this server and the core database, via remote connection. The back-up process is twofold: every 24hrs the database is backed-up to the server and every 7 days the entire server is backed up to an archival tape system.

The trial manager will document database lock prior to the final dataset being sent for analyses. A separate Data Management Plan will be prepared for the trial detailing the checks to be undertaken.

16. Publication Policy

Ongoing progress of the trial will be disseminated to the wider clinical community through relevant professional newsletters, meetings, and national and international conferences.

The final report to the funder(s) will present detailed results of the trial. The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org).

A lay persons' summary of the principal findings of the results will be sent to all patients involved in the study at their request. Participants will be asked if they wish to be informed of trial results, and if they do, they will be asked to provide a personal email address for this purpose.

Articles will be prepared for relevant professional journals as well as for peer- reviewed scientific journals.

17. Insurance / Indemnity

The trial is co-sponsored by King's College London and Guy's and St. Thomas' NHS Foundation Trust. The co-sponsors will at all times maintain adequate insurance in relation to the study independently. King's College London, through its own professional indemnity (Clinical Trials) and no fault compensation and the Trust having duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient.

18. Financial Aspects

Funding to conduct the trial is provided by J.P. Moulton Foundation (REF Tommy's Grant 81) and an NIHR Senior Investigators grant (Professor Catherine Williamson).

19. Mechanistic Sub-study: GUARD MEC

19.1 Objectives

To assess whether UDCA and metformin differentially alter gut microbiome and whether this is associated with a change in endocrine signalling, serum glucose levels and lipid composition, in women consenting to the mechanistic sub-study.

Optional: some women will also be invited to attend an MR imaging scan of their fetus to explore whether UDCA or metformin alters fetal body composition. This is a separate study, ethically approved (REC 16/LO/1573), with additional consent form.

19.2 Exploratory endpoints

- 16S sequencing to analyse gut microbiome
- Targeted metabolite profiles to measure individual bile acid composition in faeces and serum (UPLC-MS/MS)
- Hormone assays to measure: GLP-1, FGF19, C4
- Glucose-related hormones to measure: Insulin, C-peptide, Glucagon
- Serum measurements (done at the participating sites): glucose, lipid profile, free fatty acids

19.3 Design

GUARD MEC is a multi-centre nested observational study. With additional participant consent, 80 participants will be enrolled. We will measure maternal gut hormones and metabolite profiles (UPLC-MS/MS) in plasma, and gut microbes (16S rRNA sequencing) and metabolite profiles (UPLC-MS/MS) in faeces. The metabolite profiles include measurement of bile acids (including UDCA), so it will be clear if women have taken (and absorbed) the drug. The nested studies will be performed at approximately 36 week's gestation.

These patients will be identified at the same antenatal clinics and divided into four arms:

Arm 1: GDM Metformin

20 patients with GDM who were eligible and have consented to participate in the main GUARD trial and were randomised to metformin.

Arm 2: GDM UDCA

20 patients with GDM who were eligible and have consented to participate in the main GUARD trial and were randomised to UDCA.

Arm 3: GDM no pharmacotherapy

20 patients who test positive at OGTT but in whom the condition is managed with diet and lifestyle changes. These participants will have received the main GUARD Trial and sub-study PIS at diagnosis of GDM but will only be offered to take part in the sub-study.

Arm 4: healthy pregnant

Pregnant women without GDM will be invited to participate in the 'healthy' cohort of the mechanistic studies. PIS will be provided by a delegated study team member after the OGTT test has taken place, or women who haven't been diagnosed with GDM. Participants will be given sufficient time to consider the study. If deemed appropriate, consent may be taken on the same day as PIS was given.

19.4 Inclusion Exclusion criteria

Arm 1 & Arm 2:

Inclusion Criteria

1. Women who are eligible and who have consented to participate in GUARD.

Exclusion Criteria

1. Use of oral antibiotics during current pregnancy
2. Known food allergy to nuts or any of the components of the GUARD MEC breakfast.

Arm 3:

Inclusion Criteria

1. Women with GDM diagnosed at 26^{+0} to 30^{+6} weeks' gestation in accordance with the NICE guidelines (one or more glucose concentrations of ≥5.6 mmol/l fasting or ≥7.8 mmol/l 2 hours after a standard 75g OGTT, and NOT requiring pharmacological treatment).
2. Overweight or obese (Booking BMI ≥25 kg/m2)
3. Planned antenatal, birth and postpartum care at the participating centre (i.e. not planning to move before delivery).

Exclusion Criteria

1. Unwilling/unable to give written informed consent and comply with the requirements of the study protocol
2. Multiple pregnancies (twins, triplets etc) in current pregnancy
3. Congenital anomaly on ultrasound requiring fetal medicine input
4. Previous diagnosis of diabetes outside pregnancy
5. HbA1c at booking >48 mmol/mol or ≥6.5% during current pregnancy (if available)
6. Not fluent in English and absence of interpreter or translation services (ie telephone translation services)

7. Participating in another intervention study where the results could influence GDM-related endpoints, in the opinion of the responsible clinician or the CI, or participation in a CTIMP during current pregnancy.
8. Use of oral antibiotics during current pregnancy
9. Known food allergy to nuts or any of the components of the GUARD MEC breakfast.

Arm 4:

Inclusion Criteria

1. Pregnant women between 16 and 45 years of age who haven't been diagnosed with GDM by the time of the study visit.
2. Overweight or obese (Booking BMI ≥25 kg/m2)
3. Planned antenatal, birth and postpartum care at the participating centre (i.e. not planning to move before delivery).

Exclusion Criteria

1. Unwilling/unable to give written informed consent and comply with the requirements of the study protocol
2. Multiple pregnancies (twins, triplets etc) in current pregnancy
3. Congenital anomaly on ultrasound requiring fetal medicine input
4. Previous diagnosis of diabetes outside pregnancy
5. HbA1c at booking >48 mmol/mol or ≥6.5% during current pregnancy (if available)
6. Not fluent in English and absence of interpreter or translation services (ie telephone translation services)
7. Participating in another intervention study where the results could influence GDM-related endpoints, in the opinion of the responsible clinician or the CI, or participation in a CTIMP during current pregnancy.
8. Use of oral antibiotics during current pregnancy
9. Known food allergy to nuts or any of the components of the GUARD MEC breakfast.

19.5 Recruitment & assessments

Participants will be selected from the antenatal clinics at collaborating hospitals with specialist obstetric multidisciplinary teams, expert in the management of GDM.

Pregnant women who meet the eligibility criteria for any of the four arms will be approached with a PIS and be informed about the study. If willing to participant, they will be asked to provide written informed consent. For in Arms 1 & 2, only women who participate in the main GUARD trial will be invited to participate (see flowchart in Section 7).

Patients who tested negative for GDM, or those whose glucose is well controlled by diet and hence not requiring pharmacological treatment are not eligible to participate in the main GUARD Trial. These patients will be given the opportunity to participate in the sub-study GUARD MEC as controls. These women will be identified by a member of the clinical team and PIS given.

The study will be explained in detail by trained and delegated clinical or research staff, giving as much time for consideration as the patient considers necessary. Should the patient agree to participate to this part of the research, a copy of the ICF will be signed by the patient and the investigator at that point or at a later visit (or delegated member according to local standard practice), and a copy given to the patient. A copy will be filed/uploaded into the woman's electronic maternity records. The investigator's original will be filed in the ISF.

After signing informed consent, only one study visit will be required for participation in GUARD MEC. For patients in GUARD, this will coincide with Follow Up 2. Prior to this visit participants will be given a food diary to record their food intake 4 days before their appointment. Participants will attend the hospital at 36^{+0} weeks' gestation (+/-1 week), after an overnight fast and will be given a standardised breakfast with pre-determined lipid and glucose content consisting of 50 g of fat, 75 g carbohydrates, ≥750 kcal. Participants unable to ingest at least 80% of the breakfast will be withdrawn. Blood samples will be taken at four timepoints.

The following assessments will also be performed on those participants:

- Inclusion/exclusion assessment
- Demographics (including post-code), family, medical and obstetric data
- Concomitant medication
- Weight (additional weight and height from booking visit to be obtained from medical records)
- Continuous Glucose Monitoring implementation and education
- **Optional** Vascular studies: blood pressure pulse wave velocity, central arterial pressure, augmentation index.
- Blood pressure and pulse (if not consented for the vascular studies)
- **Optional** faecal sample (can be produced at the participant's home and shipped to hospital)
- Collect 4-day food diary from participant
- Study procedures-related adverse events
- eCRF completion

To coincide with the following hospital appointment:

- CGM data download and collection of device.

After birth (data to be collected from the medical notes):

- Labour and birth data: onset of labour, genital tract trauma, post-partum haemorrhage, mode of birth.

- Neonatal data: Apgar scores: 5 min post-birth, gestational age at birth, gender, NICU/SCBU admission, morbidity, feeding method at birth and discharge, inpatient night, shoulder dystocia and manoeuvres required for delivery.

- Neonatal anthropometry will be measured taken on day of delivery or as soon as feasible: birth weight and the following measurements in triplicate:

a) With blank tapes (then checked measured these with a steel rule): head circumference, chest circumference, abdominal circumference, midarm circumference

b) Skinfold thicknesses in triplicate: subscapular and triceps

c) Crown rump length, crown foot length

Visit name & approximate pregnancy week	Baseline	Follow up 2	Labour
	26^{+0}-35^{+6}	$36^{+0} \pm 1$ [b]	
Patient information	X		
Informed consent	X		
Inclusion / exclusion criteria	X		
Demographics	X		
Medical and obstetric personal and family history	X		
Adverse events		X [f]	
Concomitant medication	X	X	
Weight		X	
Blood pressure and pulse [a]		X	
Optional faecal sample [b]		X [g]	
GUARD MEC Blood samples after a breakfast b		X [h]	
Continuous Glucose Monitoring [c]		X	
Download CGM data & collect device			X
Optional vascular studies [d]		X	
4-day food diary [e]		X [e]	
Labour and birth data			X [i]
Neonatal anthropometry			X [j k]
Neonatal data			X [k l]

a - Blood pressure in triplicate and pulse only for woman who don't consent to the vascular studies. Use non-dominant arm.
b - Research samples, for storage.
c - CGM will be in place for 10 days after each study visit. Women to be trained to remove the device themselves and return it at their next visit or at labour.
d - Vascular studies: blood pressure pulse wave velocity, central arterial pressure, augmentation index.
e - Food diaries should be given to patients at baseline to be completed the 4 days prior to Follow up 2. This should occur prior to providing a faecal sample (if applicable).
f – In Arms 3 & 4, study procedure-related adverse events will be collected from the day of the procedure until the CGM is disconnected.
g -Faecal samples are optional and should be produced at approximately 36 weeks. If the woman is unable to give a faecal sample on the day, a stool sample collection kit may be provided and the sample collected by courier from the participant's home.
h - A hospital standardised breakfast will be provided. Four blood samples will be taken at the following timepoints: before breakfast, 15 minutes, 1 hour and 2 hours postprandially.
i - Labour and birth data: onset of labour, genital tract trauma, post-partum haemorrhage, mode of birth, gestational age at delivery, NICU admission, morbidity, feeding at discharge.
j - Neonatal anthropometry: birth weight and the following measurements in triplicate:

- With blank tapes (then checked measured these with a steel rule): head circumference, chest circumference, abdominal circumference, midarm circumference
- Skinfold thicknesses in triplicate: subscapular and triceps
- Crown rump length, crown foot length

k - Neonatal assessments will be performed on the day of delivery, or as soon as feasible.
l - Apgar scores: 5 min post-birth

19.6 Laboratory details

Measurement of gut hormone release and gut hormone production over a 2.5-hour period will be performed at approximately 36 weeks: participants will attend the hospital fasted and will be given a standardised breakfast with pre-determined lipid and glucose content. Blood samples will be taken when fasting, 15 minutes, 1 hour and 2 hours after breakfast finish time. These fasting samples will be evaluated in place of Follow-up 2 samples on the main GUARD participants.

The samples will be used to evaluate LFTs, lipid (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, free fatty acids) and glucose metabolism (glucose, C-peptide, insulin, HbA1c) by either standard laboratory analysis or ELISA (for gut hormone secretion, insulin, C-peptide and free fatty acids). Individual bile acid analysis will also be performed using UPLC-MS/MS. Leftover serum and plasma samples will be stored at -80^{o}C for subsequent analysis of incretins and metabolic markers.

The table below details the volume of blood and the vacutainer required for collection at each time point.

Time	FO	SST	EDTA
Fasting	4 mL	2 x 3.5 mL vacutainers (send to laboratory: 1 x liver function test and lipid profile, 1 x total bile acids) 1 x 5 mL vacutainer for storage	1 x 6 mL (1 x HbA1c, send to laboratory) 1 x 6 mL storage
0h15 postprandial	4 mL	1 x 3.5 mL vacutainer (lipid profile – send to laboratory) 1 x 5mL vacutainer for storage	1 x 6 mL (storage)
1h00 postprandial	4 mL	1 x 3.5 mL vacutainer (lipid profile – send to laboratory) 1 x 6mL vacutainer for storage	1 x 6 mL (storage)
2h00 postprandial	4 mL	1 x 3.5 mL vacutainer (lipid profile – send to laboratory) 1 x 6 mL vacutainer for storage	1 x 6 mL (storage)
Estimated total volume of blood collected:	**16 mL**	**39.5 mL**	**30 mL**

Samples collected for storage from SST vacutainers will be used to determine free fatty acids, bile acids, FGF19, C4 and other metabolic hormones of interest. Samples collected for storage from EDTA vacutainers will be used to determine HbA1c, GLP-1, insulin, C-peptide and other metabolic hormones of interest.

In total, approximately 86 mL of blood will be collected for each participant in GUARD-MEC.

19.7 Reporting of Adverse Events

For participants of GUARD MEC who are not part of the main trial, only adverse events who are related to study procedures will be collected. This includes (but is not limited to) allergic reactions to food, fainting, reactions to the CGM device, reactions of the phlebotomy, etc.

AE will be collected from the day of the study procedure at week 36, until the time the CGM device is disconnected, 10 days later. Severity will be assessed as mild, moderate and severe.

20. References

1. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and Adverse Pregnancy Outcomes. ***N Engl J Med*** 2008;**358**:1991–2002.

2. Black MH, Sacks DA, Xiang AH, Lawrence JM. Clinical Outcomes of Pregnancies Complicated by Mild Gestational Diabetes Mellitus Differ by Combinations of Abnormal Oral Glucose Tolerance Test Values. ***Diabetes Care*** 2010;**33**:2524–30.

3. Tobias DK, Stuart JJ, Li S, Chavarro J, Rimm EB, Rich-Edwards J, et al. Association of History of Gestational Diabetes With Long-term Cardiovascular Disease Risk in a Large Prospective Cohort of US Women. ***JAMA Intern Med*** 2017;**177**:1735–42.

4. Albareda M, Caballero A, Badell G, Piquer S, Ortiz A, de Leiva A, et al. Diabetes and abnormal glucose tolerance in women with previous gestational diabetes. ***Diabetes Care*** 2003;**26**:1199–205.

5. Lowe WL, Scholtens DM, Lowe LP, Kuang A, Nodzenski M, Talbot O, et al. Association of Gestational Diabetes With Maternal Disorders of Glucose Metabolism and Childhood Adiposity. ***JAMA*** 2018;**320**:1005.

6. Ryckman K, Spracklen C, Smith C, Robinson J, Saftlas A. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. ***BJOG*** 2015;**122**:643–51.

7. White SL, Pasupathy D, Sattar N, Nelson SM, Lawlor DA, Briley AL, et al. Metabolic profiling of gestational diabetes in obese women during pregnancy. ***Diabetologia*** 2017;**60**:1903–12.

8. Anderson SG, Dunn WB, Banerjee M, Brown M, Broadhurst DI, Goodacre R, et al. Evidence That Multiple Defects in Lipid Regulation Occur before Hyperglycemia during the Prodrome of Type-2 Diabetes. ***PLoS One*** 2014;**9**:e103217.

9. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational diabetes. ***Lancet Diabetes Endocrinol*** 2014;**2**:488–99.

10. Banerjee M, Anderson SG, Malik RA, Austin CE, Cruickshank JK. Small artery function 2 years postpartum in women with altered glycaemic distributions in their preceding pregnancy. ***Clin Sci*** 2012;**122**:53–61.

11. O'Sullivan EP, Avalos G, O'Reilly M, Dennedy MC, Gaffney G, Dunne F, et al. Atlantic Diabetes in Pregnancy (DIP): the prevalence and outcomes of gestational diabetes mellitus using new diagnostic criteria. ***Diabetologia*** 2011;**54**:1670–5.

12. Athukorala C, Crowther CA, Willson K, Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Women with gestational diabetes mellitus in the ACHOIS trial: Risk factors for shoulder dystocia. ***Aust New Zeal J Obstet Gynaecol*** 2007;**47**:37–41.

13. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal Lipids as Strong Determinants of Fetal Environment and Growth in Pregnancies With Gestational Diabetes Mellitus. ***Diabetes Care*** 2008;**31**:1858–63.

14. Son GH, Kwon JY, Kim YH, Park YW. Maternal serum triglycerides as predictive factors for large-forgestational age newborns in women with gestational diabetes mellitus. ***Acta Obstet Gynecol Scand*** 2010;**89**:700–4.

15. Harmon KA, Gerard L, Jensen DR, Kealey EH, Hernandez TL, Reece MS, et al. Continuous Glucose Profiles in Obese and Normal-Weight Pregnant Women on a Controlled Diet: Metabolic determinants of fetal growth. ***Diabetes Care*** 2011;**34**:2198–204.

16. Kawasaki M, Arata N, Miyazaki C, Mori R, Kikuchi T, Ogawa Y, et al. Obesity and abnormal glucose tolerance in offspring of diabetic mothers: A systematic review and meta-analysis. ***PLoS One*** 2018;**13**:e0190676.

17. Leybovitz-Haleluya N, Wainstock T, Landau D, Sheiner E. Maternal gestational diabetes mellitus and the risk of subsequent pediatric cardiovascular diseases of the offspring: a population-based cohort study with up to 18 years of follow up. ***Acta Diabetol*** 2018; doi: 10.1007/s00592-018-1176-1.

18. Sacco F, Calderone A, Castagnoli L, Cesareni G. The cell-autonomous mechanisms underlying the activity of metformin as an anticancer drug. ***Br J Cancer*** 2016;**115**:1451–6.

19. Romero R, Erez O, Hüttemann M, Maymon E, Panaitescu B, Conde-Agudelo A, et al. Metformin, the aspirin of the 21st century: its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity. ***Am J Obstet Gynecol*** 2017;**217**:282–302.

20. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, MiG Trial Investigators. Metformin versus Insulin for the Treatment of Gestational Diabetes. ***N Engl J Med*** 2008;**358**:2003–15.

21. Rowan JA, Rush EC, Obolonkin V, Battin M, Wouldes T, Hague WM. Metformin in Gestational Diabetes: The Offspring Follow-Up (MiG TOFU): Body composition at 2 years of age. ***Diabetes Care*** 2011;**34**:2279– 84.

22. Vanky E, Stridsklev S, Heimstad R, Romundstad P, Skogøy K, Kleggetveit O, et al. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. ***J Clin Endocrinol Metab*** 2010;**95**:E448–55.

23. Barbour LA, Scifres C, Valent AM, Friedman JE, Buchanan TA, Coustan D, et al. A cautionary response to SMFM statement: pharmacological treatment of gestational diabetes. ***Am J Obstet Gynecol*** 2018; doi:
10.1016/j.ajog.2018.06.013.

24. van Weelden W, Wekker V, de Wit L, Limpens J, Ijäs H, van Wassenaer-Leemhuis AG, et al. Long-Term Effects of Oral Antidiabetic Drugs During Pregnancy on Offspring: A Systematic Review and Metaanalysis of Follow-up Studies of RCTs. ***Diabetes Ther*** [Internet]. 2018 [cited 2018 Sep 12]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/30168045

25. Martis R, Crowther CA, Shepherd E, Alsweiler J, Downie MR, Brown J. Treatments for women with gestational diabetes mellitus: an overview of Cochrane systematic reviews. ***Cochrane Database Syst Rev*** 2018;**8**:CD012327.

26. Langer O, Conway DL, Berkus MD, Xenakis EM-J, Gonzales O. A Comparison of Glyburide and Insulin in Women with Gestational Diabetes Mellitus. ***N Engl J Med*** 2000;**343**:1134–8.

27. Reynolds RM, Denison FC, Juszczak E, Bell JL, Penneycard J, Strachan MWJ, et al. Glibenclamide and metfoRmin versus stAndard care in gEstational diabeteS (GRACES): a feasibility open label randomised trial. ***BMC Pregnancy Childbirth*** 2017;**17**:316.

28. Bonde L, Vilsbøll T, Nielsen T, Bagger JI, Svare JA, Holst JJ, et al. Reduced postprandial GLP-1 responses in women with gestational diabetes mellitus. ***Diabetes, Obes Metab*** 2013;**15**:713–20.

29. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. ***Cell*** 2012;**150**:470–80.

30. Crusell MKW, Hansen TH, Nielsen T, Allin KH, Rühlemann MC, Damm P, et al. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. ***Microbiome*** 2018;**6**:89.

31. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. ***Am J Clin Nutr*** 2008;**88**:894–9.

32. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. ***Nature*** 2015;**528**:262–6.

33. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. ***Diabetes*** 2009;**58**:1509–17.

34. Wikström Shemer E, Marschall HU, Ludvigsson JF, Stephansson O. Intrahepatic cholestasis of pregnancy and associated adverse pregnancy and fetal outcomes: a 12-year population-based cohort study. ***BJOG*** 2013;**120**:717–23.

35. Martineau MG, Raker C, Dixon PH, Chambers J, Machirori M, King NM, et al. The metabolic profile of intrahepatic cholestasis of pregnancy is associated with impaired glucose tolerance, dyslipidemia, and increased fetal growth. ***Diabetes Care*** 2015;**38**:243–8.

36. Kreymann B, Ghatei MA, Williams G, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. ***Lancet*** 1987;**330**:1300–4.

37. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). ***Biochem Biophys Res Commun*** 2002;**298**:714–9.

38. Shima KR, Ota T, Kato K-I, Takeshita Y, Misu H, Kaneko S, et al. Ursodeoxycholic acid potentiates dipeptidyl peptidase-4 inhibitor sitagliptin by enhancing glucagon-like peptide-1 secretion in patients with type 2 diabetes and chronic liver disease: a pilot randomized controlled and add-on study. ***BMJ Open Diabetes Res Care*** 2018;**6**:e000469.

39. Sánchez-García A, Sahebkar A, Simental-Mendía M, Simental-Mendía LE. Effect of ursodeoxycholic acid on glycemic markers: A systematic review and meta-analysis of clinical trials. ***Pharmacol Res*** 2018;**135**:144–9.

40. Foghsgaard S, Andreasen C, Vedtofte L, Andersen ES, Bahne E, Strandberg C, et al. Nonalcoholic Fatty Liver Disease Is Prevalent in Women With Prior Gestational Diabetes Mellitus and Independently Associated With Insulin Resistance and Waist Circumference. ***Diabetes Care*** 2017;**40**:109–16.

41. Murphy HR, Rayman G, Duffield K, Lewis KS, Kelly S, Johal B, et al. Changes in the Glycemic Profiles of Women With Type 1 and Type 2 Diabetes During Pregnancy. ***Diabetes Care*** 2007;**30**:2785–91.

42. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. ***Diabetes Care*** 2017;**40**:1631–40.

43. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and Adverse Pregnancy Outcomes. ***N Engl J Med*** 2008;**358**:1991–2002.

44. Méndez-Sánchez N, González V, Chávez-Tapia N, Ramos MH, Uribe M. Weight reduction and ursodeoxycholic acid in subjects with nonalcoholic fatty liver disease. A double-blind, placebo-controlled trial. ***Ann Hepatol*** 2004;**3**:108–12.

45. Chappell LC, Gurung V, Seed PT, Chambers J, Williamson C, Thornton JG. Ursodeoxycholic acid versus placebo, and early term delivery versus expectant management, in women with intrahepatic cholestasis of pregnancy: semifactorial randomised clinical trial. ***BMJ*** 2012;**344**:e3799.

46. Webster LM, Myers JE, Nelson-Piercy C, Mills C, Watt-Coote I, Khalil A, et al. Longitudinal changes in vascular function parameters in pregnant women with chronic hypertension and association with adverse outcome: a cohort study. ***Ultrasound Obstet Gynecol*** 2018; doi: 10.1002/uog.19021.

47. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic Pulse Wave Velocity Improves Cardiovascular Event Prediction. ***J Am Coll Cardiol*** 2014;**63**:636–46.

48. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. ***Eur Heart J*** 2010;**31**:1865–71.

49. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? ***Circulation*** 2002;**106**:2085–90.

50. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. ***Science*** 2006;**313**:1137– 40.

51. Gardosi J, Francis A, Williams M, Hugh O, Loi S. Customised Centile Calculator GROW v8.0.1, 2018. Gestation Network www.gestation.net.

52. White IR, Horton NJ, Carpenter J, Pocock SJ (2011). Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ 2011;342:d40 doi: 10.1136/bmj.d40.

53. Geller NL, Pocock SJ. Interim analyses in randomized clinical trials: ramifications and guidelines for practitioners. Biometrics 1987; 43:213-23.

54. Jones B, Jarvis P, Lewis JA, Ebbutt AF. Trials to assess equivalence: the importance of rigorous methods. BMJ 1996; 313: 36-39.

55. Pharmacol Res. 2018 Sep;135:144-149. doi: 10.1016/j.phrs.2018.08.008. Epub 2018 Aug 9. Effect of ursodeoxycholic acid on glycemic markers: A systematic review and meta-analysis of clinical trials. Sánchez-García A1, Sahebkar A2, Simental-Mendía M3, Simental-Mendía LE4.

56. Chappell LC, Bell J, Smith A, Linsell L, Juszczak E, Dixon PH, Chambers J, Hunter R, Dorling J, Williamson C*, Thornton JG* Ursodeoxycholic acid versus placebo in women with intrahepatic cholestasis of pregnancy (PITCHES): a randomised controlled trial. Lancet May 2019. doi: 10.1016/S0140-6736(19)31270-X. PMID: 3137839

57. Flynn et al. Dietary patterns in obese pregnant women; influence of a behavioral intervention of diet and physical activity in the UPBEAT randomized controlled trial. International Journal of Behavioral Nutrition and Physical Activity (2016) 13:124

58.

21. Appendixes

Appendix 1 - Studies of gut metabolites show conversion of UDCA to lithocholic acid (LCA), a potent TGR5 ligand.

Our data suggest that UDCA treatment causes enrichment with bile salt hydrolase-encoding bacteria of the Bacteroidetes phylum (Figure 1A). These deconjugate intestinal bile acids, enabling their modification to the secondary bile acids; in particular UDCA is converted to lithocholic acid (LCA). Of relevance to this application, secondary bile acids have greater affinity for the bile acid receptor TGR5 (also known as G protein bile acid receptor, GPBAR1), with LCA having the highest affinity[37]. Activation of TGR5 in the intestine stimulates the release of GLP-1. Our pilot data also reveal increased concentrations of LCA in the faeces of UDCA treated women (Figure 1B).

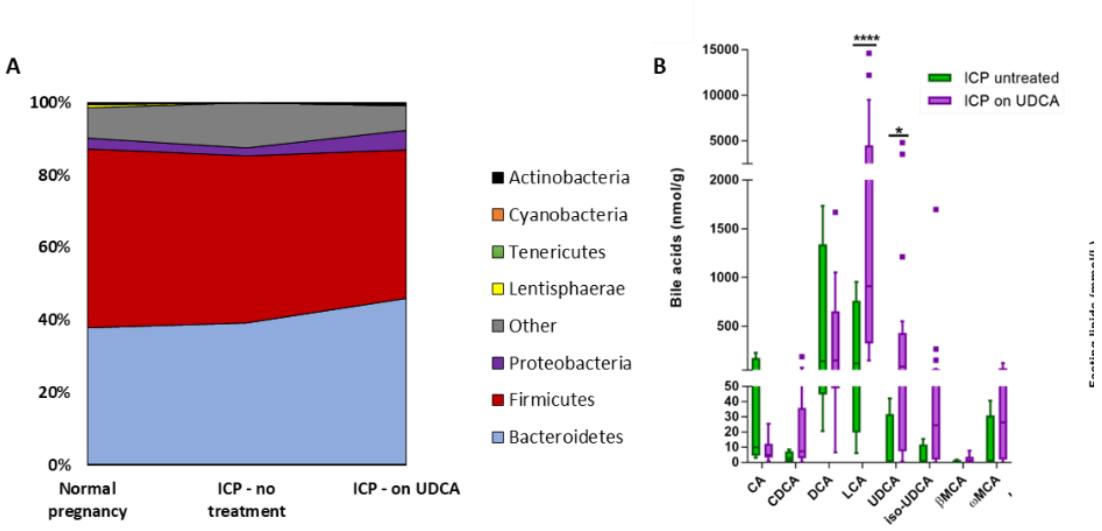


Figure 1. UDCA treatment alters the gut microbiota in ICP women. A. The ratio of Bacteroidetes to Firmicutes is increased with UDCA treatment of ICP, determined by 16S rRNA sequencing of the faecal microbiota. **B.** Secondary bile acids (LCA: lithocholic acid and UDCA) are significantly elevated in the faeces of women with ICP who were taking UDCA. **C.** The dyslipidaemia of cholestatic pregnancy is attenuated for cholesterol and low density lipoprotein (LDL) cholesterol with UDCA treatment. Groups compared with 2-way ANOVA and Tukey's multiple comparisons tests, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Appendix 2 - UDCA treatment improves ICP-associated maternal and fetal dyslipidaemia

(Figure 2A). Importantly, we also show that maternal UDCA treatment is associated with improvements in neonatal dyslipidaemia (Figure 2B).

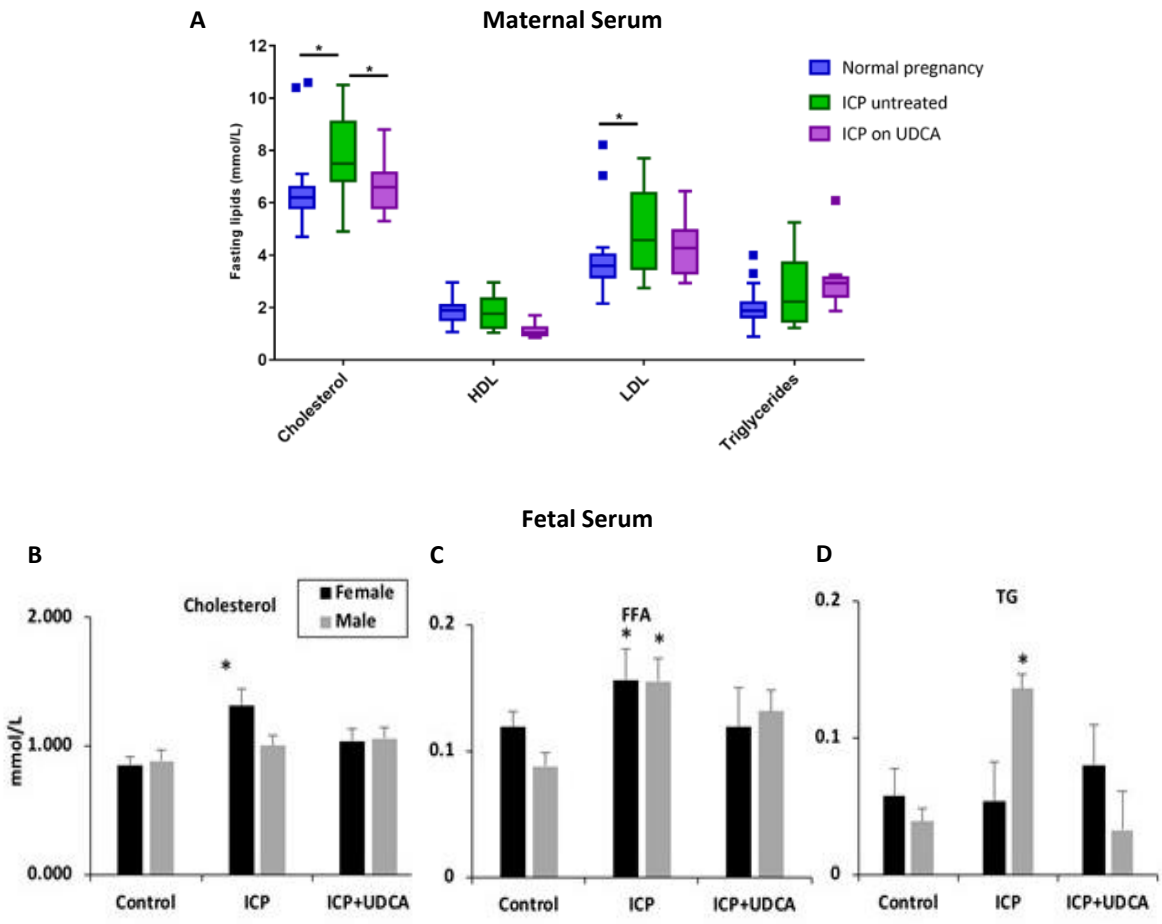


Figure 2. Impact of maternal UDCA treatment on fetal lipid profiles of women with ICP. **A.** Maternal serum lipid profile of cholesterol, HDL-Cholesterol (HDL), LDL-Cholesterol (LDL), and triglycerides in normal pregnancy, untreated ICP pregnancy, and UDCA-treated ICP pregnancy. Fetal lipid profiles from mothers who had a normal pregnancy, ICP, or ICP treated with UDCA, of **B.** Cholesterol, **C.** free fatty acids (FFA) and **D.** triglycerides (TG). Samples were measured from umbilical cord serum in female and male fetuses of women with ICP. Error bars represent standard error of the mean (SEM). Data were analysed by multiple measures of ANOVA followed by Neuman Keul's post-hoc testing. *P<0.05, n=8-10.

22. Record of protocol amendments

Amendment type, number and date	Summary of changes

23. Signatures

_____	_____
Chief Investigator *Print name*	Date
_____	_____
Statistician (if applicable) *Print name*	Date
_____	_____
Principal Investigator (if applicable) *Print name*	Date