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GUARDS Statistical Analysis Plan Version 1.1 V.2

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Maternal and Fetal Disea...

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Disclaimer

Abstract

GUARDS is a double-blind randomised placebo-controlled single site trial. The participating centre in Spain will be Clinic University Hospital “Virgen de la Arrixaca” in Murcia. Women with an abnormal oral glucose tolerance test (OGTT) screen will be offered participation. After written informed consent, eligible women will be randomly assigned to UDCA or placebo at 24^{+0}-30^{+6} weeks’ gestation until birth. All participants will be offered standard antenatal care, aiming for the GEDE guideline targets of blood glucose concentration between 3.9-7.8mmol/L (70 - 140 mg/dL). We will assess clinical efficacy, mechanisms of action and acceptability of UCDA in study participants. Clinical efficacy will be measured by maternal-infant glucose control using conventional capillary and novel continuous glucose measures and standard clinical measures for serum lipids (total, HDL- and LDL-cholesterol, triglycerides and total free fatty acids). Acceptability will be measured by patient compliance (pill counts) and confirmed by serum measurements of individual bile acids (including UDCA).

Serum samples will be taken for UPLC-MS/MS measurement of bile acids (including UDCA), so it will be clear if women have taken (and absorbed) the drug. Obstetric and neonatal outcomes will be measured. This Statistical Analysis Plan sets out how we will analyse the trial.

Troubleshooting

GUARDS

Gestational treatment with **U**rsodeoxycholic **A**cid compared to Placebo to **R**educe Severity of Gestational **D**iabetes Mellitu**s** diagnosed at 24-28 weeks' gestation (GUARDS trial)

Statistical Analysis Plan
Version 1.1 24.10.2022

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Trial protocol
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SAP revision history

24/10/2022: revision following initial blind data analysis; updated CGM target ranges included.

Authorship

The SAP was written by CW, JBC, CdPM, ML, AM and CO, with advice and input from DW (Senior Statistician). The trial CI is JBC.

Contents

1.1 Background and rationale

Approximately 10% of pregnant women develop diabetes as a new diagnosis when pregnant, a condition called gestational diabetes mellitus (GDM), which greatly increases the risk of adverse outcomes for both mother and child. Complications for the mother include increased risk of hypertensive diseases of pregnancy, including pre-

eclampsia, and higher rates of subsequent cardiovascular disease and type 2 diabetes mellitus (T2DM). 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.

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. GDM is also complicated by higher rates of preterm birth, caesarean section and birth injuries, including shoulder dystocia, which is particularly increased with LGA. 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. There is fetal dyslipidaemia, with increased free fatty acids and triglycerides in the umbilical cord blood; this is also associated with increased risk of LGA. 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.

Effectiveness of current treatments
In Spain all pregnant women are screened at 24-28 weeks' gestation using a 50-g glucose challenge test (GCT). Women who had a venous plasma glucose >7.8mmol/l are scheduled for a diagnostic, 100-g, 3-h glucose tolerance test (OGTT) and those with a positive diagnosis start self-monitoring of blood glucose (SMBG) and are given dietary and lifestyle advice. If unable to achieve recommended glucose control targets, they are prescribed subcutaneous insulin injections. At present 20% of women with GDM require insulin treatment and this results in increased hospital attendance and the need for needles, insulin therapy and regular monitoring of blood glucose concentrations by trained doctors and midwives, all of which are costly and use resources. While insulin treatment improves maternal glucose concentrations, it 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. 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 as a treatment in gestational diabetes mellitus
Our trial will compare the influence of treatment with ursodeoxycholic acid (UDCA) compared to placebo on glycaemic control (primary outcome) in women with GDM after a positive diagnosis at 24-28 weeks of gestation. We will evaluate maternal and fetal lipid and glucose metabolism. Neonatal health outcomes will also be studied, including the rate of LGA. UDCA is used to treat the commonest liver disease of pregnancy, intrahepatic cholestasis of pregnancy (ICP), and has good safety data to support its use in pregnancy.

We have generated pilot data from studies of women with ICP, a liver disorder of pregnancy that is associated with a 3-fold increase in the risk of GDM. Our studies of women with ICP either untreated or receiving UDCA treatment demonstrated that UDCA improves ICP-associated insulin resistance. We therefore propose that UDCA is a new potential treatment for GDM. 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). Furthermore, a recently published meta-analysis in people with non-

alcoholic fatty liver disease (a disorder that is commonly associated with type 2 diabetes mellitus, previous GDM and later vascular disease) 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.

1.2 Objectives

To assess the clinical efficacy and acceptability of UDCA as a treatment to improve glycaemic control in women diagnosed with GDM at 24-28 weeks' gestation.

1.3 Description of the trial

This is a double-blind randomised placebo-controlled single site trial. The participating centre in Spain will be Clinic University Hospital "Virgen de la Arrixaca" in Murcia. Women with an abnormal oral glucose tolerance test (OGTT) screen will be offered participation. After written informed consent, eligible women will be randomly assigned to UDCA or placebo at 24^{+0}-30^{+6}weeks' gestation until birth. All participants will be offered standard antenatal care, aiming for the GEDE guideline targets of blood glucose concentration between 3.9-7.8mmol/L (70 - 140 mg/dL). We will assess clinical efficacy, mechanisms of action and acceptability of UCDA in study participants. Clinical efficacy will be measured by maternal-infant glucose control using conventional capillary and novel continuous glucose measures and standard clinical measures for serum lipids (total, HDL- and LDL-cholesterol, triglycerides and total free fatty acids). Acceptability will be measured by patient compliance (pill counts) and confirmed by serum measurements of individual bile acids (including UDCA).

Serum samples will be taken for UPLC-MS/MS measurement of bile acids (including UDCA), so it will be clear if women have taken (and absorbed) the drug. Obstetric and neonatal outcomes will be measured.

The trial will be conducted in compliance with the protocol, the Declaration of Helsinki (1996), the principles of Good Clinical Practice (GCP) and applicable regulatory requirements. The study will be reviewed and approved by the National Research Ethics Committees (NREC) and competent authorities in the region of Murcia and applicable Hospital Management Boards. The FFIS will manage the sponsors' responsibilities and Quality Assurance to ensure compliance with the Clinical Trial Regulations.

1.4 Randomisation

Randomisation will be performed using an online web based system. Each participant will be assigned a study ID and randomisation code. The randomisation code will determine who receives placebo or UDCA 500mg. The

Pharmacy Manufacturing Unit and Sealed Envelopes will keep and store the randomisation code list. All participants, the PI and site clinical trial pharmacy, where used, will remain blind to trial drug allocation.

1.5 Participants

Women with GDM diagnosed at 24-28 weeks' gestation in accordance with Spanish guidelines (see inclusion criteria below) will be offered participation. After written informed consent, eligible women will be randomly assigned to UDCA or placebo at 24^{+0}-30^{+6} weeks' gestation until birth. All participants will be offered standard antenatal care, aiming for target blood concentrations of 3.9-7.8mmol/L (70 - 140 mg/dL). We will assess clinical efficacy, mechanisms of action and acceptability of UCDA in study participants. Clinical efficacy will be measured by maternal-infant glucose control using conventional capillary and novel continuous glucose measures and standard clinical measures for serum lipids (total, HDL- and LDL-cholesterol, triglycerides and total free fatty acids). Acceptability will be measured by patient compliance (pill counts) and the treatment acceptability questionnaire.
Serum samples will be taken for UPLC-MS/MS measurement of bile acids (including UDCA), so it will be clear if women have taken (and absorbed) the drug. Obstetric and neonatal outcomes will be measured.

1.5.1 Eligibility Criteria

Participant selection

The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate participants are entered. Participants not meeting the criteria should not be entered into the trial for their safety and to ensure that the trial results can be appropriately used to make future treatment decisions for other people with similar diseases or conditions. It is therefore vital that exceptions are not made to these eligibility criteria. Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

Inclusion criteria

- Women with GDM diagnosed at 24-28 weeks' gestation in accordance with Spanish guidelines (NDDG criteria), i.e. two or more glucose concentrations (fasting: ≥ 5.8 mmol/L (105 mg/dL), 1 h: ≥10.6 mmol/L, (190 mg/dL) 2 h: ≥9.2 mmol/L (165 mg/dL), 3 h: ≥ 8.1 mmol/L (145 mg/dL)) after a standard 100g OGTT, two fasting blood

glucose levels ≥ 126 mg/dl, on different days, or at random ≥ 200 mg/dL ora plasma glucose value greater than 200 mg/dL after O'Sullivan test

- Planned antenatal care at the same centre (i.e. not planning to move before delivery)
- Singleton pregnancy
- Informed and written consent

Exclusion criteria

- Age <18 years
- Multiple pregnancy in current pregnancy

- Unconscious or very ill
- Serious mental illness
- Learning difficulties
- Not fluent in local language and absence of interpreter
- Severe congenital anomaly on ultrasound
- Previous diagnosis of diabetes outside of pregnancy
- 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 (<1 year)
- 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
- Participating in another intervention study that will influence the outcome of this trial (to be advised by CI or PI)
- Known allergy/hypersensitivity/intolerance to the active substance or excipients
- Hypersensitivity to Ursodeoxycholic acid or to the following excipients: Magnesium stearate, cellulose powder, colloidal silica and sodium carboxymethyl starch, gelatin, titanium dioxide, quinoline yellow, orange yellow S, indigotine
- Patients with a non-functioning gallbladder, in patients with calcified cholesterol stones, radio-opaque stones, radiolucent gallbladder stones
- Gastric or duodenal ulcer
- Liver or intestinal disorders that interfere with the enterohepatic circulation: acute cholecystitis that does not subside, cholangitis, biliary obstruction, pancreatitis due to stones, patients with gastrointestinal-biliary fistula
- Breastfeeding.

See protocol for full details.

1.6 Principal research objectives to be addressed

Primary Outcome:

Maternal fasting glucose concentration at 35^{+0}-37^{+6}weeks

Secondary Outcomes

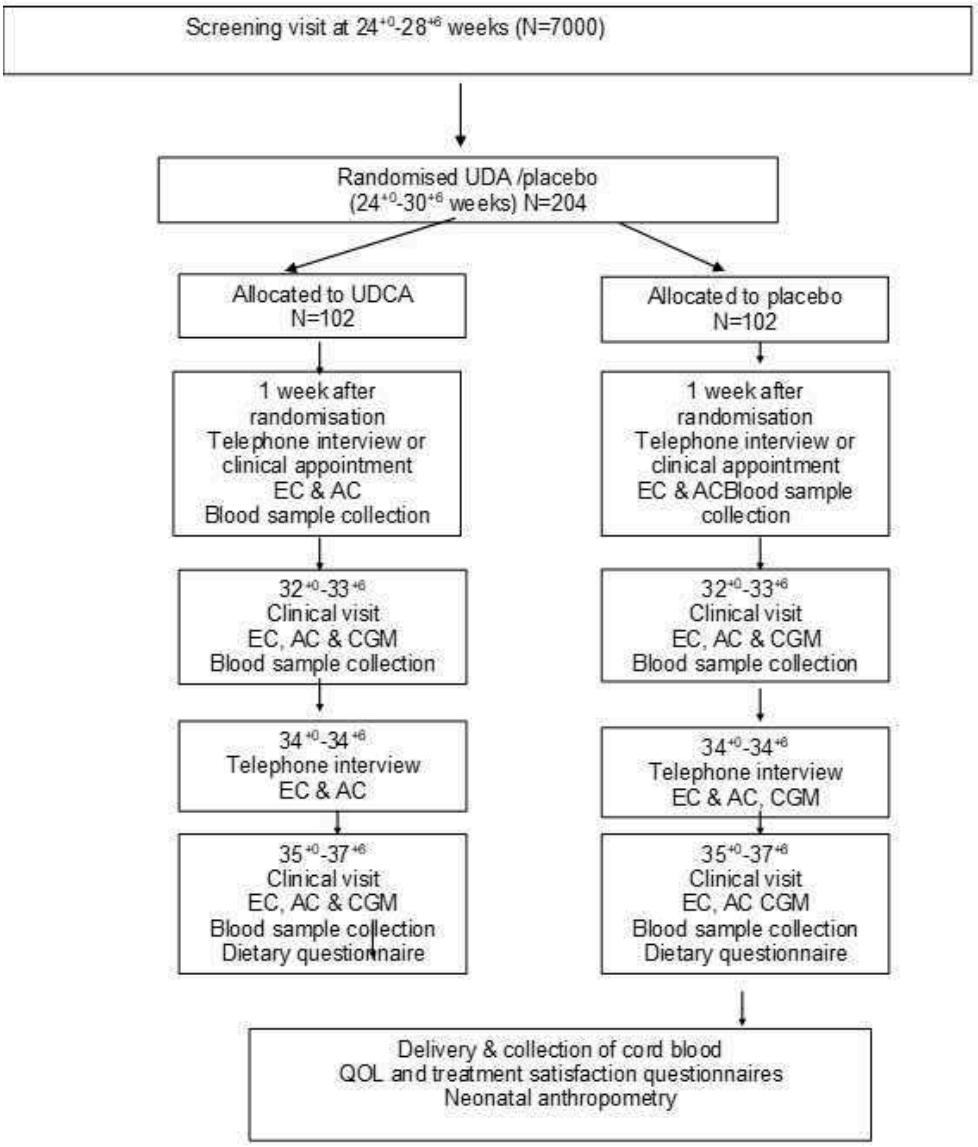
Biomedical Maternal:
1. Proportion of women requiring insulin treatment (number, time (from randomisation until treatment and total dose of insulin required (total maximal dose per day)
2. Glucose metabolism assessed by:
a) verified SMBG download (7-day mean capillary glucose, fasting glucose, 1-hr post meal glucose levels)

b) continuous glucose monitoring (CGM) to assess glycaemic control (measured at 35-37 weeks' gestation). This will determine the percentage time spent within target (glucose levels 65-120 mg/dl), percentage time spent above target (>120 mg/dl), time spent below target (<65 mg/dl) and markedly below target <50 mg/dl).
c) HbA1c concentration; a conventional marker of medium-term glycaemia
3. Lipid metabolism assessed by blood triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations
4. Biochemical analysis of maternal blood for liver function tests (24 and 36 week samples as taken in routine clinical care), bile acids, C-reactive protein (including highly sensitive analyses).
5. Maternal gestational weight change (from randomisation to 36 weeks) kg.
6. Proportion of women willing to be randomised

Biomedical Neonatal:

1. Mode of delivery (rates of primary & repeat caesarean section (CS), elective & emergency lower segment CS (LSCS) or instrumental birth (forceps or ventouse delivery).
2. Gestational age at delivery, frequency of preterm delivery
3. Infant birth weight (SD scores and customised birth weight percentile, proportion of large for gestational age (LGA), proportion of small for gestational age (SGA)), neonatal anthropometry
4. Neonatal morbidity (treatment for neonatal hypoglycaemia (glucose <40 mg/dL), neonatal jaundice (bilirubin reaching treatment threshold of 12.9 mg/dL in the first 48 hours for a term newborn that's formula fed or 15 mg/dL for a term newborn that is breast fed) and respiratory distress (requiring sufficient respiratory support to necessitate admission to the neonatal unit)
5. Neonatal unit admission (proportion and duration of hospital stay – number of days admitted, highest level care)
6. Adverse events (stillbirth, neonatal death within 6 weeks' of delivery)

1.7 Trial Diagram*



Ensure compliance (EC), address concern (AC), Continuous glucose monitoring (CGM), quality of life (QOL)

*Numbers were reduced due to COVID-related delays – see Section 1.9

1.8 Schedule of events

	Visit at Diabetes Clinic	Randomisation Visit	Telephone interview or	Visit at 32 weeks	Second telephone interview	Visit at 35-37 weeks	Delivery Umbilical cord
Gestation (weeks)	24+0-28+6	24+0-30+6	1week after randomisation	32+0-33+6	34+0-34+6	35+0-37+6	
Patient information and characteristics	Ö						
Informed consent		Ö					
Measurement of weight / height	Ö			Ö		Ö	
Measurement of MAP	Ö			Ö		Ö	
Fetal ultrasound scan	Ö			Ö		Ö	
Capillary glucose measurements	Ö			Ö		Ö	
Continuous glucose monitor				Ö		Ö	

	ing (CGM)						
	Collect data from CGM					Ö	Ö
	Analysis Glucose & lipid metabolism					Ö	
	Routine clinic bloods that will be analysed (HbA1c, liver function tests*)		Ö		Ö	Ö	
	Bile acids, 1,5-anhydroglucitol and research blood samples (for storage) and a rectal swab (for storage)		Ö		Ö	Ö	

	Fasting and post-breakfast samples to assess gut hormones						Ö	
	Check concomitant medications		Ö	Ö	Ö	Ö	Ö	
	IMP dispensing		Ö		Ö		Ö	
	Ensure compliance			Ö	Ö	Ö	Ö	
		Visit at Diabetes Clinic	**Randomisation Visit**	**Telephone interview or**	**Visit at 32 weeks**	**Second telephone interview**	**Visit at 35-37 weeks**	**Delivery Umbilical cord**
	Check side effects/ adverse events			Ö	Ö	Ö	Ö	
	Review of diary card			Ö	Ö	Ö	Ö	
	IMP retrieval						Ö	Ö
	Collection of umbilical cord blood							Ö

	Neonatal anthropometry							Ö

1.9 Sample size estimation

Initial calculations:
A study size of 204 participants will provide sufficient statistical power whilst allowing for a 40% 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), consistent with previously reported differences with UDCA treatment for non-alcoholic fatty liver disease (NAFLD),[44]and 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 0.5, 61 women per arm would be required to determine this reduction with 90% power. Thus, allowing for approximately 40% dropouts, the numbers rise to 204 women in total.

Updated calculations were performed due to COVID-related delays resulting in IMP end date approaching and manufacturer not having capacity to make more placebo. By the date the IMP could not be used we had recruited 113 participants, but 5 withdrew from the trial. In brief:

- 50 women per group required to have 82% power, $p<0.05$ to detect difference between groups
- 55 women per group required to have 85% power, $p<0.05$ to detect difference between groups

Due to the study size, interim analyses will not be performed; stopping criteria will only be applied should the TMG / CI receive reports of significant adverse events that could be attributed to trial participation. The final analysis will determine the hypothesised superiority of UDCA compared with placebo in the treatment of GDM, utilising $p<0.05$ as a threshold for significance. Given the absence of interim analysis, this significance value will not be adjusted for the final analysis.

1.10 Brief description of proposed analyses

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. Where confidence intervals are reported, the 95% confidence interval will be used. Log transformation will be performed for the primary outcome (glucose concentration), with percentage difference and geometric means reported.

The main analysis will use the intention to treat (ITT) principle, using all available data on randomised women. Patients who did not fulfil inclusion / exclusion criteria at randomisation will be excluded from the per protocol

analysis – this will include patients who were prescribed and/or received insulin on the day of randomisation.

All comparisons by treatment group will be adjusted for BMI (<30 or ≥30) as this was used to stratify the randomisation.

An initial analysis of covariables will be performed blinded to participant treatment, to enable us to finalise how they will be handled in the outcome data analysis before any data unblinding. Comparative outcome analyses between treatment groups will be blinded as to the group identity until primary analyses are complete.

Recruitment and eligibility data will be reported in a CONSORT flow diagram (http://www.consort-statement.org/consort-statement/flow-diagram).

1.11 Overview

The data analyses will commence after the last clinical visit of the final participant. Further supplementary analyses, based on the analysis of stored samples, will be performed subsequently.

1.11.1 Timing of the analysis

Data will be analysed, blinded to treatment allocation, to determine how outliers and missing data will be dealt and to decide on any grouping/transformations of data. An initial analysis of the primary outcome will be performed using random group allocation. The results of the blind data analysis will be documented in a second version of the SAP. This SAP will be approved and signed-off before any the treatment allocation is revealed.

After the SAP and approved and signed-off the treatment codes will be added to the trial data and the efficacy and safety analysis will be undertaken.

1.11.2 Baseline Data

Table 1. Baseline participant characteristics by randomised treatment group

		Treatment	Control	All women
		N = XX	N = XX	N = 113

	Maternal age	Mean (SD)	Mean (SD)	Mean (SD)
	BMI at booking	Mean (SD)	Mean (SD)	Mean (SD)
	Weight at booking	Mean (SD)	Mean (SD)	Mean (SD)
	Gestational age at diagnosis	Mean (SD)	Mean (SD)	Mean (SD)
	Ethnicity - White/ White Spanish - Asian (including Chinese) - Black/African/Caribbean - Latin American - Moroccan	n/N (%) n/N (%) n/N (%) n/N (%) n/N (%) n/N (%)	n/N (%) n/N (%) n/N (%) n/N (%) n/N (%) n/N (%)	n/N (%) n/N (%) n/N (%) n/N (%) n/N (%) n/N (%)
	Parity (number of previous pregnancies)	Mean (SD)	Mean (SD)	Mean (SD)
	Fasting glucose at OGTT	Mean (SD)	Mean (SD)	Mean (SD)
	1 hour glucose at OGTT	Mean (SD)	Mean (SD)	Mean (SD)
	2 hour glucose at OGTT	Mean (SD)	Mean (SD)	Mean (SD)
	HbA1c	Mean (SD)	Mean (SD)	Mean (SD)
	Biochemical profile			
	Total cholesterol (mmol/L),	Mean (SD)	Mean (SD)	Mean (SD)

	HDL-cholesterol (mmol/L)	Mean (SD)	Mean (SD)	Mean (SD)
	LDL-cholesterol (mmol/L)	Mean (SD)	Mean (SD)	Mean (SD)
	Triglycerides (mmol/L)	Mean (SD)	Mean (SD)	Mean (SD)
	CRP	Mean (SD)	Mean (SD)	Mean (SD)
	Ratio (s-Flt1:PLGF)	Mean (SD)	Mean (SD)	Mean (SD)
	ALT/GOT	Mean (SD)	Mean (SD)	Mean (SD)
	AST/GPT	Mean (SD)	Mean (SD)	Mean (SD)

1.11.3 Adherence to allocated treatment and treatment fidelity

Participant attendance and compliance will be recorded for all visits. This will be reported with adverse events for each group. Compliance will be shown as 'pill count/total number of pills that should be taken'. During the blinded analysis, overall treatment compliance will be used to finalise the appropriate threshold above which a participant is included in the per protocol analysis, based upon a percentage of treatment taken during the study period.

1.11.4 Loss to follow-up and other missing data

Missing data will be dealt with using multiple imputation and use MCMC (Markov chain Monte Carlo) algorithms to replace missing fasting blood glucose concentrations at baseline and 36 weeks' gestation (primary outcome) using a model adjusting for BMI (included in the original randomisation). We will generate ten datasets with different imputations, analyse each dataset and pool the results. We will report the primary outcome both with and without imputation as a sensitivity analysis. Multiple imputation will be performed in WinBUGS software (https://www.mrc-bsu.cam.ac.uk/software/bugs/the-bugs-project-winbugs/).

For the primary outcome only, if delivery occurs before week 36, participants will be excluded from analysis in case of altering underlying physiology adversely impacting the biochemical results.

Primary analysis. The primary analysis will follow the intention to treat principle, for all participants included as “per protocol” . All consenting women randomised for whom adequate data are collected will be included in the primary and main secondary endpoints.

Data validation. 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 trial team when entered on the data base; and again by the statistician if implausible at the time of analysis; and corrections made wherever possible.

Participants that withdraw from the study will also be reported, including time of withdrawal and with reasons if given, and any protocol deviations described.

1.11.5 Adverse event reporting

Adverse events will be recorded on the study database and will be reported. The incidence rates of adverse events and serious adverse events graded by Common Terminology Criteria for Adverse Event reporting (CTCAE) v4.03 and their relationship to trial drugs will be summarized by treatment group. Event rates will be compared between treatment groups using the Fishers’ exact test.

Table 2. Comparison of adverse event rate by randomised treatment group

	UDCA	Placebo	Significance
Adverse event	n (%)	n (%)	p=
e.g. diarrhoea	n (%)	n (%)	p=
No adverse event	n (%)	n (%)	p=

1.12 Main analysis of differences between baseline and later time points

Analysis populations:

The primary analysis will be an intention to treat according to the treatment allocated at randomization.

A secondary per protocol analysis, including those participants that took at least 80% of the allocated medication and who did not receive insulin on the day of randomisation, will be undertaken.

1.12.1 Analysis of primary outcome

Multiple regression will be used to compare level of fasting glucose at 36 weeks between treatment groups, with adjustment for BMI (<30 or ≥30 kg/m^2) and baseline fasting glucose concentration. Distributional assumptions underlying the analysis will be examined on the basis of the blind data review; details of any transformations applied, how outliers are dealt with, methodology for imputation, grouping of covariates, and any other aspects of the primary analysis of the primary outcome will be decided and documented in the final version of the SAP, which will be signed off prior to unblinding the data.

Once comparisons of baseline characteristics have been performed, if there are unexpected differences between groups in specific parameters, e.g. women from a specific ethnic group are over-represented in one arm of the study, we will propose additional adjustments, or secondary analyses.

Confirm primary outcome with blinded data (ITT), using multiple imputation for missing outcomes.

Secondary analyses of primary outcome – consistency of treatment effect across covariates will be determined, including interactions between treatment and covariates, and presented in a forest plot. Covariates to investigate will be: maternal BMI (<30 and ≥30), compliance at 36/40 (<80%, 80-90%, >90%), fasting blood glucose at diagnosis (<90mg/dL or ≥90mg/dL), O'Sullivan test 1 hour post load glucose concentration (<190mg/dL or ≥190mg/dL), and use of insulin before 36/40 (yes/no).

Table 3. Comparison of primary outcome by randomised treatment group

	UDCA	Placebo	Comparison (difference or Risk Ratio) With	Significance

			95% CI	
Primary endpoint				
Fasting glucose	N Mean (SD) Geometric mean	N Mean (SD) Geometric mean	Mean diff (95% CI) % difference	P=0.xxx

1.12.2 Analysis of secondary outcomes

We will use percentages and Risk Ratios for binary (Yes/No) outcomes and differences in the mean value for continuous measures; except when log transformations are needed, when the ratio of the geometric means will be used. The same principles will be applied to the design of the analysis of the secondary outcome analysis as to the primary outcome with the initial analysis of blinded results.

Table 4. Comparison of additional outcomes by randomised treatment group

	Treatment	Control	Comparison (difference/ risk Ratio) [95% CI]
Maternal outcome			
***Maternal gestational weight change at 36 weeks compared to randomisation visit			
Pre-eclampsia			

	Gestational hypertension			
	Gestational age at birth			
	Frequency of preterm birth			
	Onset of labour (spontaneous/ induction)			
	Mode of birth - Elective Caesarean Section - Emergency caesarean Section - Assisted vaginal birth - Unassisted vaginal delivery			
	Number of women requiring second line treatment to manage postpartum haemorrhage within first 24 hours of birth**			
	Proportion of women requiring insulin treatment*			
	Total dose of insulin required (maximum in one day)			
	CGM and SMBG data at 32-33 weeks:			

	CGM percentage time within target (65 – 120 mg/dL)			
	CGM percentage time above target (>120 mg/dL)			
	CGM percentage time below target (<65 mg/dL)			
	CGM percentage time markedly below target (<50 mg/dL)			
	SMBG: 7 day mean fasting glucose			
	SMBG: 7 day mean 1-hr post meal glucose			
	SMBG: 7 day mean 1-hr post breakfast glucose			
	SMBG: 7 day mean 1-hr post lunch glucose			
	SMBG: 7 day mean 1-hr post dinner glucose			
	CGM and SMBG data at 35-37 weeks:			
	CGM percentage time within target (65 – 120 mg/dL)			

	CGM percentage time above target (>120 mg/dL)			
	CGM percentage time below target (<65mg/dL)			
	CGM percentage time markedly below target (<50 mg/dl)			
	SMBG: 7 day mean fasting glucose			
	SMBG: 7 day mean 1-hr post meal glucose			
	SMBG: 7 day mean 1-hr post breakfast glucose			
	SMBG: 7 day mean 1-hr post lunch glucose			
	SMBG: 7 day mean 1-hr post dinner glucose			
	Biochemical measurements at 35-37 weeks:			
	Total cholesterol (units)			
	HDL (units)			

	LDL (units)			
	Triglyceride (units)			
	ALT			
	AST			
	Bile acids (total)			
	C-reactive protein			
	Neonatal / birth outcomes			
	Apgar scores at 1 minute			
	Apgar scores at 5 minutes			
	Apgar scores at 10 minutes			
	Occurrence of shoulder dystocia			
	Occurrence of maternal anal sphincter injury			
	Infant birth weight			
	Standard deviation scores			
	Customised birth weight percentile			

	Proportion of large for gestational age (LGA)			
	Proportion of small for gestational age (SGA)			
	Neonatal morbidity			
	Hypoglycaemia			
	Jaundice			
	Respiratory distress			
	Birth trauma			
	Neonatal intensive care and special care unit admission + duration of hospital stay			
	Stillbirth			
	Neonatal death within 6 weeks of delivery			

* Note - the decision to treat with insulin may be based upon fetal abdominal circumference.

** Blood loss at birth (>1 litre) or need for maternal blood transfusion

***Adjusted for baseline concentrations

1.13 Secondary analyses

Secondary analysis will be performed if differences are observed between groups in the pattern of raised fasting/ postprandial glucose, pattern of gestational weight gain, fetal growth (e.g. change in abdominal circumference from 28-36 weeks) or ethnic origin of participants.

A sub-analysis will be performed for patients in the UDCA arm for whom UPLC-MS/MS analysis confirms ingestion and absorption of UDCA (UDCA randomisation arm only).

Secondary analyses will also compare biochemical markers to be measured from stored samples, including maternal serum concentrations of 1,5-anhydroglucitol; a novel marker of short-term glycaemia, free fatty acid concentrations. Fasting and 1 hour post-prandial samples will be taken at $35\text{-}37^{+6}$ weeks for GLP-1, FGF19, C4, individual bile acids, insulin, C-peptide and incretins. Women will be given a voucher to buy a standardised breakfast with approximately 100g carbohydrate and 50g fat. Cord blood C-peptide, triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol and free fatty acid concentrations will also be compared.

An additional nested study will determine the fasting and 30-60 minute postprandial values after intake of a set meal (40g fat, 80g carbohydrates). These values will be determined for: total cholesterol, calculated LDL-cholesterol, HDL-cholesterol, triglycerides, free fatty acids, glucagon-like peptide 1, peptide YY, insulin, C-peptide, glucagon, FGF19, leptin, individual bile acids, C4.

Future analysis will use the CGM data to also assess 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, and less stringent CGM targets (70 - 140 mg/dL).

1.14 Statistical software

The analyses will be performed in duplicate by CO and DW, using Stata 17.0 and R software. Imputation of missing data will be performed with WinBUGS.

1.15 Decisions made based upon blind data review

a. The primary analysis will compare results obtained from the trial and samples analysed in real time. Results obtained from analysis of stored samples will be included in a subsequent secondary analysis (section 1.6, Table 1, 1.13).
b. For the primary outcome, glucose concentration will be log-transformed, and data presented in addition as geometric mean / percentage difference (section 1.10).
c.We will present graphs of log scales on the axis with data points at the measured values (section 1.10)
d. We will use % difference as the primary outcome, rather than absolute, as this is appropriate for log transformed data

e. A per protocol analysis will be performed, excluding those patients who were prescribed and/or received insulin on the same day as trial randomisation (section 1.10, 1.11.4, 1.12).

f. Missing data for the primary outcome will be handled by multiple imputation, with a sensitivity analysis comparing results both with, and without, imputation (section 1.11.4).
g. Imputation for missing data – patients that delivered before 36/40 may be a different group, so we considered not imputing data for these patients, for two reasons:
1.Type 2DM (and therefore potential pre-existing diabetes) is associated with an increased risk of preterm birth – this is not completely explained and may represent a different metabolic environment
2. UDCA reduces spontaneous preterm birth in patients with cholestasis – this may happen for other conditions (although has not been investigated previously)
h. Covariates for secondary analysis of the primary outcome, and thresholds of relevance were defined and updated (section 1.12.1). For the primary analysis, we decided today to perform the following subgroup analyses (and interaction tests):Compliance: >80%, 80-90%, >90%; BMI: <30, >=30; Fasting blood glucose <=90, >90; O'Sullivan 1 hour glucose <=190, >190; Insulin treatment before 36/40 yes/no.
i. To be clear about which patients should be included / excluded, there were 1 / 2 patients who either had prestarted insulin or started on the randomisation day (therefore before first UDCA dose), and these should be excluded based upon selection of a "per protocol population". Similarly, there were a couple of patients who chose not to participate after randomisation (0% compliance) and would form part of the ITT population
i. Analysis of the primary outcome using random group allocation will be performed before allocation of treatment groups and unblinding is performed (section 1.11.1).

1.16 References

SAP developed according to the recommendations of Gamble, Krishan, Stocken et al. Guidelines for the content of statistical analysis plan in clinical trials. JAMA 2017; 318: 2337-2343

2.1 Appendix – Data analysis plan workflow

Main principles to be applied throughout: for continuous variables, compare geometric mean values where data are not normally distributed; analyse by intention to treat; assume missing data are missing at random unless part of a separate sensitivity analysis. All analyses will be performed blinded to treatment allocation (group A versus B will not be identified).

BLIND DATA REVIEW – how co-variables are handled in the outcome data analyses will be finalised based upon these results. Summary data will be collated after completion of the blind data review.

1. **Baseline characteristics**: summary data for treatment / control / all women. Where baseline variables differ markedly between treatment groups, consider adjustment for this variable in the primary outcome regression analysis

2. **Missing data summary** – summarise number of missing data points by treatment arm for the primary outcome FINISH BLIND DATA REVIEW and collate summary data by group

1. **Primary outcome** – fasting glucose concentration at 36 weeks' gestation -
multiple regression, adjusting by baseline fasting glucose concentration (ANCOVA) and with adjustment for BMI (<30 / ≥30 kg/m^2) of (i) all women (ITT) and (ii) all women taking >80% (figure to be determined from initial blind data review) of randomised medication

1. **Secondary outcomes** – adjust as per primary outcome by maternal BMI, and by baseline values where change in a value is compared (marked *)

a.gestational weight gain at 36 weeks'*
b.proportion of patients that developed pre-eclampsia
c.proportion of patients that developed gestational hypertension
d.gestational age at birth
e.rate of preterm birth (defined as birth before 37 weeks' gestation)
f.rate of spontaneous labour onset (NB elective caesarean sections are defined at induced labour onset)
g.rate of operative modes of birth (compared with unassisted vaginal delivery as a reference rate) – for elective caesarean / emergency caesarean / assisted vaginal birth
h.proportion of patients requiring second-line treatment for postpartum haemorrhage
i.proportion of patients requiring insulin treatment
j.maximum dose of insulin per day (units) – women not requiring insulin counted as 0 units
k.mean self-monitored blood glucose (SMBG) measured fasting over 7 days**
l.mean self-monitored blood glucose (SMBG) measured 1 hour post meal over 7 days**
m.mean self-monitored blood glucose (SMBG) measured 1 hour post individual meal analysed separately over 7**
n.mean continuous blood glucose monitoring (CGM) % time in range at 35-37/40 (range is 65-120mg/dl))
o.mean CGM % time above range at 35-37/40
p.mean CGM % time below range at 35-37/40
q.mean CGM % time markedly below range (<50 mg/dL) at 35-37/40
r.mean HbA1c concentration at 35-37/40*
s.mean fasting total cholesterol concentration at 35-37/40
t.mean 30-60 minute total cholesterol concentration at 35-37/40 (absolute and with adjustment for fasting concentration)
u.mean HDL-cholesterol concentration at 35-37/40
v.mean 30-60 minute HDL-cholesterol concentration at 35-37/40 (absolute and with adjustment for fasting concentration)
w.mean LDL-cholesterol concentration at 35-37/40
x.mean 1 hourLDL-cholesterol concentration at 35-37/40 (absolute and with adjustment for fasting concentration)
y.mean triglyceride concentration at 35-37/40
z.mean 1 hourtriglyceride concentration at 35-37/40 (absolute and with adjustment for fasting concentration)
aa.mean ALT concentration at 23-25/40 and 35-37/40
bb.mean AST concentration at 23-25/40 and 35-37/40
cc.mean CRP concentration at 23-25/40 and 35-37/40
dd.mean Apgar score at 1 minute of age

ee.mean Apgar score at 5 minutes of age
ff.mean Apgar score at 10 minutes of age
gg.proportion of babies with Apgar score <7 at 1 minute of age
hh.proportion of babies with Apgar score <7 at 5 minutes of age
ii.proportion of babies with Apgar score <7 at 10 minutes of age
jj.proportion of cases of shoulder dystocia
kk.proportion of cases with maternal anal sphincter injury (3^{rd} or 4^{th} degree perineal tear)
ll.mean birthweight centile
mm.proportion of babies born large for gestational age (birthweight >90^{th} centile)
nn.proportion of babies born small for gestational age (birthweight <10^{th} centile
oo.proportion of babies with hypoglycaemia during their admission
pp.proportion of babies with jaundice requiring treatment
qq.proportion of babies born with respiratory distress sufficient to require admission to the neonatal unit for respiratory support
rr.proportion of babies sustaining a birth injury
ss.proportion of babies admitted to the neonatal unit
tt.mean duration of neonatal unit admission (score 0 for no admission)
uu.proportion of babies that were stillborn
vv.proportion of babies that had a neonatal death within 6 weeks of delivery
** the range for evaluation of CGM data is 65-120mg/dL, but we will also study the impact of treatment on time markedly below 50mg/dL (clinically relevant for hypoglycaemia).

Sensitivity analysis: for missing data

Future analyses once secondary experimental work is completed:

1. Primary outcome for women for whom UPLC-MS/MS confirms ingestion and absorption of UDCA compared with all women with receiving placebo
2. Subsequent secondary analyses are proposed based upon studies of the pattern of blood glucose abnormalities in the glucose tolerance test, to assess patterns of fetal growth, and impact of ethnicity categories, which will not be included in this primary data analysis
3. Analysis of individual bile acid concentrations and profile whilst fasting and 1 hour post prandial measured at 35-37 weeks' gestation
4. 16S sequencing of rectal swab samples.

CGM analysis of glucose variation and more stringent blood glucose