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A randomised controlled trial of preconception lifestyle intervention on maternal and offspring health in people with increased risk of gestational diabetes: study protocol for the BEFORE THE BEGINNING trial

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Abstract

Background: Gestational diabetes mellitus (GDM) is associated with increased risk for type 2 diabetes in the mother and cardiometabolic diseases in the child. The preconception period is an optimal window to adapt the lifestyle for improved outcomes for both mother and child. Our aim is to determine the effect of a lifestyle intervention, initiated before and continued throughout pregnancy, on maternal glucose tolerance and other maternal and infant cardiometabolic outcomes.

Methods: This ongoing randomised controlled trial aims to include 200 females aged 18-39 years old at increased risk for GDM who are contemplating pregnancy. The participants are randomly allocated 1:1 to an intervention or control group. The intervention consists of exercise (volume is set by a heart rate-based app and corresponds to ≥ 1 hour of weekly exercise at ≥ 80% of individual heart rate maximum), and time-restricted eating (≤ 10 hours/day window of energy intake). The primary outcome measure is glucose tolerance in gestational week 28. Maternal and offspring outcomes are measured before and during pregnancy, at delivery, and at 6-8 weeks postpartum.

Conclusion: This study will determine the effect of a novel preconception lifestyle intervention on cardiometabolic health in women with a high risk of GDM and their infants.

Ethics and dissemination: The Regional Committees for Medical and Health Research Ethics in Norway has approved the study (REK 143756). The anonymized results will be submitted for publication and posted in a publicly accessible database of clinical study results.

Abstract word count: 248

Trial registration number: Clinical trial gov NCT04585581.

Keywords: insulin resistance, time-restricted eating, aerobic exercise, glycaemic control, diet

Strengths and limitations of this study

- We will include individuals at high risk of gestational diabetes to assess if exercise and time-restricted eating are feasible and effective strategies to improve glycaemic control, and thereby improve maternal metabolic health.
- We will also explore the effects of the intervention on the cardiac function of newborns.
- Due to the difficulty of blinding investigators and participants to behavioural interventions, investigators will not be blinded for outcome assessments.
- Due to the long duration of the intervention, adherence to lifestyle modifications may be difficult for some participants despite regular monitoring and motivational support.

Introduction

The global prevalence of gestational diabetes mellitus (GDM), i.e., high plasma glucose first identified during pregnancy, continues to increase. Both environmental and genetic factors contribute to the development of GDM, and up to 14 % of live births are negatively impacted by this condition.(1) GDM typically occurs as a result of pancreatic β-cell dysfunction with pre-existing insulin resistance and increases the risk for type 2 diabetes mellitus (T2DM) and cardiovascular disease in the mother.(2, 3) Maternal obesity and hyperglycaemia affect the offspring through the egg cell quality, intrauterine environment, and foetal organ development. These metabolic conditions eventually increase the risk for cardiac dysfunction at birth, and early onset diabetes, obesity, and cardiovascular diseases later in life.(4-9) Higher maternal blood glucose concentration, even below the diagnostic criteria for GDM, is associated with increased birth weight, elevated levels of cord-blood C-peptide, childhood obesity, and elevated blood pressure, independent of maternal BMI.(10-12) Besides the inheritable risk factors, epigenetic modifications in utero, low-grade inflammation, and modifications of the gut microbiome can also negatively affect the cardiometabolic health of the offspring.(4)

Lifestyle interventions, including dietary changes, increased physical activity, and self-monitoring of blood glucose, are the first-line choice for GDM management.(13) However, many pregnant individuals fail to adhere to the recommendations for diet and exercise training(14, 15) and there is inconclusive evidence for clinically meaningful effects of diet-exercise interventions on pregnancy outcomes for the mother or child.(5, 16-18) Several recent randomised controlled trials (RCTs) and reviews conclude that pre-pregnancy lifestyle interventions are urgently needed to improve maternal health and increase the likelihood of adherence to a healthy lifestyle during pregnancy.(19-23) Alternative diet-exercise strategies, such as time-restricted eating (TRE) and high-intensity interval training (HIIT), have shown promising results on improving metabolic health among reproductive-aged females.(24-26) TRE can reduce appetite and hunger levels,(27, 28) and HIIT leads to improved exercise enjoyment and adherence in pregnancy, as well as greater and longer-lasting improvements in insulin sensitivity and cardiorespiratory fitness compared with continuous moderate-intensity training.(29)

Pre-pregnancy patterns of physical activity and exercise are important determinants of exercise during pregnancy,(30) and pre-pregnancy inception of healthy dietary habits is associated with a lower risk of GDM.(31-33) So far, there is limited evidence on the effectiveness of implementing both dietary and exercise-based lifestyle interventions before

pregnancy. It is highly relevant to find feasible and effective pre-pregnancy lifestyle interventions which can reduce maternal hyperglycaemia and its related negative consequences for mother and child.

The primary hypothesis for the BEFORE THE BEGINNING (BTB) trial is that the participants allocated to the intervention group will have improved maternal glucose tolerance in gestational week 28. We will also determine the effect of the intervention on secondary cardiometabolic outcomes in both the mothers and the newborns.

Aims

The primary aim of BEFORE THE BEGINNING

- To determine the effect of a lifestyle intervention, commenced preconception and continuing throughout pregnancy, on maternal glucose tolerance in pregnancy.

Secondary aims of BEFORE THE BEGINNING

- To determine the effect of the intervention on insulin sensitivity, blood glucose, circulating lipids, body composition, cardiorespiratory fitness, systemic inflammation, and blood pressure in the mother.
- To evaluate the effect of the intervention on cardiac function, body composition, and systemic inflammation in the newborn.
- To evaluate the adherence to the interventions, and their effects on sleep quality, appetite and hunger, physical activity, and dietary intake.

Methods

Design and study setting

This is a single-centre RCT with two parallel groups: an intervention group and a control group. We obtain data and perform assessments of the participants and/or their infants at baseline (before randomisation), after 8 weeks, twice during pregnancy (in gestational week 12 and 28), at delivery, and after 6-8 weeks postpartum (Figure 1). The trial is undertaken at the Norwegian University of Science and Technology (NTNU), in Trondheim, Norway, in collaboration with the St. Olav's Hospital, Trondheim, Norway. SPIRIT reporting guidelines were used in reporting this study.(34)

Recruitment and participants

The trial is announced through social media, hospital and university webpages, local stores, and public places. Additionally, potential participants are identified through the National Population Register, and we regularly send out electronic invitations to females aged 20-35 years in Trondheim and the surrounding area to participate in the trial. The invitation prompts them to visit the study website, which contains a short description of the trial and allows potential participants to self-screen for eligibility before further screening by telephone.

Box 1 shows the inclusion and exclusion criteria for participation in the study.

Box 1: Inclusion and exclusion criteria

Inclusion criteria

- Female
- Age: 18-39 years old
- Contemplating pregnancy within the next six months
- Understands oral and written Norwegian or English
- At least one of the following criteria must apply:
  - Body mass index ≥ 25 < 40 kg/m^2,
  - Gestational diabetes in a previous pregnancy,
  - Close relative with diabetes (either parents, siblings, or children with diabetes),
  - Fasting plasma glucose > 5.3 mmol/L,
  - Previous newborn > 4.5 kg, or
  - Non-European ethnicity (with one or both parents originating from an area outside Europe).

Exclusion criteria

- On-going pregnancy
- Trying to conceive ≥ 6 cycles at study entry
- Known diabetes (type 1 or 2)
- Shift work that includes night shifts > 2 days per week
- Previous hyperemesis
- Known cardiovascular diseases
- High-intensity exercise > 2 times per week in the last 3 months
- Habitual eating window ≤ 12 hours
- Bariatric surgery
- Any other reason which according to the researchers makes the potential participant ineligible

Randomisation and allocation

After screening and assessments at baseline, the participants are randomly allocated (1:1) to the intervention or a standard care control group, after stratifying for GDM in a previous pregnancy (yes/no). We use a computer random number generator (WebCRF3) developed and

administered at The Clinical Research Unit (Klinforsk), NTNU/St. Olav's Hospital, Trondheim, Norway to randomly allocate participants using various block sizes.

Intervention

The intervention starts before pregnancy and continues throughout pregnancy and consists of a combination of TRE and exercise. Participants are counselled to change their daily time-window of energy intake to ≤ 10 hours, ending no later than 19:00 hours, for minimum 5 days per week throughout the intervention. The remaining 2 days are "days off" when they can consume food *ad libitum* if they wish. Apart from current recommendations about preconception/pregnancy nutrition, we give no advice regarding food choices, nor do we encourage a reduced total energy intake.

We use the Personal Activity Intelligence (PAI) score, a science-backed activity metric based on heart rate (HR)(35, 36) to prescribe exercise. Since PAI is HR-based, high-intensity exercise gives substantially higher PAI scores than low-to-moderate-intensity exercise. The goal for the participants in the intervention group is to earn and maintain ≥ 100 PAI per week, which can be reached by minimum 1 hour of weekly exercise at ≥ 80% of HR maximum. One week after the baseline visit, we invite the participants for a supervised introductory exercise session and provide a brochure with exercise options (e.g., treadmill walking/running, cycling). We invite the participants for a second session 2 weeks after the introductory session. The participants can choose their mode of exercise. Once pregnant, we advise the participants to either do short work-bouts at high intensity with low-to-moderate intensity periods in-between, or longer work periods at moderate intensity (up to 85% of heart rate maximum). We contact the participants not reaching 100 PAI to offer additional supervised exercise sessions, and they can also ask for extra support and supervised exercise sessions if they want to.

Participants in the control group receive standard care and are asked to continue with their habitual physical activity and dietary habits. We contact these participants once every 8 weeks to support adherence to registrations and monitoring.

Experimental procedures and outcome measures

The study period spans from baseline assessments in the pre-pregnancy period to 6-8 weeks after delivery (Figure 2). Participants who do not become pregnant within 6 months after inclusion in the trial (changed from 12 months from December 2022, see below under modifications to the protocol after trial commencement) are excluded. For participants who

experience spontaneous abortions, we add the number of weeks that the participant was pregnant plus 4 weeks to their time in the trial before exclusion pre-pregnancy.

The participants visit the lab for assessments twice during preconception (at baseline and after 8 weeks), twice during pregnancy (in gestational weeks 12 and 28), and 6 weeks after delivery. Additionally, we sample cord blood and placenta biopsies at delivery, collect standard neonatal outcomes from the hospital records, and estimate body composition and obtain echocardiography in the newborn within 72 hours after delivery and after 6-8 weeks (Figure 2). All participants receive a brochure from the Norwegian Health Directorate with the current recommendations for physical activity, diet, and folic acid, and iodine supplements. The participants are invited to ultrasound examinations in gestational week 12, 19, and 32.

Primary outcome measure

The primary outcome measure is plasma glucose concentration obtained 2 hours after a 75 g oral glucose tolerance test (OGTT) in gestational week 28. After an overnight fast (≥ 10 hours) and no exercise for ≥ 24 hours, the participants consume 75 g of glucose (Glucosepro, Finnamedical, Finland) diluted in 250 mL water within 5 minutes. Using an indwelling catheter, we collect venous blood before the OGTT, with subsequent collections at 30, 60, 90, and 120 minutes after ingestion of glucose.

Secondary outcome measures

Secondary maternal and neonatal outcome measures are outlined in Figure 2 and detailed below.

Blood sampling and biochemistry

From all visits, fasting blood lipids, plasma glucose, and HbA1c are measured immediately after sampling, at St. Olav's Hospital, following local standardised procedures. Additional fasting plasma, serum, full blood, and urine are stored in a biobank at -80°C for later analyses. GDM is recorded at Visit 3 and 4, according to the WHO 2013 criteria (fasting plasma glucose 5.1-6.9 mmol/L and/or 2 hour plasma glucose 8.5-11.0 mmol/L after 75 g OGTT).(37) At the event of a GDM diagnosis, the participant and their general practitioner are informed for further evaluation and management. Insulin sensitivity will be calculated using homeostasis model assessment of insulin resistance (HOMA-IR)(38) and pancreatic beta cell function using HOMA-β.(38) At visit 3 and 4, the area under the curve (AUC) and incremental AUC (iAUC) from glucose and insulin concentrations will be calculated from venous blood sampling every 30 minutes during the 2 hour OGTT. Insulin Sensitivity Index, $ISI_{0,120.,(39)}$ insulinogenic index

during the first 30 minutes of the 2 hour OGTT,(40) and beta cell function (AUC_{ins}/AUC_{glu}) will be estimated(41).

Continuous glucose monitoring

The participants wear a continuous glucose monitor (CGM, FreeStyle Libre 1, Abbott Diabetes Care, Norway) for 14 days at baseline (7 days pre-intervention followed by the first 7 days of intervention/control), and for 14 days at 8 weeks from baseline. From these measurements, we will determine 24-hour glycaemic control, 3-hour postprandial glucose levels (AUC) for the first meal of the day, and nocturnal glycaemic control. The CGMs are 'masked' for the participants to avoid lifestyle changes based on their glucose levels. We also plan to explore other CGM data that can predict glycaemic control during pregnancy, using machine learning.

Height, weight, body composition, BMI, and waist circumference

Height is measured with the participants standing without shoes using a standard stadiometer. Weight and body composition are estimated in the morning after overnight fasting using bioelectrical impedance analysis (Inbody 720, Biospace CO, Korea), with participants wearing light clothes and standing barefoot. BMI is calculated as weight in kilograms divided by the square value of height in metres (kg/m^2). To account for the increase in fat-free mass hydration as pregnancy progresses, we will use a regression equation that estimates fat-free mass density as a function of gestational age.(42) Waist circumference is measured using a measuring tape at the level of the belly button with the participant standing.

Cardiorespiratory fitness

We measure peak oxygen uptake (VO_{2peak}) using indirect calorimetry (Metalyzer II, Cortex, Germany), using an individualised treadmill protocol in which the participants walk or run until volitional exhaustion. The test starts after a 10-minute warm-up. The speed or inclination is increased every 1 – 2 minutes, by 0.5 – 1.0 km/hour or 1% – 2%. VO_{2peak} is determined as the average of the three highest consecutive 10 seconds measured and will be reported as both absolute (L/min) and relative (mL/min/kg) values. We record HR throughout the exercise tests and use the peak HR recorded during the test as an estimate of the HR maximum.(43)

Blood pressure and resting heart rate

We use an automatic blood pressure device (Welch Allyn, Germany) to measure blood pressure (diastolic and systolic, in mmHg) and resting HR (beats per minute, bpm) on the participants'

left arm after they have rested in a seated position for 15 minutes. We will report the average of three measurements taken at 1-minute intervals.

Physical activity, diet, and sleep

We use activity monitors to estimate physical activity levels, energy expenditure, and sleep duration. All participants wear Sensewear Armbands (BodyMedia, Pennsylvania, USA) for 14 days at baseline (7 days pre-intervention followed by the first 7 days of intervention/control), and the participants in the intervention group wear Amazfit GTS (Huami, China) smartwatches throughout the intervention. The smartwatch is connected to the Zepp app and shares PAI data with the research team via the Memento app. Participants register their diet in an online food diary (Fatsecret app) and record the time of first and last energy intake in the project handbook for 4 days (3 weekdays and 1 weekend day) every 8 weeks. They also complete questionnaires about physical activity, sleep quality, and psychological well-being every 8 weeks throughout the study period. We use the following questionnaires: 1) International Physical Activity Questionnaire,(44) 2) Pittsburgh Sleep Quality Index,(45) and 3) Psychological General Well-Being Index.(46) At baseline, the participants fill in the Horne-Östberg Morningsness-Eveningness Questionnaire.(47) We record medication and supplements, early miscarriages, abortions, and time to pregnancy and live birth. Additionally, expectant fathers are asked to complete questionnaires at baseline and every 8 weeks throughout the trial, including questions regarding their body weight, height, physical activity, and diet. These data will be used as co-variates in later analyses.

Neonatal and other outcomes

We obtain standard clinical neonatal outcomes from hospital birth records. Midwives at St. Olav's Hospital collect umbilical cord blood immediately after birth, prior to the delivery of the placenta. Placental tissues are collected from 1) around the base of the umbilical cord on the foetal side, 2) the periphery on the maternal side (full-thickness tissue) 3) the centre of the maternal side, also for storage in RNAlater solution (Invitrogen, Thermofisher scientific, Lithuania) and 4% formaldehyde solution, and 4) the periphery on the maternal side. The samples are put in 1.8 mL cryotubes and snap-frozen immediately in liquid nitrogen, before storage at -80°C for later analyses. The samples in RNAlater solution are stored at 4°C overnight, followed by storage at -80°C for later analyses. The samples in 4% formaldehyde solution are stored under a fume hood at room temperature for 48 hours before histology slide preparation in collaboration with the CMIC Histology Lab at NTNU.

Body composition of the newborn is estimated using bioimpedance (BioScan touch i8-nano, Maltron, UK) within 72 hours of birth, and after 6-8 weeks. An experienced paediatric cardiologist examines cardiac morphology, structure, and function in the newborn, using a Vivid E95 scanner (GE Vingmed Ultrasound, Horten, Norway) and a GE 6s, and M5s phased-array transducers (GE Healthcare, Milwaukee, WI) within 72 hours of birth, and at 6-8 weeks age. A full clinical echocardiography including conventional echocardiographic parameters as well as study images with a focus on measurement of systolic and diastolic myocardial function is performed. The scanner is equipped with research software enabling high frame rate echocardiography to study cardiac flow and tissue properties as described previously.(48-50) A corresponding group of neonates (N = 30), from mothers with no known increased risk of GDM and BMI in the normal range (18.6-24.9 kg/m^2) will be used for comparison.

Adherence

We record adherence to TRE as the average daily time window for energy intake and the number of days per week that participants adhere to a ≤ 10-hour time window for energy intake. Adherence to exercise is recorded as the number of PAI points the participants get per rolling 7 days. To ensure compliance and maintain adherence, we send text messages to all participants as reminders to complete questionnaires and dietary reporting.

Modifications to the protocol after trial commencement

Since June 2021, we invite the participants to participate in a follow-up study after delivery in which we collect infant faecal samples (immediately after birth, at 6 weeks, and 6 months), maternal faecal samples (at 6 weeks and 6 months), and breast milk (at 6 weeks and 6 months). These samples are stored at -80°C for later analyses. Additionally, we started to offer supervised exercise training sessions to the participants in the intervention group. From November 2022, we started sending invitations using eFORSK (electronic form-based data collection, developed by Central Norway Regional Health Authority) and added 'Bariatric surgery' to the exclusion criteria. 'Any other reason which according to the researchers makes the potential participant ineligible' to undergo either or both interventions (e.g., traumatic foot injury, anorexia/bulimia, etc.) was also added to the exclusion criteria in November 2022. From December 2022, we removed 'Planned assisted fertilisation with female factor reason' from the exclusion criteria. In addition, we changed the maximum time before pregnancy from 12 months to 6 months to allow for the trial to be terminated in time for us to analyse the data

within the project period. In March 2023, the required number of total participants was reduced from 260 to 200 based on the revised calculation as described in the sample size calculation.

Sample size calculation

The primary outcome of this study is glucose tolerance (after a 2-hour OGTT) in gestational week 28. We consider a difference between the intervention group and the control group of 1.0 mmol/L as the minimally clinically relevant difference, based on findings from the HAPO study,(51) and have used the observed standard deviation (SD) in 2-hr plasma glucose from HAPO for the calculations. Calculation of the sample size for a two-sided t-test to detect a difference between the groups, using an SD of 1.3, a power of 0.90, and a significance level of 0.05, yields 37 participants in each group in gestational week 28. To allow for an expected exclusion from the study due to not conceiving within the study period (~50%)(52) yielding 74 per group, further drop-out during the study period (10-20%), yielding 93 per group, and to increase statistical power for secondary analyses, we initially wanted to include 260 participants in the trial. However, we will terminate the inclusion of new participants when we have reached minimum 45 participants in gestational week 12 in each group (to allow for 20% dropout during pregnancy), or at least 200 participants in total (accounting for 20% dropout before pregnancy and the observed 58.5% pregnancy rate in the ongoing trial).

Statistical analyses

The primary analysis will be done according to the 'intention to treat' principle, using all obtained data irrespective of participant adherence to the intervention and completeness of outcome measures. We plan to use linear mixed models (LMMs) to compare primary and secondary continuous outcome measures between groups, with time and group x time interactions as fixed effects variables, and subject as random factor.(53) Since no systematic baseline differences between the groups are expected in RCTs, means at baseline will be constrained to be equal in the LMMs. We will report estimates with corresponding 95% confidence intervals and p-values for differences between the intervention group and the control group. We will check the normality of residuals by visual inspection of QQ-plots and perform bootstrapping in cases of non-normal model residuals. For our primary outcome measure, we will consider a p-value < 0.05 as statistically significant. For the secondary outcome measures, p-values < 0.01 will be considered statistically significant, due to multiple comparisons, and these analyses will be explorative. We will also perform per-protocol analyses: Participants who during the preconception period accumulated an average of ≥ 75

PAI and adhered to TRE ≥ 4 days per week, will be included in the per-protocol analyses for all outcome measures. We will report additional results from all participants who were included in the trial, from the preconception period, irrespective of whether they became pregnant or not during the study period.

Blinding

The study is not blinded as it is difficult to blind participants and treatment providers to behavioural intervention. However, baseline assessments are undertaken before randomisation.

Monitoring

We do not expect any adverse effects in this study. The investigators are responsible for the documentation of any adverse or serious adverse events in the Case Report Form and the Serious Adverse Events Report Form, respectively. Participants are advised to contact the investigators if they have any unusual symptoms. All serious adverse events will be reported to the sponsor (NTNU) within 24 hours after the investigators have been informed of the event.

Patient and public involvement

We have involved users in the planning of the study and will continue involving them in the implementation and dissemination. In the planning phase, we arranged a 1-hour digital workshop with users (reproductive-aged females with overweight/obesity), in which we encouraged the audience to ask questions and give us feedback about relevant topics or issues related to research participation. Regarding the challenges of long-term adherence to lifestyle changes, we use the feedback from the user to find ways to incorporate exercise training and TRE into daily life.

Ethics and dissemination

The study is approved by the Regional Committees for Medical and Health Research Ethics in Norway (REK, reference number 143756). The comparative analysis of the neonatal echocardiography data from this study with a corresponding group of neonates from mothers with normal BMI and no increased risk of GDM is also approved (REK reference number 67584). The work is conducted according to the Declaration of Helsinki and the ICMJE Recommendations for authorship. The participants sign an informed written consent before participating in the study and can at any time withdraw from the study without further explanation. Collected data are treated following the General Data Protection Regulation. We report all protocol modifications to REK. Study-specific ID numbers are used as identifiers for

participants while entering data into an electronic case report form. Data quality is ensured by double data entry. Upon completion of the study and finalization of the study report, we will submit the results for publication and/or in a publicly accessible database of clinical study results after anonymizing the data.

Discussion

To our knowledge, the BTB study will be the first RCT to investigate the combined effects of TRE and exercise training, initiated before and continued throughout pregnancy, on cardiometabolic parameters in people at risk of GDM and their infants. We hypothesise that the combination of these two lifestyle interventions will induce an additive and clinically relevant improvement in maternal glucose tolerance, and potentially also in our secondary outcome measures in mothers and infants. As such, the initiation of lifestyle modification before pregnancy will provide a better platform for improved adherence and health outcomes, potentially breaking the intergenerational cycle of cardiometabolic disorders, and thereby reducing the risk of diabetes for future generations.

So far, there is limited data on the combination of TRE and exercise training in humans. Haganes and colleagues reported that the combination of TRE and HIIT in females with a BMI of ≥ 27 kg/m^2 for 7 weeks significantly reduced HbA1c compared with a no-intervention control group and lead to greater losses in body weight, fat mass, and visceral fat area compared with either intervention alone.(26) Since the duration of the intervention is much longer in the BTB trial, there may be lower adherence to one or both intervention strategies. The possible reasons for lower adherence are that the participants may lack motivation for such a long time, find the intervention program boring and/or difficult, or develop physical symptoms that may hinder the participant to adhere to the intervention (especially during pregnancy). Combining motivational human interaction with digital interventions can increase engagement and the effectiveness of behaviour change interventions.(54) To improve adherence throughout the study period, we offer an individualised exercise regimen and provide encouragement, support, and monitor the participants regularly, both in person and over the phone.

The incidence and risk of obesity, insulin resistance, and GDM persists through generations.(55) To disrupt this intergenerational cycle, it is urgently necessary to develop and implement effective and practical lifestyle intervention strategies which can improve the cardiometabolic health outcomes of both mother and offspring. If the preconception lifestyle interventions implemented in this study lead to favourable outcomes and prove to be feasible

and effective, it can pave the way for novel interventions that can be adopted in clinical practice during the preconception period, especially among those who are at risk of developing GDM.

Author contributions

MAJS drafted the manuscript. TM, SAN, KÅS, ACI, TF, and SLF conceived and contributed to the design of the study and the plan for analyses. GR, MAJS, and HSS coordinate the study, perform measurements on test days, monitor participants, and supervise the exercise training. SAN performs the echocardiogram on the newborns. All authors provided feedback and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available at: https://www.diabetesatlas.org
2. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. Int J Mol Sci. 2018;19(11):3342.
3. Yu Y, Soohoo M, Sørensen HT, Li J, Arah OA. Gestational Diabetes Mellitus and the Risks of Overall and Type-Specific Cardiovascular Diseases: A Population- and Sibling-Matched Cohort Study. Diabetes Care. 2021;45(1):151-9.
4. Moholdt T, Hawley JA. Maternal Lifestyle Interventions: Targeting Preconception Health. Trends Endocrinol Metab. 2020;31(8):561-9.
5. Vinter CA, Tanvig MH, Christensen MH, Ovesen PG, Jørgensen JS, Andersen MS, et al. Lifestyle Intervention in Danish Obese Pregnant Women With Early Gestational Diabetes Mellitus According to WHO 2013 Criteria Does Not Change Pregnancy Outcomes: Results From the LiP (Lifestyle in Pregnancy) Study. Diabetes Care. 2018;41(10):2079-85.
6. Nyrnes SA, Garnæs KK, Salvesen Ø, Timilsina AS, Moholdt T, Ingul CB. Cardiac function in newborns of obese women and the effect of exercise during pregnancy. A randomized controlled trial. PLoS One. 2018;13(6):e0197334.
7. Gluckman PD, Hanson MA, Buklijas T. A conceptual framework for the developmental origins of health and disease. J Dev Orig Health Dis. 2010;1(1):6-18.
8. Cai G-j, Sun X-x, Zhang L, Hong Q. Association between maternal body mass index and congenital heart defects in offspring: a systematic review. American Journal of Obstetrics and Gynecology. 2014;211(2):91-117.
9. Petropoulos A. Congenital heart disease and maternal diabetes mellitus. Евразийский Журнал Клинических Наук. 2018;1(3):32-9.
10. Lowe WL, Lowe LP, Kuang A, Catalano PM, Nodzenski M, Talbot O, et al. Maternal glucose levels during pregnancy and childhood adiposity in the Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study. Diabetologia. 2019;62(4):598-610.
11. Tam WH, Ma RCW, Ozaki R, Li AM, Chan MHM, Yuen LY, et al. In Utero Exposure to Maternal Hyperglycemia Increases Childhood Cardiometabolic Risk in Offspring. Diabetes Care. 2017;40(5):679-86.
12. Grunnet LG, Hansen S, Hjort L, Madsen CM, Kampmann FB, Thuesen ACB, et al. Adiposity, Dysmetabolic Traits, and Earlier Onset of Female Puberty in Adolescent Offspring of Women With Gestational Diabetes Mellitus: A Clinical Study Within the Danish National Birth Cohort. Diabetes Care. 2017;40(12):1746-55.
13. Management of Diabetes in Pregnancy. Diabetes Care. 2017;40(Suppl 1):S114-s9.
14. Gjestland K, Bø K, Owe KM, Eberhard-Gran M. Do pregnant women follow exercise guidelines? Prevalence data among 3482 women, and prediction of low-back pain, pelvic girdle pain and depression. Br J Sports Med. 2013;47(8):515-20.
15. Zeanah M, Schlosser SP. Adherence to ACOG Guidelines on Exercise During Pregnancy: Effect on Pregnancy Outcome. Journal of Obstetric, Gynecologic & Neonatal Nursing. 1993;22(4):329-35.
16. Tieu J, Shepherd E, Middleton P, Crowther CA. Dietary advice interventions in pregnancy for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2017;1(1):Cd006674.
17. 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(8):Cd012327.
18. Shepherd E, Gomersall JC, Tieu J, Han S, Crowther CA, Middleton P. Combined diet and exercise interventions for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2017;11(11):Cd010443.
19. Lane M, Robker RL, Robertson SA. Parenting from before conception. Science. 2014;345(6198):756-60.

20. Stephenson J, Heslehurst N, Hall J, Schoenaker D, Hutchinson J, Cade JE, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. Lancet. 2018;391(10132):1830-41.
21. Rönö K, Stach-Lempinen B, Eriksson JG, Pöyhönen-Alho M, Klemetti MM, Roine RP, et al. Prevention of gestational diabetes with a prepregnancy lifestyle intervention - findings from a randomized controlled trial. Int J Womens Health. 2018;10:493-501.
22. Raab R, Michel S, Günther J, Hoffmann J, Stecher L, Hauner H. Associations between lifestyle interventions during pregnancy and childhood weight and growth: a systematic review and meta-analysis. Int J Behav Nutr Phys Act. 2021;18(1):8-.
23. Sparks JR, Ghildayal N, Hivert M-F, Redman LM. Lifestyle interventions in pregnancy targeting GDM prevention: looking ahead to precision medicine. Diabetologia. 2022;65(11):1814-24.
24. Szumilewicz A S-RR, Worska A, Piernicka M, Yu H, Pajaujiene S, et al. How to HIIT while pregnant? The protocol characteristics and effects of high intensity interval training implemented during pregnancy – A systematic review. Baltic Journal of Health and Physical Activity. 2022;14(1):1-16.
25. Flanagan EW, Kebbe M, Sparks JR, Redman LM. Assessment of Eating Behaviors and Perceptions of Time-Restricted Eating During Pregnancy. J Nutr. 2022;152(2):475-83.
26. Haganes KL, Silva CP, Eyjólfsdóttir SK, Steen S, Grindberg M, Lydersen S, et al. Time-restricted eating and exercise training improve HbA1c and body composition in women with overweight/obesity: A randomized controlled trial. Cell Metab. 2022;34(10):1457-71.e4.
27. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early Time-Restricted Feeding Reduces Appetite and Increases Fat Oxidation But Does Not Affect Energy Expenditure in Humans. Obesity (Silver Spring). 2019;27(8):1244-54.
28. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metab. 2018;27(6):1212-21.e3.
29. Wowdzia JB, Hazell TJ, Davenport MH. Glycemic response to acute high-intensity interval versus moderate-intensity continuous exercise during pregnancy. Physiol Rep. 2022;10(18):e15454.
30. Gaston A, Cramp A. Exercise during pregnancy: a review of patterns and determinants. J Sci Med Sport. 2011;14(4):299-305.
31. Tobias DK, Zhang C, Chavarro J, Bowers K, Rich-Edwards J, Rosner B, et al. Prepregnancy adherence to dietary patterns and lower risk of gestational diabetes mellitus. The American Journal of Clinical Nutrition. 2012;96(2):289-95.
32. Gao X, Zheng Q, Jiang X, Chen X, Liao Y, Pan Y. The effect of diet quality on the risk of developing gestational diabetes mellitus: A systematic review and meta-analysis. Frontiers in Public Health. 2023;10.
33. Yong HY, Mohd Shariff Z, Mohd Yusof BN, Rejali Z, Appannah G, Bindels J, et al. The association between dietary patterns before and in early pregnancy and the risk of gestational diabetes mellitus (GDM): Data from the Malaysian SECOST cohort. PLoS One. 2020;15(1):e0227246.
34. Chan AW, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. Bmj. 2013;346:e7586.
35. Nes BM, Gutvik CR, Lavie CJ, Nauman J, Wisløff U. Personalized Activity Intelligence (PAI) for Prevention of Cardiovascular Disease and Promotion of Physical Activity. Am J Med. 2017;130(3):328-36.
36. Zisko N, Skjerve KN, Tari AR, Sandbakk SB, Wisløff U, Nes BM, et al. Personal Activity Intelligence (PAI), Sedentary Behavior and Cardiovascular Risk Factor Clustering - the HUNT Study. Prog Cardiovasc Dis. 2017;60(1):89-95.
37. World Health O. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva: World Health Organization; 2013 2013. Contract No.: WHO/NMH/MND/13.2.

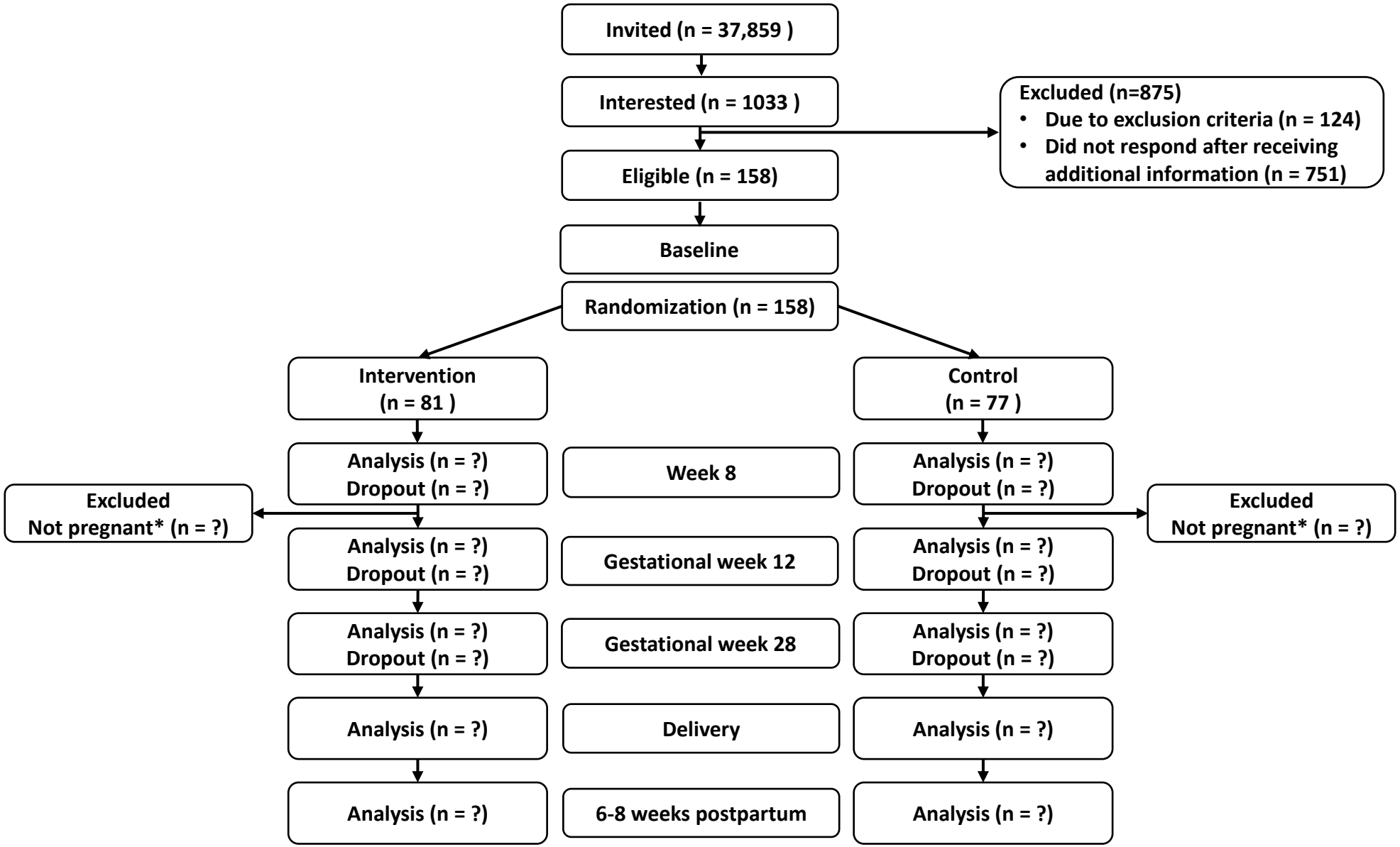
38. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
39. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract. 2000;47(3):177-84.
40. Phillips DI, Clark PM, Hales CN, Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med. 1994;11(3):286-92.
41. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295-301.
42. Most J, Marlatt KL, Altazan AD, Redman LM. Advances in assessing body composition during pregnancy. Eur J Clin Nutr. 2018;72(5):645-56.
43. Berglund IJ, Sørås SE, Relling BE, Lundgren KM, Kiel IA, Moholdt T. The relationship between maximum heart rate in a cardiorespiratory fitness test and in a maximum heart rate test. J Sci Med Sport. 2019;22(5):607-10.
44. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.
45. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
46. Grossi E, Compare A. Psychological General Well-Being Index (PGWB). In: Michalos AC, editor. Encyclopedia of Quality of Life and Well-Being Research. Dordrecht: Springer Netherlands; 2014. p. 5152-6.
47. Adan A, Almirall H. Horne & Östberg Morningness–Eveningness questionnaire: A reduced scale. Personality and Individual Differences. 1991;12:241-53.
48. Nyrnes SA, Fadnes S, Wigen MS, Mertens L, Lovstakken L. Blood Speckle-Tracking Based on High-Frame Rate Ultrasound Imaging in Pediatric Cardiology. J Am Soc Echocardiogr. 2020;33(4):493-503.e5.
49. Sørensen K, Fadnes S, Mertens L, Henry M, Segers P, Løvstakken L, et al. Assessment of Early Diastolic Intraventricular Pressure Difference in Children by Blood Speckle-Tracking Echocardiography. J Am Soc Echocardiogr. 2023.
50. Salles S, Lovstakken L, Aase SA, Bjastad TG, Torp H. Clutter Filter Wave Imaging. IEEE Trans Ultrason Ferroelectr Freq Control. 2019;66(9):1444-52.
51. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991-2002.
52. Mikkelsen EM, Riis AH, Wise LA, Hatch EE, Rothman KJ, Sørensen HT. Pre-gravid oral contraceptive use and time to pregnancy: a Danish prospective cohort study. Hum Reprod. 2013;28(5):1398-405.
53. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in randomised controlled trials. Contemp Clin Trials Commun. 2018;10:80-5.
54. Barker M, Dombrowski SU, Colbourn T, Fall CHD, Kriznik NM, Lawrence WT, et al. Intervention strategies to improve nutrition and health behaviours before conception. Lancet (London, England). 2018;391(10132):1853-64.
55. Ma RCW, Popkin BM. Intergenerational diabetes and obesity—A cycle to break? PLOS Medicine. 2017;14(10):e1002415.

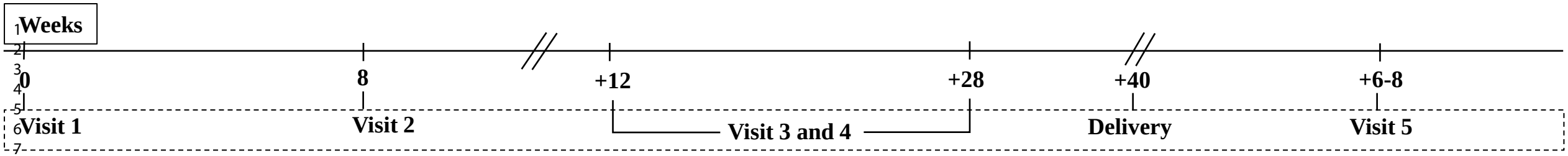
FIGURE LEGENDS

Figure 1. Consort flow diagram of the BEFORE THE BEGINNING trial (per 03.03.2023)

* If the participants are not pregnant within 12 months of inclusion, they are excluded from the study. From December 2022, the time-window for exclusion if not pregnant was reduced from 12 to 6 months.

Figure 2. Overview of time-points for assessments in the trial.





Every 8 weeks following visit 1: Food diary (4-day registration), hunger/appetite registration, time-window of energy intake, questionnaires (physical activity, sleep, psychological well-being).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not	2

		yet registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	n/a, see appendix
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 15
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	n/a, see appendix
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
Roles and responsibilities:	#5d	Composition, roles, and responsibilities of the coordinating centre, steering	n/a

committees		committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 (Box 1)
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a, see appendix
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions:	#11d	Relevant concomitant care and	7

concomitant care		interventions that are permitted or prohibited during the trial	
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5 (Figure 1) 7 (Figure 2)
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6-7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care	11

		providers, outcome assessors, data analysts), and how	
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a: no blinding
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate	11

		from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12-13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12-13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12-13
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of	n/a, see appendix

		whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a, see appendix
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board	13

		(REC / IRB) approval	
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	11-12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13-14
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the	13-14

		final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13-14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	13-14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13-14
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See Appendix
Biological specimens	#33	Plans for collection, laboratory	7-11

evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

BMJ Open

A randomised controlled trial of preconception lifestyle intervention on maternal and offspring health in people with increased risk of gestational diabetes: study protocol for the BEFORE THE BEGINNING trial

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A randomised controlled trial of preconception lifestyle intervention on maternal and offspring health in people with increased risk of gestational diabetes: study protocol for the BEFORE THE BEGINNING trial

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Word count: 4199

Abstract

Introduction: Gestational diabetes mellitus (GDM) is associated with increased risk for type 2 diabetes in the mother and cardiometabolic diseases in the child. The preconception period is an optimal window to adapt the lifestyle for improved outcomes for both mother and child. Our aim is to determine the effect of a lifestyle intervention, initiated before and continued throughout pregnancy, on maternal glucose tolerance and other maternal and infant cardiometabolic outcomes.

Methods and analysis: This ongoing randomised controlled trial has included 167 females aged 18-39 years old at increased risk for GDM who are contemplating pregnancy. The participants were randomly allocated 1:1 to an intervention or control group. The intervention consists of exercise (volume is set by a heart rate-based app and corresponds to ≥ 1 hour of weekly exercise at ≥ 80% of individual heart rate maximum), and time-restricted eating (≤ 10 hours/day window of energy intake). The primary outcome measure is glucose tolerance in gestational week 28. Maternal and offspring outcomes are measured before and during pregnancy, at delivery, and at 6-8 weeks postpartum. Primary and secondary continuous outcome measures will be compared between groups based on the "intention to treat" principle using linear mixed models.

Ethics and dissemination: The Regional Committees for Medical and Health Research Ethics in Norway has approved the study (REK 143756). The anonymised results will be submitted for publication and posted in a publicly accessible database of clinical study results.

Abstract word count: 236

Trial registration number: Clinical trial gov NCT04585581.

Keywords: insulin resistance, time-restricted eating, aerobic exercise, glycaemic control, diet

Strengths and limitations of this study

- The intervention starts before and continues throughout pregnancy to make it easier for the participants to adopt an active lifestyle before pregnancy.
- This study includes individuals at high risk of GDM from multiple ethnic backgrounds, which improves the generalisability of the findings.
- The effects of the intervention on the cardiac function and body composition of the offspring will be comprehensively evaluated.
- Due to the difficulty of blinding investigators and participants to behavioural interventions, investigators will not be blinded for outcome assessments.
- Due to the long duration of the intervention, adherence to lifestyle modifications may be difficult for some participants despite regular monitoring and motivational support.

Introduction

The global prevalence of gestational diabetes mellitus (GDM), i.e., high plasma glucose first identified during pregnancy, continues to increase. Both environmental and genetic factors contribute to the development of GDM, and up to 14 % of live births are negatively impacted by this condition.(1) GDM typically occurs because of pancreatic β-cell dysfunction with pre-existing insulin resistance and increases the risk for type 2 diabetes and cardiovascular disease in the mother.(2, 3) Maternal obesity and hyperglycaemia affect the offspring through the egg cell quality, intrauterine environment, and foetal organ development. These metabolic conditions eventually increase the risk for cardiac dysfunction at birth, and early onset diabetes, obesity, and cardiovascular diseases later in life.(4-9) Higher maternal blood glucose concentration, even below the diagnostic criteria for GDM, is associated with increased birth weight, elevated levels of cord-blood C-peptide, childhood obesity, and elevated blood pressure, independent of maternal body mass index (BMI).(10-12) Besides the inheritable risk factors, epigenetic modifications in utero, low-grade inflammation, and modifications of the gut microbiome can also negatively affect the cardiometabolic health of the offspring.(4)

Lifestyle interventions, including dietary changes, increased physical activity, and self-monitoring of blood glucose, are the first-line choice for GDM management.(13) However, many pregnant individuals fail to adhere to the recommendations for diet and exercise training(14, 15) and there is inconclusive evidence for clinically meaningful effects of diet-exercise interventions on pregnancy outcomes for the mother or child.(5, 16-18) Several recent randomised controlled trials (RCTs) and reviews conclude that pre-pregnancy lifestyle interventions are urgently needed to improve maternal health and increase the likelihood of adherence to a healthy lifestyle during pregnancy.(19-23) Alternative diet-exercise strategies, such as time-restricted eating (TRE) and high-intensity interval training (HIIT), have shown promising results on improving metabolic health among reproductive-aged females.(24-26) TRE is a safe and feasible intervention in individuals with overweight, obesity, prediabetes, and type 2 diabetes.(27) It has been shown to improve glucose tolerance, and insulin sensitivity, and reduce appetite, hunger, HbA1c, and total body and fat mass in this population.(28-36) While data on the effects of TRE in pregnancy are scarce, observational data suggest that longer maternal night-fasting intervals are associated with decreased fasting glucose.(37) The safety of HIIT is not yet established during pregnancy, but recent publications indicate that HIIT is safe, with higher enjoyment and improved adherence than continuous moderate-intensity

training,(38) and may provide cardiometabolic benefits for both mothers and their offspring.(39-41)

Pre-pregnancy patterns of physical activity and exercise are important determinants of exercise during pregnancy,(42) and pre-pregnancy inception of healthy dietary habits is associated with a lower risk of GDM.(43-45) So far, there is limited evidence on the effectiveness of implementing both dietary and exercise-based lifestyle interventions before pregnancy. It is highly relevant to find feasible and effective pre-pregnancy lifestyle interventions which can reduce maternal hyperglycaemia and its related negative consequences for mother and child.

The primary hypothesis for the BEFORE THE BEGINNING (BTB) trial is that the participants allocated to the intervention group (time-restricted eating and exercise) will have improved maternal glucose tolerance in gestational week 28, compared with participants in the control group. We will also determine the effect of the intervention on secondary cardiometabolic outcomes in both the mothers and their newborns.

Aims

The primary aim of BEFORE THE BEGINNING

- To determine the effect of a lifestyle intervention, commenced preconception and continuing throughout pregnancy, on maternal glucose tolerance in pregnancy.

Secondary aims of BEFORE THE BEGINNING

- To evaluate the effect of the intervention on insulin sensitivity, blood glucose, circulating lipids, body composition, cardiorespiratory fitness, systemic inflammation, and blood pressure in the mothers.
- To evaluate the effect of the intervention on cardiac function, body composition, and systemic inflammation in the newborns.
- To evaluate the adherence to the interventions, and their effects on sleep quality, appetite and hunger, physical activity, and dietary intake.

Methods

Design and study setting

This is an ongoing single-centre RCT with two parallel groups: an intervention group and a control group, undertaken at the Norwegian University of Science and Technology (NTNU) in

Trondheim, Norway, in collaboration with the St. Olav's Hospital, Trondheim, Norway. SPIRIT reporting guidelines were used in reporting this study protocol.(46)

Recruitment and participants

The trial was announced through social media, hospital and university webpages, local stores, and public places. Additionally, potential participants were identified through the National Population Register, and we regularly sent out electronic invitations to females aged 20-35 years in Trondheim and the surrounding area to participate in the trial. The invitation prompted them to visit the study website, which contains a short description of the trial and allows potential participants to self-screen for eligibility before further screening by telephone. The first participant was included on 25th September 2020 and the last participant was included on 28th April 2023.

Box 1 shows the inclusion and exclusion criteria for participation in the study.

Box 1: Inclusion and exclusion criteria

Inclusion criteria

- Female
- Age: 18-39 years old
- Contemplating pregnancy within the next six months
- Understands oral and written Norwegian or English
- At least one of the following criteria must apply:
  - Body mass index ≥ 25 < 40 kg/m^2,
  - Gestational diabetes in a previous pregnancy,
  - Close relative with diabetes (either parents, siblings, or children with diabetes),
  - Fasting plasma glucose > 5.3 mmol/L,
  - Previous newborn > 4.5 kg, or
  - Non-European ethnicity (with one or both parents originating from an area outside Europe).

Exclusion criteria

- On-going pregnancy
- Trying to conceive ≥ 6 cycles at study entry
- Known diabetes (type 1 or 2)
- Shift work that includes night shifts > 2 days per week
- Previous hyperemesis
- Known cardiovascular diseases
- High-intensity exercise > 2 times per week in the last 3 months
- Habitual eating window ≤ 12 hours
- Bariatric surgery
- Any other reason which according to the researchers makes the potential participant ineligible

Randomisation and allocation

After screening and assessments at baseline, the participants were randomly allocated (1:1) to the intervention or a standard care control group, after stratifying for GDM in a previous pregnancy (yes/no). At the first visit, the study procedures, equipment, and applications were set up and explained to the participants.

We used a computer random number generator (WebCRF3) developed and administered at The Clinical Research Unit (Klinforsk), NTNU/St. Olav's Hospital, Trondheim, Norway to randomly allocate participants using various block sizes.

Intervention

The intervention starts before pregnancy and continues throughout pregnancy and consists of a combination of TRE and exercise. Participants are counselled to change their daily time-window of energy intake to ≤ 10 hours, ending no later than 19:00 hours, for minimum 5 days per week throughout the intervention. The remaining 2 days are "days off" when they can consume food *ad libitum* if they wish. Apart from current recommendations about preconception/pregnancy nutrition, we give no advice regarding food choices, nor do we encourage a reduced total energy intake.

We use the Personal Activity Intelligence (PAI) score, a science-backed activity metric based on heart rate (HR)(47, 48) to prescribe exercise. Since PAI is HR-based, high-intensity exercise gives substantially higher PAI scores than low-to-moderate-intensity exercise. The goal for the participants in the intervention group is to earn and maintain ≥ 100 PAI per week, which can be reached by minimum 1 hour of weekly exercise at ≥ 80% of HR maximum. One week after the baseline visit, we invite the participants for a supervised introductory exercise session and provide a brochure with exercise options (e.g., treadmill walking/running, cycling). We invite the participants for a second session 2 weeks after the introductory session. The participants can choose their mode of exercise. Once pregnant, we advise the participants to either do short work-bouts at high intensity with low-to-moderate intensity periods in-between, or longer work periods up to 85% of heart rate maximum. We contact the participants not reaching 100 PAI to offer additional supervised exercise sessions, and they can also ask for extra support and supervised exercise sessions if they want to.

Participants in the control group receive standard care and are asked to continue with their habitual physical activity and dietary habits. We contact these participants once every 8 weeks to support adherence to registrations and monitoring.

Experimental procedures and outcome measures

The study period spans from baseline assessments in the pre-pregnancy period to 6-8 weeks after delivery (Figure 1). Participants who do not become pregnant within 6 months after inclusion in the trial (changed from 12 months from December 2022, see below under modifications to the protocol after trial commencement) are excluded. For participants who experience spontaneous abortions, we add the number of weeks that the participant was pregnant plus 4 weeks to their time in the trial before exclusion pre-pregnancy.

Assessments of the participants are performed twice during preconception (at baseline before randomisation, and after 8 weeks), and twice during pregnancy (in gestational weeks 12 and 28). Outcomes in the newborns are assessed within 72 hours after delivery and at age 6-8 weeks (Figure 2). All participants receive a brochure from the Norwegian Health Directorate with the current recommendations for physical activity, diet, and folic acid, and iodine supplements. The participants are invited to ultrasound examinations in gestational weeks 12, 19, and 32.

Primary outcome measure

The primary outcome measure is plasma glucose concentration obtained 2 hours after a 75 g oral glucose tolerance test (OGTT) in gestational week 28. After an overnight fast (≥ 10 hours) and no exercise for ≥ 24 hours, the participants consume 75 g of glucose (Glucosepro, Finnamedical, Finland) diluted in 250 mL water within 5 minutes. Using an indwelling catheter, we collect venous blood before the OGTT, with subsequent collections at 30, 60, 90, and 120 minutes after ingestion of glucose.

Secondary outcome measures

Secondary maternal and neonatal outcome measures (Figure 2) are described below.

Blood sampling and biochemistry

From all visits, fasting blood lipids, plasma glucose, and HbA1c are measured immediately after sampling, at St. Olav's Hospital, following local standardised procedures. Additional fasting plasma, serum, full blood, and urine are stored in a biobank at -80°C for later analyses. GDM is recorded at Visit 3 and 4, according to the WHO 2013 criteria (fasting plasma glucose 5.1-6.9 mmol/L and/or 2-hour plasma glucose 8.5-11.0 mmol/L after 75 g OGTT).(49) At the event of a GDM diagnosis, the participant and their general practitioner are informed for further evaluation and management. Insulin sensitivity will be calculated using homeostasis model assessment of insulin resistance (HOMA-IR)(50) and pancreatic beta cell function using

HOMA-β.(50) At visit 3 and 4, the area under the curve (AUC) and incremental AUC (iAUC) from glucose and insulin concentrations will be calculated from venous blood sampling every 30 minutes during the 2-hour OGTT. Insulin Sensitivity Index, $ISI_{0,120}$,(51) insulinogenic index during the first 30 minutes of the 2-hour OGTT,(52) and beta cell function (AUC_{ins}/AUC_{glu}) will be estimated.(53)

Continuous glucose monitoring

The participants wear a continuous glucose monitor (CGM, FreeStyle Libre 1, Abbott Diabetes Care, Norway) for 14 days at baseline (7 days pre-intervention followed by the first 7 days of intervention/control), and for 14 days starting at 8 weeks from baseline. From these measurements, we will determine 24-hour glycaemic control, 3-hour postprandial glucose levels (AUC) for the first meal of the day, and nocturnal glycaemic control. The screens of the CGM readers are taped over to avoid lifestyle changes based on the participants' glucose levels. We also plan to explore other CGM data that can predict glycaemic control during pregnancy, using machine learning.

Height, weight, body composition, BMI, and waist circumference

Height is measured with the participants standing without shoes using a standard stadiometer. Weight and body composition are estimated in the morning after overnight fasting using bioelectrical impedance analysis (Inbody 720, Biospace CO, Korea), with participants wearing light clothes and standing barefoot. BMI is calculated as weight in kilograms divided by the square value of height in metres (kg/m^2). To account for the increase in fat-free mass hydration as pregnancy progresses, we will use a regression equation that estimates fat-free mass density as a function of gestational age.(54) Waist circumference is measured using a measuring tape at the level of the belly button with the participant standing.

Cardiorespiratory fitness

We measure peak oxygen uptake (VO_{2peak}) using indirect calorimetry (Metalyzer II, Cortex, Germany), using an individualised treadmill protocol in which the participants walk or run until volitional exhaustion. The test starts after a 10-minute warm-up. The speed or inclination is increased every 1 – 2 minutes, by 0.5 – 1.0 km/hour or 1% – 2%. VO_{2peak} is determined as the average of the three highest consecutive 10 seconds measured and will be reported as both absolute (L/min) and relative (mL/min/kg) values. We record HR throughout the exercise tests and use the peak HR recorded during the test as an estimate of the HR maximum.(55)

Blood pressure and resting heart rate

We use an automatic blood pressure device (Welch Allyn, Germany) to measure blood pressure (diastolic and systolic, in mmHg) and resting HR (beats per minute, bpm) on the participants' left arm after they have rested in a seated position for 15 minutes. We will report the average of three measurements taken at 1-minute intervals.

Physical activity, diet, and sleep

We use activity monitors to estimate physical activity levels, energy expenditure, and sleep duration. All participants wear Sensewear Armbands (BodyMedia, Pennsylvania, USA) for 14 days at baseline (7 days pre-intervention followed by the first 7 days of intervention/control), and the participants in the intervention group wear Amazfit GTS (Huami, China) smartwatches throughout the intervention. The smartwatch is connected to the Zepp app and shares PAI data with the research team via the Memento app. Participants register their diet in an online food diary (Fatsecret app) and record the time of first and last energy intake in the project handbook for 4 days (3 weekdays and 1 weekend day) every 8 weeks. They also complete questionnaires about physical activity, sleep quality, and psychological well-being every 8 weeks throughout the study period. We use the following questionnaires: 1) International Physical Activity Questionnaire,(56) 2) Pittsburgh Sleep Quality Index,(57) and 3) Psychological General Well-Being Index.(58) At baseline, the participants fill in the Horne-Östberg Morningsness-Eveningness Questionnaire.(59) We record medication and supplements, early miscarriages, abortions, and time to pregnancy and live birth. Additionally, expectant fathers are asked to complete questionnaires at baseline and every 8 weeks throughout the trial, including questions regarding their body weight, height, physical activity, and diet. These data will be used as co-variates in later analyses.

Neonatal and other outcomes

We obtain standard clinical neonatal outcomes from hospital birth records. Midwives at St. Olav's Hospital collect umbilical cord blood immediately after birth, prior to the delivery of the placenta. Placental tissues are collected from 1) around the base of the umbilical cord on the foetal side, 2) the periphery on the maternal side (full-thickness tissue), 3) the centre of the maternal side, also for storage in RNAlater solution (Invitrogen, Thermofisher scientific, Lithuania) and 4% formaldehyde solution, and 4) the periphery on the maternal side. The samples are put in 1.8 mL cryotubes and snap-frozen immediately in liquid nitrogen, before storage at -80°C for later analyses. The samples in RNAlater solution are stored at 4°C

overnight, followed by storage at -80°C for later analyses. The samples in 4% formaldehyde solution are stored under a fume hood at room temperature for 48 hours before histology slide preparation in collaboration with the CMIC Histology Lab at NTNU.

Within 72 hours of birth, and at age 6-8 weeks, body composition of the newborn is estimated using bioimpedance (BioScan touch i8-nano, Maltron, UK). Additionally, an experienced paediatric cardiologist examines cardiac morphology, structure, and function in the newborn, using a Vivid E95 scanner (GE Vingmed Ultrasound, Horten, Norway) and a GE 6s, and M5s phased-array transducers (GE Healthcare, Milwaukee, WI). A full clinical echocardiography including conventional echocardiographic parameters as well as study images with a focus on measurement of systolic and diastolic myocardial function is performed. The scanner is equipped with research software enabling high frame rate echocardiography to study cardiac flow and tissue properties as described previously.(60-62) A corresponding group of neonates (N = 30), from mothers with no known increased risk of GDM and BMI in the normal range (18.6-24.9 kg/m^2) will be used for comparison.

Adherence

We record adherence to TRE as the average daily time-window for energy intake for 4 days every 8 weeks. Additionally, we categorise participants as adherent if they report a ≤ 10-hour time-window for energy intake on ≥ 2 of these 4 days. Adherence to exercise is recorded as the number of PAI points the participants get per rolling 7 days. To ensure compliance and maintain adherence, we send text messages to all participants as reminders to complete questionnaires and dietary reporting. We also announce friendly competitions such as "Who can keep 100 weekly PAI points or more for a whole month?" in a Facebook group for the participants. The data are only accessible to the researchers and a gift card is awarded to the winners.

Modifications to the protocol after trial commencement

Since June 2021, we invite the participants to participate in a follow-up study after delivery in which we collect infant faecal samples (immediately after birth, at 6 weeks, and 6 months), maternal faecal samples (at 6 weeks and 6 months), and breast milk (at 6 weeks and 6 months). These samples are stored at -80°C for later analyses. Additionally, we started to offer supervised exercise training sessions to the participants in the intervention group. From November 2022, we started sending invitations using eFORSK (electronic form-based data collection, developed by Central Norway Regional Health Authority) and added 'Bariatric

surgery' to the exclusion criteria. 'Any other reason which according to the researchers makes the potential participant ineligible' to undergo either or both interventions (e.g., traumatic foot injury, anorexia/bulimia, etc.) was also added to the exclusion criteria in November 2022. From December 2022, we removed 'Planned assisted fertilisation with female factor reason' from the exclusion criteria. In addition, we changed the maximum time before pregnancy from 12 months to 6 months to allow for the trial to be terminated in time for us to analyse the data within the project period. In March 2023, the required number of total participants was reduced from 260 to 200 based on the revised calculation as described in the sample size calculation, with additional specification of stopping before 200 if we had sufficient pregnant participants for the primary outcome measure. In June 2023, we changed from Amazfit GTS to Polar Ignite 2 (Polar, Finland) smartwatch and from Zepp and Memento to Polar Flow and Mia app.

Sample size calculation

The primary outcome of this study is glucose tolerance (after a 2-hour OGTT) in gestational week 28. The HAPO study results(63) indicate strong, continuous associations of maternal glucose levels, even below the diagnostic level of GDM with adverse maternal and offspring outcomes. Based on the increasing risk of adverse maternal and offspring outcomes across 2-hour plasma glucose categories with a change of ~1 mmol/L, we consider a difference of 1 mmol/L in 2-hour plasma glucose after OGTT between the intervention and control group as clinically relevant. We also used the observed standard deviation (1 SD = 1.3 mmol/L) in 2-hour plasma glucose after OGTT in the HAPO study for the sample size calculations. Calculation of the sample size for a two-sided t-test to detect a difference of 1 mmol/L between the groups, using an SD of 1.3 mmol/L, a power of 0.90, and a significance level of 0.05, yields 37 participants in each group in gestational week 28. To allow for an expected exclusion from the study due to not conceiving within the study period (~50%)(64) yielding 74 per group, further drop-out during the study period (10-20%), yielding 93 per group, and to increase statistical power for secondary analyses, we initially wanted to include 260 participants in the trial.

However, we terminated the inclusion of new participants at 167 participants since we had reached 47 participants in each group who were pregnant in gestational week 12. With this number of participants, we foresee that we will have at least 37 participants in each group in gestational week 28, allowing for up to 20% dropout during pregnancy. We expect more participants who are already included to become pregnant in the upcoming period, which will increase the number of pregnant participants.

Statistical analyses

The primary analysis will be done according to the 'intention to treat' principle, using all obtained data irrespective of participant adherence to the intervention and completeness of outcome measures. We plan to use linear mixed models (LMMs) to compare primary and secondary continuous outcome measures between groups, with time and group x time interactions as fixed effects variables, and subject as random factor.(65) Since no systematic baseline differences between the groups are expected in RCTs, means at baseline will be constrained to be equal in the LMMs. We will report estimates with corresponding 95% confidence intervals and p-values for differences between the intervention group and the control group. We will check the normality of residuals by visual inspection of QQ-plots and bootstrapping, transformations or non-parametric methods will be used in cases of non-normal model residuals. For the primary outcome measure, we will consider a p-value < 0.05 as statistically significant. For the secondary outcome measures, p-values < 0.01 will be considered statistically significant, due to multiple comparisons, and these analyses will be explorative. We will also perform per-protocol analyses: Participants with an average of ≥ 75 PAI per rolling week and adherence to TRE (as per definition above) during the preconception period will be included in the per-protocol analyses for all outcome measures. We will report additional results from all participants who were included in the trial, from the preconception period, irrespective of whether they became pregnant or not during the study period.

Blinding

The study is not blinded as it is difficult to blind participants and treatment providers to behavioural intervention. However, baseline assessments are undertaken before randomisation.

Monitoring

We do not expect any adverse effects in this study. If pregnant women are worried about foetal safety during exercise, we have experienced personnel available in the research group to monitor foetal heart rate during exercise sessions. The investigators are responsible for the documentation of any adverse or serious adverse events in the Case Report Form and the Serious Adverse Events Report Form, respectively. Participants are advised to contact the investigators if they have any unusual symptoms. All serious adverse events will be reported to the sponsor (NTNU) within 24 hours after the investigators have been informed of the event.

Patient and public involvement

We have involved users in the planning of the study and will continue involving them in the implementation and dissemination. In the planning phase, we arranged a 1-hour digital workshop with users (reproductive-aged females with overweight/obesity), where we encouraged the audience to ask questions and give us feedback about relevant topics or issues related to participation. Regarding the challenges of long-term adherence, we use these feedbacks to find ways to incorporate exercise training and TRE into daily life.

Ethics and dissemination

The Regional Committees for Medical and Health Research Ethics in Norway approved the study (REK, reference number 143756). The comparative analysis of the neonatal echocardiography data from this study with a corresponding group of neonates from mothers with normal BMI and no increased risk of GDM is also approved (REK reference number 67584). The work is conducted according to the Declaration of Helsinki and the ICMJE Recommendations for authorship. The participants sign an informed written consent before participating in the study and can at any time withdraw from the study without further explanation. Study specific ID numbers are used as participants' identification. We ensure data quality by double data entry into an electronic CRF and treat the collected data following the General Data Protection Regulation. All protocol modifications are reported to REK. Upon completion of the study and finalization of the study report, we will submit the results for publication and/or in a publicly accessible database of clinical study results after anonymizing the data.

Discussion

Based on a thorough literature search, the BTB study will be the first RCT to investigate the combined effects of TRE and exercise training, initiated before and continued throughout pregnancy, on cardiometabolic parameters in people at risk of GDM and their infants. We hypothesise that the combination of these two lifestyle interventions will induce an additive and clinically relevant improvement in maternal glucose tolerance, and potentially also in our secondary outcome measures in mothers and infants. As such, the initiation of lifestyle modification before pregnancy will provide a better platform for improved adherence and health outcomes, potentially breaking the intergenerational cycle of cardiometabolic disorders, and thereby reducing the risk of diabetes for future generations.

So far, there is limited data on the combination of TRE and exercise training in humans. Haganes and colleagues reported that the combination of TRE and HIIT in females with a BMI of ≥ 27 kg/m^2 for 7 weeks significantly reduced HbA1c compared with a no-intervention control group and lead to greater losses in body weight, fat mass, and visceral fat area compared with either intervention alone.(26) Since the duration of the intervention is much longer in the BTB trial, there may be lower adherence to one or both intervention strategies. The possible reasons for lower adherence are that the participants may lack motivation for such a long time, find the intervention program boring and/or difficult, or develop physical symptoms that may hinder the participant to adhere to the intervention (especially during pregnancy). Combining motivational human interaction with digital interventions can increase engagement and the effectiveness of behaviour change interventions.(66) To improve adherence throughout the study period, we offer an individualised exercise regimen and provide encouragement, support, and monitor the participants regularly, both in person and over the phone.

The incidence and risk of obesity, insulin resistance, and GDM persists through generations.(67) To disrupt this intergenerational cycle, it is urgently necessary to develop and implement effective and practical lifestyle intervention strategies which can improve the cardiometabolic health outcomes of both mother and offspring. If the preconception lifestyle interventions implemented in this study lead to favourable outcomes and prove to be feasible and effective, it can pave the way for novel interventions that can be adopted in clinical practice during the preconception period, especially among those who are at risk of developing GDM.

Author contributions

MAJS drafted the manuscript. TM, SAN, KÅS, ACI, TF, and SLF conceived and contributed to the design of the study and the plan for analyses. GR, MAJS, and HSS coordinate the study, perform measurements on test days, monitor participants, and supervise the exercise training. SAN performs the echocardiogram on the newborns. All authors provided feedback and approved the final manuscript.

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Facility, St. Olavs Hospital. We would also like to thank the midwives at the Women and Children's Centre, St. Olavs Hospital for the collection of samples related to birth. eFORSK, a stand-alone form-based information and communications technology solution for electronic collection of data, developed by Central Norway Regional Health Authority is used for sending invitations to the study.

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Competing interests

The authors declare that they have no competing interests.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available at: https://www.diabetesatlas.org
2. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. Int J Mol Sci. 2018;19(11):3342.
3. Yu Y, Soohoo M, Sørensen HT, Li J, Arah OA. Gestational Diabetes Mellitus and the Risks of Overall and Type-Specific Cardiovascular Diseases: A Population- and Sibling-Matched Cohort Study. Diabetes Care. 2021;45(1):151-9.
4. Moholdt T, Hawley JA. Maternal Lifestyle Interventions: Targeting Preconception Health. Trends Endocrinol Metab. 2020;31(8):561-9.
5. Vinter CA, Tanvig MH, Christensen MH, Ovesen PG, Jørgensen JS, Andersen MS, et al. Lifestyle Intervention in Danish Obese Pregnant Women With Early Gestational Diabetes Mellitus According to WHO 2013 Criteria Does Not Change Pregnancy Outcomes: Results From the LiP (Lifestyle in Pregnancy) Study. Diabetes Care. 2018;41(10):2079-85.
6. Nyrnes SA, Garnæs KK, Salvesen Ø, Timilsina AS, Moholdt T, Ingul CB. Cardiac function in newborns of obese women and the effect of exercise during pregnancy. A randomized controlled trial. PLoS One. 2018;13(6):e0197334.
7. Gluckman PD, Hanson MA, Buklijas T. A conceptual framework for the developmental origins of health and disease. J Dev Orig Health Dis. 2010;1(1):6-18.
8. Cai G-j, Sun X-x, Zhang L, Hong Q. Association between maternal body mass index and congenital heart defects in offspring: a systematic review. American Journal of Obstetrics and Gynecology. 2014;211(2):91-117.
9. Petropoulos A. Congenital heart disease and maternal diabetes mellitus. Евразийский Журнал Клинических Наук. 2018;1(3):32-9.
10. Lowe WL, Lowe LP, Kuang A, Catalano PM, Nodzenski M, Talbot O, et al. Maternal glucose levels during pregnancy and childhood adiposity in the Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study. Diabetologia. 2019;62(4):598-610.
11. Tam WH, Ma RCW, Ozaki R, Li AM, Chan MHM, Yuen LY, et al. In Utero Exposure to Maternal Hyperglycemia Increases Childhood Cardiometabolic Risk in Offspring. Diabetes Care. 2017;40(5):679-86.
12. Grunnet LG, Hansen S, Hjort L, Madsen CM, Kampmann FB, Thuesen ACB, et al. Adiposity, Dysmetabolic Traits, and Earlier Onset of Female Puberty in Adolescent Offspring of Women With Gestational Diabetes Mellitus: A Clinical Study Within the Danish National Birth Cohort. Diabetes Care. 2017;40(12):1746-55.
13. Management of Diabetes in Pregnancy. Diabetes Care. 2017;40(Suppl 1):S114-s9.
14. Gjestland K, Bø K, Owe KM, Eberhard-Gran M. Do pregnant women follow exercise guidelines? Prevalence data among 3482 women, and prediction of low-back pain, pelvic girdle pain and depression. Br J Sports Med. 2013;47(8):515-20.
15. Zeanah M, Schlosser SP. Adherence to ACOG Guidelines on Exercise During Pregnancy: Effect on Pregnancy Outcome. Journal of Obstetric, Gynecologic & Neonatal Nursing. 1993;22(4):329-35.
16. Tieu J, Shepherd E, Middleton P, Crowther CA. Dietary advice interventions in pregnancy for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2017;1(1):Cd006674.
17. 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(8):Cd012327.
18. Shepherd E, Gomersall JC, Tieu J, Han S, Crowther CA, Middleton P. Combined diet and exercise interventions for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2017;11(11):Cd010443.
19. Lane M, Robker RL, Robertson SA. Parenting from before conception. Science. 2014;345(6198):756-60.

20. Stephenson J, Heslehurst N, Hall J, Schoenaker D, Hutchinson J, Cade JE, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. Lancet. 2018;391(10132):1830-41.
21. Rönö K, Stach-Lempinen B, Eriksson JG, Pöyhönen-Alho M, Klemetti MM, Roine RP, et al. Prevention of gestational diabetes with a prepregnancy lifestyle intervention - findings from a randomized controlled trial. Int J Womens Health. 2018;10:493-501.
22. Raab R, Michel S, Günther J, Hoffmann J, Stecher L, Hauner H. Associations between lifestyle interventions during pregnancy and childhood weight and growth: a systematic review and meta-analysis. Int J Behav Nutr Phys Act. 2021;18(1):8-.
23. Sparks JR, Ghildayal N, Hivert M-F, Redman LM. Lifestyle interventions in pregnancy targeting GDM prevention: looking ahead to precision medicine. Diabetologia. 2022;65(11):1814-24.
24. Szumilewicz A S-RR, Worska A, Piernicka M, Yu H, Pajaujiene S, et al. How to HIIT while pregnant? The protocol characteristics and effects of high intensity interval training implemented during pregnancy – A systematic review. Baltic Journal of Health and Physical Activity. 2022;14(1):1-16.
25. Flanagan EW, Kebbe M, Sparks JR, Redman LM. Assessment of Eating Behaviors and Perceptions of Time-Restricted Eating During Pregnancy. J Nutr. 2022;152(2):475-83.
26. Haganes KL, Silva CP, Eyjólfsdóttir SK, Steen S, Grindberg M, Lydersen S, et al. Time-restricted eating and exercise training improve HbA1c and body composition in women with overweight/obesity: A randomized controlled trial. Cell Metab. 2022;34(10):1457-71.e4.
27. Termannsen AD, Varming A, van Elst C, Bjerre N, Nørgaard O, Hempler NF, et al. Feasibility of time-restricted eating in individuals with overweight, obesity, prediabetes, or type 2 diabetes: A systematic scoping review. Obesity (Silver Spring). 2023;31(6):1463-85.
28. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early Time-Restricted Feeding Reduces Appetite and Increases Fat Oxidation But Does Not Affect Energy Expenditure in Humans. Obesity (Silver Spring). 2019;27(8):1244-54.
29. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metab. 2018;27(6):1212-21.e3.
30. Chair SY, Cai H, Cao X, Qin Y, Cheng HY, Ng MT. Intermittent Fasting in Weight Loss and Cardiometabolic Risk Reduction: A Randomized Controlled Trial. J Nurs Res. 2022;30(1):e185.
31. Hutchison AT, Regmi P, Manoogian ENC, Fleischer JG, Wittert GA, Panda S, et al. Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial. Obesity (Silver Spring). 2019;27(5):724-32.
32. Jamshed H, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Clock, Aging, and Autophagy in Humans. Nutrients. 2019;11(6).
33. Wilkinson MJ, Manoogian ENC, Zadourian A, Lo H, Fakhouri S, Shoghi A, et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell Metab. 2020;31(1):92-104.e5.
34. Andriessen C, Fealy CE, Veelen A, van Beek SMM, Roumans KHM, Connell NJ, et al. Three weeks of time-restricted eating improves glucose homeostasis in adults with type 2 diabetes but does not improve insulin sensitivity: a randomised crossover trial. Diabetologia. 2022;65(10):1710-20.
35. Che T, Yan C, Tian D, Zhang X, Liu X, Wu Z. Time-restricted feeding improves blood glucose and insulin sensitivity in overweight patients with type 2 diabetes: a randomised controlled trial. Nutr Metab (Lond). 2021;18(1):88.
36. Parr EB, Devlin BL, Lim KHC, Moresi LNZ, Geils C, Brennan L, et al. Time-Restricted Eating as a Nutrition Strategy for Individuals with Type 2 Diabetes: A Feasibility Study. Nutrients. 2020;12(11).
37. Loy SL, Chan JK, Wee PH, Colega MT, Cheung YB, Godfrey KM, et al. Maternal Circadian Eating Time and Frequency Are Associated with Blood Glucose Concentrations during Pregnancy. J Nutr. 2017;147(1):70-7.

38. Wowdzia JB, Hazell TJ, Davenport MH. Glycemic response to acute high-intensity interval versus moderate-intensity continuous exercise during pregnancy. Physiol Rep. 2022;10(18):e15454.
39. Szumilewicz A, Santos-Rocha R, Worska A, Piernicka M, Yu H, Pajaujiene S, et al. How to HIIT while pregnant? The protocol characteristics and effects of high intensity interval training implemented during pregnancy: A systematic review. Baltic journal of health and physical activity. 2022;14(1):1-16.
40. Yu H, Santos-Rocha R, Radzimiński Ł, Jastrzębski Z, Bonisławska I, Szwarc A, et al. Effects of 8-Week Online, Supervised High-Intensity Interval Training on the Parameters Related to the Anaerobic Threshold, Body Weight, and Body Composition during Pregnancy: A Randomized Controlled Trial. Nutrients. 2022;14(24):5279.
41. Wowdzia J, Hazell T, Vanden Berg E, Labrecque L, Brassard P, Davenport M. Maternal and Fetal Cardiovascular Responses to Acute High-Intensity Interval and Moderate-Intensity Continuous Training Exercise During Pregnancy: A Randomized Crossover Trial. Sports Medicine. 2023:1-15.
42. Gaston A, Cramp A. Exercise during pregnancy: a review of patterns and determinants. J Sci Med Sport. 2011;14(4):299-305.
43. Tobias DK, Zhang C, Chavarro J, Bowers K, Rich-Edwards J, Rosner B, et al. Prepregnancy adherence to dietary patterns and lower risk of gestational diabetes mellitus. The American Journal of Clinical Nutrition. 2012;96(2):289-95.
44. Gao X, Zheng Q, Jiang X, Chen X, Liao Y, Pan Y. The effect of diet quality on the risk of developing gestational diabetes mellitus: A systematic review and meta-analysis. Frontiers in Public Health. 2023;10.
45. Yong HY, Mohd Shariff Z, Mohd Yusof BN, Rejali Z, Appannah G, Bindels J, et al. The association between dietary patterns before and in early pregnancy and the risk of gestational diabetes mellitus (GDM): Data from the Malaysian SECOST cohort. PLoS One. 2020;15(1):e0227246.
46. Chan AW, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. Bmj. 2013;346:e7586.
47. Nes BM, Gutvik CR, Lavie CJ, Nauman J, Wisløff U. Personalized Activity Intelligence (PAI) for Prevention of Cardiovascular Disease and Promotion of Physical Activity. Am J Med. 2017;130(3):328-36.
48. Zisko N, Skjerve KN, Tari AR, Sandbakk SB, Wisløff U, Nes BM, et al. Personal Activity Intelligence (PAI), Sedentary Behavior and Cardiovascular Risk Factor Clustering - the HUNT Study. Prog Cardiovasc Dis. 2017;60(1):89-95.
49. World Health O. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva: World Health Organization; 2013 2013. Contract No.: WHO/NMH/MND/13.2.
50. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
51. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract. 2000;47(3):177-84.
52. Phillips DI, Clark PM, Hales CN, Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med. 1994;11(3):286-92.
53. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295-301.
54. Most J, Marlatt KL, Altazan AD, Redman LM. Advances in assessing body composition during pregnancy. Eur J Clin Nutr. 2018;72(5):645-56.
55. Berglund IJ, Sørås SE, Relling BE, Lundgren KM, Kiel IA, Moholdt T. The relationship between maximum heart rate in a cardiorespiratory fitness test and in a maximum heart rate test. J Sci Med Sport. 2019;22(5):607-10.

56. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.
57. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
58. Grossi E, Compare A. Psychological General Well-Being Index (PGWB). In: Michalos AC, editor. Encyclopedia of Quality of Life and Well-Being Research. Dordrecht: Springer Netherlands; 2014. p. 5152-6.
59. Adan A, Almirall H. Horne & Östberg Morningness–Eveningness questionnaire: A reduced scale. Personality and Individual Differences. 1991;12:241-53.
60. Nyrnes SA, Fadnes S, Wigen MS, Mertens L, Lovstakken L. Blood Speckle-Tracking Based on High-Frame Rate Ultrasound Imaging in Pediatric Cardiology. J Am Soc Echocardiogr. 2020;33(4):493-503.e5.
61. Sørensen K, Fadnes S, Mertens L, Henry M, Segers P, Løvstakken L, et al. Assessment of Early Diastolic Intraventricular Pressure Difference in Children by Blood Speckle-Tracking Echocardiography. J Am Soc Echocardiogr. 2023.
62. Salles S, Lovstakken L, Aase SA, Bjastad TG, Torp H. Clutter Filter Wave Imaging. IEEE Trans Ultrason Ferroelectr Freq Control. 2019;66(9):1444-52.
63. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991-2002.
64. Mikkelsen EM, Riis AH, Wise LA, Hatch EE, Rothman KJ, Sørensen HT. Pre-gravid oral contraceptive use and time to pregnancy: a Danish prospective cohort study. Hum Reprod. 2013;28(5):1398-405.
65. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in randomised controlled trials. Contemp Clin Trials Commun. 2018;10:80-5.
66. Barker M, Dombrowski SU, Colbourn T, Fall CHD, Kriznik NM, Lawrence WT, et al. Intervention strategies to improve nutrition and health behaviours before conception. Lancet (London, England). 2018;391(10132):1853-64.
67. Ma RCW, Popkin BM. Intergenerational diabetes and obesity—A cycle to break? PLOS Medicine. 2017;14(10):e1002415.

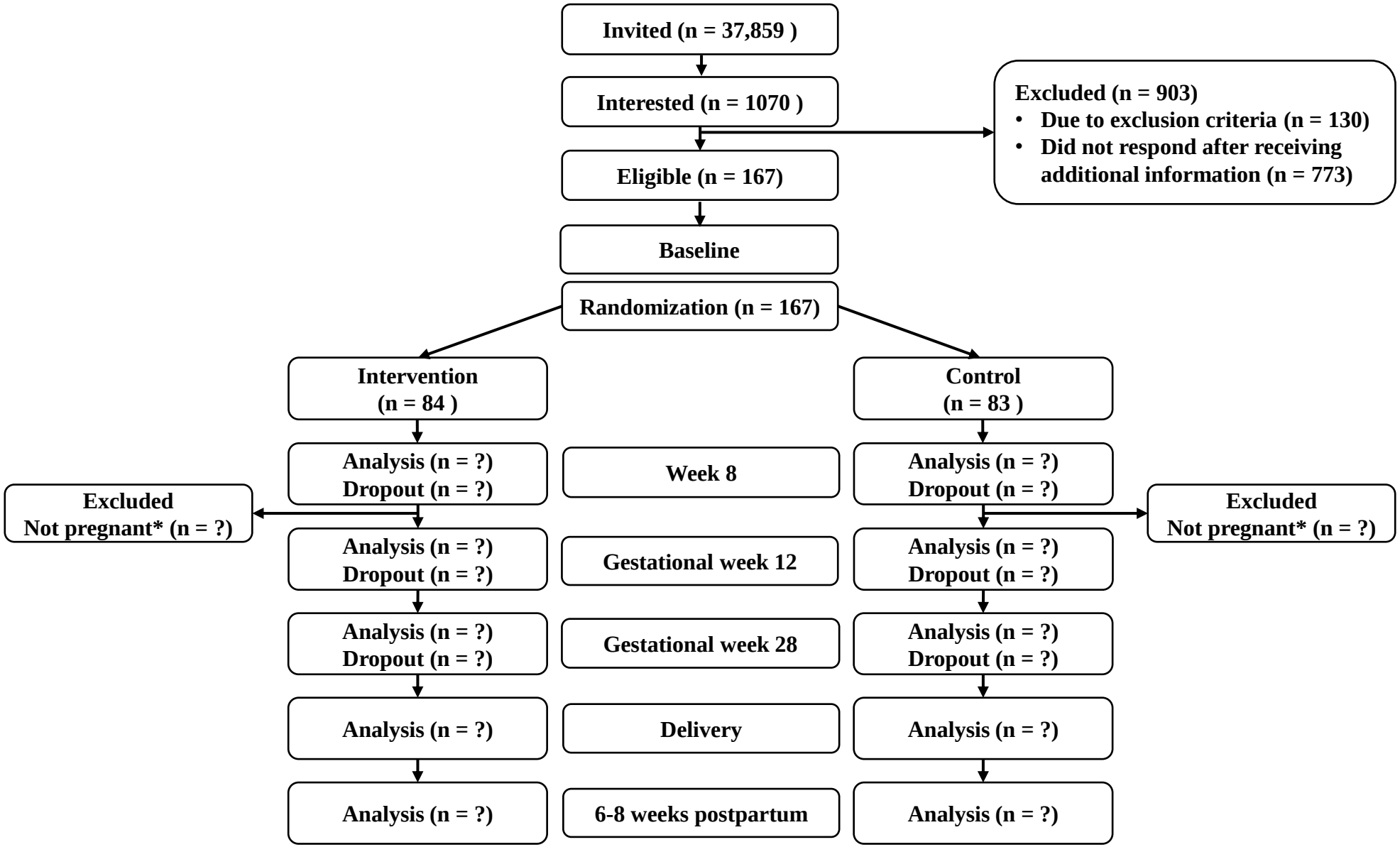
FIGURE LEGENDS

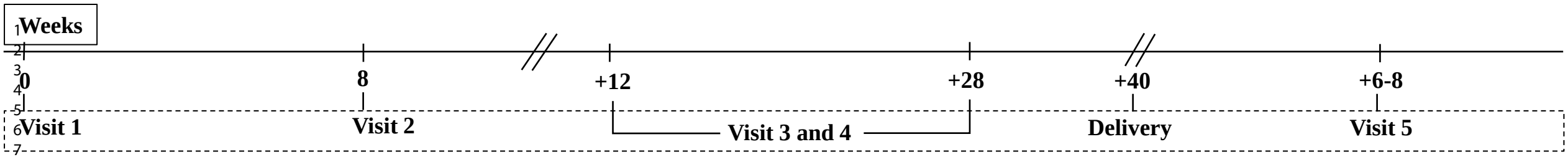
Figure 1. Consort flow diagram of the BEFORE THE BEGINNING trial

(Ongoing study: status 25.09.2020 – 17.07.2023)

* If the participants are not pregnant within 12 months of inclusion, they are excluded from the study. From December 2022, the time-window for exclusion if not pregnant was reduced from 12 to 6 months.

Figure 2. Overview of time-points for assessments in the trial.


Invited (n = 37,859)
Interested (n = 1070)
Excluded (n = 903)
• Due to exclusion criteria (n = 130)
• Did not respond after receiving additional information (n = 773)
Eligible (n = 167)
Baseline
Randomization (n = 167)
Intervention (n = 84)
Control (n = 83)
Analysis (n = ?) Dropout (n = ?)
Week 8
Analysis (n = ?) Dropout (n = ?)
Excluded Not pregnant* (n = ?)
Excluded Not pregnant* (n = ?)
Analysis (n = ?) Dropout (n = ?)
Gestational week 12
Analysis (n = ?) Dropout (n = ?)
Analysis (n = ?) Dropout (n = ?)
Gestational week 28
Analysis (n = ?) Dropout (n = ?)
Analysis (n = ?)
Delivery
Analysis (n = ?)
Analysis (n = ?)
6-8 weeks postpartum
Analysis (n = ?)



Every 8 weeks following visit 1: Food diary (4-day registration), hunger/appetite registration, time-window of energy intake, questionnaires (physical activity, sleep, psychological well-being).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not	2

		yet registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	n/a, see appendix
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 15
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	n/a, see appendix
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities:	#5d	Composition, roles, and responsibilities of the coordinating centre, steering	n/a

committees		committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5,6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 (Box 1)
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a, see appendix
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions:	#11d	Relevant concomitant care and	7

concomitant care		interventions that are permitted or prohibited during the trial	
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7 (Figure 1) 7 (Figure 2)
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care	13

		providers, outcome assessors, data analysts), and how	
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a: no blinding
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate	12

		from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of	n/a, see appendix

		whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a, see appendix
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board	14

		(REC / IRB) approval	
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	11-12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	14
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Data access	#29	Statement of who will have access to the	14

		final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See Appendix
Biological specimens	#33	Plans for collection, laboratory	8ssss-11

evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

BMJ Open

A randomised controlled trial of preconception lifestyle intervention on maternal and offspring health in people with increased risk of gestational diabetes: study protocol for the BEFORE THE BEGINNING trial

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A randomised controlled trial of preconception lifestyle intervention on maternal and offspring health in people with increased risk of gestational diabetes: study protocol for the BEFORE THE BEGINNING trial

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Word count: 4199

Abstract

Introduction: Gestational diabetes mellitus (GDM) is associated with increased risk for type 2 diabetes in the mother and cardiometabolic diseases in the child. The preconception period is an optimal window to adapt the lifestyle for improved outcomes for both mother and child. Our aim is to determine the effect of a lifestyle intervention, initiated before and continued throughout pregnancy, on maternal glucose tolerance and other maternal and infant cardiometabolic outcomes.

Methods and analysis: This ongoing randomised controlled trial has included 167 females aged 18-39 years old at increased risk for GDM who are contemplating pregnancy. The participants were randomly allocated 1:1 to an intervention or control group. The intervention consists of exercise (volume is set by a heart rate-based app and corresponds to ≥ 1 hour of weekly exercise at ≥ 80% of individual heart rate maximum), and time-restricted eating (≤ 10 hours/day window of energy intake). The primary outcome measure is glucose tolerance in gestational week 28. Maternal and offspring outcomes are measured before and during pregnancy, at delivery, and at 6-8 weeks postpartum. Primary and secondary continuous outcome measures will be compared between groups based on the "intention to treat" principle using linear mixed models.

Ethics and dissemination: The Regional Committees for Medical and Health Research Ethics in Norway has approved the study (REK 143756). The anonymised results will be submitted for publication and posted in a publicly accessible database of clinical study results.

Abstract word count: 236

Trial registration number: Clinical trial gov NCT04585581.

Keywords: insulin resistance, time-restricted eating, aerobic exercise, glycaemic control, diet

Strengths and limitations of this study

- The intervention starts before and continues throughout pregnancy to make it easier for the participants to adopt an active lifestyle before pregnancy.
- This study includes individuals at high risk of GDM from multiple ethnic backgrounds, which improves the generalisability of the findings.
- The effects of the intervention on the cardiac function and body composition of the offspring will be comprehensively evaluated.
- Due to the difficulty of blinding investigators and participants to behavioural interventions, investigators will not be blinded for outcome assessments.
- Due to the long duration of the intervention, adherence to lifestyle modifications may be difficult for some participants despite regular monitoring and motivational support.

Introduction

The global prevalence of gestational diabetes mellitus (GDM), i.e., high plasma glucose first identified during pregnancy, continues to increase. Both environmental and genetic factors contribute to the development of GDM, and up to 14 % of live births are negatively impacted by this condition.(1) GDM typically occurs because of pancreatic β-cell dysfunction with pre-existing insulin resistance and increases the risk for type 2 diabetes and cardiovascular disease in the mother.(2, 3) Maternal obesity and hyperglycaemia affect the offspring through the egg cell quality, intrauterine environment, and foetal organ development. These metabolic conditions eventually increase the risk for cardiac dysfunction at birth, and early onset diabetes, obesity, and cardiovascular diseases later in life.(4-9) Higher maternal blood glucose concentration, even below the diagnostic criteria for GDM, is associated with increased birth weight, elevated levels of cord-blood C-peptide, childhood obesity, and elevated blood pressure, independent of maternal body mass index (BMI).(10-12) Besides the inheritable risk factors, epigenetic modifications in utero, low-grade inflammation, and modifications of the gut microbiome can also negatively affect the cardiometabolic health of the offspring.(4)

Lifestyle interventions, including dietary changes, increased physical activity, and self-monitoring of blood glucose, are the first-line choice for GDM management.(13) However, many pregnant individuals fail to adhere to the recommendations for diet and exercise training(14, 15) and there is inconclusive evidence for clinically meaningful effects of diet-exercise interventions on pregnancy outcomes for the mother or child.(5, 16-18) Several recent randomised controlled trials (RCTs) and reviews conclude that pre-pregnancy lifestyle interventions are urgently needed to improve maternal health and increase the likelihood of adherence to a healthy lifestyle during pregnancy.(19-23) Alternative diet-exercise strategies, such as time-restricted eating (TRE) and high-intensity interval training (HIIT), have shown promising results on improving metabolic health among reproductive-aged females.(24-26) TRE is a safe and feasible intervention in individuals with overweight, obesity, prediabetes, and type 2 diabetes.(27) It has been shown to improve glucose tolerance, and insulin sensitivity, and reduce appetite, hunger, HbA1c, and total body and fat mass in this population.(28-36) While data on the effects of TRE in pregnancy are scarce, observational data suggest that longer maternal night-fasting intervals are associated with decreased fasting glucose.(37) The safety of HIIT is not yet established during pregnancy, but recent publications indicate that HIIT is safe, with higher enjoyment and improved adherence than continuous moderate-intensity

training,(38) and may provide cardiometabolic benefits for both mothers and their offspring.(39-41)

Pre-pregnancy patterns of physical activity and exercise are important determinants of exercise during pregnancy,(42) and pre-pregnancy inception of healthy dietary habits is associated with a lower risk of GDM.(43-45) So far, there is limited evidence on the effectiveness of implementing both dietary and exercise-based lifestyle interventions before pregnancy. It is highly relevant to find feasible and effective pre-pregnancy lifestyle interventions which can reduce maternal hyperglycaemia and its related negative consequences for mother and child.

The primary hypothesis for the BEFORE THE BEGINNING (BTB) trial is that the participants allocated to the intervention group (time-restricted eating and exercise) will have improved maternal glucose tolerance in gestational week 28, compared with participants in the control group. We will also determine the effect of the intervention on secondary cardiometabolic outcomes in both the mothers and their newborns.

Aims

The primary aim of BEFORE THE BEGINNING

- To determine the effect of a lifestyle intervention, commenced preconception and continuing throughout pregnancy, on maternal glucose tolerance in pregnancy.

Secondary aims of BEFORE THE BEGINNING

- To evaluate the effect of the intervention on insulin sensitivity, blood glucose, circulating lipids, body composition, cardiorespiratory fitness, systemic inflammation, and blood pressure in the mothers.
- To evaluate the effect of the intervention on cardiac function, body composition, and systemic inflammation in the newborns.
- To evaluate the adherence to the interventions, and their effects on sleep quality, appetite and hunger, physical activity, and dietary intake.

Methods

Design and study setting

This is an ongoing single-centre RCT with two parallel groups: an intervention group and a control group, undertaken at the Norwegian University of Science and Technology (NTNU) in

Trondheim, Norway, in collaboration with the St. Olav's Hospital, Trondheim, Norway. SPIRIT reporting guidelines were used in reporting this study protocol.(46)

Recruitment and participants

The trial was announced through social media, hospital and university webpages, local stores, and public places. Additionally, potential participants were identified through the National Population Register, and we regularly sent out electronic invitations to females aged 20-35 years in Trondheim and the surrounding area to participate in the trial. The invitation prompted them to visit the study website, which contains a short description of the trial and allows potential participants to self-screen for eligibility before further screening by telephone. The first participant was included on 25th September 2020 and the last participant was included on 28th April 2023.

Box 1 shows the inclusion and exclusion criteria for participation in the study.

Box 1: Inclusion and exclusion criteria

Inclusion criteria

- Female
- Age: 18-39 years old
- Contemplating pregnancy within the next six months
- Understands oral and written Norwegian or English
- At least one of the following criteria must apply:
  - Body mass index ≥ 25 < 40 kg/m^2,
  - Gestational diabetes in a previous pregnancy,
  - Close relative with diabetes (either parents, siblings, or children with diabetes),
  - Fasting plasma glucose > 5.3 mmol/L,
  - Previous newborn > 4.5 kg, or
  - Non-European ethnicity (with one or both parents originating from an area outside Europe).

Exclusion criteria

- On-going pregnancy
- Trying to conceive ≥ 6 cycles at study entry
- Known diabetes (type 1 or 2)
- Shift work that includes night shifts > 2 days per week
- Previous hyperemesis
- Known cardiovascular diseases
- High-intensity exercise > 2 times per week in the last 3 months
- Habitual eating window ≤ 12 hours
- Bariatric surgery
- Any other reason which according to the researchers makes the potential participant ineligible

Randomisation and allocation

After screening and assessments at baseline, the participants were randomly allocated (1:1) to the intervention or a standard care control group, after stratifying for GDM in a previous pregnancy (yes/no). At the first visit, the study procedures, equipment, and applications were set up and explained to the participants.

We used a computer random number generator (WebCRF3) developed and administered at The Clinical Research Unit (Klinforsk), NTNU/St. Olav's Hospital, Trondheim, Norway to randomly allocate participants using various block sizes.

Intervention

The intervention starts before pregnancy and continues throughout pregnancy and consists of a combination of TRE and exercise. Participants are counselled to change their daily time-window of energy intake to ≤ 10 hours, ending no later than 19:00 hours, for minimum 5 days per week throughout the intervention. The remaining 2 days are "days off" when they can consume food *ad libitum* if they wish. Apart from current recommendations about preconception/pregnancy nutrition, we give no advice regarding food choices, nor do we encourage a reduced total energy intake.

We use the Personal Activity Intelligence (PAI) score, a science-backed activity metric based on heart rate (HR)(47, 48) to prescribe exercise. Since PAI is HR-based, high-intensity exercise gives substantially higher PAI scores than low-to-moderate-intensity exercise. The goal for the participants in the intervention group is to earn and maintain ≥ 100 PAI per week, which can be reached by minimum 1 hour of weekly exercise at ≥ 80% of HR maximum. One week after the baseline visit, we invite the participants for a supervised introductory exercise session and provide a brochure with exercise options (e.g., treadmill walking/running, cycling). We invite the participants for a second session 2 weeks after the introductory session. The participants can choose their mode of exercise. Once pregnant, we advise the participants to either do short work-bouts at high intensity with low-to-moderate intensity periods in-between, or longer work periods up to 85% of heart rate maximum. We contact the participants not reaching 100 PAI to offer additional supervised exercise sessions, and they can also ask for extra support and supervised exercise sessions if they want to.

Participants in the control group receive standard care and are asked to continue with their habitual physical activity and dietary habits. We contact these participants once every 8 weeks to support adherence to registrations and monitoring.

Experimental procedures and outcome measures

The study period spans from baseline assessments in the pre-pregnancy period to 6-8 weeks after delivery (Figure 1). Participants who do not become pregnant within 6 months after inclusion in the trial (changed from 12 months from December 2022, see below under modifications to the protocol after trial commencement) are excluded. For participants who experience spontaneous abortions, we add the number of weeks that the participant was pregnant plus 4 weeks to their time in the trial before exclusion pre-pregnancy.

Assessments of the participants are performed twice during preconception (at baseline before randomisation, and after 8 weeks), and twice during pregnancy (in gestational weeks 12 and 28). Outcomes in the newborns are assessed within 72 hours after delivery and at age 6-8 weeks (Figure 2). All participants receive a brochure from the Norwegian Health Directorate with the current recommendations for physical activity, diet, and folic acid, and iodine supplements. The participants are invited to ultrasound examinations in gestational weeks 12, 19, and 32.

Primary outcome measure

The primary outcome measure is plasma glucose concentration obtained 2 hours after a 75 g oral glucose tolerance test (OGTT) in gestational week 28. After an overnight fast (≥ 10 hours) and no exercise for ≥ 24 hours, the participants consume 75 g of glucose (Glucosepro, Finnamedical, Finland) diluted in 250 mL water within 5 minutes. Using an indwelling catheter, we collect venous blood before the OGTT, with subsequent collections at 30, 60, 90, and 120 minutes after ingestion of glucose.

Secondary outcome measures

Secondary maternal and neonatal outcome measures (Figure 2) are described below.

Blood sampling and biochemistry

From all visits, fasting blood lipids, plasma glucose, and HbA1c are measured immediately after sampling, at St. Olav's Hospital, following local standardised procedures. Additional fasting plasma, serum, full blood, and urine are stored in a biobank at -80°C for later analyses. GDM is recorded at Visit 3 and 4, according to the WHO 2013 criteria (fasting plasma glucose 5.1-6.9 mmol/L and/or 2-hour plasma glucose 8.5-11.0 mmol/L after 75 g OGTT).(49) At the event of a GDM diagnosis, the participant and their general practitioner are informed for further evaluation and management. Insulin sensitivity will be calculated using homeostasis model assessment of insulin resistance (HOMA-IR)(50) and pancreatic beta cell function using

HOMA-β.(50) At visit 3 and 4, the area under the curve (AUC) and incremental AUC (iAUC) from glucose and insulin concentrations will be calculated from venous blood sampling every 30 minutes during the 2-hour OGTT. Insulin Sensitivity Index, $ISI_{0,120}$,(51) insulinogenic index during the first 30 minutes of the 2-hour OGTT,(52) and beta cell function (AUC_{ins}/AUC_{glu}) will be estimated.(53)

Continuous glucose monitoring

The participants wear a continuous glucose monitor (CGM, FreeStyle Libre 1, Abbott Diabetes Care, Norway) for 14 days at baseline (7 days pre-intervention followed by the first 7 days of intervention/control), and for 14 days starting at 8 weeks from baseline. From these measurements, we will determine 24-hour glycaemic control, 3-hour postprandial glucose levels (AUC) for the first meal of the day, and nocturnal glycaemic control. The screens of the CGM readers are taped over to avoid lifestyle changes based on the participants' glucose levels. We also plan to explore other CGM data that can predict glycaemic control during pregnancy, using machine learning.

Height, weight, body composition, BMI, and waist circumference

Height is measured with the participants standing without shoes using a standard stadiometer. Weight and body composition are estimated in the morning after overnight fasting using bioelectrical impedance analysis (Inbody 720, Biospace CO, Korea), with participants wearing light clothes and standing barefoot. BMI is calculated as weight in kilograms divided by the square value of height in metres (kg/m^2). To account for the increase in fat-free mass hydration as pregnancy progresses, we will use a regression equation that estimates fat-free mass density as a function of gestational age.(54) Waist circumference is measured using a measuring tape at the level of the belly button with the participant standing.

Cardiorespiratory fitness

We measure peak oxygen uptake (VO_{2peak}) using indirect calorimetry (Metalyzer II, Cortex, Germany), using an individualised treadmill protocol in which the participants walk or run until volitional exhaustion. The test starts after a 10-minute warm-up. The speed or inclination is increased every 1 – 2 minutes, by 0.5 – 1.0 km/hour or 1% – 2%. VO_{2peak} is determined as the average of the three highest consecutive 10 seconds measured and will be reported as both absolute (L/min) and relative (mL/min/kg) values. We record HR throughout the exercise tests and use the peak HR recorded during the test as an estimate of the HR maximum.(55)

Blood pressure and resting heart rate

We use an automatic blood pressure device (Welch Allyn, Germany) to measure blood pressure (diastolic and systolic, in mmHg) and resting HR (beats per minute, bpm) on the participants' left arm after they have rested in a seated position for 15 minutes. We will report the average of three measurements taken at 1-minute intervals.

Physical activity, diet, and sleep

We use activity monitors to estimate physical activity levels, energy expenditure, and sleep duration. All participants wear Sensewear Armbands (BodyMedia, Pennsylvania, USA) for 14 days at baseline (7 days pre-intervention followed by the first 7 days of intervention/control), and the participants in the intervention group wear Amazfit GTS (Huami, China) smartwatches throughout the intervention. The smartwatch is connected to the Zepp app and shares PAI data with the research team via the Memento app. Participants register their diet in an online food diary (Fatsecret app) and record the time of first and last energy intake in the project handbook for 4 days (3 weekdays and 1 weekend day) every 8 weeks. They also complete questionnaires about physical activity, sleep quality, and psychological well-being every 8 weeks throughout the study period. We use the following questionnaires: 1) International Physical Activity Questionnaire,(56) 2) Pittsburgh Sleep Quality Index,(57) and 3) Psychological General Well-Being Index.(58) At baseline, the participants fill in the Horne-Östberg Morningsness-Eveningness Questionnaire.(59) We record medication and supplements, early miscarriages, abortions, and time to pregnancy and live birth. Additionally, expectant fathers are asked to complete questionnaires at baseline and every 8 weeks throughout the trial, including questions regarding their body weight, height, physical activity, and diet. These data will be used as co-variates in later analyses.

Neonatal and other outcomes

We obtain standard clinical neonatal outcomes from hospital birth records. Midwives at St. Olav's Hospital collect umbilical cord blood immediately after birth, prior to the delivery of the placenta. Placental tissues are collected from 1) around the base of the umbilical cord on the foetal side, 2) the periphery on the maternal side (full-thickness tissue), 3) the centre of the maternal side, also for storage in RNAlater solution (Invitrogen, Thermofisher scientific, Lithuania) and 4% formaldehyde solution, and 4) the periphery on the maternal side. The samples are put in 1.8 mL cryotubes and snap-frozen immediately in liquid nitrogen, before storage at -80°C for later analyses. The samples in RNAlater solution are stored at 4°C

overnight, followed by storage at -80°C for later analyses. The samples in 4% formaldehyde solution are stored under a fume hood at room temperature for 48 hours before histology slide preparation in collaboration with the CMIC Histology Lab at NTNU.

Within 72 hours of birth, and at age 6-8 weeks, body composition of the newborn is estimated using bioimpedance (BioScan touch i8-nano, Maltron, UK). Additionally, an experienced paediatric cardiologist examines cardiac morphology, structure, and function in the newborn, using a Vivid E95 scanner (GE Vingmed Ultrasound, Horten, Norway) and a GE 6s, and M5s phased-array transducers (GE Healthcare, Milwaukee, WI). A full clinical echocardiography including conventional echocardiographic parameters as well as study images with a focus on measurement of systolic and diastolic myocardial function is performed. The scanner is equipped with research software enabling high frame rate echocardiography to study cardiac flow and tissue properties as described previously.(60-62) A corresponding group of neonates (N = 30), from mothers with no known increased risk of GDM and BMI in the normal range (18.6-24.9 kg/m^2) will be used for comparison.

Adherence

We record adherence to TRE as the average daily time-window for energy intake for 4 days every 8 weeks. Additionally, we categorise participants as adherent if they report a ≤ 10-hour time-window for energy intake on ≥ 2 of these 4 days. Adherence to exercise is recorded as the number of PAI points the participants get per rolling 7 days. To ensure compliance and maintain adherence, we send text messages to all participants as reminders to complete questionnaires and dietary reporting. We also announce friendly competitions such as "Who can keep 100 weekly PAI points or more for a whole month?" in a Facebook group for the participants. The data are only accessible to the researchers and a gift card is awarded to the winners.

Modifications to the protocol after trial commencement

Since June 2021, we invite the participants to participate in a follow-up study after delivery in which we collect infant faecal samples (immediately after birth, at 6 weeks, and 6 months), maternal faecal samples (at 6 weeks and 6 months), and breast milk (at 6 weeks and 6 months). These samples are stored at -80°C for later analyses. Additionally, we started to offer supervised exercise training sessions to the participants in the intervention group. From November 2022, we started sending invitations using eFORSK (electronic form-based data collection, developed by Central Norway Regional Health Authority) and added 'Bariatric

surgery' to the exclusion criteria. 'Any other reason which according to the researchers makes the potential participant ineligible' to undergo either or both interventions (e.g., traumatic foot injury, anorexia/bulimia, etc.) was also added to the exclusion criteria in November 2022. From December 2022, we removed 'Planned assisted fertilisation with female factor reason' from the exclusion criteria. In addition, we changed the maximum time before pregnancy from 12 months to 6 months to allow for the trial to be terminated in time for us to analyse the data within the project period. In March 2023, the required number of total participants was reduced from 260 to 200 based on the revised calculation as described in the sample size calculation, with additional specification of stopping before 200 if we had sufficient pregnant participants for the primary outcome measure. In June 2023, we changed from Amazfit GTS to Polar Ignite 2 (Polar, Finland) smartwatch and from Zepp and Memento to Polar Flow and Mia app.

Sample size calculation

The primary outcome of this study is glucose tolerance (after a 2-hour OGTT) in gestational week 28. The HAPO study results(63) indicate strong, continuous associations of maternal glucose levels, even below the diagnostic level of GDM with adverse maternal and offspring outcomes. Based on the increasing risk of adverse maternal and offspring outcomes across 2-hour plasma glucose categories with a change of ~1 mmol/L, we consider a difference of 1 mmol/L in 2-hour plasma glucose after OGTT between the intervention and control group as clinically relevant. We also used the observed standard deviation (1 SD = 1.3 mmol/L) in 2-hour plasma glucose after OGTT in the HAPO study for the sample size calculations. Calculation of the sample size for a two-sided t-test to detect a difference of 1 mmol/L between the groups, using an SD of 1.3 mmol/L, a power of 0.90, and a significance level of 0.05, yields 37 participants in each group in gestational week 28. To allow for an expected exclusion from the study due to not conceiving within the study period (~50%)(64) yielding 74 per group, further drop-out during the study period (10-20%), yielding 93 per group, and to increase statistical power for secondary analyses, we initially wanted to include 260 participants in the trial.

However, we terminated the inclusion of new participants at 167 participants since we had reached 47 participants in each group who were pregnant in gestational week 12. With this number of participants, we foresee that we will have at least 37 participants in each group in gestational week 28, allowing for up to 20% dropout during pregnancy. We expect more participants who are already included to become pregnant in the upcoming period, which will increase the number of pregnant participants.

Statistical analyses

The primary analysis will be done according to the 'intention to treat' principle, using all obtained data irrespective of participant adherence to the intervention and completeness of outcome measures. We plan to use linear mixed models (LMMs) to compare primary and secondary continuous outcome measures between groups, with time and group x time interactions as fixed effects variables, and subject as random factor.(65) Since no systematic baseline differences between the groups are expected in RCTs, means at baseline will be constrained to be equal in the LMMs. We will report estimates with corresponding 95% confidence intervals and p-values for differences between the intervention group and the control group. We will check the normality of residuals by visual inspection of QQ-plots and bootstrapping, transformations or non-parametric methods will be used in cases of non-normal model residuals. For the primary outcome measure, we will consider a p-value < 0.05 as statistically significant. For the secondary outcome measures, p-values < 0.01 will be considered statistically significant, due to multiple comparisons, and these analyses will be explorative. We will also perform per-protocol analyses: Participants with an average of ≥ 75 PAI per rolling week and adherence to TRE (as per definition above) during the preconception period will be included in the per-protocol analyses for all outcome measures. We will report additional results from all participants who were included in the trial, from the preconception period, irrespective of whether they became pregnant or not during the study period.

Blinding

The study is not blinded as it is difficult to blind participants and treatment providers to behavioural intervention. However, baseline assessments are undertaken before randomisation.

Monitoring

We do not expect any adverse effects in this study. If pregnant women are worried about foetal safety during exercise, we have experienced personnel available in the research group to monitor foetal heart rate during exercise sessions. The investigators are responsible for the documentation of any adverse or serious adverse events in the Case Report Form and the Serious Adverse Events Report Form, respectively. Participants are advised to contact the investigators if they have any unusual symptoms. All serious adverse events will be reported to the sponsor (NTNU) within 24 hours after the investigators have been informed of the event.

Patient and public involvement

We have involved users in the planning of the study and will continue involving them in the implementation and dissemination. In the planning phase, we arranged a 1-hour digital workshop with users (reproductive-aged females with overweight/obesity), where we encouraged the audience to ask questions and give us feedback about relevant topics or issues related to participation. Regarding the challenges of long-term adherence, we use these feedbacks to find ways to incorporate exercise training and TRE into daily life.

Ethics and dissemination

The Regional Committees for Medical and Health Research Ethics in Norway approved the study (REK, reference number 143756). The comparative analysis of the neonatal echocardiography data from this study with a corresponding group of neonates from mothers with normal BMI and no increased risk of GDM is also approved (REK reference number 67584). The work is conducted according to the Declaration of Helsinki and the ICMJE Recommendations for authorship. The participants sign an informed written consent before participating in the study and can at any time withdraw from the study without further explanation. Study specific ID numbers are used as participants' identification. We ensure data quality by double data entry into an electronic CRF and treat the collected data following the General Data Protection Regulation. All protocol modifications are reported to REK. Upon completion of the study and finalization of the study report, we will submit the results for publication and/or in a publicly accessible database of clinical study results after anonymizing the data.

Discussion

Based on a thorough literature search, the BTB study will be the first RCT to investigate the combined effects of TRE and exercise training, initiated before and continued throughout pregnancy, on cardiometabolic parameters in people at risk of GDM and their infants. We hypothesise that the combination of these two lifestyle interventions will induce an additive and clinically relevant improvement in maternal glucose tolerance, and potentially also in our secondary outcome measures in mothers and infants. As such, the initiation of lifestyle modification before pregnancy will provide a better platform for improved adherence and health outcomes, potentially breaking the intergenerational cycle of cardiometabolic disorders, and thereby reducing the risk of diabetes for future generations.

So far, there is limited data on the combination of TRE and exercise training in humans. Haganes and colleagues reported that the combination of TRE and HIIT in females with a BMI of ≥ 27 kg/m^2 for 7 weeks significantly reduced HbA1c compared with a no-intervention control group and lead to greater losses in body weight, fat mass, and visceral fat area compared with either intervention alone.(26) Since the duration of the intervention is much longer in the BTB trial, there may be lower adherence to one or both intervention strategies. The possible reasons for lower adherence are that the participants may lack motivation for such a long time, find the intervention program boring and/or difficult, or develop physical symptoms that may hinder the participant to adhere to the intervention (especially during pregnancy). Combining motivational human interaction with digital interventions can increase engagement and the effectiveness of behaviour change interventions.(66) To improve adherence throughout the study period, we offer an individualised exercise regimen and provide encouragement, support, and monitor the participants regularly, both in person and over the phone.

The incidence and risk of obesity, insulin resistance, and GDM persists through generations.(67) To disrupt this intergenerational cycle, it is urgently necessary to develop and implement effective and practical lifestyle intervention strategies which can improve the cardiometabolic health outcomes of both mother and offspring. If the preconception lifestyle interventions implemented in this study lead to favourable outcomes and prove to be feasible and effective, it can pave the way for novel interventions that can be adopted in clinical practice during the preconception period, especially among those who are at risk of developing GDM.

Author contributions

MAJS drafted the manuscript. TM, SAN, KÅS, ACI, TF, and SLF conceived and contributed to the design of the study and the plan for analyses. GR, MAJS, and HSS coordinate the study, perform measurements on test days, monitor participants, and supervise the exercise training. SAN performs the echocardiogram on the newborns. All authors provided feedback and approved the final manuscript.

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Facility, St. Olavs Hospital. We would also like to thank the midwives at the Women and Children's Centre, St. Olavs Hospital for the collection of samples related to birth. eFORSK, a stand-alone form-based information and communications technology solution for electronic collection of data, developed by Central Norway Regional Health Authority is used for sending invitations to the study.

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Competing interests

The authors declare that they have no competing interests.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: 2021. Available at: https://www.diabetesatlas.org
2. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. Int J Mol Sci. 2018;19(11):3342.
3. Yu Y, Soohoo M, Sørensen HT, Li J, Arah OA. Gestational Diabetes Mellitus and the Risks of Overall and Type-Specific Cardiovascular Diseases: A Population- and Sibling-Matched Cohort Study. Diabetes Care. 2021;45(1):151-9.
4. Moholdt T, Hawley JA. Maternal Lifestyle Interventions: Targeting Preconception Health. Trends Endocrinol Metab. 2020;31(8):561-9.
5. Vinter CA, Tanvig MH, Christensen MH, Ovesen PG, Jørgensen JS, Andersen MS, et al. Lifestyle Intervention in Danish Obese Pregnant Women With Early Gestational Diabetes Mellitus According to WHO 2013 Criteria Does Not Change Pregnancy Outcomes: Results From the LiP (Lifestyle in Pregnancy) Study. Diabetes Care. 2018;41(10):2079-85.
6. Nyrnes SA, Garnæs KK, Salvesen Ø, Timilsina AS, Moholdt T, Ingul CB. Cardiac function in newborns of obese women and the effect of exercise during pregnancy. A randomized controlled trial. PLoS One. 2018;13(6):e0197334.
7. Gluckman PD, Hanson MA, Buklijas T. A conceptual framework for the developmental origins of health and disease. J Dev Orig Health Dis. 2010;1(1):6-18.
8. Cai G-j, Sun X-x, Zhang L, Hong Q. Association between maternal body mass index and congenital heart defects in offspring: a systematic review. American Journal of Obstetrics and Gynecology. 2014;211(2):91-117.
9. Petropoulos A. Congenital heart disease and maternal diabetes mellitus. Евразийский Журнал Клинических Наук. 2018;1(3):32-9.
10. Lowe WL, Lowe LP, Kuang A, Catalano PM, Nodzenski M, Talbot O, et al. Maternal glucose levels during pregnancy and childhood adiposity in the Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study. Diabetologia. 2019;62(4):598-610.
11. Tam WH, Ma RCW, Ozaki R, Li AM, Chan MHM, Yuen LY, et al. In Utero Exposure to Maternal Hyperglycemia Increases Childhood Cardiometabolic Risk in Offspring. Diabetes Care. 2017;40(5):679-86.
12. Grunnet LG, Hansen S, Hjort L, Madsen CM, Kampmann FB, Thuesen ACB, et al. Adiposity, Dysmetabolic Traits, and Earlier Onset of Female Puberty in Adolescent Offspring of Women With Gestational Diabetes Mellitus: A Clinical Study Within the Danish National Birth Cohort. Diabetes Care. 2017;40(12):1746-55.
13. Management of Diabetes in Pregnancy. Diabetes Care. 2017;40(Suppl 1):S114-s9.
14. Gjestland K, Bø K, Owe KM, Eberhard-Gran M. Do pregnant women follow exercise guidelines? Prevalence data among 3482 women, and prediction of low-back pain, pelvic girdle pain and depression. Br J Sports Med. 2013;47(8):515-20.
15. Zeanah M, Schlosser SP. Adherence to ACOG Guidelines on Exercise During Pregnancy: Effect on Pregnancy Outcome. Journal of Obstetric, Gynecologic & Neonatal Nursing. 1993;22(4):329-35.
16. Tieu J, Shepherd E, Middleton P, Crowther CA. Dietary advice interventions in pregnancy for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2017;1(1):Cd006674.
17. 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(8):Cd012327.
18. Shepherd E, Gomersall JC, Tieu J, Han S, Crowther CA, Middleton P. Combined diet and exercise interventions for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2017;11(11):Cd010443.
19. Lane M, Robker RL, Robertson SA. Parenting from before conception. Science. 2014;345(6198):756-60.

20. Stephenson J, Heslehurst N, Hall J, Schoenaker D, Hutchinson J, Cade JE, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. Lancet. 2018;391(10132):1830-41.
21. Rönö K, Stach-Lempinen B, Eriksson JG, Pöyhönen-Alho M, Klemetti MM, Roine RP, et al. Prevention of gestational diabetes with a prepregnancy lifestyle intervention - findings from a randomized controlled trial. Int J Womens Health. 2018;10:493-501.
22. Raab R, Michel S, Günther J, Hoffmann J, Stecher L, Hauner H. Associations between lifestyle interventions during pregnancy and childhood weight and growth: a systematic review and meta-analysis. Int J Behav Nutr Phys Act. 2021;18(1):8-.
23. Sparks JR, Ghildayal N, Hivert M-F, Redman LM. Lifestyle interventions in pregnancy targeting GDM prevention: looking ahead to precision medicine. Diabetologia. 2022;65(11):1814-24.
24. Szumilewicz A S-RR, Worska A, Piernicka M, Yu H, Pajaujiene S, et al. How to HIIT while pregnant? The protocol characteristics and effects of high intensity interval training implemented during pregnancy – A systematic review. Baltic Journal of Health and Physical Activity. 2022;14(1):1-16.
25. Flanagan EW, Kebbe M, Sparks JR, Redman LM. Assessment of Eating Behaviors and Perceptions of Time-Restricted Eating During Pregnancy. J Nutr. 2022;152(2):475-83.
26. Haganes KL, Silva CP, Eyjólfsdóttir SK, Steen S, Grindberg M, Lydersen S, et al. Time-restricted eating and exercise training improve HbA1c and body composition in women with overweight/obesity: A randomized controlled trial. Cell Metab. 2022;34(10):1457-71.e4.
27. Termannsen AD, Varming A, van Elst C, Bjerre N, Nørgaard O, Hempler NF, et al. Feasibility of time-restricted eating in individuals with overweight, obesity, prediabetes, or type 2 diabetes: A systematic scoping review. Obesity (Silver Spring). 2023;31(6):1463-85.
28. Ravussin E, Beyl RA, Poggiogalle E, Hsia DS, Peterson CM. Early Time-Restricted Feeding Reduces Appetite and Increases Fat Oxidation But Does Not Affect Energy Expenditure in Humans. Obesity (Silver Spring). 2019;27(8):1244-54.
29. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metab. 2018;27(6):1212-21.e3.
30. Chair SY, Cai H, Cao X, Qin Y, Cheng HY, Ng MT. Intermittent Fasting in Weight Loss and Cardiometabolic Risk Reduction: A Randomized Controlled Trial. J Nurs Res. 2022;30(1):e185.
31. Hutchison AT, Regmi P, Manoogian ENC, Fleischer JG, Wittert GA, Panda S, et al. Time-Restricted Feeding Improves Glucose Tolerance in Men at Risk for Type 2 Diabetes: A Randomized Crossover Trial. Obesity (Silver Spring). 2019;27(5):724-32.
32. Jamshed H, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Clock, Aging, and Autophagy in Humans. Nutrients. 2019;11(6).
33. Wilkinson MJ, Manoogian ENC, Zadourian A, Lo H, Fakhouri S, Shoghi A, et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell Metab. 2020;31(1):92-104.e5.
34. Andriessen C, Fealy CE, Veelen A, van Beek SMM, Roumans KHM, Connell NJ, et al. Three weeks of time-restricted eating improves glucose homeostasis in adults with type 2 diabetes but does not improve insulin sensitivity: a randomised crossover trial. Diabetologia. 2022;65(10):1710-20.
35. Che T, Yan C, Tian D, Zhang X, Liu X, Wu Z. Time-restricted feeding improves blood glucose and insulin sensitivity in overweight patients with type 2 diabetes: a randomised controlled trial. Nutr Metab (Lond). 2021;18(1):88.
36. Parr EB, Devlin BL, Lim KHC, Moresi LNZ, Geils C, Brennan L, et al. Time-Restricted Eating as a Nutrition Strategy for Individuals with Type 2 Diabetes: A Feasibility Study. Nutrients. 2020;12(11).
37. Loy SL, Chan JK, Wee PH, Colega MT, Cheung YB, Godfrey KM, et al. Maternal Circadian Eating Time and Frequency Are Associated with Blood Glucose Concentrations during Pregnancy. J Nutr. 2017;147(1):70-7.

38. Wowdzia JB, Hazell TJ, Davenport MH. Glycemic response to acute high-intensity interval versus moderate-intensity continuous exercise during pregnancy. Physiol Rep. 2022;10(18):e15454.
39. Szumilewicz A, Santos-Rocha R, Worska A, Piernicka M, Yu H, Pajaujiene S, et al. How to HIIT while pregnant? The protocol characteristics and effects of high intensity interval training implemented during pregnancy: A systematic review. Baltic journal of health and physical activity. 2022;14(1):1-16.
40. Yu H, Santos-Rocha R, Radzimiński Ł, Jastrzębski Z, Bonisławska I, Szwarc A, et al. Effects of 8-Week Online, Supervised High-Intensity Interval Training on the Parameters Related to the Anaerobic Threshold, Body Weight, and Body Composition during Pregnancy: A Randomized Controlled Trial. Nutrients. 2022;14(24):5279.
41. Wowdzia J, Hazell T, Vanden Berg E, Labrecque L, Brassard P, Davenport M. Maternal and Fetal Cardiovascular Responses to Acute High-Intensity Interval and Moderate-Intensity Continuous Training Exercise During Pregnancy: A Randomized Crossover Trial. Sports Medicine. 2023:1-15.
42. Gaston A, Cramp A. Exercise during pregnancy: a review of patterns and determinants. J Sci Med Sport. 2011;14(4):299-305.
43. Tobias DK, Zhang C, Chavarro J, Bowers K, Rich-Edwards J, Rosner B, et al. Prepregnancy adherence to dietary patterns and lower risk of gestational diabetes mellitus. The American Journal of Clinical Nutrition. 2012;96(2):289-95.
44. Gao X, Zheng Q, Jiang X, Chen X, Liao Y, Pan Y. The effect of diet quality on the risk of developing gestational diabetes mellitus: A systematic review and meta-analysis. Frontiers in Public Health. 2023;10.
45. Yong HY, Mohd Shariff Z, Mohd Yusof BN, Rejali Z, Appannah G, Bindels J, et al. The association between dietary patterns before and in early pregnancy and the risk of gestational diabetes mellitus (GDM): Data from the Malaysian SECOST cohort. PLoS One. 2020;15(1):e0227246.
46. Chan AW, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. Bmj. 2013;346:e7586.
47. Nes BM, Gutvik CR, Lavie CJ, Nauman J, Wisløff U. Personalized Activity Intelligence (PAI) for Prevention of Cardiovascular Disease and Promotion of Physical Activity. Am J Med. 2017;130(3):328-36.
48. Zisko N, Skjerve KN, Tari AR, Sandbakk SB, Wisløff U, Nes BM, et al. Personal Activity Intelligence (PAI), Sedentary Behavior and Cardiovascular Risk Factor Clustering - the HUNT Study. Prog Cardiovasc Dis. 2017;60(1):89-95.
49. World Health O. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva: World Health Organization; 2013 2013. Contract No.: WHO/NMH/MND/13.2.
50. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
51. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract. 2000;47(3):177-84.
52. Phillips DI, Clark PM, Hales CN, Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med. 1994;11(3):286-92.
53. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295-301.
54. Most J, Marlatt KL, Altazan AD, Redman LM. Advances in assessing body composition during pregnancy. Eur J Clin Nutr. 2018;72(5):645-56.
55. Berglund IJ, Sørås SE, Relling BE, Lundgren KM, Kiel IA, Moholdt T. The relationship between maximum heart rate in a cardiorespiratory fitness test and in a maximum heart rate test. J Sci Med Sport. 2019;22(5):607-10.

56. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.
57. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.
58. Grossi E, Compare A. Psychological General Well-Being Index (PGWB). In: Michalos AC, editor. Encyclopedia of Quality of Life and Well-Being Research. Dordrecht: Springer Netherlands; 2014. p. 5152-6.
59. Adan A, Almirall H. Horne & Östberg Morningness–Eveningness questionnaire: A reduced scale. Personality and Individual Differences. 1991;12:241-53.
60. Nyrnes SA, Fadnes S, Wigen MS, Mertens L, Lovstakken L. Blood Speckle-Tracking Based on High-Frame Rate Ultrasound Imaging in Pediatric Cardiology. J Am Soc Echocardiogr. 2020;33(4):493-503.e5.
61. Sørensen K, Fadnes S, Mertens L, Henry M, Segers P, Løvstakken L, et al. Assessment of Early Diastolic Intraventricular Pressure Difference in Children by Blood Speckle-Tracking Echocardiography. J Am Soc Echocardiogr. 2023.
62. Salles S, Lovstakken L, Aase SA, Bjastad TG, Torp H. Clutter Filter Wave Imaging. IEEE Trans Ultrason Ferroelectr Freq Control. 2019;66(9):1444-52.
63. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991-2002.
64. Mikkelsen EM, Riis AH, Wise LA, Hatch EE, Rothman KJ, Sørensen HT. Pre-gravid oral contraceptive use and time to pregnancy: a Danish prospective cohort study. Hum Reprod. 2013;28(5):1398-405.
65. J T, L B, T H, J R, M W, M H. Different ways to estimate treatment effects in randomised controlled trials. Contemp Clin Trials Commun. 2018;10:80-5.
66. Barker M, Dombrowski SU, Colbourn T, Fall CHD, Kriznik NM, Lawrence WT, et al. Intervention strategies to improve nutrition and health behaviours before conception. Lancet (London, England). 2018;391(10132):1853-64.
67. Ma RCW, Popkin BM. Intergenerational diabetes and obesity—A cycle to break? PLOS Medicine. 2017;14(10):e1002415.

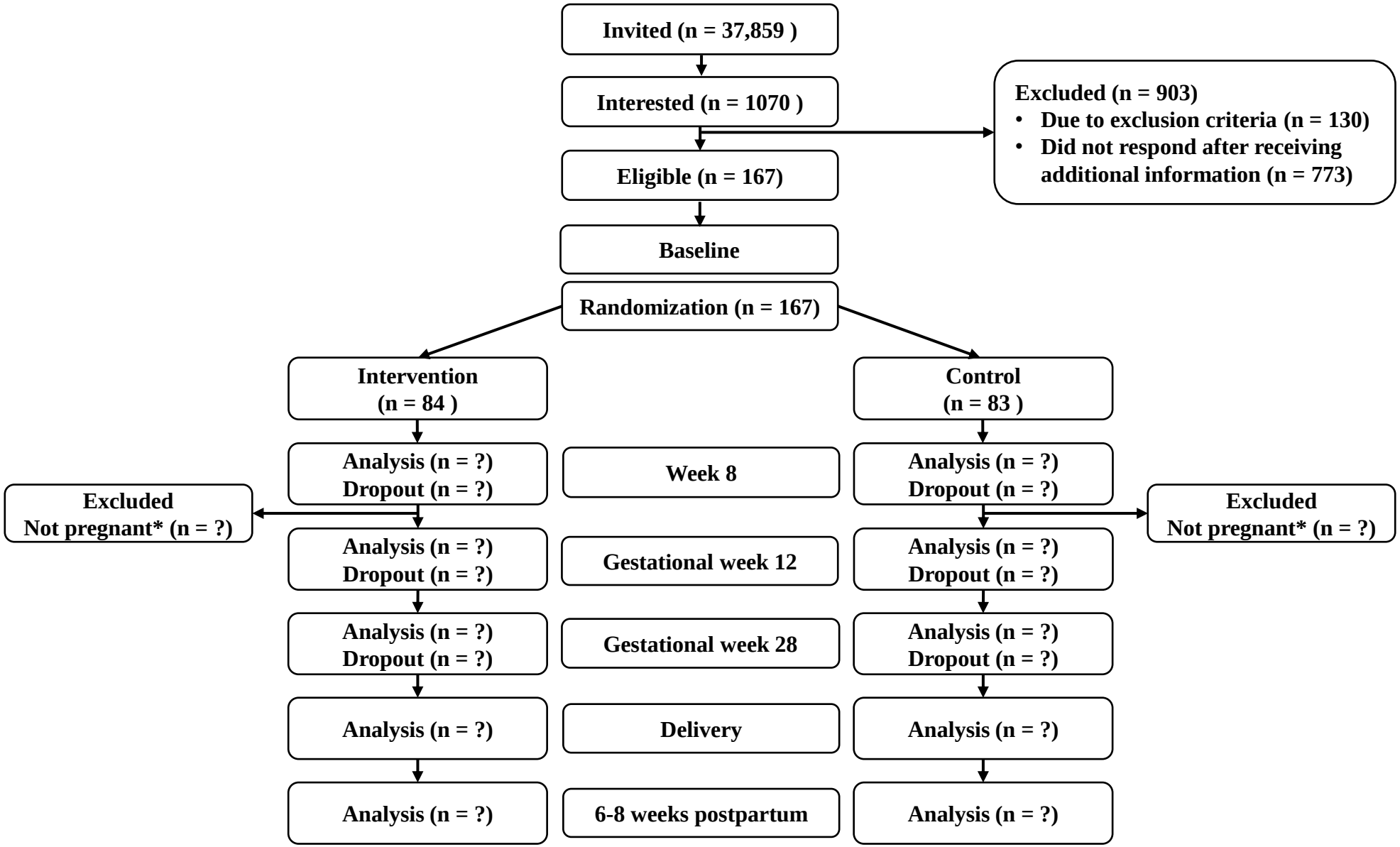
FIGURE LEGENDS

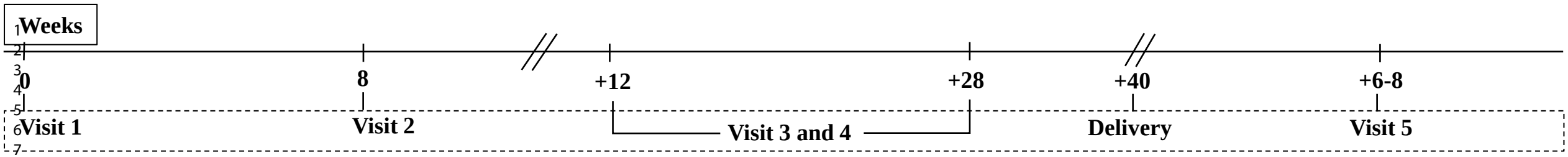
Figure 1. Consort flow diagram of the BEFORE THE BEGINNING trial

(Ongoing study: status 25.09.2020 – 17.07.2023)

* If the participants are not pregnant within 12 months of inclusion, they are excluded from the study. From December 2022, the time-window for exclusion if not pregnant was reduced from 12 to 6 months.

Figure 2. Overview of time-points for assessments in the trial.


Invited (n = 37,859)
Interested (n = 1070)
Excluded (n = 903)
• Due to exclusion criteria (n = 130)
• Did not respond after receiving additional information (n = 773)
Eligible (n = 167)
Baseline
Randomization (n = 167)
Intervention (n = 84)
Control (n = 83)
Analysis (n = ?) Dropout (n = ?)
Week 8
Analysis (n = ?) Dropout (n = ?)
Excluded Not pregnant* (n = ?)
Excluded Not pregnant* (n = ?)
Analysis (n = ?) Dropout (n = ?)
Gestational week 12
Analysis (n = ?) Dropout (n = ?)
Analysis (n = ?) Dropout (n = ?)
Gestational week 28
Analysis (n = ?) Dropout (n = ?)
Analysis (n = ?)
Delivery
Analysis (n = ?)
Analysis (n = ?)
6-8 weeks postpartum
Analysis (n = ?)



Every 8 weeks following visit 1: Food diary (4-day registration), hunger/appetite registration, time-window of energy intake, questionnaires (physical activity, sleep, psychological well-being).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not	2

		yet registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	n/a, see appendix
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 15
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	n/a, see appendix
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
Roles and responsibilities:	#5d	Composition, roles, and responsibilities of the coordinating centre, steering	n/a

committees		committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5,6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6 (Box 1)
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a, see appendix
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions:	#11d	Relevant concomitant care and	7

concomitant care		interventions that are permitted or prohibited during the trial	
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7 (Figure 1) 7 (Figure 2)
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care	13

		providers, outcome assessors, data analysts), and how	
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a: no blinding
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate	12

		from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of	n/a, see appendix

		whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a, see appendix
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board	14

		(REC / IRB) approval	
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	11-12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	14
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Data access	#29	Statement of who will have access to the	14

		final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See Appendix
Biological specimens	#33	Plans for collection, laboratory	8ssss-11

evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable