PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Sujan, Md Abu Jafar; Skarstad, Hanna; Rosvold, Guro; Fougner, Stine; Nyrnes, Siri; Iversen, Ann-Charlotte; Follestad, Turid; Salvesen, Kjell; Moholdt, Trine

VERSION 1 – REVIEW

REVIEWER	Yanting Wu Obstetrics and Gynecology Hospital of Fudan University, Obstetrics and Gynecology Hospital, Institute of Reproduction and Development,Fudan University
REVIEW RETURNED	18-Apr-2023

GENERAL COMMENTS	Summary: The main objective of this study is to evaluate the effect of the combination of Time-restricted eating (TRE) and High-intensity interval training (HIT) commencing before pregnancy on maternal glucose tolerance in pregnancy. A single-centre RCT involving a minimum of 200 women is designed. It is hypothesized that this novel diet-exercise strategy would reduce the incidence of gestational diabetes (GDM) in high-risk populations. Strengths: 1) This is the first RCT to investigate the combined effects of TRE and HIT on maternal glucose levels throughout pregnancy. 2)This study commenced interventions Limited data on preconception interventions on GDM were reported. 3)Multiple strategies and standards have been developed for assessing and enhancing participants' adherence to the intervention. 4) Various questionnaires concerning sleep quality, psychological well-being and father's background was also collected. 5) Children were evaluated comprehensively including body composition, cardiac function, etc. Questions: 1)The primary outcome of this study is plasma glucose concentration obtained 2 hours after a 75g oral glucose tolerance test (OGTT). Could you please provide specific reasons for choosing 2-h glucose level as the primary outcome? 2)The sample size calculation was based on a difference of 1.0mmol/L in glucose level, which seems to be a relatively dramatic change in clinical practice. Thus, it is worrying whether the sample size of 200 women would be powerful enough for evaluation since lifestyle intervention studies tend to have a higher dropout rate than

	others as well. 3)Participants were asked to wear a continuous glucose monitor (CGM) during the study. The CGMs are "masked" for the participants. Could you please provide further details on how it works exactly for glucose monitoring？ 4)How does "friendly competition" among participants work to increase adherence on the internet? Do they have a ranking list anonymously? 5)It is said that if pregnant women were worried about fetal safety during exercise, they could perform it in the hospital under medical surveillance. How often could they achieve and is it practical？ 6)Has the safety of HIT in this study been evaluated among pregnant women, especially in those who went through IVF in other research before? 7)Any prespecified subgroup analyses stated in the manuscript？ 8)If diet registration in an online food diary is required every 8 weeks, how to assess their adherence to TRE (≤10-hour time window） every week？ 9)Pregnant women are less tolerant of hunger and will develop excessive ketones if starving, which could have adverse effects on infants. And if no energy intake after 19:00, nocturnal hypoglycemia might occur. Could you please specify how to deal with this kind of situation in your study? 10)It is noticed that several equipments and applications have been used in this study, including CGM, Sensewear Armbands(BodyMedia) for activity estimation, Amazfit GTS smartwatches connected to the Zepp app, Memento app for PAI data, Fatsecret app for diet registration. Will it be difficult and time-consuming to explain and teach participants to use all these equipments during recruitment？ Do they have to wear all of them throughout the study?

REVIEWER	Kristiina Rönö Helsinki University Central Hospital Department of Obstetrics and Gynaecology
REVIEW RETURNED	14-May-2023

GENERAL COMMENTS	The authors present impressive and ambitious protocol for ongoing pre-pregnancy lifestyle intervention study. They have taken in to account the problems that have arisen from the few pre-pregnancy lifestyle interventions up to date. The methods are adequately described including modifications to the protocol after trial commencement. One thing that need clarification are the dates of the study period. When was the study commenced? participant included? When will the recruitment end or has it already ended? The dates are presented in the protocol synopsis, but it would be helpful if they would be included in the manuscript.

	Is 158 the final number of participants? Or should the flow chart figure legend be clarified with addition of the time period (between xxx and March 3rd 2023?)?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Yanting Wu, Obstetrics and Gynecology Hospital of Fudan University, International Peace Maternity and Child Health Hospital

Comments to the Author:

Summary: The main objective of this study is to evaluate the effect of the combination of Time-restricted eating (TRE) and High-intensity interval training (HIT) commencing before pregnancy on maternal glucose tolerance in pregnancy. A single-centre RCT involving a minimum of 200 women is designed. It is hypothesized that this novel diet-exercise strategy would reduce the incidence of gestational diabetes (GDM) in high-risk populations.

Strengths: 1) This is the first RCT to investigate the combined effects of TRE and HIT on maternal glucose levels throughout pregnancy.
2)This study commenced interventions Limited data on preconception interventions on GDM were reported.
3)Multiple strategies and standards have been developed for assessing and enhancing participants' adherence to the intervention.
4) Various questionnaires concerning sleep quality, psychological well-being and father's background was also collected.
5) Children were evaluated comprehensively including body composition, cardiac function, etc.

Questions:
1)The primary outcome of this study is plasma glucose concentration obtained 2 hours after a 75g oral glucose tolerance test (OGTT). Could you please provide specific reasons for choosing 2-h glucose level as the primary outcome?

- The primary hypothesis for the BEFORE THE BEGINNING (BTB) trial is that the participants allocated to the intervention group (TRE and exercise) will have improved maternal glucose tolerance in gestational week 28, compared with participants in the control group.
Intervention studies on women at risk of gestational diabetes mellitus (GDM) usually include GDM diagnosis as a primary outcome but that doesn't reflect the changes in glucose levels in women without GDM. The alternatives for the primary outcome in the this study were fasting or 2-hour plasma glucose after OGTT in gestation week 28 since these variables are routinely used in GDM diagnosis. There is not enough knowledge to know which one of the two is the better choice for the primary outcome. Therefore, we chose to use 2-hour plasma glucose after OGTT as the primary outcome and basis for sample size calculation based on the glucose categories used in the HAPO study (revised in the manuscript, page 12).

2)The sample size calculation was based on a difference of 1.0mmol/L in glucose level, which seems to be a relatively dramatic change in clinical practice. Thus, it is worrying whether the sample size of 200 women would be powerful enough for evaluation since lifestyle intervention studies tend to have a higher dropout rate than others as well.

- The explanation is revised in the manuscript. Since the original submission, we have ended inclusion of new participants. An explaination of this is also added (page 12).

The HAPO study results (Metzger BE et al., N Engl J Med 2008) indicate strong, continuous associations of maternal glucose levels, even below the diagnostic level of GDM with adverse maternal and offspring outcomes. The risk of adverse maternal and offspring outcomes increased

with increasing 2-hour plasma glucose around gestational week 28 categorized by a change of ~1 mmol/L. Since the published data on finding the clinically significant difference are limited, we chose to use this difference of 1 mmol/L in 2-hour plasma glucose after OGTT between the intervention and control group. We also used the observed 1 SD (1.3 mmol/L) in 2-hour plasma glucose after OGTT in the HAPO study for the sample size calculations.
We have a power of 0.90 to detect such a difference between groups if we have 37 participants in each group (74 in total) with data for the primary outcome measure in gestational week 28. We have taken into account up to 20% dropout during the study period, in addition to exclusions because some participants will not become pregnant. We have currently 101 pregnant participants in the study, out of 167 included, and there are still a few of the included participants who may fall pregnant within the next months, meaning that we expect to have the statistical power to detect what are considered clinically meaningful difference between groups.

3)Participants were asked to wear a continuous glucose monitor (CGM) during the study. The CGMs are "masked" for the participants. Could you please provide further details on how it works exactly for glucose monitoring？

- The explanation is revised in the manuscript (page 9).
The screens of the CGM readers are taped over to avoid lifestyle changes based on the participants' glucose levels.

4)How does "friendly competition" among participants work to increase adherence on the internet? Do they have a ranking list anonymously?

- A short explanation is added in the manuscript (page 11).
This section is in the original protocol and was not detailed in the article because of word count restraints.
The participants are invited to join a Facebook group for the study, where we announce competitions such as "Who can keep 100 weekly PAI points or more for a whole month?" The winners are offered a gift card. There is no ranking list, and the participants cannot access other participants' data.

5)It is said that if pregnant women were worried about fetal safety during exercise, they could perform it in the hospital under medical surveillance. How often could they achieve and is it practical？

- A line about available foetal heart rate monitoring during exercise is added in the manuscript (monitoring, page 13).
This section is also in the main protocol and not detailed in the article due to word count restraints. So far, none of the participants has asked for such surveillance. If it is necessary, we have experienced personnel available in the research group to monitor fetal heart rate during exercise sessions.

6)Has the safety of HIT in this study been evaluated among pregnant women, especially in those who went through IVF in other research before?

- We have not mentioned the safety of HIIT during IVF-induced pregnancy in the manuscript. The data on the role of HIIT in IVF-induced pregnancy are also scarce. One study showed that moderate to high-intensity exercise during pregnancy with IVF has been shown to reduce the risk of adverse maternal and fetal outcomes.
(https://www.tandfonline.com/doi/full/10.1080/02701367.2019.1639601.)

-A systematic review of 12 pregnancy studies concluded that HIIT is safe during pregnancy with variable maternal and offspring benefits. We have added more details about the safety of HIIT in the introduction and provided additional references (page 4,5). HIIT protocols that we advise our

participants to follow during pregnancy are modified to avoid raising maternal heart rate above 85% of heart rate maximum (please see page 7).

7)Any prespecified subgroup analyses stated in the manuscript？

- We have described an additional per-protocol analysis (please see page 13).

8) If diet registration in an online food diary is required every 8 weeks, how to assess their adherence to TRE (≤10-hour time window） every week？

- We have revised the manuscript accordingly, please see page 8.
Thank you for making us aware of this shortcoming in our recording of adherence to the TRE. Since the duration of the study is very long (10-16 months), we decided to only record diet data and time-window for energy intake every eight weeks. As the participants in the intervention group are asked to follow TRE at least 5 days per week, and we register the time-window for energy intake only for 4 days (3 weekdays and 1 weekend day) every eight weeks, we will use the following definition of adherence to TRE: Participants who report eating windows ≤ 10-h at 2 or more days at each time-point for registration will be classified as adherent.

9)Pregnant women are less tolerant of hunger and will develop excessive ketones if starving, which could have adverse effects on infants. And if no energy intake after 19:00, nocturnal hypoglycemia might occur. Could you please specify how to deal with this kind of situation in your study?

- We have revised the TRE section in introduction in the manuscript (page 4) with new references.

Time-restricted eating (TRE) does not imply starvation. TRE is considered safe in T2DM patients without any risk of hypoglycemia (https://pubmed.ncbi.nlm.nih.gov/35684097/). While data on the effects of TRE in pregnancy are scarce, observational data suggest that longer night-time fasting can be beneficial for maternal glycaemic control.
Moreover, a systematic review of Ramadan fasting in pregnancy concluded that maternal fasting during Ramadan was associated with favourable short-term maternal outcomes (lower gestational weight gain and fasting blood glucose) but had no association with birth outcomes (birth weight, gestational age, odds of low birth weight or preterm delivery) (Chen YE et al., Nutrients 2023, PMID: 36771469).
We have so far not been notified about nocturnal hypoglycaemia from any of the participants and do not expect that this will happen. In the event of unusual symptoms, the participant will notify the investigators. The investigators will report the incident to the sponsor (NTNU) within 24 hours, advise the participant to seek medical attention, and assess the intervention and discontinue if necessary.

10) It is noticed that several equipments and applications have been used in this study, including CGM, Sensewear Armbands(BodyMedia) for activity estimation, Amazfit GTS smartwatches connected to the Zepp app, Memento app for PAI data, Fatsecret app for diet registration. Will it be difficult and time-consuming to explain and teach participants to use all these equipments during recruitment？ Do they have to wear all of them throughout the study?

- At the first visit, the study procedures, equipment, and applications were set up and explained to the participants. (Newly added, page 7)
The baseline visit takes about an hour, during which baseline measurements followed by randomization are done, and the study procedure and all equipment and applications are explained and set up. We provide help during the study period if the participants need this to maintain good adherence to the monitoring.

All participants wear the CGM (page 7) and the Sensewear Armband (page 10) for two weeks at baseline and the CGM again at eight weeks. The participants in the intervention group wear smartwatches during the entire study period (page 10).

Reviewer: 2
Dr. Kristiina Rönö, Helsinki University Central Hospital Department of Obstetrics and Gynaecology, Helsingin yliopisto
Comments to the Author:
The authors present impressive and ambitious protocol for ongoing pre-pregnancy lifestyle intervention study. They have taken in to account the problems that have arisen from the few pre-pregnancy lifestyle interventions up to date.

The methods are adequately described including modifications to the protocol after trial commencement.

One thing that need clarification are the dates of the study period. When was the study commenced? participant included? When will the recruitment end or has it already ended? The dates are presented in the protocol synopsis, but it would be helpful if they would be included in the manuscript.
- The study commenced in September 2020. The dates of the study period are updated in the manuscript (page 6). Since the original submission, we have ended the recruitment of participants. Additional information about the end of recruitment is also mentioned in the sample size section (page 12).

Is 158 the final number of participants? Or should the flow chart figure legend be clarified with addition of the time period (between xxx and March 3rd 2023?)?

- We have changed the flow chart figure and its legend to show an updated flow of participants in the trial. The final number of participants is 167.

VERSION 2 – REVIEW

REVIEWER	Yanting Wu Obstetrics and Gynecology Hospital of Fudan University, Obstetrics and Gynecology Hospital, Institute of Reproduction and Development,Fudan University
REVIEW RETURNED	18-Jul-2023

GENERAL COMMENTS	This PROTOCOL is detailed and I don't have any particular questions. But allow me to make a suggestion. Different studies have come to different conclusions about the prevention of GDM in high-risk groups or about the benefits of early treatment of GDM for mothers and babies; in my humble opinion, I am pessimistic about the prevention of GDM (and I hope I am wrong). GDM should not be viewed as a disease specific to pregnancy, but rather as a special period of time for type 2 diabetes. Any attempt to completely block the onset and progression of a chronic disease with just a few months of intervention should be treated with caution, and it is good to know that the duration of the intervention in this study was relatively long term. In particular, don't forget that GDM is also a genetically predisposed disease, which you covered in the background. Thus, secondary outcomes can be as numerous as possible, which helps the reader to understand the overall impact of interventions that begin early in the preconception period on maternal and infant outcomes, even if the primary outcome is negative.

	Another macro-level issue is that persistent interventions are highly dependent on the self-consciousness of the study participants, which then inevitably affects the extrapolation of this study's findings to real-world replication. Also, even if the mothers' blood glucose in mid to late pregnancy did decrease after the intervention compared to the control group, it is worth thinking about what this decrease actually means. Previous studies have shown a positive correlation between blood glucose values and increased maternal and infant outcomes with no clear threshold. However, a purely mathematical decrease in blood glucose values is still not convincing, so I would have expected a statistically significant difference in maternal and infant outcomes in the results of this project. Forgive me for being abrupt and presumptuous, but I think it would be interesting if the above issues were reflected in the discussion when the findings of this study are formally published.

VERSION 2 – AUTHOR RESPONSE

Dear Dr. Yanting Wu,

Thank you for your valuable input. We appreciate the questions and issues you mentioned regarding the limitations of the intervention for improving glucose tolerance/prevention of GDM and real-world application. As per your concluding remarks, we will reflect on these issues when we formally publish our study results. Therefore, if we understood your comment correctly, no further revisions to the protocol article are necessary.