PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Analysis of nutritional habits and levels of physical activity during pregnancy, birth and the postpartum period of women in health area no. 1, Toledo (Spain). The PrePaN study protocol.
AUTHORS	Muñóz Muñóz, Aránzazu; Cantarino, Sagrario; De Dios Aguado, María de las Mercedes; Velasco Abellán, Minerva; González López, Beatríz; Molina Gallego, Brigida; González Pascual, Juan Luis; Arias Palencia, Natalia María

VERSION 1 – REVIEW

REVIEWER	Stacey Ellery Hudson Institute of Medical Research Australia
REVIEW RETURNED	01-Apr-2019

GENERAL COMMENTS	This is an observational study, assessing the nutrition and physical activity habits of pregnant women in Toledo, Spain. The study intends to track women during the three trimesters of pregnancy as well as in the postpartum period. The primary outcome is to simply describe the habits. The secondary outcomes are to describe any relationships between nutrition and physical activity with pregnancy and birth outcomes. Major Comments • Title: The title does not make sense. I would remove "together with the consequences of the same" • Abstract: need to state "the primary objective is ....." if you want to then want to say secondly, and thirdly. • Pre-eclampsia is mentioned in the abstract but not as an outcome. Considering the pathophysiology of PE I doubt it is a relevant pregnancy complication in the context of diet and exercise. If you wish to include it you will need to discuss it and provide references in the introduction section of the manuscript. • The strengths and limitations statements should be specific to your study design. In this regard, statements 4 and 5 are relevant, but 1-3 are not. You also do not address the limitations to your study design, which is necessary. • Not clear whether diet and exercise will be assessed independently or as one entity, in relation to pregnancy and birth outcomes. If its separately these should be stated as individual outcomes. If you intend to combine you need to state how you are going to do that statistically. • Whilst your approach to sample size calculations in justified you need to state what your recruitment goals are for the two-year study, based on those calculations and then provide

	information about the feasibility of recruiting that many women across your sites. • You refer to biochemical parameters (mainly glucose measurements) in your discussion and in Figure 1, however no detail appears in the variables section. Even include this detail or remove those statements from the discussion. • It would be useful to state specifically what dietary information you obtain from the questionnaires will be used for your regression analysis. For example, will you use total energy? Or will it be broken down into carbohydrates, proteins, fats etc? • It is not clear how some of the birth outcomes and neonatal data you intend to collect and analyse can be related back to the PA and dietary habits of the women. An e.g. being cord pinching. Please provide rationale or remove. • The flow of the discussion is disjointed. I think paragraph one should actually be paragraph 3. It is also unclear as to whether the “larger studies that are needed” which you refer to in the final paragraph are in fact this study, or that more work will need to be done, but that your study will guide these larger studies. A more conclusive statement about your study at the end of the discussion is needed. It would also be useful to address any limitations to your study design in the discussion. Minor comments • Abstract, Line 35, you state postpartum twice • Foetal should be spelt “fetal” throughout • Need to state “in the” or “during the” postpartum period throughout • Page 5, line 42 trimesters should be trimesters • Page 6, line 38, please use the conventions intrauterine growth restriction (IUGR) or fetal growth restriction (FGR)

REVIEWER	Dr Thomas J Cade Royal Women's Hospital Melbourne, Australia
REVIEW RETURNED	06-Apr-2019

GENERAL COMMENTS	Thank you for your proposal on this prospective study examining an unimportant and under-reported area of health. I think it is topical, particularly with reference to gestational diabetes and (if you chose to do so) a potential follow-up economic analysis with regards to "cases averted" for those who undertake sufficient physical activity (PA). Although I have ticked "no" to a number of boxes, this is only because I am unclear on these issues in the current format. I am sure you have them adequately addressed in the study design and this particular manuscript just needs some tightening to make it clear to the reader exactly what you are examining. My specific comments are: Abstract - It comes across as if you are recruiting women AFTER they have completed pregnancy and the post-partum period. However, in the body of the article you wish to recruit them before 14 weeks. This needs to be made a little more clear: presumably you mean you will exclude women who do not complete their pregnancy and postpartum visits? If so, it may be worthwhile to specifically mention what you plan to do with those lost to follow-up.

	- You mention biochemical parameters: I'm still slightly unclear which ones these are other than possibly maternal glycaemia and umbilical cord measurements. - There are multiple primary outcomes mentioned here: are they intended as a composite outcome? If not: perhaps one should be the primary outcome and the others secondary. If this is not appropriate, you will need to elaborate exactly which you chose for your sample size calculation (see later). - Umbilical cord pH is a secondary outcome. Is this routinely taken in your institutions? Are will only study participants have this recorded? What occurs if the mother wishes delayed cord clamping? Introduction - You mention the "main outcome" but then refer to what I believe are your main aims. This sentence doesn't seem to refer back to your aforementioned primary outcomes in the abstract. Indeed here they are listed as secondary outcomes. I found it confusing. Inclusion criteria - You wish to recruit women less than 14 weeks with a singleton pregnancy. I presume a dating scan of some kind is mandated: who performs this and at what gestation? Variables - I was a little unclear when the 1st, 2nd and 3rd antenatal visits were until I found your informative flow-chart at the end. Perhaps direct the reader to this explicitly. Pregnancy data - You mention "the possibility of a preterm birth". This doesn't read well: either preterm birth is an outcome or it is not. If it is: specify what gestation you define. - You also use the term "amongst others". I think you should list all your outcomes of interest specifically and take care not to have too many: it come sometimes come across as data trawling. - With regards to this latter point: there are many variables listed that are not defined as primary or secondary outcomes and don't really strike me as potential confounders to these nor as important demographics. It may be prudent to either trim the list or state how/why you will report these and what analysis you plan to do with them. Statistical Analysis - I cannot tell from these two paragraphs which outcome you have chosen to define your sample size. I presume it is either one of the primary outcomes defined in the abstract or perhaps a composite of all of them. I honestly have found it very difficult to understand what the prevalences of 14% and 3% are referring to. I would suggest it would be much easier to state the outcome you have used, the presumed background proportions/prevalence (with a reference or some pilot data) and what level of change in proportions you define as significant and the sample size you have thus calculated. - The "regression models" paragraph is a little simplistic. Could you perhaps define which univariate analyses you plan (and how) and then which multivariate analyses you will then undertake specifically targeting which outcomes? Here I presume "patient health" is the outcome but I am unclear how you have defined that. Perhaps I am simply not reading your plans correctly: I am sure they are robust and you have a sound basis for your study. You clearly have been planning it meticulously and have passed your institutional research and ethics committee(s). Therefore, if these small things are tidied up, making it very clear to the reader which

	exact outcomes are of most interest, I think your manuscript would be greatly improved.

VERSION 1 – AUTHOR RESPONSE

REVIEWER'S COMMENTS TO AUTHORS:

Reviewer #1:
Major Comments

- Title: The title does not make sense. I would remove "together with the consequences of the same".
- Abstract: need to state "the primary objective is ....." if you want to then want to say secondly, and thirdly.

Authors
Thank you for your suggestions and corrections. We apologize for the mistakes that have already been corrected.

- Pre-eclampsia is mentioned in the abstract but not as an outcome. Considering the pathophysiology of PE I doubt it is a relevant pregnancy complication in the context of diet and exercise. If you wish to include it you will need to discuss it and provide references in the introduction section of the manuscript.

Authors
Thank you for your suggestions and corrections. Pre-eclampsia is mentioned as a primary outcome in abstract section. We have included the next sentence in Introduction (page 3):
“The scientific literature contains relevant information on the effects of moderate and regular PA during pregnancy: mothers gain less weight and reduce the risk of pregnancy-related diabetes, pre-eclampsia and hypertension (Muktabhant et al., 2015; Aune et al., 2014)”. We have included a new specific reference about pre- eclampsia (Aune et al., 2014).

- The strengths and limitations statements should be specific to your study design. In this regard, tatements 4 and 5 are relevant, but 1-3 are not. You also do not address the limitations to your study design, which is necessary.

Authors
Thank you for the review’s comment. We agree with the reviewer about the strenghs and limitations paragraph. We have removed the first three statements. Also, we have included the next sentences in Strengths and limitations (page 2):

“The Yana- C questionnaire has not been validated in pregnant women.
To control for the limitation mentioned above we will use a self-administered questionnaire about good adhesion to the Mediterranean diet (PREDIMED). Also, we will validate the Yana-C questionnaire in pregnant women.”

- Not clear whether diet and exercise will be assessed independently or as one entity, in relation to pregnancy and birth outcomes. If its separately these should be stated as individual outcomes. If you intend to combine you need to state how you are going to do that statistically.

Authors
Thank you for your suggestion. Diet and exercise will be assessed independently. We have included the next sentence (please, see Introduction, last paragraph in page 3):

“The main outcomes of this research are to examine independently the nutritional habits and the levels of PA in women during pregnancy and the postpartum period by means of validated questionnaires and accelerometers.”

- Whilst your approach to sample size calculations in justified you need to state what your recruitment goals are for the two-year study, based on those calculations and then provide information about the feasibility of recruiting that many women across your sites.

Authors
We apologize for the mistakes that has already been corrected. We have reviewed and modified the Statistical analysis paragraph (please, see page 7).
“Sample size has been calculated using Epidat 4.1, with an exposed/non-exposed ratio of 1. The outcome variable will be gestational diabetes mellitus. A prevalence of 14% is assumed in the group of exposed (sedentary) women and a prevalence of 3% is assumed in the group of non-exposed women (who are active according to the current criteria of the American College of Sports Medicine- 30 minutes/day of moderately intense PA every day or almost every day, accumulating at least 150 min/week). A 5% alpha error and 80% statistical power will be assumed. Following these premises, it will be estimated that 194 pregnant women should be included in the study. Approximately 5% should be added to these premises to account for possible non-responders (women who do not wish to take part in the study) and drop-outs.
Descriptive statistics with precision estimates will be used to report the prevalence of each parameter using a cross- sectional data. Mixed regression models will be used to examine the relationship between dependent variables and patient health, measured using one or more explanatory variables that express exposure to a risk factor and controlling for baseline values. The results will be expressed as absolute differences in changes in variables between the baseline and final measurements (95% confidence interval)
All statistical analyses will be performed with the statistical software IBM® SPSS® Statistics 24, and the level of significance will be set at $p<0.05$.”

- You refer to biochemical parameters (mainly glucose measurements) in your discusión and in Figure 1, however no detail appears in the variables section. Even include this detail or remove those statements from the discussion.

Authors
Thank you for the review’s comment. We have added this paragraph in the Variables to clarify the question (please, see page 5):

“Biochemical parameters:

Study data will be collected by trained research staff. All blood samples will be taken from the right or left cubital fossa, after an 8–12 h fast, between 08:00–10:00 am. We will determine glucose, insulin and O´Sullivan. In the study population women will be routinely screened for gestational diabetes at 22-26 weeks of gestation with a nonfasting oral glucose challenge test in which venous blood will be sampled 1 hour after a 50-g oral glucose load. If the 1-hour glucose result are at least 140 mg / dL, the participant will be referred to a 100-g fasting glucose 3-hour tolerance test. Normal results will be a blood glucose below 95 mg / dL at baseline, below 180 mg / dL at 1 hour, below 155 mg / dL at 2 hours, and below 140 mg / dL at 3 hours.”

- It would be useful to state specifically what dietary information you obtain from the questionnaires will be used for your regression analysis. For example, will you use total energy? Or will it be broken down into carbohydrates, proteins, fats etc?

Authors
Thank you for the review's comment. We have added this paragraph in the Variables to clarify the question (please, see page 6):

"Energy (kcal) and macronutrient intake (percentages) will be measured by two non-consecutive 24-h recalls (weekday and weekend day), using YANA-C software program. Percentages of Energy intake from carbohydrate, protein, fat and macronutrients (g) relative to weight (kg) will be calculated."

- It is not clear how some of the birth outcomes and neonatal data you intend to collect and analyse can be related back to the PA and dietary habits of the women. An e.g. being cord pinching. Please provide rationale or remove.

Authors
We agree with the reviewer's comment and we have deleted the following neonatal data: "cord pinching (early/late), commencement of breast feeding in the first two hours of life (yes/no)."
We believe that the results of birth can affect the physical activity and mother's nutrition in the postpartum period.

- The flow of the discussion is disjointed. I think paragraph one should actually be paragraph 3. It is also unclear as to whether the "larger studies that are needed" which you refer to in the final paragraph are in fact this study, or that more work will need to be done, but that your study will guide these larger studies. A more conclusive statement about your study at the end of the discussion is needed. It would also be useful to address any limitations to your study design in the discusión.

Authors
Totally agree with reviewer. We have modified the Discussion section to clarify the question (please, see pages 7 and 8):

"Adherence to an exercise routine is influenced by factors such as: the habit of exercising prior to pregnancy, sociocultural level, equality and the insistence by healthcare workers that pregnant women undertake PA. The study by Nascimento et al. (2015) showed that half of the women taking part ceased doing physical activity during pregnancy.
It is therefore important that healthcare personnel offer information on the risks and benefits of PA, while also setting personalised guidelines adapted to the specific needs of each woman. All of the information supplied to future mothers must be supported by scientific evidence. The aim is to guide future mothers towards a healthy lifestyle and to change their habits. This is not only to prevent pathologies during pregnancy and in the postpartum period, but rather to ensure that their new habits last throughout their life.

The study results will make it possible to better advise pregnant women about recommendable PA and nutrition. They will also make it possible to update health education programs for this population group, leading to many benefits for mothers as well as their children.
The study will also record biochemical parameters which, in association with the data gathered using the scales and accelerometer, will make it possible to prevent the risk of non-transmissible diseases during pregnancy and in the postpartum period. Biochemical changes are closely linked to the amount

of mothers' PA and the quality and type of their diet during pregnancy and in the postpartum period. An example of this relationship is the use of the biochemical parameter of glucose as a gestational diabetes marker. Pérez-Ferre et al. (2015) intervened in a group of women with gestational diabetes, changing their dietary habits and encouraging physical exercise. They found that the risk of developing type 2 diabetes in the future fell in the intervention group in comparison with the control group (Perez-Ferre et al., 2015).
Several limitations to our study should be considered. First of all, as with any observational study, we cannot eliminate residual confounding by unmeasured factors. However, we will be able include information on previously identified factors such as age, BMI before pregnancy and race / ethnicity, and consider other sociodemographic characteristics. Secondly, it is possible that accelerometers may produce some reactivity by the participants (Hawthorne effect) in wearing the device; however, unlike self-reports, accelerometer estimates do not suffer from bias due to social desirability and recall problems. Finally, the Yana- C questionnaire has not been validated in pregnant women. To control this limitation, we will use a self-administered questionnaire about good adhesion to the Mediterranean diet (PREDIMED).

Larger studies like ours are therefore necessary which quantity the PA of women during pregnancy and in the postpartum period, to set guidelines based on scientific evidence. The present study will help identify the frequency, duration, intensity and type of PA in pregnant women and their impact on delivery, mother and new-born outcomes. This information will promote education for health by health professionals and involve practice in these women. "

Minor comments

- Abstract, Line 35, you state postpartum twice
- Foetal should be spelt "fetal" throughout
- Need to state "in the" or "during the" postpartum period throughout
- Page 5, line 42 trimesters should be trimesters
- Page 6, line 38, please use the conventions intrauterine growth restriction (IUGR) or fetal growth restriction (FGR)

Authors
We thanked a lot the reviewer effort to correct the paper. In addition, we have reviewed the manuscript and already correct the gramatical erros. We apologize for the inconvenience.

Reviewer: 2
Abstract
- It comes across as if you are recruiting women AFTER they have completed pregnancy and the post-partum period. However, in the body of the article you wish to recruit them before 14 weeks. This needs to be made a little more clear: presumably you mean you will exclude women who do not complete their pregnancy and postpartum visits? If so, it may be worthwhile to specifically mention what you plan to do with those lost to follow-up.

Authors
Thank you for the review's comment. We have changed this sentence to clarify the question (please, see Abstract, page 2):
"The participants will be pregnant women aged from 18 to 40 years old who should attend all the check-ups during their pregnancy and the postpartum period."

Also, we have added the next sentence in Exclusion criteria (page 4):
"iv) Women who do not complete the follow-up."

- You mention biochemical parameters: I'm still slightly unclear which ones these are other than possibly maternal glycaemia and umbilical cord measurements.

Authors
This question has already answered before to reviewer 1.
- There are multiple primary outcomes mentioned here: are they intended as a composite outcome? If not: perhaps one should be the primary outcome and the others secondary. If this is not appropriate, you will need to elaborate exactly which you chose for your sample size calculation (see later).
Authors
This question has already answered before to reviewer 1.
- Umbilical cord pH is a secondary outcome. Is this routinely taken in your institutions? Are will only study participants have this recorded? What occurs if the mother wishes delayed cord clamping?
Authors
Comments are highly appreciated. Umbilical cord blood pH in the newborn babies is routinely taken in our institutions. If the mother wishes delayed cord clamping, we will also take the pH data and later we will consider whether to take them into account as a function of the time elapsed since the birth of the newborn.
Introduction
- You mention the "main outcome" but then refer to what I believe are your main aims. This sentence doesn't seem to refer back to your aforementioned primary outcomes in the abstract. Indeed here they are listed as secondary outcomes. I found it confusing.
Authors
Thank you for your concern. We have changed the word “objective” instead of “outcome” (please, see Introduction page 3).
Inclusion criteria
- You wish to recruit women less than 14 weeks with a singleton pregnancy. I presume a dating scan of some kind is mandated: who performs this and at what gestation?
Authors
We have included this information in the document (please, see Methods/Design page 4):
“The sample will be recruited by matrons by means of non-probabilistic consecutive sampling in Primary Care facilities.”
Variables
- I was a little unclear when the 1st, 2nd and 3rd antenatal visits were until I found your informative flow-chart at the end. Perhaps direct the reader to this explicitly.
Authors
Done. Thank you.
Pregnancy data
- You mention "the possibility of a preterm birth". This doesn't read well: either preterm birth is an outcome or it is not. If it is: specify what gestation you define.
- You also use the term "amongst others". I think you should list all your outcomes of interest specifically and take care not to have too many: it come sometimes come across as data trawling.
Authors
Thank you for your suggestion. We have modified that paragraph to clarify the question (please, see Pregnancy data, page 6):
“duration of pregnancy (weeks and days of amenorrhea, according to the date of the last period and confirmation by ultrasound scan), medical problems during pregnancy (Yes/No) premature birth (fewer than 37 weeks' gestational age), arterial hypertension, gestational diabetes (defined according to the criteria of the American Diabetes Association (ADA) (2015)), fetal growth restriction and a reduction in amniotic fluid).”
- With regards to this latter point: there are many variables listed that are not defined as primary or secondary outcomes and don't really strike me as potential confounders to these nor as important demographics. It may be prudent to either trim the list or state how/why you will report these and what analysis you plan to do with them.
Authors

Comments are highly appreciated. In our opinion, these variables could be potential confounders and it could negatively affect the practice of physical activity during this phase.
Statistical Analysis
- I cannot tell from these two paragraphs which outcome you have chosen to define your sample size. I presume it is either one of the primary outcomes defined in the abstract or perhaps a composite of all of them. I honestly have found it very difficult to understand what the prevalences of 14% and 3% are referring to. I would suggest it would be much easier to state the outcome you have used, the presumed background proportions/prevalence (with a reference or some pilot data) and what level of change in proportions you define as significant and the sample size you have thus calculated.
- The "regression models" paragraph is a little simplistic. Could you perhaps define which univariate analyses you plan (and how) and then which multivariate analyses you will then undertake specifically targeting which outcomes? Here I presume "patient health" is the outcome but I am unclear how you have defined that.
Authors
We apologize for the mistakes that has already answered before to reviewer 1.

Perhaps I am simply not reading your plans correctly: I am sure they are robust and you have a sound basis for your study. You clearly have been planning it meticulously and have passed your institutional research and ethics committee(s). Therefore, if these small things are tidied up, making it very clear to the reader which exact outcomes are of most interest, I think your manuscript would be greatly improved.

Authors
Thank you for the interesting comments.

VERSION 2 – REVIEW

REVIEWER	Stacey Ellery Hudson Institute of Medical Research
REVIEW RETURNED	13-May-2019

GENERAL COMMENTS	Abstract -Need to say "The primary objective of this study...." or else the following sentences do not make sense. -Statistical Analysis section is written in future tense when it should be past. You have already completed the sample size calculations. Can the authors please reference where the prevalence rates of GDM (i.e. 14% for sedentary and 3% for active) have been derived from? Are they specific to the Spanish population? -Overall, there are still a few minor flow issues throughout, particularly in the discussion. The proofing team of the journal should be able to help you select the appropriate phrasing to better link some of your paragraphs.

REVIEWER	Thomas Cade Royal Women's Hospital, Melbourne, Australia
REVIEW RETURNED	17-May-2019

GENERAL COMMENTS	Well done: I think you have addressed all of my initial concerns and I am looking forward to reading the eventual results of your study.

VERSION 2 – AUTHOR RESPONSE

Reviewer #1:

The reviewer provided a marked copy with additional comments. Please contact the publisher for full details.