ARTICLE DETAILS

TITLE (PROVISIONAL)  The CLIMB (Complex Lipids In Mothers and Babies) study: protocol for a multi-centre, three-group, parallel randomized controlled trial to investigate the effect of supplementation of complex lipids in pregnancy, on maternal ganglioside status and subsequent cognitive outcomes in the offspring.

AUTHORS  Huang, Shuai; Mo, Ting-Ting; Norris, Tom; Sun, Si; Zhang, Ting; Han, Ting-Li; Rowan, Angela; Xia, Yinyin; Zhang, Hua; Qi, Hong-Bo; Baker, Philip

VERSION 1 - REVIEW

REVIEWER  Jacqueline F Gould  South Australian Health and Medical Research Institute, Australia

REVIEW RETURNED  18-Apr-2017

GENERAL COMMENTS  This is a protocol for a large randomised trial of complex lipids in pregnancy.

Can more information be presented about the intervention? For example when the intervention commences and ceases, how much women are instructed to consume each day, what form (liquid/powder) the intervention comes in? If women do not attend appointments during pregnancy, will they run out of the intervention? Are women still allowed to drink normal milk during the study? What is the primary outcome and hypothesis for the study? what was used for the sample size calculation? What happens if there are withdrawals or loss to follow-up? Clarification is needed for the role of the sponsor-they had a role in the design and writing of the protocol, will they also have a role in the analysis and reporting of the data? What will happen if the results show a null or adverse effect? The study appears as though it will be undertaken at one centre-please remove multi-centre from the title. (please note: enrolment and follow-up at different centres does not make it a multi-centre study, this term refers to number of enrolment sites) Blinding-line 200: “staff performing the assessments will be aware of which subjects have been randomized to standard care” please clarify why the assessments are not blinded?

Minor comments:
Please provide website for the trial registry line 54

Methods and design—note this should be in present tense, not past tense (please amend throughout the manuscript)

Line 213—consider including additional information on planned epigenetic analyses

REVIEWER
Hans Demmelmaier
University of Munich Medical Center
Hauner Children’s Hospital
Germany

REVIEW RETURNED
10-Jun-2017

GENERAL COMMENTS
The authors describe a very interesting study. The manuscript is well written and good to read.
There are only some small points, I would like to raise:
It was not fully clear to me, whether the study is mono centric or multi centric (i.e. is it only different sites of one Institution or different institutions, where recruitment takes place)
As primary maternal outcome serum complex lipid levels are given, but no further details. These analyses are complex and there are different valid approaches possible, thus it seems important to give more details here (e.g. is total sialic acid analyzed or specific species).
In the conclusion the authors speculate about the reduction of neurological abnormalities by the supplementation with complex milk lipids. It is not clear to me, how this can be tested from the data to be collected. It seems a good and important idea to me, but it would be good to have a systematic procedure to collect corresponding data.
That subjects are only recruited in one area may limit generalizability, but might be an advantage as it limits the variability of the subjects. On the other hand the study design implies that any other nutritional factor than complex milk lipids might limit infant development and they might show their positive effect only in combination with that dietary component (e.g. n-3 long chain polyunsaturated fatty acids).
Two other points I would find interesting:
In studies including milk fat globule membranes in infant nutrition mixed finding in relation to allergies have occurred. Is this of relevance for this study?
It would be interesting to read in the introduction about the content of the tested lipids in the habitual diet of the women and its variability, if such data are available.

VERSION 1 – AUTHOR RESPONSE

Part A (Reviewer #1)

Comment 1: This is a protocol for a large randomised trial of complex lipids in pregnancy. Can more information be presented about the intervention? For example when the intervention commences and ceases, how much women are instructed to consume each day, what form (liquid/powder) the intervention comes in?
Response: According to the reviewer’s comment, we have added more details and rewritten the sentences.

(page 7, para 4): “The period of intervention is from recruitment (at a gestation of 11-14 weeks) until the baby is born, allowing the impact of maternal and fetal nutrition during gestation, a sensitive window for brain development, to be investigated. Pregnant women will be assigned to either milk powder of product A or product B. The only difference between products A and B, both reduced-fat formulations, is the amount of CML contained in each, with product B having a restored level of CML to that of full-fat milk (Table 1). Both treatment groups are instructed to consume 37.5 g of the milk powder twice a day, with clear instructions written on the milk powder. These instructions are verbally communicated by the research nurse. ”

Comment 2: If women do not attend appointments during pregnancy, will they run out of the intervention?

Response 2: Any women who do not attend appointments will be considered as dropouts. To be more precise, we have rewritten the sentence.

(page 7, para 2): “If a participant does not return for a scheduled visit, every effort (e.g. phone call, WeChat and QQ) will be made to contact them. If the participant refuses to attend, she will be considered as having dropped out. If the participant withdraws from the study and withdraws consent, no intervention will be performed and no additional data will be collected. ”

Comment 3: Are women still allowed to drink normal milk during the study?

Response 3: Yes, all participants are allowed to drink any kinds of milks during the study. We have used food frequency questionnaire and 24 hour food recall in an attempt to adjust for this.

Comment 4: What is the primary outcome and hypothesis for the study?

Response 4: Our primary outcome is listed in the measures and samples section, and our hypothesis is written in the introduction.

(page 10, para 2): “The primary maternal outcomes are serum complex lipid (e.g. GA) levels in maternal blood.”

“The primary offspring outcomes are infant cognitive development (assessed by BSID-I) and general infant health (infant growth, e.g. height, weight and skinfold thickness).”

(Page 4, para 4): “Therefore, we hypothesize that a higher maternal intake of CML in pregnancy, serving as an additional source of GA, will increase GA status in both mother and offspring, with potential benefits relating to brain development and general health in the infant, especially during the first year of life.”

Comment 5: what was used for the sample size calculation?

Response 5: We calculate the sample size based on the primary maternal outcomes (serum ganglioside level).

\[ n = \frac{2\sigma^2 (Z\alpha + Z\beta)^2}{d^2} \]

where:
- \( n \) = Subject number for each group
- \( d \) = The standardized difference between means, measured in units of the standard deviation
- \( \sigma \) = Pooled standard deviation
- \( Z\alpha \) and \( Z\beta \) derived from standard normal distribution, for a significance level of 5% (\( Z\alpha=1.960 \)) and study power of 80% (\( Z\beta=0.842 \)).

According to the reference 30, we use sample variance to estimate the population variance:
\[ \sigma^2 = \frac{(S_a^2 + S_b^2)}{2} = 0.273 \]

- \( S_a \) = Standard deviation of the treatment group
- \( S_b \) = Standard deviation of the reference group

# Mean differences

- \( d = \mu_a - \mu_b = 0.936 \)
- \( \mu_a \) = Mean of the treatment group
- \( \mu_b \) = Mean of the reference group

- \( n \) is calculated to be 5.

To detect a more precise difference between two groups, \( d = 0.15 \) is chose, and \( n \) is calculated to be 191.

- The loss to follow-up rate is expected to be 20%.
- \[ N = n + n \times 0.2 \]
- \( N \) = Final total subject number for each group.

- \( N \) then is calculated to be 229.

Because we calculates the sample size based on our primary outcome, to facilitate measurements and analysis of our various second outcomes, we enlarge \( N \) to 500.

**Comment 6:** What happens if there are withdrawals or loss to follow-up?

**Response 6:** Thank you for the comment. We have rewritten the sentences and answered this question in the withdrawal section (page 7 para 1).

“All participants are free to withdraw from the study at any time, for any reason, and without any impact on future medical care. If a participant is withdrawn before completing the study, the date and reason of withdrawal will be entered into the case report form (CRF), and all her previous information (including measures and samples) will still be stored for later experiments and analysis. If a participant does not return for a scheduled visit, every effort (e.g. phone call, WeChat and QQ) will be made to contact them. If the participant refuses to attend, she will be considered as having dropped out. If the participant withdraws from the study and withdraws consent, no intervention will be performed and no additional data will be collected.”

**Comment 7:** Clarification is needed for the role of the sponsor-they had a role in the design and writing of the protocol, will they also have a role in the analysis and reporting of the data? What will happen if the results show a null or adverse effect?

**Response 7:** In the CLIMB study, the sponsor have no role in any analysis and reporting of the data. If we find a null or adverse effect, we will still report it faithfully.

**Comment 8:** The study appears as though it will be undertaken at one centre-please remove multi-centre from the title. (please note: enrolment and follow-up at different centres does not make it a multi-centre study, this term refers to number of enrolment sites)

**Response 8:** We have two different enrolment sites, the First Affiliated Hospital of Chongqing Medical University (FCQMU) and Chongqing Health Center for Women and Children (CHC). To avoid confusion, we have written the sentences.

**(page 5, last para):** “Study enrolment will take place at the First Affiliated Hospital of Chongqing Medical University (FCQMU) and Chongqing Health Center for Women and Children (CHC). To avoid confusion, we have written the sentences. (page 5, last para): “Study enrolment will take place at the First Affiliated Hospital of Chongqing Medical University (FCQMU) and Chongqing Health Center for Women and Children (CHC). After delivery, infant follow up will take place at the CHC. In total there are five clinic visits (see Tables 2 & 3) – three will take place during pregnancy (11-34 weeks, at both the FCQMU and CHC) and two will take place in the infant period (6 weeks and, 12 months, exclusively at CHC).”

**(page 6, para 2):** “Recruitment posters promoting the study will be placed in the maternity clinics of two centres (FCQMU and CHC).”
Comment 9: Blinding-line 200: “staff performing the assessments will be aware of which subjects have been randomized to standard care” please clarify why the assessments are not blinded?

Response 9: At clinic visit 1 all eligible participants will be randomized via the MedSciNet AB (Stockholm, Sweden) Interactive Web Response System, to either control, intervention or reference group (standard care). After randomization, the participant will be allocated to a specific research nurse for the duration of the study, in order to facilitate one-to-one communication between nurses and participants. Therefore, the nurse performing the nutritional assessments (the food frequency questionnaire and 24 hour food recall), the specific research nurse assigned, will be aware of which subjects have been randomized to standard care, because the standard care group do not receive milk powder every time they attend the appointment. However, it is performing biophysical tests (including ultra-sonography) and investigators undertaking the laboratory assessments and biochemical assays, analysis of the metabolome, epigenome, etc. were blinded as to which group (A, B or C) the participant was in.

To avoid confusion, we have rewritten the sentence.

Comment 10: Please provide website for the trial registry line 54.

Response 10: According to the reviewer’s comment, we have added the website for the trial registry.

Comment 11: Methods and design—note this should be in present tense, not past tense (please amend throughout the manuscript).

Response 11: According to the reviewer’s comment, we have rewritten the whole manuscript.

Comment 12: Line 213—consider including additional information on planned epigenetic analyses.

Response 12: According to the reviewer’s comment, information has been added.

Part B (Reviewer #2)

Comment 1: It was not fully clear to me, whether the study is mono centric or multi-centric (i.e. is it only different sites of one Institution or different institutions, where recruitment takes place)

Response 1: This study has two different recruitment sites, the First Affiliated Hospital of Chongqing Medical University (FCQMU) and Chongqing Health Center for Women and Children (CHC). To avoid confusion, we have written the sentences.

Comment: Study enrolment will take place at the First Affiliated Hospital of Chongqing Medical University (FCQMU) and Chongqing Health Center for Women and Children (CHC). After delivery, infant follow-up will take place at the CHC. In total there are five clinic visits (see Tables 2 & 3) – three will take place during pregnancy (11-34 weeks, at both the FCQMU and CHC) and two will...
take place in the infant period (6 weeks and 12 months, exclusively at CHC)."

Comment 2: As primary maternal outcome serum complex lipid levels are given, but no further details. These analyses are complex and there are different valid approaches possible, thus it seems important to give more details here (e.g. is total sialic acid analyzed or specific species).

Response 2: According to the reviewer's comment, details have been added.

Comment 3: In the conclusion the authors speculate about the reduction of neurological abnormalities by the supplementation with complex milk lipids. It is not clear to me, how this can be tested from the data to be collected. It seems a good and important idea to me, but it would be good to have a systematic procedure to collect corresponding data.

Response 3: Our primary hypothesis is that supplementation with complex milk lipids during pregnancy will improve brain development of the offspring, whose outcome will be measured by the Bayley Scales of Infant Development I. However, incidence of congenital abnormality in China is about 5% and neurological abnormality is even lower. Even though we suspect that supplementation with complex milk lipids might reduce neurological abnormalities in infant, as we recruit only 1500 pregnant women in total, we might not able to address the problem well, therefore to be more precise, we have deleted “reduce neurological abnormalities” and rewritten the sentence.

Response 4: Chongqing is a major city of western China with its population of 30 million (2015), and is also one of China’s four direct-controlled municipalities (Chongqing, Tianjin, Beijing and Shanghai). As the only inland municipality in China, Chongqing has its own advantage in serving as the economic centre of the upstream Yangtze River basin. It is one of China’s major manufacturing center, transportation hub and emerging megacities. Attracted by its economic prosperity, Chongqing has a growing size of population migration from all parts of the country. Different people from different areas of China come to Chongqing along with their different culture and dietary patterns, which therefore, increases the generalizability and representativeness of Chongqing in this study. Therefore, we choose Chongqing because it is a representative city of western China, and it provides enough population from all over the country, which allows us to study the Chinese population as a whole. Furthermore, with the use of nutrition assessment (food frequency questionnaire and 24 hour food recall), we manage to eliminate any potential dietary confounding factors.

Response 5: In this study, we use Food frequency questionnaire and 24 hour food recall for nutritional assessment of both mother and infant, therefore to eliminate potential dietary differences and to analyze any unexpected effect of other components.

Comment 6: Two other points I would find interesting: In studies including milk fat globule membranes in infant nutrition mixed finding in relation to allergies have occurred. Is this of relevance for this
Response 6: The intervention with supplementation of complex milk lipids is for the mother only and not for the infant. To avoid confusion, we have rewritten the sentence. (page 7, para 4): “The period of intervention is from recruitment (at a gestation of 11-14 weeks) until the baby is born”

Comment 7: It would be interesting to read in the introduction about the content of the tested lipids in the habitual diet of the women and its variability, if such data are available.

Response 7: We agree that this is a pertinent and important point to consider. We sought to obtain this information from the literature, but were unable to find any relevant data.

VERSION 2 – REVIEW

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Hans Demmelmaier</th>
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<tbody>
<tr>
<td>LMU München, Germany</td>
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| REVIEW RETURNED | 24-Jul-2017 |

| GENERAL COMMENTS | From the clinical side the methods are adequately described, but from the laboratory side, I still miss details about the determination of gangliosides. This does not seem essential to me, but would strengthen the manuscript. |

VERSION 2 – AUTHOR RESPONSE

(Reviewer #2)

Comment 1: From the clinical side the methods are adequately described, but from the laboratory side, I still miss details about the determination of gangliosides. This does not seem essential to me, but would strengthen the manuscript.

Response: According to the reviewer’s comment, method of the determination of gangliosides has been added. (page 10, paragraph 2): “The primary maternal outcomes are serum complex lipid (e.g. GA determined by UPLC-MS) levels in maternal blood.”
The CLIMB (Complex Lipids In Mothers and Babies) study: protocol for a multicentre, three-group, parallel randomised controlled trial to investigate the effect of supplementation of complex lipids in pregnancy, on maternal ganglioside status and subsequent cognitive outcomes in the offspring

Shuai Huang, Ting-Ting Mo, Tom Norris, Si Sun, Ting Zhang, Ting-Li Han, Angela Rowan, Yin-Yin Xia, Hua Zhang, Hong-Bo Qi and Philip N Baker

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