PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cow's Milk Fat Obesity pRevention Trial (CoMFORT): a primary care embedded randomised controlled trial protocol to determine the effect of cow's milk fat on child adiposity
AUTHORS	Vanderhout, Shelley; Aglipay, Mary; Birken, Catherine; Li, Patricia; O'Connor, Deborah L; Thorpe, Kevin; Constantin, Evelyn; Davis, Marie-Adele; Feldman, Mark; Ball, Geoff; Janus, Magdalena; Jüni, Peter; Junker, Anne; Laupacis, Andreas; L'Abbé, Mary; Manson, Heather; Moretti, Myla; Persaud, Nav; Omand, Jessica; Relton, Clare; Wong, Peter; Yamashiro, Hirotaka; Tavares, Erika; Weir, Shannon; Maguire, Jonathon L

VERSION 1 – REVIEW

REVIEWER	Karen Mativenko-Sikar University College Cork, Ireland
REVIEW RETURNED	03-Dec-2019

GENERAL COMMENTS	Well done on designing this trial. I have a number of comments and recommendations that need to be addressed for publication of this protocol. Abstract Line 18- development is a relatively vague term so would clarify or delete. Strengths and limitations Unclear how both arms are 'usual care' as the abstract states that the current recommendation is to move to reduced fat. This is clarified in the introduction, but needs clarification earlier if presenting in this way here. Introduction Page 6, line 7. Remove reference to British milk consumption as it is not the context of the study and the recommendations for milk consumption is different in Britain Page 6, line 42, change the word 'revealed' to 'reported' Page 6, line 48, suggest removing "though this relationship seems paradoxical" as it is not needed. Page 6, lines 53-56: sentence needs to be revised for clarity and a clearer and more detailed discussion of how full fat milk consumption can minimise odds of overweight, is needed here. Page 7. Line 17. Clarify what is meant by 'developmental' A full discussion of potential mechanisms by which full milk consumption is expected to influence other outcomes in this trial is

	essential. For instance, how is differential milk fat content expected to impact on cognitive and cardiometabolic outcomes? Objectives Objective 5- there is no earlier reference to costs, this needs to be introduced in the introduction and clearly explained. There is logical for costs related to health care system if type of milk consumption reduces adverse health outcomes- and therefore likelihood of needing to utilise health care services for these outcomes. However I find it hard to imagine these costs would manifest after 24 months. As noted for the introduction, the expected hypotheses and outcomes are unclear as the majority of them have not been introduced or explained in the introduction. Please fully explain each of these and the proposed mechanisms. Methods and analysis Page 8, line 48- If children are recruited up to 2.99 years, how you will the study handle instances where children have already been introduced to recommendations for reduced fat at age 2 years, prior to entering the trial? Page 9, lines 2-5: Please state how many affiliated primary healthcare providers are in the network if possible for clarity. Page 10 Inclusion criteria- how do parents report if the children are healthy- please specify the question(s) asked to determine this (e.g. is it on a scale, is it dichotomous healthy/unhealthy) Exclusion criteria- please operationalize 'severe developmental delay' for the purposes of this study. Can the authors please comment on potential implications of physicians making recommendations with which they may not agree, e.g. if a patient is randomised to one recommendation but the physician does not agree with this it may impact intervention implementation/fidelity etc. Whole milk recommendation- please clarify if the recommendation to not switch to reduced fat is explicitly stated to parents, or if they are only told what they should do in this experimental condition? Please include what the email reminders will say, either in the text or as an appendix Reduced fat milk recommendation- please clarify if it will be explained to parents that these are current guidelines- if so it may influence their adherence. For both conditions, clarity about what is said is needed. Adherence- the authors refer to reaching children before 1.5 but do not refer to recruitment of children up to nearly 3 years of age, please address. Also please note that the age of children at recruitment is a recruitment point and does not ensure adherence, only increases likelihood of timely message delivery. Page 11. Line 2- how frequent are the well-child visits, and how

	many visits (and therefore intervention exposures) will participants receive during the study? Page 11, line 5- what do the magnets say? Page 11- Baseline Participant Characteristics: Are any developmental outcomes measured? Waist circumference referred to later but not included here, please correct Page 11, line 42-43: because 2 years post-randomisation has been used in other studies is insufficient rationale for your choice here. Please give an appropriate rationale based on scientific hypotheses about expected effects on primary (and secondary) outcomes over time Please present timing of measurements more clearly. At present you have a baseline measurement section that does not include all measures taken at enrolment. Page 14: Sample size- why is 20% attrition expected, please justify this figure. Page 14: Recruitment- how will presence of research assistants lower burden and ease trial entry? Please be sure to explain and justify all such comments. -Points 1- 3 could be combined as a single point that participants will be recruited in person by a trained research assistant who is known to them through the TARGet Kids program. - Having the blood sampling later than the surveys and anthropometrics will not necessarily improve maximise recruitment because participants will still be expecting and undergoing the blood draw in the same appointment. Page 15 Randomisation- Please include a final statement of how blinding will be communicated to physicians. Page 15 Blinding- You have rightly stated that participant and healthcare professional blinding cannot occur, so please clarify for whom the allocation concealment is relevant/ Page 15 line 42-45: Reinforcement of recommendations is the intervention; it is not a retention strategy. Remove this statement from here. Page 15, lines 45- 48: what does every reasonable attempt mean? You need to explain this. Page 16 line 2-5: Why is it expected that a home visit will work where other approaches have not? And from where does the figure of 10% emerge? Page 16 Data management: more information is needed here about how data will be processed, stored, and accessed. Page 17 Ethics: Please give ethics approval number if possible and please outline how ethical issues are addressed in this study.

	More general comment: Any behaviour change theory/techniques or use of specific implementation strategies based on a framework approach? These discussed to provide confidence that the intervention is empirically and theoretically based, with appropriate methods chosen for delivery.

REVIEWER	Professor Ian Givens Institute for Food, Nutrition and Health University of Reading United Kingdom
REVIEW RETURNED	10-Jan-2020

GENERAL COMMENTS	This is a protocol paper describing the plans for a study on an important topic. Overall, the paper describes the protocol in clear details but it would be helpful to have further clarity on the following points: 1. For protocol papers it seems that the journal requires the planned dates of the study which are not given. 2. p 7, line 46, re the primary outcome. It would have been good to also have a direct measure of adiposity for a primary outcome but presumably this was regarded as impractical with a large cohort of children? 3. p 7, line 52, re secondary outcome, would 'Better cardiovascular health' be more correct as 'Lower risk of cardiovascular disease' ? 4. p 8, line 49, and p 9, line 42 It is said that children aged 1.5 to 2.99 years will be recruited and randomised to the study treatments which seems at odds with the objective of examining the effect of consuming whole vs. fat reduced milk from the age of 2 years. Also, under Adherence it says that children from 1.5 years will be included in the recruitment phase to so as to involve families before milk choices are formed. The last point is clearly how things should be but more clarity on the earlier statements about recruiting and randomisation would remove confusion. 5. Under Reduced Fat Milk Recommendation, (page numbering at bottom of pages has gone back to 1.) it is said that children on the reduced milk fat treatment would be provided with the same nutritional recommendations as those in whole milk treatment. It is not clear what 'provided with' means. Crucially does it mean that as far as possible the children on the reduced fat milk will have the same total fat intake as those in the whole milk group? If yes how would this be achieved and with fat source? 6. Randomisation. There is no mention of how sex is dealt with. Presumably the aim would be have essentially the same proportion of males and females in both treatment groups? 7. Minor points. In several places the term 'caloric intake' is mentioned which would be better as 'energy intake'. Also concentrations of fat in milk are given in non-SI units (%) rather than g/100g.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Comment: Line 18- development is a relatively vague term so would clarify or delete.
Response: Thank you. We have replaced this with “growth.”

Comment: Unclear how both arms are ‘usual care’ as the abstract states that the current recommendation is to move to reduced fat. This is clarified in the introduction, but needs clarification earlier if presenting in this way here.
Response: We have changed “usual care” to “nutritional” in this section.

Comment: Page 6, line 7. Remove reference to British milk consumption as it is not the context of the study and the recommendations for milk consumption is different in Britain

Response: Thank you, we have removed this.

Comment: Page 6, line 42, change the word ‘revealed’ to ‘reported’

Response: We have made this change.

Comment: Page 6, line 48, suggest removing “though this relationship seems paradoxical” as it is not needed.

Response: Thank you. We have removed this.

Comment: Page 6, lines 53-56: sentence needs to be revised for clarity and a clearer and more detailed discussion of how full fat milk consumption can minimise odds of overweight, is needed here.

Response: Thank you for this suggestion. We have added more detail from page 6 line 48 to page 7 line 16: “Proposed mechanisms include higher satiety offered by whole milk due to hormonal responses to dietary milk fat consumption thus displacing nutrient-poor foods or sugary beverages. Another theory is that a low fat, high protein diet in early childhood may program a “thrifty metabolism,” where the body adapts by storing excess energy as fat. Conversely, a higher fat diet may metabolically program higher energy utilization and lower energy storage. Fatty acids found in cow’s milk, such as trans-palmitoleic acid and conjugated linoleic acid, may be protective against excess adiposity. Reverse causality could also explain this relationship, where parents may choose milk with a fat content to counter-balance the adiposity of their child (i.e. higher fat milk for a leaner child.”

Comment: Page 7. Line 17. Clarify what is meant by ‘developmental’

Response: We have added the word “cognitive” to provide more specificity.

Comment: A full discussion of potential mechanisms by which full milk consumption is expected to influence other outcomes in this trial is essential. For instance, how is differential milk fat content expected to impact on cognitive and cardiometabolic outcomes?

Response: As noted above, we have added more detail to this topic. We have also added rationale for the secondary outcomes on page 7 lines 26-53: “It is possible that cow’s milk fat may also result in other beneficial health effects. Higher circulating levels of trans-palmitoleic acid have been associated with lower adiposity, LDL cholesterol, insulin resistance, and triglycerides, and positively associated with HDL cholesterol, in several large adult cohort studies. During early childhood, dietary fat consumption is known to support cognitive development, which usually concludes around six years of age. Cow’s milk fat may promote brain development due to its essential fatty acid content (e.g. linoleic acid) which may manifest in gains across multiple developmental domains including social, emotional, and physical. The ratio of essential fatty acids linoleic to alpha-linoleic acid (n-6 to n-3) in whole cow’s

milk is believed to optimize circulating DHA (docosahexaeonic acid), which is an important fatty acid to brain growth and function."

Comment: Objective 5- there is no earlier reference to costs, this needs to be introduced in the introduction and clearly explained. There is logical for costs related to health care system if type of milk consumption reduces adverse health outcomes- and therefore likelihood of needing to utilise health care services for these outcomes. However I find it hard to imagine these costs would manifest after 24 months.

Response: Thank you for this comment. We have added to page 7 lines 18-24: "Given the financial burden of overweight and obesity on healthcare systems worldwide, determining which milk fat recommendation in childhood is effective in lowering a child's risk of developing excess adiposity, may result in substantial healthcare savings in the future."

Comment: As noted for the introduction, the expected hypotheses and outcomes are unclear as the majority of them have not been introduced or explained in the introduction. Please fully explain each of these and the proposed mechanisms.

Response: Thank you for this suggestion. We have clarified as noted above, pages 6-7.

Comment: Page 8, line 48- If children are recruited up to 2.99 years, how you will the study handle instances where children have already been introduced to recommendations for reduced fat at age 2 years, prior to entering the trial?

Response: Thank you for this comment. We will provide the same intervention to all children regardless of prior recommendations, for consistency. Randomization should ensure equal distribution of prior recommendations between the two groups.

Comment: Page 9, lines 2-5: Please state how many affiliated primary healthcare providers are in the network if possible for clarity.

Response: We have added "over 100" university affiliated primary care physicians to page 10 line 2.

Comment: Page 10: Inclusion criteria- how do parents report if the children are healthy- please specify the question(s) asked to determine this (e.g. is it on a scale, is it dichotomous healthy/unhealthy)

Response: Thank you. We have added the following clarification to page 10 lines 18-21: "healthy by parental report (characterized as not living with chronic or acute illness, except for asthma)."

Comment: Exclusion criteria- please operationalize 'severe developmental delay' for the purposes of this study.

Response: Thank you for this comment. We have added "which impacts on daily functioning" to this page 10 line 34.

Comment: Can the authors please comment on potential implications of physicians making recommendations with which they may not agree, e.g. if a patient is randomised to one recommendation but the physician does not agree with this it may impact intervention implementation/fidelity etc.

Response: Thank you for this comment. All participating physicians have reviewed the study protocol and have provided consent to administer both interventions. Any physicians who are not comfortable

will not enroll participants in the study. We have added “All participating healthcare providers have provided consent to participate in the randomization process” to page 11 lines 10-12.

Comment: Whole milk recommendation- please clarify if the recommendation to not switch to reduced fat is explicitly stated to parents, or if they are only told what they should do in this experimental condition?
Please include what the email reminders will say, either in the text or as an appendix

Response: Thank you for this suggestion. We have included the email reminder and intervention scripts in a Supplementary File. In both groups, parents will be told that they have been randomized to their respective milk fat recommendation as part of the study during enrollment.

Comment: Reduced fat milk recommendation- please clarify if it will be explained to parents that these are current guidelines- if so it may influence their adherence. For both conditions, clarity about what is said is needed.

Response: Thank you for this comment. We have included the intervention scripts in a Supplementary File: “Your child is recommended to consume 2 cups or 500 mL of [whole or 3.25%/1%] cow’s milk each day. Do you have any questions about that?”

Comment: Adherence- the authors refer to reaching children before 1.5 but do not refer to recruitment of children up to nearly 3 years of age, please address. Also please note that the age of children at recruitment is a recruitment point and does not ensure adherence, only increases likelihood of timely message delivery.

Response: Thank you for this comment. We have removed the sentence about recruitment.

Comment: Page 11. Line 2- how frequent are the well-child visits, and how many visits (and therefore intervention exposures) will participants receive during the study?

Response: Thank you. We have changed this to “Primary healthcare providers will be reminded to repeat milk recommendations at up to two subsequent well-child visits during study participation” on page 12 lines 5-10.

Comment: Page 11, line 5- what do the magnets say?

Response: We have included copies of magnets in the Supplemental File for reference.

Comment: Page 11- Baseline Participant Characteristics: Are any developmental outcomes measured? Waist circumference referred to later but not included here, please correct.

Response: Thank you for this comment. We have added “waist circumference, cognitive development using the Ages and Stages Questionnaire” to baseline characteristics on page 12 lines 29 and 42-45.

Comment: Page 11, line 42-43: because 2 years post-randomisation has been used in other studies is insufficient rationale for your choice here. Please give an appropriate rationale based on scientific hypotheses about expected effects on primary (and secondary) outcomes over time

Response: Thank you for this comment. 2 years of follow-up was chosen to be long enough to identify meaningful differences in children’s adiposity, which has been demonstrated in the cited studies.

Comment: Please present timing of measurements more clearly. At present you have a baseline measurement section that does not include all measures taken at enrolment.

Response: Thank you. We have corrected this as noted above, page 12 lines 29 and 42-45.

Comment: Page 14: Sample size- why is 20% attrition expected, please justify this figure.

Response: We expect 20% to be a conservative estimate of attrition. However, recent TARGet Kids! clinical trials have experienced 10% attrition. We have cited this study in the protocol after "20% loss to follow-up" on page 15 line 38.

Comment: Page 14: Recruitment- how will presence of research assistants lower burden and ease trial entry? Please be sure to explain and justify all such comments.

Response: Thank you for this comment. We have modified this section according to your suggestion below. The Recruitment section now reads "Recruitment strategies include recruitment in person by a trained research assistant who is known to them through the TARGet Kids! program at a routine primary healthcare visit" on page 15 lines 49 to 57.

Comment: Points 1- 3 could be combined as a single point that participants will be recruited in person by a trained research assistant who is known to them through the TARGet Kids program.

Response: Thank you. We have made this change.

Comment: Having the blood sampling later than the surveys and anthropometrics will not necessarily improve maximise recruitment because participants will still be expecting and undergoing the blood draw in the same appointment.

Response: Thank you. We have removed this.

Comment: Page 15 Blinding- You have rightly stated that participant and healthcare professional blinding cannot occur, so please clarify for whom the allocation concealment is relevant/

Response: Thank you for this comment. We have added "Allocation concealment is preserved for research assistants and parents who may enroll participants depending on randomization sequence if they are aware of it" to page 16 lines 32-34.

Comment: Page 15 line 42-45: Reinforcement of recommendations is the intervention; it is not a retention strategy. Remove this statement from here.

Response: Thank you. We have removed this.

Comment: Page 15, lines 45- 48: what does every reasonable attempt mean? You need to explain this.

Response: Thank you. We have added "including phone calls and emails" to page 16 line 40.

Comment: Page 16 line 2-5: Why is it expected that a home visit will work where other approaches have not? And from where does the figure of 10% emerge?

Response: We have removed this.

Comment: Page 16 Data management: more information is needed here about how data will be processed, stored, and accessed.

Response: Thank you. We have added to page 17 line 7-26 “Study data were collected and managed using REDCap electronic data capture tools hosted at St. Michael’s Hospital. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.”

Comment: Page 17 Ethics: Please give ethics approval number if possible and please outline how ethical issues are addressed in this study.

Response: Thank you. We have added these to page 19 lines 7 and 10.

Comment: Any behaviour change theory/techniques or use of specific implementation strategies based on a framework approach? These discussed to provide confidence that the intervention is empirically and theoretically based, with appropriate methods chosen for delivery.

Response: Thank you for this comment. The intervention was designed to mimic the current primary healthcare milk fat recommendation which is currently recommended to be provided by Canadian primary healthcare providers at the 2 year health maintenance visit (see: www.rourkebabyrecord.ca).

Reviewer: 2

Comment: 1. For protocol papers it seems that the journal requires the planned dates of the study which are not given.

Response: Thank you for this suggestion. We have added “Recruitment started in February 2020 and is expected to take 24 months to complete enrollment” to page 10 lines 10-12.

Comment: 2. p 7, line 46, re the primary outcome. It would have been good to also have a direct measure of adiposity for a primary outcome but presumably this was regarded as impractical with a large cohort of children?

Response: Thank you for this suggestion. We agree that a more granular measure of adiposity would be advantageous, but this is unfeasible in the primary healthcare setting due to the amount of available time and space for data collection during primary healthcare visits.

Comment: 3. p 7, line 52, re secondary outcome, would 'Better cardiovascular health' be more correct as 'Lower risk of cardiovascular disease' ?

Response: Thank you. We have made this change.

Comment: 4. p 8, line 49, and p 9, line 42 It is said that children aged 1.5 to 2.99 years will be recruited and randomised to the study treatments which seems at odds with the objective of examining the effect of consuming whole vs. fat reduced milk from the age of 2 years. Also, under Adherence it says that children from 1.5 years will be included in the recruitment phase to so as to involve families before milk choices are formed. The last point is clearly how things should be but more clarity on the earlier statements about recruiting and randomisation would remove confusion.

Response: Thank you for this suggestion. We have modified the Adherence section to clarify this by removing “1) Children from 1.5 years of age will be included during the recruitment phase so that

recommendations reach families before milk choices have been formed."

Comment: 5. Under Reduced Fat Milk Recommendation, (page numbering at bottom of pages has gone back to 1.) it is said that children on the reduced milk fat treatment would be provided with the same nutritional recommendations as those in whole milk treatment. It is not clear what 'provided with' means. Crucially does it mean that as far as possible the children on the reduced fat milk will have the same total fat intake as those in the whole milk group? If yes how would this be achieved and with fat source?

Response: Thank you for this comment. We have changed this to "Children who receive the reduced fat recommendation will be provided with the same age-appropriate nutritional recommendations for foods other than cow's milk" to clarify on page 11 lines 29 and 51.

Comment: 6. Randomisation. There is no mention of how sex is dealt with. Presumably the aim would be have essentially the same proportion of males and females in both treatment groups?

Response: Thank you for this concern. We expect that randomization will result in an equal proportion of males and females in both groups and zBMI is a standardized measure of body weight adjusted for child sex. We have included the following statement in the Statistical Analysis section on page 17 lines 40-45: "Although randomisation is expected to balance the covariates, important variables such as age, sex and baseline zBMI, that demonstrate, by chance, a potentially clinically meaningful imbalance, will be considered as adjusting covariates."

Comment: 7. Minor points. In several places the term 'caloric intake' is mentioned which would be better as 'energy intake'. Also concentrations of fat in milk are given in non-SI units (%) rather than g/100g.

Response: Thank you. We have changed "caloric" to "energy" throughout. We have used percentages because of the way cow's milk is presented to consumers (i.e., in 1%, 2%, 3.25% formats) and would be best understood by parents.

VERSION 2 – REVIEW

REVIEWER	Karen Matvienko-Sikar University College Cork, Ireland
REVIEW RETURNED	11-Feb-2020

GENERAL COMMENTS	The authors have sufficiently addressed all of my queries, I look forward to seeing the protocol in print and to hearing about the the outcomes of the trial in due course.

REVIEWER	Professor Ian Givens Institute for Food, Nutrition and Health University of Reading Reading RG6 6AR United Kingdom
REVIEW RETURNED	13-Feb-2020

GENERAL COMMENTS	I am happy with the responses to all of my earlier comments except one. Re Reviewer 2, comment 5, I remain unclear as how the total

	diets are going to be managed. The response says that 'We have changed this to “Children who receive the reduced fat recommendation will be provided with the same age-appropriate nutritional recommendations for foods other than cow’s milk”' The specific question about whether it is the intention that children receiving the reduced fat milk will have the same total fat intake as those in the whole milk group is still not clear. Given that the key intention is to compare reduced fat milk with whole milk it is important to have clear dietary strategy especially for dietary fat. As things are it seems that whilst the parents will be given guidance on nutritional recommendations for foods other than cow’s milk but there is not a specific plan regarding nutrient/fat intake. It would be helpful if the authors could make the dietary strategy clear and justify it especially about equalizing or not fat (and hence energy) intake in both treatments.

VERSION 2 – AUTHOR RESPONSE

Please accept our revised manuscript “Cow’s Milk Fat Obesity pRevention Trial (CoMFORT): a primary care embedded randomised controlled trial to determine the effect of cow’s milk fat on child adiposity” for consideration of publication in the BMJ Open. We kindly thank the thoughtful reviewers for their careful suggestions and have made all corrections as advised.

The one outstanding comment has been addressed:

Comment: It remains unclear as how the total diets are going to be managed. The specific question about whether it is the intention that children receiving the reduced fat milk will have the same total fat intake as those in the whole milk group is still not clear. It would be helpful if the authors could make the dietary strategy clear and justify it especially about equalizing or not fat (and hence energy) intake in both treatments.

Response: Thank you for clarifying this. We plan to analyse total fat intake through a 24-hour dietary recall at age 4 years, collected through the Automated Self-Administered 24-hour recall tool, which will capture total fat intake from both cow’s milk fat and other foods. We have added the following to page 17 line 18-21, in the “Sugary Beverage and Total Energy Intake” section: “Differences in fat intake from both cow’s milk and other dietary sources of fat will be evaluated.”

Thank you for taking the time to review our manuscript. We appreciate your consideration and look forward to your response.