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)	The association between Caesarean Section Delivery and Obesity in Childhood: A Longitudinal Cohort Study in the Republic of Ireland
AUTHORS	Masukume, Gwinyai; McCarthy, F. P.; Baker, Philip; Kenny, Louise; Morton, Susan; Murray, Deidre; Hourihane, Jonathan; Khashan, Ali

VERSION 1 – REVIEW

REVIEWER	Rodrigo M Carrill-Larco CRONICAS Centre of Excellence in Chronic Diseases, Universidad Peruana Cayetano Heredia, Lima, Peru.
REVIEW RETURNED	11-Jul-2018

GENERAL COMMENTS	Article summary: Last bullet point. Would you consider specifying what “cases” you meant? Perhaps: the number of overweight and obesity cases… Methods – exposure and outcome ascertainment: last paragraph, potential confounders. Would you consider that antibiotics or any maternal infection near the birth to be a confounder as well? Provided the pathway between birth and obesity is by microbiota flora, infections or antibiotics (prescribed to the mother or to the baby early in life) could influence this association. Probably the study population had low infection rates? Be as it may, not including infections or antibiotic use could be a limitation or an issue worth discussing briefly. Lastly, would you consider giving further detail about some variables? For example “smoking before and during pregnancy” and “pre-eclampsia” were included as confounders. Additional details about how these were assessed would be appreciated. Methods – statistical analysis: did the regression models accounted for multiple records of the same subject (i.e., clustered data)? The regression models seem to have been adjusted for birth weight. Was there a strong correlation between this and the outcomes? In this line, have you considered adjusting the models for BMI at different ages (e.g., outcome at two years adjusted for BMI at age one) and the use of other models that account for multiple observations in time? This may be outside the scope of this paper, but given the repeated measures available in the data, modelling them would give more robust results than only looking at one outcome at the time. Results – regression models: would you consider re-phrasing some sentences to make them clearer? For example, in the last paragraph (4th line) it reads: …results in the association between

	prelabour… However, there was no strong association (was not significant). Maybe this could read “results in a non-significant association…”. Results – Table 1: it seems that the first variable is the age of the mother. If so, would you consider modifying the label to clearly reflect this is indeed the mother’s age? In addition, some labels could be clearer, and would help to better understand the table without referring to the text. For example, “maternal BMI at 155 weeks…” If this refers to 15 weeks of pregnancy, would you consider making this label more specific? Results – Table 1, body composition (at two months): there seems to be quite a significant amount of missing data. Would you please consider checking if there were any relevant differences between included and excluded (missing data) subjects? Were there any differences between these groups that could have affected the results or conclusions? If appropriate or relevant, a supplementary table could be included. On the other hand, if they were excluded due to any other reasons (e.g., medical condition), would you please comment this on the methods section? Discussion – main findings: would you consider briefly describing the direction of the significant differences? For example where it reads “…significant difference in BMI at age six months was observed between infants born by…”. Would you consider briefly explaining which group had the positive or negative difference? Discussion – strengths and limitations, last paragraph: it seems that the low number of cases was a limitation. Would you please consider discussing if this was expected, i.e., the prevalence/incidence seems to agree with other studies in the country or similar populations? If there were fewer cases in your sample, in comparison to other literature, would you discuss this discrepancy and its implications on your results? In line with the last paragraph before the conclusion, what would be the implications of this “low risk population” in the results? Discussion: page 12, lines 37-44. Would you consider revising the statement “Us finding an association at age five…”? First, it appears that membranes are more likely to have ruptured in LSCS; however, it could also happen (and sometime highly frequent) in prelabour CS. In fact, it could be the case that an early membrane rupture could lead to CS (either pre-labour or during labour). Second, the reference to causation seems to be rather strong. Studying causation would perhaps require other methods and a different approach (e.g., casual inference methods), if not another study design. Please, if possible, consider rewording these lines (i.e., tone down). Data sharing statement: it would be useful to know if the data used for this work, and other collected as part of this study, are available and how it can be accessed.

REVIEWER	Tina Lavin Research Associate, School of Population and Global Health, University of Western Australia, Perth, Australia
REVIEW RETURNED	19-Jul-2018

GENERAL COMMENTS	Reviewer 2 comments “The association between Caesarean Section Delivery and

	Obesity in Childhood: A Longitudinal Cohort Study" This study adds to the limited literature around CS and obesity later in life. The major strength of the this study is the analysis around pre-labour CS as compared to CS after a trial of labour and childhood obesity risk of which is there a paucity of published literature. Please find my comments below: Abstract: 1. Objectives: state at what age the outcome was measured e.g. early childhood - Minor comment around terminology: Childhood implies time-points up to 18 years. Your analysis lends itself to infancy and early childhood. Perhaps refer to 'early childhood' - In your abstract (and throughout manuscript – see comments below) you present data on BMI, however your most robust measurement was body fat % especially at early ages Background: 2. The relevancy of paragraph 3 (line 28) is a little confusing as this was not a focus of your study. i.e. that neonatal body fat % is a better predictor of CS risk than birthweight. You did not use neonatal body fat % as an outcome measure and did not adjust for neonatal body fat % as a confounder for CS (i.e. confounding by medical indication) in your models. The rationale behind this paragraph is thus a little confusing. 3. The rest of background is well-written and relevant. Methods: 4. I'm a little confused why you focused on BMI as measure of overweight/obesity rather than %BF given that %BF is a more robust measure of overweight/obesity (as you have highlighted in your manuscript) especially at such young ages. There are some issues with use of BMI at birth, 2 months and 6 months as the consensus is that this is not a valid measure for overweight/obesity at such young ages. This is due to issues such as known variability in growth trajectory between 0-2 years. This measure also has limited clinical value at 6 months (and younger). As stated in your manuscript IOTF classification begins at 2 years, yet you have applied it to BMI at 6 months and younger. The biological plausibility of observing obesity at birth, 2 months and 6 months due to birth through CS is also a little unclear. You mentioned in your manuscript that neonatal body fat % is a better predictor of CS than birthweight, could this also be a better measure for overweight/obesity (over BMI)? Can you please comment or provide rationale for validity regarding the use of BMI at such a young ages? I'm also wondering why BF% was not used in place of BMI? I think there also needs to be some rationale provided as to why obesity at birth, 2 months and 6 months would be associated with CS birth given the biological mechanisms at play. 5. Do you think confounding by indication was an issue? For example were the women who delivered pre-labour CS different to post-labour CS/vaginal birth (you have outlined in Table 1 but not discussed).

	Results: 6. Figure 1 and Figure 2 – as mentioned previously BMI at birth, 2 months and 6 months are not generally considered valid measurements – therefore I don't think should be presented in your figures or a main focus of your discussion. Would it be possible to present and plot BF% at these ages instead? As well as use this as your main outcome measure?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 comment	**Response**
Article summary: Last bullet point. Would you consider specifying what "cases" you meant? Perhaps: the number of overweight and obesity cases…	Thank you for highlighting the ambiguity of this statement. Specification has been done as suggested.
Methods – exposure and outcome ascertainment: last paragraph, potential confounders. Would you consider that antibiotics or any maternal infection near the birth to be a confounder as well? Provided the pathway between birth and obesity is by microbiota flora, infections or antibiotics (prescribed to the mother or to the baby early in life) could influence this association. Probably the study population had low infection rates? Be as it may, not including infections or antibiotic use could be a limitation or an issue worth discussing briefly. Lastly, would you consider giving further detail about some variables? For example "smoking before and during pregnancy" and "pre-eclampsia" were included as confounders. Additional details about how these were assessed would be appreciated.	The reviewer makes a valid and important point. Thus to the discussion as suggested the following sentence has been added: "It has been suggested that any association between CS birth and childhood obesity is due to antibiotics administered during CS, with CS delivery serving as a proxy, nonetheless this proposition has not been supported by evidence.[1 2]" Thanks for requesting more detail. We agree that including both smoking before and during pregnancy can create misunderstanding. We have removed the aspect referring to smoking before pregnancy because, as an example, one of the final regression (a method to control for confounding) models we found that the variance inflation factor was > 4. Smoking before and during pregnancy were highly correlated thus only one them (smoking during pregnancy) was included in the final model(s).

	We have also added the following sentence to further illustrate what we mean by a confounder: "For instance smoking cigarettes is a potential confounder because it is a risk factor for both CS birth[3] and for childhood obesity.[4]"
Methods – statistical analysis: did the regression models accounted for multiple records of the same subject (i.e., clustered data)? The regression models seem to have been adjusted for birth weight. Was there a strong correlation between this and the outcomes? In this line, have you considered adjusting the models for BMI at different ages (e.g., outcome at two years adjusted for BMI at age one) and the use of other models that account for multiple observations in time? This may be outside the scope of this paper, but given the repeated measures available in the data, modelling them would give more robust results than only looking at one outcome at the time.	We agree with the reviewer that this may be outside the scope of the paper. However, regarding the birth weight issue: As mentioned in the abstract (and paper), the adjusted relative risk ratio (aRRR) changed from =1.37; [95% CI 0.69-2.69]) to (aRRR=0.86; [95% CI 0.36-2.08]) after exclusion of non-macrosomic infants. This was a > 10% change in the aRRR, specifically towards the null, leading to the conclusion that macrosomia (birth weight > 4000g) is a confounder. So yes birth weight was adjusted for and further stratification showed it is birth weight of a certain magnitude (> 4000g) which is confounding the association. This is in keeping with: "We wanted, in particular, to examine the potential confounding effect of macrosomia, as this is both a risk factor for CS, and for long term obesity." Background. In addition at later ages, we did not find an association therefore we did not conduct further analysis.
Results – regression models: would you consider re-phrasing some sentences to make them clearer? For example, in the last paragraph (4th line) it reads: …results in the association between prelabour… However, there was no strong association (was not significant). Maybe this could read "results in a non-significant association…".	This observation is very helpful. That sentence has been rephrased as recommended.

Results – Table 1: it seems that the first variable is the age of the mother. If so, would you consider modifying the label to clearly reflect this is indeed the mother's age? In addition, some labels could be clearer, and would help to better understand the table without referring to the text. For example, "maternal BMI at 155 weeks…" If this refers to 15 weeks of pregnancy, would you consider making this label more specific?	Thank you, the mother's age label has been clarified. We have double-checked and the original label read "Maternal BMI at 15 weeks".
Results – Table 1, body composition (at two months): there seems to be quite a significant amount of missing data. Would you please consider checking if there were any relevant differences between included and excluded (missing data) subjects? Were there any differences between these groups that could have affected the results or conclusions? If appropriate or relevant, a supplementary table could be included. On the other hand, if they were excluded due to any other reasons (e.g., medical condition), would you please comment this on the methods section?	It is correct that there is a significant amount of missing data for body composition at two months. We have therefore added, "Thus missing data was unlikely to have affected the results or conclusions (Supplementary Table 1).".
Discussion – main findings: would you consider briefly describing the direction of the significant differences? For example where it reads "…significant difference in BMI at age six months was observed between infants born by…". Would you consider briefly explaining which group had the positive or negative difference?	The group with the positive difference has been mentioned thus: "Infants born by CS had a higher mean BMI."
Discussion – strengths and limitations, last paragraph: it seems that the low number of cases was a limitation. Would you please consider discussing if this was expected, i.e., the prevalence/incidence seems to agree with other studies in the country or similar populations? If there were fewer cases in your sample, in comparison to other literature, would you discuss this discrepancy and its implications on your results? In line with the last paragraph before the conclusion, what would be the implications of this "low risk population" in the results?	The reviewer brings up an important consideration. We had made a comparison of the prevalence to that in the country in terms of magnitude. As the reviewer suggests, we have now gone further by clarifying our statement on the study's external validity as follows: "This suggests the generalizability of findings to the Irish population, particularly 'low risk' first time mothers."
Discussion: page 12, lines 37-44. Would you consider revising the statement "Us finding an association at age five…"? First, it appears that membranes are more likely to have ruptured in	We agree with the reviewer that membranes are more likely to have ruptured in LSCS (in labour) than with prelabour CS and that we need to tone

LSCS; however, it could also happen (and sometime highly frequent) in prelabour CS. In fact, it could be the case that an early membrane rupture could lead to CS (either pre-labour or during labour). Second, the reference to causation seems to be rather strong. Studying causation would perhaps require other methods and a different approach (e.g., casual inference methods), if not another study design. Please, if possible, consider rewording these lines (i.e., tone down).	down. We have thus rephrased and reworded that sentence. Causal was removed and replaced. In addition we have cited a recent study – May 2018 - showing that before the onset of contractions (prelabour) the amniotic fluid is essentially free of bacteria in most cases. Reference 19. https://www.ncbi.nlm.nih.gov/pubmed/29852156
Data sharing statement: it would be useful to know if the data used for this work, and other collected as part of this study, are available and how it can be accessed.	We have added: "Data may be accessed by request from the Babies After SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE) study. Contact details are available on the study website http://www.baselinestudy.net/."

Reviewer: 2 comments	**Response**
Abstract: 1. Objectives: state at what age the outcome was measured e.g. early childhood - Minor comment around terminology: Childhood implies time-points up to 18 years. Your analysis lends itself to infancy and early childhood. Perhaps refer to 'early childhood'	Being more precise is indeed better. Therefore reference to early childhood has been made.
- In your abstract (and throughout manuscript – see comments below) you present data on BMI, however your most robust measurement was body fat % especially at early ages Background: 2. The relevancy of paragraph 3 (line 28) is a little confusing as this was not a focus of your study. i.e. that neonatal body fat % is a better predictor of CS risk than birthweight. You did not use neonatal body fat % as an outcome measure and did not adjust for neonatal body fat % as a confounder for CS (i.e. confounding by medical indication) in your models. The rationale behind this paragraph is thus a little confusing.	The rationale for mentioning neonatal body fat % has been added: "Therefore conversely changes in body fat percentage could be an early and more sensitive indicator of future health."
3. The rest of background is well-written and relevant.	Your kind comment is much appreciated.

4. I'm a little confused why you focused on BMI as measure of overweight/obesity rather than %BF given that %BF is a more robust measure of overweight/obesity (as you have highlighted in your manuscript) especially at such young ages.	Unfortunately %BF was available at only age two months.
There are some issues with use of BMI at birth, 2 months and 6 months as the consensus is that this is not a valid measure for overweight/obesity at such young ages. This is due to issues such as known variability in growth trajectory between 0-2 years. This measure also has limited clinical value at 6 months (and younger). As stated in your manuscript IOTF classification begins at 2 years, yet you have applied it to BMI at 6 months and younger. The biological plausibility of observing obesity at birth, 2 months and 6 months due to birth through CS is also a little unclear. You mentioned in your manuscript that neonatal body fat % is a better predictor of CS than birthweight, could this also be a better measure for overweight/obesity (over BMI)? Can you please comment or provide rationale for validity regarding the use of BMI at such a young ages? I'm also wondering why BF% was not used in place of BMI? I think there also needs to be some rationale provided as to why obesity at birth, 2 months and 6 months would be associated with CS birth given the biological mechanisms at play.	We agree with the reviewer that BMI at birth, 2 months and 6 months by consensus is not a valid measure for overweight/obesity at such young ages. We therefore did not apply the IOTF classification below age two as the reviewer contends we did. We had mentioned in the exposure and outcome ascertainment section, "At age two and five years, BMI was classified as thin, normal, overweight or obese, according to the International Obesity Task Force (IOTF) criteria.[5 6]" In the, interpretation section, we had written and drawn from the paper by Vinding RK *et al*. "Our findings are similar to those of infants, born in 2010, from a Danish prospective cohort study which found that the largest BMI difference by delivery mode, from birth to five years of age, occurred at six months' age and that this difference did not track into later childhood at age five.[2]" This paper by Vinding RK *et al.* also plotted BMI (Figure 1) at time points below two years e.g. at birth, 2 months and 6 months so that the BMI's natural history could be visualised from birth. https://www.ncbi.nlm.nih.gov/pubmed/28814549 In the discussion we have added this sentence to explain the importance and consequent focus on the first two years of life: "It is worth highlighting that the first two years of life have been identified as a critical developmental window during which perturbations in growth and development are more likely to result in lifelong sequelae.[7]"
5. Do you think confounding by indication was an issue? For example were the women who delivered pre-labour CS different to post-labour CS/vaginal birth (you have outlined in Table 1 but not discussed).	This comment is appreciated like the others. We have now added: "The exact indications for CS were not available for this cohort."
Results: 6. Figure 1 and Figure 2 – as mentioned previously BMI at birth, 2 months and 6 months are not generally considered valid measurements – therefore I don't think should be presented in your figures or a main focus of your discussion. Would it be possible to present and plot BF% at these ages instead? As well as use this as your main outcome measure?	As mentioned in the response above: In the, interpretation section, we had written and drawn from the paper by Vinding RK *et al*. "Our findings are similar to those of infants, born in 2010, from a Danish prospective cohort study which found that the largest BMI difference by delivery mode, from birth to five years of age, occurred at six months' age and that this difference did not track into later childhood at age five.[2]"

	This paper by Vinding *et al*. also plotted BMI (Figure 1) at time points below two years e.g. at birth, 2 months and 6 months so that the BMI's natural history could be visualised from birth. https://www.ncbi.nlm.nih.gov/pubmed/28814549 In the discussion we have added this sentence to explain the importance and consequent focus on the first two years of life: "It is worth highlighting that the first two years of life have been identified as a critical developmental window during which perturbations in growth and development are more likely to result in lifelong sequelae.[7]" Unfortunately it is not possible to present and plot BF% as suggested because it was not available at those ages.

References

1. Mueller NT, Mao G, Bennet WL, et al. Does vaginal delivery mitigate or strengthen the intergenerational association of overweight and obesity? Findings from the Boston Birth Cohort. International journal of obesity (2005) 2017;**41**(4):497-501.
2. Vinding RK, Sejersen TS, Chawes BL, et al. Cesarean Delivery and Body Mass Index at 6 Months and Into Childhood. Pediatrics 2017;**139**(6).
3. Sinnott SJ, Brick A, Layte R, et al. National Variation in Caesarean Section Rates: A Cross Sectional Study in Ireland. PLoS One 2016;**11**(6):e0156172.
4. Magriplis E, Farajian P, Panagiotakos DB, et al. Maternal smoking and risk of obesity in school children: Investigating early life theory from the GRECO study. Preventive medicine reports 2017;**8**:177-82.
5. Cole TJ, Bellizzi MC, Flegal KM, et al. Establishing a standard definition for child overweight and obesity worldwide: international survey. Bmj 2000;**320**(7244):1240-3.
6. Cole TJ, Flegal KM, Nicholls D, et al. Body mass index cut offs to define thinness in children and adolescents: international survey. Bmj 2007;**335**(7612):194.
7. Barker DJ. Sir Richard Doll Lecture. Developmental origins of chronic disease. Public health 2012;**126**(3):185-9.

VERSION 2 – REVIEW

REVIEWER	Tina Lavin School of Population and Global Health University of Western Australia, Perth ustralia
REVIEW RETURNED	17-Aug-2018

GENERAL COMMENTS	Thank you for addressing my concerns.