PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Maternal Multivitamin Intake and Orofacial Clefts in Offspring: Japan Environment and Children's Study (JECS) cohort study
AUTHORS	Yoshida, Satomi; Takeuchi, Masato; Kawakami, Chihiro; Kawakami, Koji; Ito, Shuichi

VERSION 1 – REVIEW

REVIEWER	Jose Suazo Universidad de Chile
REVIEW RETURNED	18-Jul-2019

GENERAL COMMENTS	This manuscript reveals new evidence showing the potential teratogenic effects of periconceptional intake of multivitamin supplements in a national wide study. These findings are contrary to previous reports supporting the protective role against orofacial clefts of certain micronutrients plus folic acid. The discussion explaining this discrepancy is based on retinoic acid and its teratogenic effects. The authors must comment about the different multivitamin supplement available in Japan and if there is some of them designed for pregnancy and compare the retinoic acid content among them. A minor issue: page 6, line 45 says "periconceptual" and may say "periconceptional".

REVIEWER	Kari Klungsøyr Nasjonalt folkehelseinstitutt, Division of Mental and Physical Health
REVIEW RETURNED	04-Aug-2019

GENERAL COMMENTS	This study is based on data from the Japan Environment and Children's Study (JECS), a nationwide, prospective birth cohort study with data from close to 100 000 pregnant women and their offspring. The study aimed at examining the relation between maternal nutrition and supplement intake and oral clefts. Maternal nutrition was based on information from a semi-quantitative food frequency questionnaire answered in the first trimester. Information on the use of supplements was collected from interviews at two time points during pregnancy about intake before pregnancy was confirmed, from the first trimester and from the period after 12 weeks. The oral clefts were based on diagnoses made within one month after birth by two pediatricians at unit centers of the JECS. Selected variables were included as covariates in models analyzing the relation between maternal supplement use and oral clefts.

	The paper is well written and is based on a large cohort study, however, the results are unexpected and need to be evaluated carefully. Most studies looking at the present associations have found different results (multivitamin supplements associated with a reduced risk of oral clefts or no association). The present study does further not find an association between folate supplements and oral clefts, whereas many studies find that folate supplements may also be associated with a reduced risk of oral clefts. The paper lacks some important information which is needed to evaluate the results, and the analyses do not fully investigate what is defined as the study aim: “to examine the relationship between nutrition and CL alone, CP alone and CL with CP, with a focus on micronutrient and supplement intake….”. I have the following main comments: * First, the authors have important data on nutritional intake, and one of the aims was to investigate the relationship between nutrition and oral clefts. However, comparing intake in mothers to infants with cleft palate only (CP), cleft lip with or without cleft palate (CL/P) and unaffected infants, they state that there were no significant differences in nutrient intake (Table 1). However, since CP and CL/P may be viewed as separate entities with a different embryological basis and likely different causal factors, it would be good to compare each of these groups (one at a time) with the reference group. This would also be more in line with the logistic regression analyses where CL/P and CP are included as dependent variables, one at a time. If there are differences in nutrient intake between the mothers to one of the affected groups and the reference mothers, these nutrients should be included in the corresponding logistic regression analysis (e.g. by modelling intake as a categorical variable based on quartiles or quintiles). This would be especially interesting regarding the intake of retinol among mothers to CL/P infants and mothers to unaffected infants, since the p-value comparing this nutrient between all three groups was relatively low, p=0.176, and the median intake was 445 µg in CL/P mothers, relative 406 µg in mothers to unaffected infants. Also, the authors assume that it is retinol/ vitamin A from the multivitamin supplements that drive the associations found, thus nutritional intake of retinol should be included in a full model. * I am concerned that the authors do not have any information about the content of the multivitamin supplements. It should be possible to at least give some information about the content of the most commonly used multivitamin supplements in Japan during the study years. As it is now, the authors make assumptions about the contents likely driving the associations without any supporting information. E.g. the statement on page 20, lines 24-25: “whereas excess vitamin A intake may be detrimental” cannot be based on the current study. Although this problem is mentioned as a limitation of the study, the authors base much of the discussion on the assumption that vitamin A / retinol is driving the association found. Without knowledge of the true content of the supplements, this should be modified or rewritten. Adjustment for nutritional retinol would be good here. The authors further assume that the multivitamin supplements are without folate since “questions addressed to mothers about multivitamin intake were independent of those about intake of folic acid supplements” (Discussion, page 18). This is not sufficient to assume that multivitamins do not contain folate.

	In Norway, nearly all multivitamin supplements contain some folic acid and has done so since the association between folic acid and neural tube defects was established. The JECS included study participants during 2011-2014, so this association was well known and I would be surprised if multivitamin supplements in Japan do not include any folate. * The authors do not say anything about syndromic cases. CP is more often syndromic than CL/P. How many of the cleft cases were isolated (each oral cleft group), and how many were associated with other major congenital anomalies? If associated with other anomalies, what were the associated anomalies? If the cleft cases include cases with associated anomalies, the authors should also include analyses on isolated cases. * Totally, 98,787 mother-child pairs were included in the Study population. How many women had more than one child? The authors have not taken account of correlation between infants born to the same mother, which should be done. * Covariates evaluated for association with maternal nutrition and oral clefts included gestational diabetes, gestational week (at birth, I suppose) and birth weight. However, none of these variables should be considered potential confounders, as they all occur after the lip and palate have developed, with the possible exception of gestational diabetes. However, birth weight and gestational week were not included in the logistic regression analyses, which is good. Pre-gestational diabetes mellitus, however, could be a potential confounder, as some studies find it to be associated with offspring oral clefts, and it has evident associations with maternal nutrition. Why have the authors included gestational diabetes but not pre-gestational diabetes mellitus? * All the cases included were live born children. Do the authors have information on stillborn cases? What about terminations for fetal anomaly? Were there any terminations of pregnancy for cases with oral clefts? These would most likely be for syndromic cases, and of less interest in the present study, but it would be good to know the numbers to complete the picture. * Page 20, last sentence: “due to the rarity of the study outcome (orofacial clefts), our choice of a cohort design allowed us to better detect the investigated association compared with a case-control design”. This is not correct. When the outcome is rare, the best design is the case-control study, since very large cohorts are needed to allow the study of rare outcomes. Even in the current large cohort study, only 52 infants with CP were included. Had the authors chosen a case control design, they could have included more CP infants and preferably had an equal number of CP and CL/P infants. The wide confidence intervals around the estimates for the CP group indicates that even in this large cohort there is likely a lack of power for the evaluation of multivitamin supplements and cleft palate only. For instance, the point estimate for supplement use in the first trimester suggests a more than doubled risk associated with CP, but was not statistically significant : OR 2.43 (0.88-6.74). * Genetic factors are important for the occurrence of oral clefts. It is therefore a limitation that the authors do not have information on

	possible parental oral clefts. This is, however, discussed by the authors as one of the limitations of the study. Specific comments throughout: * Abstract, Results, line 45: "… 182 (0.18%) were diagnosed with cleft lip and/or cleft palate": should be cleft lip with/without cleft palate. * Strengths and limitations, page 5, bullet point three: As mentioned above, the best study design for rare outcomes is the case-control study. However, when the cohort is sufficiently large enough, the cohort study can also be used. Even though the present analyses are based on a large cohort study, there were only 52 cleft palate cases, and a likely lack of power when analyzing this group. * Methodology, page 7, line 27: rather use "aimed" than "aims" since the recruitment period is over. * Methodology, page 7, line 35-36: The study is based on the JECS, and the authors refer to other papers regarding detailed study profile. Please give a SHORT summary of the most important details (e.g. how and when were women invited to participate, the participation rate and major differences among those participating and the general pregnant population in Japan) also in the present paper. * Information on use of drugs and supplements was collected through two interviews (InT1 and InT2); page 8, line 48. Do T1 and T2 indicate trimester 1 and 2? If so, please insert "…answers given in interviews during the first and second trimester regarding the use of drugs and supplements (InT1, InT2)." * Page 8, line 50: "…and supplement consumption was queried during three periods: before pregnancy confirmation,…" Change "during" with "for". (I suppose the interviews were not carried out during these periods). * Results, page 11, lines 42-45: "There were no differences in median nutrient intake estimated from FFQs among the CL and/or CP, and CP groups." Include the unaffected group. * Table 2: The column comment "Either term" under the multivitamin heading, needs explanation. Please include a footnote explaining what is meant. Further, table 2 could include statistical testing (e.g. with p-values as footnotes) between the groups with and without multivitamin supplement use, for each variable shown. * Table 3, logistic regression analyses: None of the models included socioeconomic factors although there are significant differences between the supplement users and the non-users for both education and income. I would at least include maternal education in one of the models, since educational level is associated with life style factors that may not be captured by the other variables included in the models, and may also be associated with the risk of offspring birth defects. I would also include micronutrients, especially retinol, in one of the models, see comments above. This is especially needed since the

	study aim was “to examine the relationship between nutrition and CL alone, CP alone and CL with CP, with a focus on micronutrient and supplement intake….” As for now, no models include micronutrients as the exposure in a model adjusting for multivitamins, folate supplements and relevant confounders with CL/P or CP as outcomes. (CL alone is not investigated in any models). Regarding the use of “Either term”: please include a footnote explaining the meaning (I suppose it means independent of timing?) * Discussion, page 21: “we did not observe a clear association between multivitamin intake during pregnancy and the incidence of CL only”: I suppose the authors mean CP only?

REVIEWER	Carissa Rocheleau Centers for Disease Control and Prevention National Institute for Occupational Safety and Health USA
REVIEW RETURNED	06-Aug-2019

GENERAL COMMENTS	As the authors note, little work has been performed in Asian countries to examine the relationship between nutritional supplementation and birth defects; thus this work has the potential to provide an important contribution to the literature. However, given the extensive and increasingly sophisticated work that has already been published on this topic to inform current research, my expectations from a new analysis are fairly high: I expect a new analysis to address the complexities and biases that have already been characterized in order to advance our understanding. I have several major concerns with this analysis and its interpretation, and recommend substantial revisions (some of which would require re-analysis) before this work reviewed further. First, the classification of any multivitamin use with a binary (yes/no) variable can be problematic. I do not know what the regulations surrounding multivitamins look like in Japan, but in the U.S. many multivitamins are no adequate (e.g. do not contain 400mcg of folic acid, which has been demonstrated to reduce neural tube defects). Some multivitamins contain exceedingly large doses of certain vitamins (e.g. 40 or more times the RDA); excesses of certain micronutrients, including vitamin A, have been associated with some birth defects. Other multivitamins contain herbal or probiotic supplements (note: probiotic supplements can be contaminated with potentially harmful bacteria or fungi unless rigorous quality control has been conducted), the safety of which during pregnancy is not established. Other studies have addressed this issue by looking at the active ingredients of multivitamin products and classifying them as either a multivitamin that meets recommendations (meets 100% of RDA for pregnant women without excess > 200% or additional unrecommended products), or multivitamin not meeting recommendations; both groups can be compared to no multivitamin use. Evaluating the data in this way is far more informative than a simple yes/no for multivitamin use. Secondly, I am concerned that the authors have not addressed pregnancy survival bias as a competing hypothesis that could explain their findings. This issue is widely understood in birth

	defects epidemiology, and works thusly: when cases are ascertained from among liveborn infants (as was done in this study), these are prevalent cases and not incident cases. Case pregnancies ending in miscarriage, stillbirth, or abortion are censored from the data when outcomes are ascertained only at live birth. This can create a particular form or survival bias for pregnancies, in which a factor that improves the survival of a fetus with a malformation will appear to be positively associated with the malformation when we look only at liveborn infants. In short, the authors' data could potentially be interpreted as "multivitamins increase the survival of a fetus with an orofacial cleft to birth" rather than a risk due to the multivitamin. If information on pregnancies ending in outcomes other than live birth is available, this bias can be reduced with a fetuses-at-risk approach. If only livebirths are available, evaluating defect severity, preterm birth, or infants that are small-for-gestational-age by exposure status can at least give us some indication of whether this alternative interpretation seems likely or not. At a minimum, the authors need to carefully and thoroughly address this potential explanation for their data in the manuscript. Finally, the authors make broad clinical recommendations that are well beyond the scope of this study, recommending that women be counseled on the potential risks of multivitamin use. This recommendation is not supported by the data the authors present, and could result in more harm than benefit. Even if the authors' findings are completely accurate and perfect, avoiding multivitamin use would only result in the prevention of 1 orofacial cleft per 10,000 pregnancies in Japan. Taking a multivitamin containing 400mcg of folic acid, on the other hand, has been repeatedly demonstrated to reduce neural tube defects by around 50%. Neural tube defects affect a little over 8 in 10,000 births in Japan. Thus, for every 10,000 mothers who decided to avoid a multivitamin based on the authors' warning, 1 orofacial cleft would be traded for 4 neural tube defects (which have a higher associated infant mortality and long-term disability risk). When the public reads a paper like this, and is not presented with full data on competing risks and benefits, they may make decisions that cause tangible harm. Because these three issues will require substantial revision, I will hold off on additional minor comments as these might change in the next version.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Jose Suazo

Institution and Country: Universidad de Chile, Chile

Please state any competing interests or state 'None declared': No competing interest

Comment 1: This manuscript reveals new evidence showing the potential teratogenic effects of periconceptional intake of multivitamin supplements in a national wide study. These findings are contrary to previous reports supporting the protective role against orofacial clefts of certain micronutrients plus folic acid. The discussion explaining this discrepancy is based on retinoic acid and its teratogenic effects. The authors must comment about the different multivitamin supplement available in Japan and if there is some of them designed for pregnancy and compare the retinoic acid content among them.

Response: We thank the reviewer for the useful comments. As the Reviewer indicated, though our hypothesis is based on retinoic acid and its teratogenic effects, different multivitamin supplements are available in Japan. Actually, multivitamin products sold in Japan are categorized as “health food products,” and these health food products are not regulated. Generally, multivitamins contain the following: vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, pantothenic acid, biotin, folic acid, vitamin C, and vitamin E. If the women tend to take in enough folic acid for prevention of spina bifida resulting from multivitamin intake, that may lead to overdose or excessive intake of other vitamins and minerals that have been reported to be teratogens. The knowledge of this risk among the public is low, thus, pregnant women in Japan may take in multivitamin supplements without knowledge of the risk of multivitamin supplement intake.

We have described multivitamin products sold in Japan in the Discussion section.

Comment 2: A minor issue: page 6, line 45 says "periconceptual" and may say "periconceptional".

Response: Thank you for pointing this out. We have used “periconceptional” throughout the manuscript.

Reviewer: 2

Reviewer Name: Kari Klungsøyr

Institution and Country: Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway and Division of Mental and Physical Health, Norwegian Institute of Public Health, Bergen, Norway

Please state any competing interests or state ‘None declared’: None declared

This study is based on data from the Japan Environment and Children’s Study (JECS), a nationwide, prospective birth cohort study with data from close to 100 000 pregnant women and their offspring. The study aimed at examining the relation between maternal nutrition and supplement intake and oral clefts. Maternal nutrition was based on information from a semi-quantitative food frequency questionnaire answered in the first trimester. Information on the use of supplements was collected from interviews at two time points during pregnancy about intake before pregnancy was confirmed, from the first trimester and from the period after 12 weeks. The oral clefts were based on diagnoses made within one month after birth by two pediatricians at unit centers of the JECS. Selected variables

were included as covariates in models analyzing the relation between maternal supplement use and oral clefts.

The paper is well written and is based on a large cohort study, however, the results are unexpected and need to be evaluated carefully. Most studies looking at the present associations have found different results (multivitamin supplements associated with a reduced risk of oral clefts or no association). The present study does further not find an association between folate supplements and oral clefts, whereas many studies find that folate supplements may also be associated with a reduced risk of oral clefts. The paper lacks some important information which is needed to evaluate the results, and the analyses do not fully investigate what is defined as the study aim: “to examine the relationship between nutrition and CL alone, CP alone and CL with CP, with a focus on micronutrient and supplement intake….”.

I have the following main comments:

Comment 1: First, the authors have important data on nutritional intake, and one of the aims was to investigate the relationship between nutrition and oral clefts. However, comparing intake in mothers to infants with cleft palate only (CP), cleft lip with or without cleft palate (CL/P) and unaffected infants, they state that there were no significant differences in nutrient intake (Table 1). However, since CP and CL/P may be viewed as separate entities with a different embryological basis and likely different causal factors, it would be good to compare each of these groups (one at a time) with the reference group. This would also be more in line with the logistic regression analyses where CL/P and CP are included as dependent variables, one at a time. If there are differences in nutrient intake between the mothers to one of the affected groups and the reference mothers, these nutrients should be included in the corresponding logistic regression analysis (e.g. by modelling intake as a categorical variable based on quartiles or quintiles). This would be especially interesting regarding the intake of retinol among mothers to CL/P infants and mothers to unaffected infants, since the p-value comparing this nutrient between all three groups was relatively low, p=0.176, and the median intake was 445 µg in CL/P mothers, relative 406 µg in mothers to unaffected infants. Also, the authors assume that it is retinol/ vitamin A from the multivitamin supplements that drive the associations found, thus nutritional intake of retinol should be included in a full model.

Response: We thank the reviewer for the useful comments. Based on the Reviewer’s comment, we compared each of cleft lip only, cleft lip and palate, and cleft palate only with the reference group. Accordingly, we have revised the results of Table 1, Table 3, and supplementary table with the outcome categorized by cleft lip only, cleft lip and palate, and cleft palate only. Furthermore, we included retinol by food intake in the multinomial logistic regression analyses, by modelling intake as a categorical variable based on quartiles. This was done because there were differences in nutrient intake between the mothers of one of the affected groups and the mothers in the reference group, as the Reviewer indicated.

Comment 2: I am concerned that the authors do not have any information about the content of the multivitamin supplements. It should be possible to at least give some information about the content of the most commonly used multivitamin supplements in Japan during the study years. As it is now, the authors make assumptions about the contents likely driving the associations without any supporting

information. E.g. the statement on page 20, lines 24-25: “whereas excess vitamin A intake may be detrimental” cannot be based on the current study.

Although this problem is mentioned as a limitation of the study, the authors base much of the discussion on the assumption that vitamin A / retinol is driving the association found. Without knowledge of the true content of the supplements, this should be modified or rewritten. Adjustment for nutritional retinol would be good here. The authors further assume that the multivitamin supplements are without folate since “questions addressed to mothers about multivitamin intake were independent of those about intake of folic acid supplements” (Discussion, page 18). This is not sufficient to assume that multivitamins do not contain folate. In Norway, nearly all multivitamin supplements contain some folic acid and has done so since the association between folic acid and neural tube defects was established. The JECS included study participants during 2011-2014, so this association was well known and I would be surprised if multivitamin supplements in Japan do not include any folate.

Response: Thank you for your comments. Like in Norway, nearly all multivitamin supplements in Japan contain some folic acid, therefore, folic acid intake among women during pre- or initial pregnancy is recommended, due to its preventive effect against spina bifida. Actually, the different multivitamin supplements available and sold in Japan are categorized as “health food products”. Generally, multivitamin products sold in Japan contain vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, pantothenic acid, biotin, folic acid, vitamin C, and vitamin E. Compared with purely folic acid supplements (100% folic acid supplement), the dose of folic acid contained in multivitamins sold in Japan is not sufficient. If the women tend to take in enough folic acid for the prevention of spina bifida resulting from multivitamin intake, it may lead to overdose or excessive intake of other vitamins and minerals that have been reported as teratogens. The Food Safety Commission of Japanese government alerted that “Though multivitamins contain folic acid, which has a preventive effect against spina bifida, pregnant women should pay attention when they take in multivitamin supplements because they contain vitamin A, which may cause malformation at birth” [1]. However, this is only an official statement and does not prevail among the public. Due to the low level of consciousness among the public, pregnant women in Japan may consume multivitamin supplements without knowledge of the risk of multivitamin supplement intake.

The reviewer also indicated the problem of the sentence: “questions addressed to mothers about multivitamin intake were independent of those about intake of folic acid supplements”. We are sorry for this confusing expression. We have revised and described this in the Discussion.

[1] Food Safety Commission in Japan. http://www.fsc.go.jp/osirase/kenkosyokuhin.data/kenkosyokuhin_datakenkosyokuhin_houkoku.pdf (in Japanese).

Comment 3: The authors do not say anything about syndromic cases. CP is more often syndromic than CL/P. How many of the cleft cases were isolated (each oral cleft group), and how many were associated with other major congenital anomalies? If associated with other anomalies, what were the associated anomalies? If the cleft cases include cases with associated anomalies, the authors should also include analyses on isolated cases.

Response: As the reviewer indicated, our outcome could contain syndromic and non-syndromic cases. However, our data contained no information about syndromic and non-syndromic cases (the isolated cases). Though JECS data contain the information about congenital malformation of children,

it is difficult to determine syndromic or non-syndromic CL/CP, because we could not check all congenital malformations related to the CL/CP. Therefore, we have stated in the limitation subsection in the Discussion that our outcome measure did not consider syndromic and non-syndromic cases.

Comment 4: Totally, 98,787 mother-child pairs were included in the Study population. How many women had more than one child? The authors have not taken account of correlation between infants born to the same mother, which should be done.

Response: Thank you for pointing this out. We have checked the data, and over 99.1% of the children were first-borns. The remaining 0.9% of the children had siblings. This may be because our survey is a prospective cohort study, which depends on patients' voluntary participation. Furthermore, we performed additional analyses which adjusted for siblings, but the results were virtually unchanged. We decided not to include siblings in the analyses because this could introduce bias in view of the very small proportion of children with siblings.

Comment 5: Covariates evaluated for association with maternal nutrition and oral clefts included gestational diabetes, gestational week (at birth, I suppose) and birth weight. However, none of these variables should be considered potential confounders, as they all occur after the lip and palate have developed, with the possible exception of gestational diabetes. However, birth weight and gestational week were not included in the logistic regression analyses, which is good. Pre-gestational diabetes mellitus, however, could be a potential confounder, as some studies find it to be associated with offspring oral clefts, and it has evident associations with maternal nutrition. Why have the authors included gestational diabetes but not pre-gestational diabetes mellitus?

Response: Thank you for pointing this out. We have checked the data, and 201 (0.2%) out of 98,787 mothers had pre-gestational diabetes mellitus. As the reviewer indicated, pre-gestational diabetes mellitus may affect oral clefts, compared to diabetes mellitus during pregnancy [1]. We have revised the analyses that included pre-gestational diabetes mellitus as a potential confounder. Furthermore, we excluded gestational week and birth weight from the covariates of primary analyses.

[1] Stott-Miller M, Heike CL, Kratz M, Starr JR. Increased risk of orofacial clefts associated with maternal obesity: case-control study and Monte Carlo-based bias analysis. Paediatric and perinatal epidemiology. 2010;24(5):502-12.

Comment 6: All the cases included were live born children. Do the authors have information on stillborn cases? What about terminations for fetal anomaly? Were there any terminations of pregnancy for cases with oral clefts? These would most likely be for syndromic cases, and of less interest in the present study, but it would be good to know the numbers to complete the picture.

Response: Thank you for pointing this out. The initial participants for JECS study were 104,102 mothers and 3,954 cases were still born. We have checked the still birth cases (n=3954); among them, at least 9 children (0.23%) had orofacial clefts, and this percentage of occurrence of orofacial

clefts was similar to that of the live-birth group (0.24%). Regretfully, we could not precisely determine the end of gestational week among still births, as about 70% had missing data. As a result, we could not know whether the still birth cases were caused by miscarriages (spontaneous abortion) or by supplement use. We described the survival bias in our study as a limitation in the Discussion.

Comment 7: Page 20, last sentence: “due to the rarity of the study outcome (orofacial clefts), our choice of a cohort design allowed us to better detect the investigated association compared with a case-control design”. This is not correct. When the outcome is rare, the best design is the case-control study, since very large cohorts are needed to allow the study of rare outcomes. Even in the current large cohort study, only 52 infants with CP were included. Had the authors chosen a case control design, they could have included more CP infants and preferably had an equal number of CP and CL/P infants. The wide confidence intervals around the estimates for the CP group indicates that even in this large cohort there is likely a lack of power for the evaluation of multivitamin supplements and cleft palate only. For instance, the point estimate for supplement use in the first trimester suggests a more than doubled risk associated with CP, but was not statistically significant: OR 2.43 (0.88-6.74).

Response: Thank you for pointing this out. We have revised the sentence in the section where the strengths of our study are discussed as follows: “Our study used a large size cohort study. However, the occurrence of the outcome was rare, and therefore, a case-control study may be more desirable since very large cohorts are needed to allow for the study of rare outcomes.”

Comment 8: Genetic factors are important for the occurrence of oral clefts. It is therefore a limitation that the authors do not have information on possible parental oral clefts. This is, however, discussed by the authors as one of the limitations of the study.

Response: We could not gather information on parental orofacial clefts, and have discussed this as a limitation in the Discussion section, in that we did not consider familial history and genetic factors, which may be associated with orofacial clefts in offspring.

Comment 9: Specific comments throughout:

* Abstract, Results, line 45: “… 182 (0.18%) were diagnosed with cleft lip and/or cleft palate”: should be cleft lip with/without cleft palate.

Response: Thank you for pointing this out. We have revised the words “cleft lip and/or cleft palate” to “cleft lip with/without cleft palate” in our manuscript.

* Strengths and limitations, page 5, bullet point three: As mentioned above, the best study design for rare outcomes is the case-control study. However, when the cohort is sufficiently large enough, the cohort study can also be used. Even though the present analyses are based on a large cohort study, there were only 52 cleft palate cases, and a likely lack of power when analyzing this group.

Response: Thank you for pointing this out. As the reviewer indicated, the best design for analysis may be case-control. However, as the reviewer indicated, there may be a likely lack of statistical power when we conduct a case-control analysis because of the small number of cleft palate cases. This has been discussed in the Discussion.

* Methodology, page 7, line 27: rather use “aimed” than “aims” since the recruitment period is over.

Response: Thank you for pointing this out. We have revised “aims” to “aimed”.

* Methodology, page 7, line 35-36: The study is based on the JECS, and the authors refer to other papers regarding detailed study profile. Please give a SHORT summary of the most important details (e.g. how and when were women invited to participate, the participation rate and major differences among those participating and the general pregnant population in Japan) also in the present paper.

Response: Thank you for pointing this out. Based on the reviewer’s advice, we have added a short summary of JECS study regarding study area, recruitment methods, coverage rate of children for study area, and representativeness of the Study in the Methods section.

* Information on use of drugs and supplements was collected through two interviews (InT1 and InT2); page 8, line 48. Do T1 and T2 indicate trimester 1 and 2? If so, please insert “…answers given in interviews during the first and second trimester regarding the use of drugs and supplements (InT1, InT2).”

Response: Thank you for pointing this out. T1 means first trimester and T2 means in the second or third trimester (T2 include both second and third trimesters). We have added this description to the Methods.

* Page 8, line 50: “…and supplement consumption was queried during three periods: before pregnancy confirmation,…” Change “during” with “for”. (I suppose the interviews were not carried out during these periods).

Response: We have changed the word “during” to “for”.

* Results, page 11, lines 42-45: “There were no differences in median nutrient intake estimated from FFQs among the CL and/or CP, and CP groups.” Include the unaffected group.

Response: We have added the description “unaffected group”.

* Table 2: The column comment “Either term” under the multivitamin heading, needs explanation. Please include a footnote explaining what is meant.

Further, table 2 could include statistical testing (e.g. with p-values as footnotes) between the groups with and without multivitamin supplement use, for each variable shown.

Response: Either term means multivitamin intake at any term of "before pregnancy confirmation", "Up to 12 weeks", or "After 12 weeks". We have added the explanation in the footnote of Table 2. Furthermore, we have added p values in Table 2.

* Table 3, logistic regression analyses: None of the models included socioeconomic factors although there are significant differences between the supplement users and the non-users for both education and income. I would at least include maternal education in one of the models, since educational level is associated with life style factors that may not be captured by the other variables included in the models, and may also be associated with the risk of offspring birth defects.

I would also include micronutrients, especially retinol, in one of the models, see comments above. This is especially needed since the study aim was “to examine the relationship between nutrition and CL alone, CP alone and CL with CP, with a focus on micronutrient and supplement intake….” As for now, no models include micronutrients as the exposure in a model adjusting for multivitamins, folate supplements and relevant confounders with CL/P or CP as outcomes. (CL alone is not investigated in any models).

Response: As the reviewer’s advised, we included maternal education and retinol use in the revised multinomial logistic regression models.

Regarding the use of “Either term”: please include a footnote explaining the meaning (I suppose it means independent of timing?)

Response: We have added the explanation about “Either term” in the footnotes of Table 2. Either term means multivitamin intake at any term of "before pregnancy confirmation", "Up to 12 weeks", or "After 12 weeks".

* Discussion, page 21: “we did not observe a clear association between multivitamin intake during pregnancy and the incidence of CL only”: I suppose the authors mean CP only?

Response: Thank you for pointing this out. We are sorry for our mistake and have revised to “we did not observe a clear association between multivitamin intake during pregnancy and the incidence of CP only”.

Reviewer: 3

Reviewer Name: Carissa Rocheleau

Institution and Country: Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, USA

Please state any competing interests or state ‘None declared’: None declared

As the authors note, little work has been performed in Asian countries to examine the relationship between nutritional supplementation and birth defects; thus this work has the potential to provide an important contribution to the literature. However, given the extensive and increasingly sophisticated work that has already been published on this topic to inform current research, my expectations from a new analysis are fairly high: I expect a new analysis to address the complexities and biases that have already been characterized in order to advance our understanding. I have several major concerns with this analysis and its interpretation, and recommend substantial revisions (some of which would require re-analysis) before this work reviewed further.

First, the classification of any multivitamin use with a binary (yes/no) variable can be problematic. I do not know what the regulations surrounding multivitamins look like in Japan, but in the U.S. many multivitamins are no adequate (e.g. do not contain 400mcg of folic acid, which has been demonstrated to reduce neural tube defects). Some multivitamins contain exceedingly large doses of certain vitamins (e.g. 40 or more times the RDA); excesses of certain micronutrients, including vitamin A, have been associated with some birth defects. Other multivitamins contain herbal or probiotic supplements (note: probiotic supplements can be contaminated with potentially harmful bacteria or fungi unless rigorous quality control has been conducted), the safety of which during pregnancy is not established. Other studies have addressed this issue by looking at the active ingredients of multivitamin products and classifying them as either a multivitamin that meets recommendations (meets 100% of RDA for pregnant women without excess > 200% or additional unrecommended products), or multivitamin not meeting recommendations; both groups can be compared to no multivitamin use. Evaluating the data in this way is far more informative than a simple yes/no for multivitamin use.

Response: Thank you for pointing this out. As the reviewer indicated, the assessment of multivitamin use among mothers was a critical point of our study. In Japan, compared with the US, regulation of supplements, including the display of those products, is not restricted. Actually, multivitamin products sold in Japan are categorized as “health food products”, although multivitamins generally sold in Japan contain vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, pantothenic acid, biotin, folic acid, vitamin C, and vitamin E. The dose of folic acid contained in multivitamins sold in Japan is also not sufficient (<400 mcg of folic acid). If the women tend to consume enough folic acid for prevention of spina bifida resulting from multivitamin use, that may lead to overdose or excessive

intake of other vitamins and minerals that have been reported to be teratogens. For this purpose, the Food Safety Commission of the Japanese government alerted that “Though multivitamins contain folic acid, which have a preventive effect against spina bifida, pregnant women should pay attention when they take in multivitamin supplements because they contain vitamin A, which may cause malformation at birth” [1]. However, this is only an official statement and does not prevail among the public. Due to the low level of consciousness among the public, pregnant women in Japan may consume multivitamin supplements without knowledge of the risk of multivitamin supplement intake.

If the periconceptional intake of multivitamin supplements has potential teratogenic effects, pregnant women should be alerted of over dose intake of multivitamin supplements or the risk and benefit involved their use.

[1] Food safety commission in Japan. http://www.fsc.go.jp/osirase/kenkosyokuhin.data/kenkosyokuhin_datakenkosyokuhin_houkoku.pdf

Secondly, I am concerned that the authors have not addressed pregnancy survival bias as a competing hypothesis that could explain their findings. This issue is widely understood in birth defects epidemiology, and works thusly: when cases are ascertained from among liveborn infants (as was done in this study), these are prevalent cases and not incident cases. Case pregnancies ending in miscarriage, stillbirth, or abortion are censored from the data when outcomes are ascertained only at live birth. This can create a particular form or survival bias for pregnancies, in which a factor that improves the survival of a fetus with a malformation will appear to be positively associated with the malformation when we look only at liveborn infants. In short, the authors’ data could potentially be interpreted as “multivitamins increase the survival of a fetus with an orofacial cleft to birth” rather than a risk due to the multivitamin. If information on pregnancies ending in outcomes other than live birth is available, this bias can be reduced with a fetuses-at-risk approach. If only livebirths are available, evaluating defect severity, preterm birth, or infants that are small-for-gestational-age by exposure status can at least give us some indication of whether this alternative interpretation seems likely or not. At a minimum, the authors need to carefully and thoroughly address this potential explanation for their data in the manuscript.

Response: Thank you for pointing this out. As the Reviewer indicated, the better approach may be the fetuses-at-risk approach for reducing the survival bias. We checked the still birth cases (n=3954); among them, at least 9 children (0.23%) had orofacial clefts, and this percentage of occurrence of orofacial clefts was similar to that of the live birth group (0.24%). Regretfully, we could not precisely determine the end of gestational week among still births, as about 70% had missing data. As a result, we could not know whether the still birth cases were caused by miscarriages (spontaneous abortion) or by supplement use. Consequently, we described the survival bias in our study as a limitation in the Discussion as follows: “Our study only considered live-born children, and this could potentially cause a survival bias for pregnancies. This means multivitamins increase the survival of a fetus with an orofacial cleft to birth rather than a risk due to the multivitamin.”

Finally, the authors make broad clinical recommendations that are well beyond the scope of this study, recommending that women be counseled on the potential risks of multivitamin use. This recommendation is not supported by the data the authors present, and could result in more harm than benefit. Even if the authors’ findings are completely accurate and perfect, avoiding multivitamin use would only result in the prevention of 1 orofacial cleft per 10,000 pregnancies in Japan. Taking a

multivitamin containing 400mcg of folic acid, on the other hand, has been repeatedly demonstrated to reduce neural tube defects by around 50%. Neural tube defects affect a little over 8 in 10,000 births in Japan. Thus, for every 10,000 mothers who decided to avoid a multivitamin based on the authors' warning, 1 orofacial cleft would be traded for 4 neural tube defects (which have a higher associated infant mortality and long-term disability risk). When the public reads a paper like this, and is not presented with full data on competing risks and benefits, they may make decisions that cause tangible harm.

Because these three issues will require substantial revision, I will hold off on additional minor comments as these might change in the next version.

Response: Thank you for pointing this out. As the reviewer indicated, our recommendation may mislead mothers. Like in other countries, the preventive effect of folic acid against neural tube defects has been established in Japan. Therefore, our message should be the caution against excessive intake of multivitamins that contain folic acid and other effective micronutrients during pregnancy. Consequently, we revised this description in the Discussion, as our study did not provide evidence of this association. However, it is necessary to bring the knowledge of the risk and benefit to the attention of pregnant women.

VERSION 2 – REVIEW

REVIEWER	Jose Suazo Universidad de Chile, Chile
REVIEW RETURNED	18-Nov-2019

GENERAL COMMENTS	The authors include all of my suggestion in the current version of this manuscript.

REVIEWER	Kari Klungsøyr University of Bergen, Norway and Norwegian Institute of Public Health, Norway
REVIEW RETURNED	15-Dec-2019

GENERAL COMMENTS	This study is based on data from the Japan Environment and Children's Study (JECS), a nationwide, prospective birth cohort study with data from close to 100 000 pregnant women and their offspring. The study aimed at examining the relation between maternal nutrition and supplement intake and oral clefts. I have reviewed this paper previously, this is a resubmission to the same journal. The authors have answered most of my previous comments, and the paper has much improved. The most important is that the authors are now more careful about the conclusions that vitamin A / retinol is driving the association found. Without knowledge of the true content of the supplements, this needed to be modified, which has been done. The authors have also now included more information about the supplements available in Japan, and show that most of these also contain some folic acid. In the third paragraph of the Discussion, the authors write: "If the women tend to take enough folic acid for prevention of spina bifida from

	multivitamin, this may lead to overdose or excessive intake of other vitamins and minerals, which are reported as teratogens. For this purpose, the Food Safety Commission of Japanese government alerted that “Though multivitamins contain folic acid, which have preventive effects against spina bifida, pregnancy women should pay attention when they take multivitamin supplements because they contain vitamin A, which causes malformation at birth”.[24]” I do suppose that there official recommendations also in Japan that pregnant women should take pure folic acid supplements periconceptionally to prevent NTD, and not use multivitamins as the folic acid supplementation source? One sentence about this could be included. I replied "no" to the question "Is the study design appropriate to answer the research question?" This is because 1) the design is a cohort study, and the outcomes are rare and few, and 2) the exact contents of the multivitamins were not known. To study this question, even larger cohorts or a well designed case control study where exposures were captured accurately, including information on the content of multivitamin supplements, would be better. However, this is commented on by the authors in the Discussion. I have some minor remaining comments: This should have been commented in my previous review, but why do the authors adjust for infant sex? Sex of the infant is not a confounder, and should therefore not be adjusted for. In table 2: The gestational age of infants to mothers who take and do not take multivitamins, is in both groups 38.8 weeks. The p-value of the difference is 0.002. Is this correct? In table 3, there is an error in the first line showing the association between multivitamin intake Before pregnancy confirmation and cleft palate only: the RR is 0.82 (2.00–3.38); the lower confidence limit should probably be 0.2?) In my previous comment nu 4, I ask How many women had more than one child, and whether the authors have taken account of correlations between siblings. The authors answer that over 99.1% of the children were first-borns. When looking at table 2, mothers report that their child is a first born in 30.5% among those not taking multivitamins, and 37.7% among those taking multivitamins. So I guess the authors mean to say that 99.1% of mothers are participating in the JECS with only one child (not with first-born children). This could be stated in the paragraph where the authors describe the JECS, as it shows that concern about dependent data is not a big problem.

REVIEWER	Russell Kirby University of South Florida, Tampa, Florida, USA
REVIEW RETURNED	19-Jan-2020

GENERAL COMMENTS	The previous version of this manuscript generated numerous questions, in part due to the seemingly unexpected result that periconceptional use of multivitamin supplements was associated with increased risk of orofacial clefts. The authors have attempted to respond to these many comments and critiques, and the manuscript has improved as a result.

	However, this reviewer still has concerns and feels the study might be improved. Among other things, there are newer population based US estimates (Mai et al, BDR 2019;111:1420-1435 which show both that the prevalence of orofacial clefts has not changed very much from 1999-2001 through 2011-2014, and at somewhere in the 16-17 cases per 10000 births range, is considerably lower than that found in the present study (23/10,000)) - incidentally, most birth defects epidemiologists report prevalence per 10,000 births or deliveries, and that should be done in this manuscript as well. Its very likely that the population experience in Japan differs, and that the baseline rate is in fact approximately 50% higher, and this may be due to genetic, environmental or maternal behavioral factors or interactions among these. This reviewer has concerns that gene-environment interactions may be important in the observed association. As the authors note, there are many genetic pathways to orofacial clefts - more than 250 distinct genetic diseases and disorders may have these in their phenotype - and its possible that some of these conditions are more frequent in the Japanese population. More attention should be paid to measurement as well, especially for folic acid consumption in the periconceptional period. Its not surprising that no associations were found with after 12 week usage, based on timing of organ development, but the main analyses (Table 3) are based on a dichotomous measure of multivitamin intake alone. Table 3 also raises some analytical questions. How were maternal age and BMI measured? Neither of these variables has a linear trend with birth defects prevalence, and should be analyzed as categorical variables. It would also be helpful to see the RRs and CIs for the covariates, so that the reader can assess whether any variables exhibit potential confounding, interaction or effect modification. It's fine to focus on the main association of interest, but readers also need to see these details. The authors should also scan the previous literature more clearly; while not as strong methodologically, Canfield et al. BDRA 2006 examined prevalence of a range of birth defects pre- and post-fortification in the US, and did not find evidence for an association similar to that observed here. Undoubtedly there are other studies as well.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Jose Suazo

Institution and Country: Universidad de Chile, Chile

Please state any competing interests or state ‘None declared’: None declared

The authors include all of my suggestion in the current version of this manuscript.

Reviewer: 2

Reviewer Name: Kari Klungsøyr

Institution and Country: University of Bergen, Norway and Norwegian Institute of Public Health, Norway

Please state any competing interests or state ‘None declared’: None declared

Comment 1: The authors have answered most of my previous comments, and the paper has much improved. The most important is that the authors are now more careful about the conclusions that vitamin A / retinol is driving the association found. Without knowledge of the true content of the supplements, this needed to be modified, which has been done. The authors have also now included more information about the supplements available in Japan, and show that most of these also contain some folic acid. In the third paragraph of the Discussion, the authors write: “If the women tend to take enough folic acid for prevention of spina bifida from multivitamin, this may lead to overdose or excessive intake of other vitamins and minerals, which are reported as teratogens. For this purpose, the Food Safety Commission of Japanese government alerted that “Though multivitamins contain folic acid, which have preventive effects against spina bifida, pregnancy women should pay attention when they take multivitamin supplements because they contain vitamin A, which causes malformation at birth”.[24]”

I do suppose that there official recommendations also in Japan that pregnant women should take pure folic acid supplements periconceptionally to prevent NTD, and not use multivitamins as the folic acid supplementation source? One sentence about this could be included.

Response: Regretfully, there is no official recommendation on the intake of pure folic acid for pregnant women, except for the Food Safety Commission of Japanese government. Japan is behind in folic acid intake for periconceptional periods, and the regulation for supplementation of Japanese government is not as strict as countries such as the US. Therefore, we hope that our results could be the trigger for appropriate intake for folic acid among Japanese women. We have added the explanation about lack of official statement about the use of pure folic acid by pregnant women instead of multivitamin in Japan in the discussion (page 22, line 2-3).

Comment 2:

I replied "no" to the question "Is the study design appropriate to answer the research question?" This is because 1) the design is a cohort study, and the outcomes are rare and few, and 2) the exact contents of the multivitamins were not known. To study this question, even larger cohorts or a well designed case control study where exposures were captured accurately, including information on the content of multivitamin supplements, would be better. However, this is commented on by the authors in the Discussion.

Response: As the Review indicated, larger cohorts or a well-designed case-control study would be better for our research question. Though the recruitment period of JECS study has elapsed and we could not expand to a larger cohort, nested case-control study could be designed for our study. However, nested case-control and cohort studies would provide the similar conclusion. Either way, information on the content of multivitamin supplements would be critical in our study. Since our study is a part of JECS cohort study, in which the central hypothesis was the exposure of chemical substance during pregnancy and development in children, multivitamin supplements for pregnant women were not the focus. Therefore, we have revised the explanation in the limitation section of discussion with more specific submission as follows: “the outcome of our study was rare and therefore, larger cohorts or a well-designed case control study where exposures will be captured accurately, including information on the content of multivitamin supplements, would be necessary in future” (page 24, line 4-7).

Comment 3: I have some minor remaining comments:

This should have been commented in my previous review, but why do the authors adjust for infant sex? Sex of the infant is not a confounder, and should therefore not be adjusted for.

Response: We have revised the adjusted models of Table 3 (page 18-19) and supplementary table 1, in which we did not adjusted for sex of the child. However, the models almost did not change the point estimates of the analysis after excluding sex. We have revised the results section to reflect this on page 17, line 9-10.

Comment 4: In table 2: The gestational age of infants to mothers who take and do not take multivitamins, is in both groups 38.8 weeks. The p-value of the difference is 0.002. Is this correct?

Response: We have reconfirmed the analysis, but p-value of the difference for gestational age was 0.002. We think this could be caused by the skewed distribution of gestational age stratified with not taking multivitamins vs. taking multivitamins. For this, we have revised and showed the results with median and IQR [interquartile range] by Mann Whitney tests though the results were not changed (median 39 weeks, IQR [38, 40] at both group) with < 0.001 p-value.

Comment 5: In table 3, there is an error in the first line showing the association between multivitamin intake Before pregnancy confirmation and cleft palate only: the RR is 0.82 (2.00–3.38); the lower confidence limit should probably be 0.2?)

Response: We are sorry for our mistake the confidence interval of cleft palate only. The RR and CI was 0.82 (CI: 0.20–3.38) not 0.82 (CI: 2.00–3.38). We have revised the result of crude model of cleft palate only at Table 3 (page 18).

Comment 6: In my previous comment nu 4, I ask How many women had more than one child, and whether the authors have taken account of correlations between siblings. The authors answer that

over 99.1% of the children were first-borns. When looking at table 2, mothers report that their child is a first born in 30.5% among those not taking multivitamins, and 37.7% among those taking multivitamins. So I guess the authors mean to say that 99.1% of mothers are participating in the JECS with only one child (not with first-born children). This could be stated in the paragraph where the authors describe the JECS, as it shows that concern about dependent data is not a big problem.

Response: After checking the data again, 99.1% was the first-born with multiple birth. As the Reviewer indicated, 99.1% did not show the percentage of fist-born children. Though JECS study obtains the detailed information of previous pregnancy for mothers, it is not easy to specify the birth order form only pregnancy information. Instead, we have added the explanation that sibling was not considered in analysis to the limitation in discussion section (page 24, line 8).

Reviewer: 3

Reviewer Name: Russell Kirby

Institution and Country: University of South Florida, Tampa, Florida, USA

Please state any competing interests or state 'None declared': None declared

Comment 1: However, this reviewer still has concerns and feels the study might be improved. Among other things, there are newer population based US estimates (Mai et al, BDR 2019;111:1420-1435 which show both that the prevalence of orofacial clefts has not changed very much from 1999-2001 through 2011-2014, and at somewhere in the 16-17 cases per 10000 births range, is considerably lower than that found in the present study (23/10,000)) - incidentally, most birth defects epidemiologists report prevalence per 10,000 births or deliveries, and that should be done in this manuscript as well. Its very likely that the population experience in Japan differs, and that the baseline rate is in fact approximately 50% higher, and this may be due to genetic, environmental or maternal behavioral factors or interactions among these.

Response: We have added the incidence per 10,000 into the Results "23.7 per 10,000 births in our study" and this incidence was higher compared with that of western countries. On the other hand, this incidence was similar with the previous report of IPDTOC Working Group (reference 3 in our manuscript) which reported the prevalence of CL with or without CP in Japan is 20.0 per 10,000 births. As the Reviewer indicated, this may be due to genetic, environmental, or maternal behavioral factors or interactions among them. Wu et al. [1] and some reports have found gene and environmental interaction with maternal exposures of multivitamin supplementation for the orofacial clefts. However, their results showed not exposed maternal multivitamin supplementation was associated with slightly increased risk of CL with or without CP. We think adverse effect of multivitamin in our subjects could be caused by teratogen nutrients, taken excessively during pregnancy. Either way, there could be gene-environmental interaction among them; therefore, we have added these explanations to the discussion on page 22, line 13-20 with the following reference.

[1] Wu T, Liang KY, Hetmanski JB, et al. Evidence of gene-environment interaction for the IRF6 gene and maternal multivitamin supplementation in controlling the risk of cleft lip with/without cleft palate. Hum Genet. 2010;128(4):401-10. (Page 30, line 15-17)

Comment 2: This reviewer has concerns that gene-environment interactions may be important in the observed association. As the authors note, there are many genetic pathways to orofacial clefts - more than 250 distinct genetic diseases and disorders may have these in their phenotype - and its possible that some of these conditions are more frequent in the Japanese population.

Response: As the Reviewer indicated, the gene-environment interactions could be important for the incidence of orofacial cleft. As the response to Comment 1 of the Reviewer, we have added the explanation of the gene-environment interactions with the reference to the discussion (page 22, line 13-20).

Comment 3: More attention should be paid to measurement as well, especially for folic acid consumption in the periconceptional period. Its not surprising that no associations were found with after 12 week usage, based on timing of organ development, but the main analyses (Table 3) are based on a dichotomous measure of multivitamin intake alone.

Response: As the Reviewer indicated, the effect or folic acid could be limited to periconceptional period (before pregnancy and up to 12 weeks) in the view of organ development. It is the same as in Japan, there is a recommendation for taking folic acid for pregnant women to prevent NTD (neural tube defect), and we did not think folic acid intake is problematic for orofacial clefts. Actually, folic acid was not associated with the incidence of outcomes in our study. However, multivitamin intake was unexpectedly associated with the incidence of outcomes.

Either way, the information on the content of multivitamin supplements would be critical to our study. Because our study was conducted as a part of JECS cohort study, in which the central hypothesis was the exposure of chemical substance in utero for the development in children, the use of multivitamin supplements for pregnant women was not the focus. Therefore, we have added the explanations to the limitation section as follows: “larger cohorts or a well-designed case control study where exposures would be captured accurately, including information on the content of multivitamin supplements, would be necessary in future” (page 24, line 4-7).

Comment 4: Table 3 also raises some analytical questions. How were maternal age and BMI measured? Neither of these variables has a linear trend with birth defects prevalence, and should be analyzed as categorical variables. It would also be helpful to see the RRs and CIs for the covariates, so that the reader can assess whether any variables exhibit potential confounding, interaction or effect modification. It's fine to focus on the main association of interest, but readers also need to see these details.

Response: As the Reviewer indicated, maternal age, and BMI did affect the incidence of birth defect with non-linear. We performed additional analyses which adjusted for maternal age (<20, 20 to 35, ≥35) and BMI (<18.5, 18.5 to 25, ≥25) as categories but the results were virtually unchanged. Regarding the covariates, we are sorry, the official rule of JECS requires us not to show the RR except for targeted exposure and outcome because of the priority for other researchers. Therefore, we could not show the detailed RR of covariates for outcome, some covariates showed slightly increased risk for orofacial clefts but those statistical significance no significance and not needed for report, we think.

Comment 5: The authors should also scan the previous literature more clearly; while not as strong methodologically, Canfield et al. BDRA 2006 examined prevalence of a range of birth defects pre- and post-fortification in the US, and did not find evidence for an association similar to that observed here. Undoubtedly there are other studies as well.

Response: In response to the reviewer's comments, we again performed literature search extensively. As the Reviewer indicated, some studies reported that folic acid fortification could decrease the prevalence of specific birth defects. However, the focus of our study was on the effect of multivitamin supplementation, and there were limited studies on the risk of multivitamin and orofacial clefts. Furthermore, as mentioned in our manuscript, the results of maternal multivitamin use and adverse birth outcomes were not consistent. These discrepancies among the results of previous studies may be attributable to the differences in the types of multivitamin supplements assessed. We think our results could be caused by excessive intake of fat-soluble vitamins, such as vitamin A, contained in multivitamins; and have been reported as teratogens. (If women tend to take enough folic acid for prevention of spina bifida from multivitamin, this may lead to an overdose or excessive intake of other vitamins and minerals.) Either way, well-designed future study where exposures would be captured accurately, including information on the content of multivitamin supplements, would be necessary.

VERSION 3 – REVIEW

REVIEWER	Kari Klungsøyr University of Bergen, Norway and The Norwegian Institute of Public Health, Norway
REVIEW RETURNED	09-Mar-2020

GENERAL COMMENTS	The authors have answered my comments from the second review. I now only have some minor comments: 1) To my first comment in the second review, concerning official recommendations on the use of pure folic acid supplements in the periconceptional period, the authors say that "Regretfully, there is no official recommendation on the intake of pure folic acid for pregnant women, ….." and "Therefore, we hope that our results could be the trigger for appropriate intake for folic acid among Japanese women." In line with this, I recommend that the Conclusion (page 25, lines 1-2) which now reads "Accordingly, pregnant women and those intending to become pregnant should be advised of the potential risks of multivitamin supplementation." could be changed to "Accordingly, pregnant women and those intending to become pregnant should be informed about the benefits of taking pure folic acid supplements and advised about the potential risks of excess multivitamin supplement intake.." 2) Page 22, line 3: Add "s" to "multivitamin" 3) Page 17, line 4: "IQR: interquartile range" seems odd here? 4) Page 21: The authors write about folic acid and its preventive effect on spina bifida. Spina bifida should be changed to Neural tube defects.

	5) Page 21, lines 15-17: As in other countries, the preventive effect of folic acid against spina bifida among women in the pre- or initial pregnancy stage are recommended for folic acid intake. I suggest to rewrite to e.g.: “As in other countries, women in the pre- or initial pregnancy stage are recommended to take folic acid due to its preventive effect on Neural tube defects”, 6) Again, I have replied "no" to the question "Is the study design appropriate to answer the research question?" This is because 1) the design is a cohort study, and the outcomes are rare and few, and 2) the exact contents of the multivitamins were not known. However, this is adequately discussed by the authors, so no further changes are needed here.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 2
Reviewer name : Kari Klungsøyr.
Institution and Country: University of Bergen, Norway, and the Norwegian Institute of Public Health, Norway
Please state any competing interests or state None declared: None declared.

Comment 1: To my first comment in the second review, concerning official recommendations on the use of pure folic acid supplements in the periconceptional period, the authors say that “Regretfully, there is no official recommendation on the intake of pure folic acid for pregnant women, …..“ and “Therefore, we hope that our results could be the trigger for appropriate intake for folic acid among Japanese women.”
In line with this, I recommend that the Conclusion (page 25, lines 1-2), which now reads “Accordingly, pregnant women and those intending to become pregnant should be advised of the potential risks of multivitamin supplementation.” could be changed to “Accordingly, pregnant women and those intending to become pregnant should be informed about the benefits of taking pure folic acid supplements and advised about the potential risks of excess multivitamin supplement intake..”

Response: We appreciate the reviewer’s pertinent comments. Accordingly, we have revised the description of the intake recommendations of multivitamin supplements for pre-pregnant and pregnant women in the Conclusion section (page 25, lines 1-3).

Comment 2: Page 22, line 3: Add “s” to “multivitamin”

Response: We have added “s” to “multivitamin” as instructed (Page 22, line 3).

Comment 3: Page 17, line 4: “IQR: interquartile range” seems odd here?

Response: Thank you for bringing this to our attention. We apologize for this error, and we have made the necessary correction (Page 17, lines 3-4).

Comment 4: Page 21: The authors wrote about folic acid and its preventive effect on spina bifida. Spina bifida should be changed to neural tube defects.

Response: We have changed ‘spina bifida’ to ‘neural tube defects’ as per your suggestion (page 21).

Comment 5: Page 21, lines 15-17: As in other countries, the preventive effect of folic acid against spina bifida among women in the pre- or initial pregnancy stage are recommended for folic acid intake. I suggest to rewrite to e.g.: “As in other countries, women in the pre- or initial pregnancy stage are recommended to take folic acid due to its preventive effect on Neural tube defects”,

Response: With respect to the reviewer’s comment, we have revised the description of the recommendation for folic acid consumption in Japan (Page 21, lines 15-17).

Comment 6: Again, I have replied "no" to the question "Is the study design appropriate to answer the research question?" This is because 1) the design is a cohort study, and the outcomes are rare and few, and 2) the exact contents of the multivitamins were not known. However, this is adequately discussed by the authors, so no further changes are needed here.

Response: Thank you for your valuable suggestion. We will definitely consider this in future JECS studies.