Author’s response to reviews

Title: Association between Pre-and Postnatal Growth and Longitudinal Trends in Serum Uric Acid Levels and Blood Pressure in Children aged 3 to 7 years

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Author’s response to reviews:

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Dear Dr. Samuel Harris:

Thank you very much for reviewing our manuscript titled “Association between Pre-and Postnatal Growth and Longitudinal Trends in Serum Uric Acid Levels and Blood Pressure in Children aged 3 to 7 years” (reference number BPED-D-18-00674R1). We greatly appreciate the constructive comments and suggestions provided by the reviewers, which we have carefully reviewed, and have revised our manuscript accordingly. Our point-by-point responses are given below.

We hope that the revised manuscript is suitable for publication in the BMC Pediatrics. I look forward to hearing from you.

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Sincerely,
Response to Reviewer 1:

1. The lack of a clear definition of catch up growth

Response: This study defined catch-up growth as a change in weight (between birth and age 3) with a z-score > 0.67 for low-birth-weight (LBW) subjects. Age- and gender- specific z-scores were calculated using the criteria from the 2007 Korean Children and Adolescents Growth Standards. (p.7, lines 148-151)

2. The mix of preterm and term infants with BW <2500 gram

Response: The first analysis included preterm and term infants with birthweights lower than 2500 g to assess whether there was a difference in the uric acid level over time between normal-birth-weight (NBW) children and LBW children who experienced catch-up growth. However, to assess the effect of LBW due only to intrauterine growth restriction, preterm children born with LBW were excluded from the second analysis, which included only LBW-at-term children (p.8, lines 167-171). The analysis showed that LBW-at-term children who experienced catch-up growth had higher uric acid levels than NBW children over the entire follow-up period.

3. The large loss to follow up of >50% without description of who is lost to follow up.

Response: Because we included only children who had their weight measured at 3 years of age, the study included more data from 3-year-olds. However, when we compared the characteristics between children who did and did not participate in the 5-year-follow-up visit using the t-test or chi-square test, there were no differences in the proportion of LBWCU (p=0.1193), sex (p=0.7364), and the mean BMI at 3 years of age (p=0.5087). In addition, the analysis included 57 children who did not participate in the 5-year-follow-up, but participated in the 7-year-follow-up because we used a linear mixed-model analysis. In repeated measures situations, the mixed model approach used in PROC MIXED provides a better mechanism for handling missing values. PROC MIXED uses all observations for which there are no missing or invalid independent variables, including those for which there are missing dependent variables without deleting missing data.

4. The lack of numbers of individuals at each measure in table 1.

Response: As suggested, the numbers of individuals at each measurement point have been added to Table 1.

5. The lack of normal values for uric acid in childhood and the number of children that have too high levels.

Response: As suggested, we have added the following related information to the revised manuscript.
Sixteen children had SUA concentrations greater than normal (2.0~5.5 mg/dL) at any follow-up point.

6. The lack of information about the relation of uric acid to BMI (since uric acid is dependent on intake of protein, one would think that it is higher in those of higher BMI).

Response: As suggested, a possible mechanism for the relationship between uric acid and BMI has been added to the revised manuscript (p.16, lines 323-327):

“In this study, the results were adjusted for BMI because there is evidence for a relationship between BMI and uric acid. Visceral fat accumulation, which is associated with a higher BMI, induces elevated plasma free fatty acids and stimulates triglyceride synthesis, which is followed by UA overproduction. In addition, the decrease in urinary uric acid excretion was investigated in obese or overweight people.”

7. Because LBW infants have similar BMI's compared to those born NBW, LBW preterm infants might just have grown appropriately and gaining weight and height at a normal pace while reaching term age; then they are misclassified. Therefore, I would recommend separating the cohort from the start of the analyses in term SGA and preterm children.

Response: Regarding the reviewer’s concern about possible misclassification, we conducted a subgroup analysis comparing the NBW- and LBW-at-term children by excluding LBW-preterm children. This has been described in the Method as follows (p. 8, lines 167-171):

“The first model assessed whether there was a difference in uric acid level over time between normal-birth-weight (NBW) children and LBW children who experienced catch-up growth. LBW can be caused by either preterm birth or intrauterine growth restriction. Therefore, to assess the effects of LBW from intrauterine growth restriction only, we excluded children born with LBW due to preterm birth in the second model.”

Response to Reviewer 2:

1. Are there any LBW children without catch up growth? There appear no LBW term or preterm group without catch up growth included for comparison. Hence any difference found could be related to LBW/Preterm rather than due to having catch-up growth as speculated. In the LBW-preterm group, how many would remain LBW at full term equivalent (corrected to EDC)? Should they be considered as preterm and "NBW corrected for prematurity"?

Response: LBW children who did not exhibit catch-up growth were excluded from the analysis, as only two LBW children did not experience catch-up growth. Consequently, LBW children who experienced catch-up growth were not compared with LBW children without catch-up growth, which remains a study limitation. Instead, SUA levels were compared with the degree of catch-up growth among LBW-at-term children. The results of this analysis have been described in the manuscript as follows (p.12, lines 234-242):

“To assess whether the SUA level is associated with the degree of catch-up growth, the LBW-at-term children were divided into two subgroups according to their change in weight z-scores from birth to age 3 using the median value. Throughout the follow-up period, the group with a weight change greater than the median value had consistently higher SUA levels than NBW children, with an increasing difference as the children aged (mean difference at age 7 = 1.03, p = 0.028). The unadjusted model indicated that group (p = 0.009), time (p = 0.001), and the interaction between group and time (p = 0.046) significantly influenced the SUA levels. The effects of group and time remained significant after
adjusting for sex, gestational age, and BMI, with p-values of 0.037 and 0.009, respectively.”

2. The number of children followed up at 5 years was 188 (NBW: 166, LBWCUG: 22), and the number followed up at 7 years was (NBW: 145, LBWCUG: 17) vs the initial age 3 year cohort of 364 (NBW:308, LBWCUG: 56). LBWCUG is a combined investigation group of LBW-term and LBW-preterm infants. The number for each subgroup at follow up would likely be small due to a high loss rate in follow up. Nonetheless, this should be declared that the reader could at least know if there was over-representation of one subgroup at each follow up age. Table 1 could be reformatted to include more data on the group numbers to clarify. Table 1 in the current form could give the impression that there were 308 NBW and 56 LBWCUG children through to 7 years of age (though noted in the table footnotes).

Response: As suggested, Table 1 has been modified to show the number of children in each subgroup at each follow-up age more clearly.

3. Table 2 shows the LSM from the modelling adjusted for sex, GA and BMI at age of uric acid measurement. I suggest to include the raw data with mean, SD/range as the top panel and LSM below for readers to compare and understand the differences before and after the adjustment. It was noted that "when modelled without adjustment, LBWCUG children and consistently higher SUA levels between 3 and 7 years of age (p-0.031 and 0.027)".

Response: As suggested, the raw data with mean (95% CI) have been added to Table 2.

4. The investigators examined the LSM of SBP between 3 and 7 year of age based on the multivariable generalized linear model adjusted for sex, gestational age, and height, weight, and uric acid at 3 years of age, and found a higher SBP by 7.89 mmHg with borderline significance (p=0.082) in children who experienced low birth weight and catch-up growth compared with those who had a normal birth weight. Should a sensitivity test with or without uric acid be performed as uric acid is associated with SBP. In Model 1 (Table 3), results were adjusted for height and weight at baseline but not with sex and gestation. Again, it would be desirable to include the raw data in table.

Response: As suggested, the unadjusted results and adjusted results without adjustment for uric acid have been added to Table 3.

5. Figure 1 shows the uric acid trajectories from age 3 to 7. The plots are from the estimated LSM. Again it would be important to know the data breakdown of the age 5 and 7 LBSUCG to give confidence of the plot b regarding the 20 term LBW with catch up growth.

Response: Figure 1 has been modified to provide more information at the ages of 5 and 7 years.

Response to Reviewer 3:

1. Page 7: 164-165: The authors said that "Means and standard deviations were calculated for continuous variables". How did they ensure that Mean is the correct descriptive statistics to be used for the analysis and not median which is peculiar to the case of non-normality?

Response: The manuscript has been revised as follows (p.8, lines 159-160): “The means with the standard deviation or median and interquartile range were calculated for
continuous variables after performing the normality test.”
The normality of each variable was tested, and the descriptive statistics were calculated accordingly (Table 1).

2. Linear mixed models are an extension of simple linear models that allows both fixed and random effects, and are particularly used when there is non-independence in the data, such as arises from a hierarchical structure. The model has both fixed effects and random effects and are useful in a wide variety of disciplines but of more importance in settings where repeated measurements are made on the same statistical units (longitudinal study), or where measurements are made on clusters of related statistical units. Therefore, the use of linear mixed models in this study is in order. However, there are various assumptions that must be satisfied before the models can be used correctly and effectively. I encourage the authors to address in their paper how these assumptions were handled to prevent them from being violated. This is pertinent to the analysis, the results and findings from this study.

(1) In the application of any mixed model, its random variables add the assumption that observations within a level, the random variable groups, are correlated. The assumption can only be relaxed if observations are independent of other observations except where there is correlation specified by the random variable groups.

(2) Independence assumption: Independence shows the effects connected with the random variable groups that are uncorrelated with the means of the fixed effect from the random variable groups.

(3) Linearity: For each of the independent variables in a mixed model, there is an assumption that the models are similar. This assumption can be checked with plots of the residuals versus each of the variables.

(4) Normality of the residuals: This is often addressed with a quantile-quantile plot where a substantial deviation from linearity of the observations is an indication of a deviation from residuals normality.

Response: In this study, the REPEATED statement was used in PROC MIXED to specify the R matrix in the mixed model, because the uric acid for the same subject was measured at three equally spaced time points. In repeated measures modeling, observations within the same subject are assumed to be correlated and observations from different subjects are assumed to be independent. Therefore, the first and second assumptions were satisfied based on the nature of the analysis. We also specified an unstructured covariance matrix for the covariance structure of the errors, but did not specify the random effects.

The normality and linearity assumptions were met, and the method used to check these assumptions has been added to the revised manuscript as follows (p. 9, lines 181-182):
“The assumptions for the mixed model were checked and confirmed to be satisfied in all models.”

3. Diagnostic check:
Appropriate diagnostic check must be used to ascertain that the model built is not influenced by one or a small set of observations, an evidence that the model over fits or sensitive to those observations included in the model.

Inter Class Correlation must also be checked to show how much of the variation in the response variable, that is not attributed to fixed effects, but accounted for by a random effect. This is done by computing the ratio of the variance of the random effect to the total random variation.

Mixed models function by providing some contraction to the random effects and the amount of this contraction is based on how much information is domicile in a random effect groups. In order to know which of the observations used in the model constitutes stress to the model, creating both the fixed effect model and the model with the random effects is necessary. There might be need to evaluate both models for those observations with high leverage or Cook's distance. Thereafter, it is also necessary to
evaluate the coefficients' change in the mixed model by dropping some observations marked by the linear or generalized model.
The authors are to provide statistical evidence in the paper on how these diagnostic checks were accomplished.

Response: Because we did not specify random effects in the mixed model, computing the ratio of the variance of the random effects to the total random variation is not applicable to our study. We evaluated the change in the coefficients in the mixed model by dropping some observations with high leverage or Cook’s distance. The change in the coefficients to the SE was 9~31%, and the results of the diagnostic test have been described in the revised manuscript as follows (p. 9, lines 182-186):
“In addition to ascertaining that the model is not influenced by the small set of observations, the change in coefficients was evaluated after dropping observations with high Cook’s distances. The coefficients changed by as much as 9~31% depending on the models, and there were no differences in the direction or significance of the results after dropping observations. Therefore, we decided to keep the observations to maintain the statistical power.”

4. Title: The design of the study does not provide any indication that can convince the scientific world that pre- and postnatal growth are the main causes of the change in the longitudinal trends in Serum Uric Acid Levels (a product of purine catabolism, and hyperuricemia) and Blood Pressure in Children as emphasized in the title. Therefore, the use of the word "effects" in the title should be revised.

Response: As suggested, the title of the revised manuscript has been changed to “Association between Pre-and Postnatal Growth and Longitudinal Trends in Serum Uric Acid Levels and Blood Pressure in Children aged 3 to 7 years”

5. Abstract: Problem statement and objectives of the study are missing in the background section.

Response: As suggested, the Background section of the Abstract has been revised as follows (p. 3, lines 50-55):
“Uric acid has been identified as an important factor in the development of hypertension. If low birth weight (LBW) combined with catch-up growth (CUG) is associated with continuously elevated serum uric acid levels (SUA) level trajectories, LBW children who experience CUG may have an increased risk of hypertension later in life. Therefore, this cohort study analyzed longitudinal trends in SUA levels and changes in blood pressure in relation to pre- and postnatal growth over an extended follow-up period.”

6. Abstract: The author should specify whether the study is retrospective or prospective cohort study. The specific type of generalized linear model used should be mentioned. The word catch-up must be defined clearly in the method since a result was generated based on the word in the abstract.

Response: As suggested, the Method section of the Abstract has been revised as follows (p. 3, lines 57-62):
“This prospective cohort study of 364 children from the Ewha Birth and Growth Cohort assessed the effects of pre- and postnatal growth status on SUA at 3, 5, and 7 years of age using a linear mixed model and the change in blood pressure over the 7-year follow-up period using a generalized linear model (analysis of covariance). CUG was defined as a change in weight (between birth and age 3) with a z-score > 0.67 for LBW subjects. The multivariate model considered sex, gestational age, and uric acid, height, and weight at 3 years of age.”
7. Abstract: The conclusion must include a statement that depicts the main finding from this study. The included sentences as currently presented are recommendations which ought to have come from the main finding. Consequently, this section should be revised.

Response: As suggested, the Conclusion of the Abstract has been revised as follows (p. 4, lines 73-76):
“The uric acid levels and changes in systolic blood pressure were consistently higher among LBW children who experienced CUG compared with NBW children for the first 7 years of life. LBW children who experienced greater weight gain from birth to age 3 had even higher uric acid levels compared with NBW children.”

8. Introduction: Despite the importance of this study and its associated relevance to the scientific world, many studies have been conducted using the same approach in some settings around the world. I doubt if the findings from this new study will significantly vary from those obtained from these previous studies. It is understood that cultural differences in terms of food intake and environmental conditions can make a difference in Uric Acid Levels and Blood Pressure in Children between settings. It is essential that the authors make a good case for the motivation and justification for this study in Ewha. The possible explanation could be in terms of the design of the study, in terms of study subjects including modes of selection and data analyses approaches utilized. The gap in knowledge should be essentially indicated in the introduction.

Response: Previous cross-sectional studies indicated that low birth weight is associated with elevated uric acid levels. However, no prospective study has investigated both the trajectory of serum uric acid levels and changes in blood pressure in relation to pre- and postnatal growth over an extended follow-up period. To highlight this gap in knowledge in the Introduction, we have modified the manuscript as follows (p. 5, lines 110-117):
“A study reported that preterm birth combined with accelerated postnatal growth is associated with higher SUA levels at age 3. Other studies have investigated the association between birth weight and SUA levels in children and adolescents, but they measured SUA levels at one time point only. To our knowledge, no studies have prospectively investigated SUA level trajectories. We do not know how long birth weight and catch-up growth are associated with SUA levels or blood pressure. Therefore, in this cohort study, we analyzed longitudinal trends in both SUA levels and changes in blood pressure in relation to pre- and postnatal growth over an extended follow-up period.”