PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents
AUTHORS	Ellul, Susan; Wake, Melissa; Clifford, Susan; Lange, Katherine; Wurtz, Peter; Juonala, Markus; Dwyer, Terry; Carlin, John; Burgner, David; Saffery, Richard

VERSION 1 - REVIEW

REVIEWER	Dr Raphaële Castagné, Research Fellow LEASP, UMR 1027, Inserm-Université Toulouse III Paul Sabatier, Toulouse, France
REVIEW RETURNED	23-Jan-2018

GENERAL COMMENTS	Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents The manuscript examines metabolic profiles in children and their parents in term of sex and age differences, they further explore the metabolites correlation in parents-child dyad. They used high throughput NMR data collected from the Child Health Checkpoint study nested within the Longitudinal Study of Australian Children. While strengths of the work include the good sample size, the study design and quality of the data, at its current state the manuscript have several problems that need to be addressed. One important concern is the lack of multiple testing correction in the analytical strategy and the unadjusted nature of the results (except the partial correlation). Introduction: 1/ 3rd paragraph: The authors mentioned the “it remains unclear how the serum metabolome responds to [….] hormonal-specific factor in childhood”: a point that is not addressed in the subsequent analyses nor in the discussion 2/ 4th paragraph: Sentences are needed to justify the assumptions that metabolites profiles are shared between generations 3/The order used in the introduction is metabolomics profiling analyses in children, their parents and parent child concordance, keep this order all the way through to ease the understanding of the reader Methods: 1/ How the informative subset of 70 lipid and metabolites were defined ? It is written that they “capture the majority of the variation

	within the dataset", do they results from a principal components analyse, if such a supplementary figure should be added with the contribution of each variable to the first dimension. 2/ A study overflow would be very helpful to understand what type of analyses has been done in children, adults and dyad 3/ 70 tests are performed, there is no mention of multiple testing correction 4/ Others measures are given but are included only in the partial correlation analyses between parents and children 5/ The section statistical analysis should be organised in sub-sections a) Gender differences in children: there is no rational to investigate age differences since children are approximately the same age, the t-test used to compare the mean metabolite concentration does not allow to include other variables, probably a more generic model such as a general linear model may allow you to control for confounding effect (i.e. body mass index, socioeconomic disadvantage, time of blood collection and fasting time) b) Gender and age differences in adult: as above, the t-test to compare the mean metabolite concentration does not allow to include other variables and the observed differences might not be due only to gender and/or age For both children and adult analyses: Where indication of disease, being under medication available in the dataset? Since 70 metabolites are tested, it is needed to apply a multiple testing correction c) Parent-child correlation: the authors used 2 approaches (paired t-tests and correlation), I am not sure to understand the rationale behind that, probably a linear mixed model will allow you to (1) account for within family correlation (2) control for potential confounders. Results: 1/ The results section should be re-organised to be consistent with the introduction and method section 2/ In general, the authors gave indication through "more pronounced", "many metabolites", this should be rephrased taking into account the statistical parameters. 3/ P12 "correlations overlapped": what does it mean ? Same metabolites? Same correlation coefficients ?

REVIEWER	Joanne Sordillo Harvard Medical School
REVIEW RETURNED	23-Feb-2018

GENERAL COMMENTS	Major Comments 1. How was the panel of 70 biomarkers (chosen to capture most of the variability in metabolites) selected out of the 200+ metabolites? The selection process behind choosing these 70 isn't explained.

	2. Did the authors construct correlation matrices for children separately? For Adults separately? Were there differences in the most correlated metabolites for children vs. adults? 3. Why did the authors use Pearson correlations, rather than intra-class correlations? It seems like the intra-class correlation would be more appropriate for identifying how closely children resemble their parents in terms of metabolite profiles. 4. Did the authors have information about puberty in the children? 5. Figures 2-4 could not be evaluated, because they failed to convert to images in the PDF file (an error message was listed instead of the actual figure). Same issue for supplemental figures. 6. The authors have basic subject characteristics like age and BMI, but chose not to examine those as predictors of the metabolites. Why? This is a relatively large sample size for a metabolomics study, and report on the relationships between these basic characteristics and metabolites levels would be interesting. It would also be interesting to compare metabolite associations with BMI in children with those observed in the adults. (For example, BMI may be associated with particular metabolites in children but not adults and vice versa). For the adults (who have a wider age range than the children), it would be interesting to see the relationship between increasing age and metabolites levels. Minor Comments 1. Line 10, should say “with the potential to improve” not “with the potential to improving” 2. Range of correlations in abstract should report the lowest statistically significant correlation as the minimum, the maximum correlation listed can be kept as is. 3. Page 9, line 4; correct spelling of word “focused”

REVIEWER	Diana L. Santos Ferreira, Senior R. Associate in Metabolomics University of Bristol, UK
REVIEW RETURNED	28-Feb-2018

GENERAL COMMENTS	This study reports children-parent metabolic profile differences alongside sex-differences in both parents and children separately, using data from 1133 Australian parent-child pairs. The 70 metabolic traits were measured in serum by a Nuclear Magnetic Resonance metabolomic platform. It is a well-written, nicely presented descriptive paper and your rationale for conducting this research is clear. My comments are below. 1. From Table 2 and the Methods section, it is reported that participant’s Body Mass Index (BMI) was collected, since BMI is well known to influence metabolic trait levels could a rational be provided why BMI was not used to adjust the analysis? 2. Page 4, line 27, when the authors write “all cholesterol” do they mean “all non-HDL” cholesterol instead? 3. I praise the careful and detailed description of the pre-analytical phase (sample collection, preparation, etc..) which is crucial for interpretation of the results. 3.1. For future reference: to avoid contamination by anticoagulants (EDTA, heparin) it is advisable to collect serum first. 3.2. Could centrifugation details be provided? 3.3. Was blood clothing allowed at room temperature or other?

	4. Page 7, line 38, Table 1, the authors mention “12 lipids in each 14 subclasses”, to my knowledge each of the 14 lipoprotein subclasses is characterized by lipoprotein particle concentration and 6 lipid variables (total lipids, phospholipids, total cholesterol, cholesterol esters, free cholesterol and triglycerides). Are the authors also referring to the 4 lipoprotein ratios (phospholipids, cholesterol esters, free cholesterol and triglycerides over total lipids)? Could the authors name the 12 lipids? 5. Page 8, line 1-2, “We excluded glucose and lactate (…) and processing variables”, if the authors suspect that the concentration of these two metabolites were affected by pre-analytical conditions, results for pyruvate and alanine should be interpreted with caution. 6. STROBE statement: it would be useful to include paragraph excerpts instead of page and line numbers as these might change if the manuscript is accepted for publication. Minor detail: 1. Page 6, line 20, do the authors mean “-80oC” instead of “-809 oC”?

REVIEWER	Andrew Vincent University of Adelaide Australia
REVIEW RETURNED	26-Apr-2018

GENERAL COMMENTS	Statistical Review of “Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents”. The manuscript is very well written and easy to follow. However there are a couple of areas regarding the statistical methods that should be addressed. Major Issues. 1: Inference regarding correlations appear to have been made by visual inspection of point estimates and confidence intervals. - Page 12 lines 25-26 “Correlations for all parents and all children showed similar patterns to that observed for mother and child by sex.” - Page 12 line 27 “Confidence intervals (95%) for all mother-son and mother-daughter correlations overlapped.” Please quantify the strength of associations (correlations) via multivariable linear regressions, with appropriate interaction terms for the different dyads/groups. For example constructing a linear regression for each metabolite using mother values as outcomes and child values as the continuous predictor then an interaction with child sex would quantify the difference correlation strength between mother-son and mother-daughter dyads. Similarly in the first example using sex of parent as the interaction term. 2: A substantial number of comparisons are being made, and while for some conclusions the differences are clear (eg differences in means - child v adult), there are other analyses where the differences are less pronounced (eg ApoA-1 being lower in girls than boys).

	Please perform an analysis (ie a multiple testing adjustment or FDR) to show that the less pronounced results that are explicitly reported (eg page 12 lines 5, 8, 28-32) are beyond what would be expected by chance. Minor Issues 3: Page 7 lines 16-19, Please specify what methods were used to select the 70 metabolites. 4: Please explain why Glycerol has roughly half the sample size of the other factors. 5: Page 12 line 21 please use lower case for "correlation coefficient".

VERSION 1 – AUTHOR RESPONSE

Editor/Reviewer Comments	Author's Response	Reference page
Reviewer 1 : Dr Raphaële Castagné, LEASP, UMR 1027, Inserm-Université Toulouse III Paul Sabatier, Toulouse, France		
R.1.1. 3rd paragraph: The authors mentioned the "it remains unclear how the serum metabolome responds to [….] hormonal-specific factor in childhood": a point that is not addressed in the subsequent analyses nor in the discussion	We thank the reviewer for bringing this to our attention. We have removed the reference to "hormonal-specific factors in childhood" in the 3rd paragraph. The text now reads "However, it remains unclear how the serum metabolome differs in adults compared to children and by sex particularly in childhood."	Page 4
R.1.2. 4th paragraph: Sentences are needed to justify the assumptions that metabolites profiles are shared between generations	We have added the following text "Considerable evidence exists that the metabolomic profile is regulated, at least in part, by genetic factors [1] [2] and is also influenced by dietary and lifestyle factors. Each of these influences is likely to be shared between parents and their offspring to varying degrees, however, parent-child correlations of metabolites from NMR-based platforms have not been reported previously."	Page 5
R.1.3. The order used in the introduction is metabolomics profiling analyses in children, their parents and parent child concordance, keep this order all the way through to ease the	We thank the reviewer for this suggestion and apologise if this was unclear. The last paragraph of the introduction lists the aims as to present (1) NMR-based metabolomics analysis of a population-based cohort of 11-12 year old children and their parents, (2) identify age and sex-specific metabolomic profiles and (3) report sex-specific parent-child concordance.	Pages 5, 11, 13

Editor/Reviewer Comments	Author's Response	Reference page
understanding of the reader	We believe that the statistical methods also follow the same order as do the results and reviewer 3 and 4 noted that the manuscript was well written and easy to follow. However, we have modified the text in the introduction to make the aims clearer. "Here, we describe (1) the distribution of NMR-based metabolite measures in a population-based cohort of 11-12 year old children and their parents, differences in metabolite concentrations (2) by age (adults compared to children) and (3) by sex in children and adults; and (4) report sex-specific parent-child concordance." and we have clarified in the statistical analysis section (Page 11) and results (Page 13) what methods were used to address each aim by including sub-section headings.	
R.1.4. How the informative subset of 70 lipid and metabolites were defined ? It is written that they "capture the majority of the variation within the dataset", do they results from a principal components analyse, if such a supplementary figure should be added with the contribution of each variable to the first dimension.	We agree that this should be clarified and have amended the text to carefully describe how the subset of metabolites were chosen. We have amended the text in the methods to read: "We eliminated the 5 ratio measures for each of the 14 lipoprotein subclass particles. In addition, the 7 other measures within each of the lipoproteins (esterified cholesterol, free cholesterol, total cholesterol, triglycerides, phospholipids, total lipids and particle concentration) are all highly correlated and therefore we only reported total lipids for each of the lipoprotein subclass particles."	Page 9
R.1.5. A study overflow would be very helpful to understand what type of analyses has been done in children, adults and dyad	The paper does include an abbreviated participant flow chart consistent with the other papers in the series (figure 1) and other details are included in the methods paper.[3] Detail about what analyses were conducted on which samples was included in the methods section "Participants were included in the current analyses if metabolomic data from CheckPoint were available (figure 1). Venous blood was not available for home-visit participants, but was collected at all city and most regional assessment centres. Participant pairs were excluded from the concordance analyses in this study if the attending parent was not the biological parent."(Page 6). In addition, we have added sub-section headings in the statistical analyses section of the methods to clarify what analyses were undertaken to	figure 1 Pages 6, 11

Editor/Reviewer Comments	Author's Response	Reference page
	address each aim (Page 11) and we believe this is helpful in clarifying what analyses were undertaken in children, adults and dyads.	
R.1.6. 70 tests are performed, there is no mention of multiple testing correction	We acknowledge the reviewers' suggestion that multiple testing correction be undertaken and therefore we have amended the paper to account for multiple comparisons using Benjamini-Hochberg with a FDR of 10% for (a) mean differences - adult versus child and (b) differences in means by sex in children and adults. We have amended the statistical methods and results sections accordingly. The following text has been added to the methods: "P-values were adjusted using Benjamini-Hochberg (B-H) with a false discovery rate (FDR) of 10% to account for multiple comparisons." (Page 12) We have updated Figure captions accordingly to include the text "Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10)" (Page 23) The overarching aim of the paper (and the special series within which this paper belongs) is to describe the data that is available and is intended to be primarily of a descriptive nature therefore we have not made adjustments for multiple comparisons for the parent-child correlations - instead interpreting with caution; presenting correlations and confidence intervals and focusing on patterns enabling readers to draw their own conclusions (Page 15, 16)	Pages 12, 23, 15, 16
R.1.7. Others measures are given but are included only in the partial correlation analyses between parents and children	The aims of the paper have been clarified in the introduction as "Here, we describe (1) the distribution of NMR-based metabolite measures in a population-based cohort of 11-12 year old children and their parents, differences in metabolite concentrations (2) by age (adults compared to children) and (3) by sex in children and adults; and (4) report sex-specific parent-child concordance." Thus, for aim (1) we describe the distribution of the metabolite measures for children and adults separately by sex and overall as detailed in the methods section. (Page 11) Given the number of	Pages 5, 11

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	metabolites, in aim (2) we describe the mean difference between the adult and child measures and in aim (3) the difference in means by sex in children and adults separately in order to visually describe and present our results. Therefore for aims (1-3) we do not feel that additional adjustments are necessary in keeping with the descriptive aims of the paper and of the special series to which this paper belongs.	
R.1.8. The section statistical analysis should be organised in sub-sections	We thank the reviewer for the suggestion and have included sub-section headings in the statistical analysis section to clarify what methods were used to address each aim.	Page 11, 12
R.1.9. Gender differences in children: there is no rational to investigate age differences since children are approximately the same age, the t-test used to compare the mean metabolite concentration does not allow to include other variables, probably a more generic model such as a general linear model may allow you to control for confounding effect (i.e. body mass index, socioeconomic disadvantage, time of blood collection and fasting time)	We apologise for the confusion. There was no intention to look at age differences in children separately because as the reviewer correctly states the children are of similar age. When we refer to age difference, we mean describing the difference in metabolite concentration for adults compared to children. We agree that this could have been clearer and have updated the manuscript accordingly to clarify the aim in the introduction with the following text to report: "differences in metabolite concentrations (2) by age (adults compared to children) and (3) by sex in children and adults…." (Page 5) as well as clarifying in the statistical analysis section of the methods (Page 11). We have also updated the subheading in results section (Page 14) to make this clearer. While we understand that the use of a linear model would allow us to include potential confounders, we do not feel that additional adjustments are necessary in keeping with the descriptive aims of the paper. (see also R.1.7)	Pages 5, 11, 14
R.1.10. Gender and age differences in adult: as above, the t-test to compare the mean metabolite concentration does not allow to include other variables and the observed differences might not be due only to gender and/or age	We apologise if this was unclear. There was no intention to look at age differences in adults separately. Please see response R.1.7 and R.1.9.	Page 5

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R.1.11. For both children and adult analyses: Where indication of disease, being under medication available in the dataset?	Parents reported on their child's current medications/supplements use and lifetime hospitalisations, however, equivalent data was not collected for parents. Data on current disease status was not systematically collected. Given that the focus was to describe the metabolomics data available these measures were not included in this paper.	No change to manuscript
R.1.12. Since 70 metabolites are tested, it is needed to apply a multiple testing correction	Please see R.1.6.	Pages 12, 23, 15, 16
R.1.13. Parent-child correlation: the authors used 2 approaches (paired t-tests and correlation), I am not sure to understand the rationale behind that, probably a linear mixed model will allow you to (1) account for within family correlation (2) control for potential confounders.	We apologise for any confusion; we have added sub-sections in the statistical analysis section of the methods to help clarify what methods were used to address each aim (Page 11). The t-tests were used to describe the difference between adult and child metabolite concentrations and were not used for parent-child concordance. As the paper was intended to be descriptive (as is the aim of the series) we were not seeking to make adjustment for potential confounders. We are also not seeking to fully explain why there are differences in this paper as more targeted papers looking at these aspects are planned. Parent-child concordance is examined using correlations (and partial correlations) and not via t-test. Our focus is simply on the simple description of patterns of association between parent and child measures. We have modified the text in the paper (introduction) to make the aims clearer by amending the text to read "Here, we describe (1) the distribution of NMR-based metabolite measures in a population-based cohort of 11-12 year old children and their parents, differences in metabolite concentrations (2) by age (adults compared to children) and (3) by sex in children and adults; and (4) report sex-specific parent-child concordance." (Page 5) and have clarified in the statistical methods the methods used to address each aim. (Page 11)	Page 5, 11
R.1.14. The results section should be re-organised to be consistent with the	We thank the reviewer for this suggestion. The last paragraph of the introduction has been amended to clarify the aims. Please also see R.1.3 and R.1.13.	Pages 5, 11, 14

Editor/Reviewer Comments	Author's Response	Reference page
introduction and method section		
R.1.15. In general, the authors gave indication through "more pronounced", "many metabolites", this should be rephrased taking into account the statistical parameters.	Due to the descriptive nature of the paper we have chosen to focus on describing our findings in terms of overall patterns as well as presenting coefficients with confidence intervals in figures. There is a large emerging body of literature critiquing the arbitrary dichotomization of evidence using statistical thresholds[4 5 6] so we have placed less emphasis on p-values for these reasons; rather describing the general patterns and directing to the figures to enable readers to draw their own conclusions.	No change to manuscript
R.1.16. P12 "correlations overlapped": what does it mean ? Same metabolites? Same correlation coefficients ?	We agree that this is unclear and we have excluded reference to "correlations overlapped" in this paper.	Page 17
Reviewer 2: Joanne Sordillo, Harvard Medical School, US		
R.2.1. How was the panel of 70 biomarkers (chosen to capture most of the variability in metabolites) selected out of the 200+ metabolites? The selection process behind choosing these 70 isn't explained.	We agree that this should be clarified and have amended the text to carefully describe how the subset of metabolites were chosen. We have amended the text in the methods to read: "We eliminated the 5 ratio measures for each of the 14 lipoprotein subclass particles. In addition, the 7 other measures within each of the lipoproteins (esterified cholesterol, free cholesterol, total cholesterol, triglycerides, phospholipids, total lipids and particle concentration) are all highly correlated and therefore we only reported total lipids for each of the lipoprotein subclass particles."	Page 9
R.2.2. Did the authors construct correlation matrices for children separately? For Adults separately? Were there differences in the most correlated metabolites for children vs. adults?	Correlation matrices were provided in the paper for adults (supplementary figure 1) and children (Supplementary figure 2) although R.2.5 suggests that the reviewer was unable to view the images and we therefore apologise. The supplementary figures were included for descriptive purposes only and therefore we did not do any formal comparison for children vs adults. We have added text which reads "and the pattern of correlations were similar for adults and children."	Page 9
R.2.3. Why did the authors use Pearson correlations, rather than intra-class correlations? It	We are not sure that we understand this point because intra-class correlation (ICC) applies to measuring association within unstructured clusters or groups. As our goal was to describe the association between parent and children	No change to manuscript

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seems like the intra-class correlation would be more appropriate for identifying how closely children resemble their parents in terms of metabolite profiles.	measures we feel that Pearson's correlation coefficient is appropriate.	
R.2.4. Did the authors have information about puberty in the children?	Children self-reported pubertal status using the Sexual Maturity Scale and the Pubertal Development Scale. In addition, girls were asked if they were currently menstruating. However, given that the focus was to describe the metabolomics data available these measures were not included in this paper. We plan more targeted analyses in subsequent papers, but they were not within our a priori hypotheses for this paper.	No change to manuscript
R.2.5. Figures 2-4 could not be evaluated, because they failed to convert to images in the PDF file (an error message was listed instead of the actual figure). Same issue for supplemental figures.	Apologies, our understanding was that the images were also made available to reviewers separately to the PDF file. We have compressed the file size of this image and include the updated files as part of this revision.	Figures 2-4 and Supplementary Figures
R.2.6. The authors have basic subject characteristics like age and BMI, but chose not to examine those as predictors of the metabolites. Why? This is a relatively large sample size for a metabolomics study, and report on the relationships between these basic characteristics and metabolites levels would be interesting. It would also be interesting to compare metabolite associations with BMI in children with those observed in the adults. (For example, BMI may be	We understand that analyses exploring the metabolomic associations with BMI and with continuous age in adults is interesting but the intention of this paper is primarily to describe the metabolomic measures available for Child Health CheckPoint. For clarity we have therefore excluded BMI from the paper and we plan to examine these associations (which were not within our a priori hypotheses for this paper) in subsequent papers.	Pages 10, 14

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associated with particular metabolites in children but not adults and vice versa). For the adults (who have a wider age range than the children), it would be interesting to see the relationship between increasing age and metabolites levels.		
R.2.7. Line 10, should say "with the potential to improve" not "with the potential to improving"	We have replaced "improving" with "improve".	Page 2
R.2.8. Range of correlations in abstract should report the lowest statistically significant correlation as the minimum, the maximum correlation listed can be kept as is.	There is a large emerging body of literature critiquing the arbitrary dichotomization of evidence using statistical thresholds. [4] [5] [6] We thank the reviewer for the suggestion however we felt it important to present a fair and accurate portrayal of the range of correlations observed (whether they meet cut offs for conventional statistical significance or not) and the uncertainty surrounding these to enable readers to make their own conclusions. However, we have amended the text in the abstract to be more succinct "Positive correlations were observed for the majority of metabolites including for isoleucine (CC 0.33, 95% CI 0.27 to 0.38), total cholesterol (CC 0.30, 95% CI 0.24 to 0.35) and omega 6 fatty acids (CC 0.28, 95% CI 0.23 to 0.34) in parent-child comparisons."	Page 2
R.2.9. Page 9, line 4; correct spelling of word "focused"	We have updated the text to say "focused" not "focussed"	Page 9
Reviewer 3: Diana L. Santos Ferreira, University of Bristol, UK		
R.3.1. From Table 2 and the Methods section, it is reported that participant's Body Mass Index (BMI) was collected, since BMI is well known to influence metabolic trait levels could a rational be provided why BMI was not	The intention of this paper is primarily to describe the metabolomic measures available hence we did not adjust for BMI. For clarity we have therefore excluded BMI from the paper and we plan to examine these associations (which were not within our a priori hypotheses for this paper) in subsequent papers.	Pages 10, 14

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used to adjust the analysis?		
R.3.2. Page 4, line 27, when the authors write "all cholesterol" do they mean "all non-HDL" cholesterol instead?	We thank the reviewer for bringing this to our attention. We have updated the text to read "all non-HDL" rather than "all cholesterol".	Page 4
R.3.3. I praise the careful and detailed description of the pre-analytical phase (sample collection, preparation, etc..) which is crucial for interpretation of the results.	Thank you. The Child Health CheckPoint study was carefully planned with all procedures documented with high quality Standard Operating Procedures (SOPs). More detail is available in the cohort summary paper[3] and SOPs describing biospecimen processing will be made available on the study website by Quarter 3 2018.	NA
R.3.4. For future reference: to avoid contamination by anticoagulants (EDTA, heparin) it is advisable to collect serum first.	We thank the reviewer for the suggestion. Indeed EDTA was collected before serum and serum was collected prior to Li-heparin. The reason for this is that the most precious samples were collected first (for Child Health CheckPoint this is EDTA) to ensure viable cells. In some cases, only one tube was able to be collected from some participants. We also note that the UK Biobank order of collection has two different anticoagulant tubes as first collected.[7]	No change to manuscript
R.3.5. Could centrifugation details be provided?	The sample tubes were spun at 550g relative centrifugal force (RCF) for 10 minutes at room temperature. We have added the centrifugation details to the description of the pre-analytical stage in the methods section by including the following text "The sample tubes were spun at 550g relative centrifugal force for 10 minutes at room temperature…." This information is also detailed in the bioprocessing SOP to be made available on the study website by Quarter 3 2018.	Page 6
R.3.6. Was blood clothing allowed at room temperature or other?	Yes, blood clotting was allowed at room temperature for at least 30 minutes after collection. We have added the information regarding blood clotting to the description of the pre-analytical stage in the methods section by including the following text "Blood clotting was allowed at room temperature for at least 30 minutes after collection".	Page 6

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	This information is also detailed in the bioprocessing SOP to be made available on the study website by Quarter 3 2018.	
R.3.7. Page 7, line 38, Table 1, the authors mention "12 lipids in each 14 subclasses", to my knowledge each of the 14 lipoprotein subclasses is characterized by lipoprotein particle concentration and 6 lipid variables (total lipids, phospholipids, total cholesterol, cholesterol esters, free cholesterol and triglycerides). Are the authors also referring to the 4 lipoprotein ratios (phospholipids, cholesterol esters, free cholesterol and triglycerides over total lipids)? Could the authors name the 12 lipids?	As the reviewer has stated, each of the 14 lipoprotein subclasses is characterised by 7 measures: (1) a lipoprotein particle concentration and (2) 6 other lipid measures (total lipids, phospholipids, total cholesterol, cholesterol esters, free cholesterol and triglycerides). In Table 1, as the reviewer has suggested we had indeed also included the lipoprotein ratios. There are five lipoprotein ratios and they are: esterified cholesterol/total lipids (%), free cholesterol/total lipids (%), total cholesterol/total lipids (%), triglycerides/total lipids (%) and phospholipids/total lipids (%). We have therefore updated Table 1 to clarify the 12 lipid measures available for each lipoprotein subclass.	Page 9
R.3.8. Page 8, line 1-2, "We excluded glucose and lactate (…) and processing variables", if the authors suspect that the concentration of these two metabolites were affected by pre-analytical conditions, results for pyruvate and alanine should be interpreted with caution.	Although glucose and lactate are the metabolites most likely to be affected by pre-analytical conditions, we agree that pyruvate and alanine should be interpreted with caution if pre-analytical conditions are of concern. However, we note that collection and processing of blood specimens followed a strict, high quality SOP including limiting processing time generally to within 2 hours. We have therefore updated the paper to include glucose and lactate.	Page 10
R.3.9. STROBE statement: it would be useful to include paragraph excerpts instead of page and line numbers as these might change if the	We thank the reviewer for the helpful suggestion and will consider using paragraph excerpts for future work. However for this paper we have updated the page and line numbers in the STROBE statement.	STROBE statement

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manuscript is accepted for publication.		
R.3.10. Minor detail: 1. Page 6, line 20, do the authors mean "-80oC" instead of "-809 oC"?	We have corrected the text to read "-80ºC" rather than "-809C".	Page 6
Reviewer 4: Andrew Vincent, University of Adelaide, Australia		
R.4.1. Inference regarding correlations appear to have been made by visual inspection of point estimates and confidence intervals: Page 12 lines 25-26 "Correlations for all parents and all children showed similar patterns to that observed for mother and child by sex."	Due to the descriptive nature of the paper (and the papers in the series to which this paper belongs) our intention is to describe the patterns observed with less emphasis on statistical significance. There is a large emerging body of literature critiquing the arbitrary dichotomization of evidence using statistical thresholds [4] [5] [6] so we believe that the reporting of correlations and confidence intervals and describing patterns in the absence of p-values is appropriate and enables readers to draw their own conclusions.	No change to manuscript
R.4.2. Page 12 line 27 "Confidence intervals (95%) for all mother-son and mother-daughter correlations overlapped."	We agree that it is inappropriate to make any judgements from whether the confidence intervals overlapped. We have therefore removed the text from the manuscript.	Page 17
R.4.3. Please quantify the strength of associations (correlations) via multivariable linear regressions, with appropriate interaction terms for the different dyads/groups. For example constructing a linear regression for each metabolite using mother values as outcomes and child values as the continuous predictor then an interaction with child sex would quantify the difference correlation strength between mother-son and mother-daughter dyads. Similarly in the first example using sex of parent as the interaction term.	We thank the reviewer for the suggestion and understand their concerns. However, we re-emphasise the descriptive aims of the paper and as such we do not feel that formal testing via inclusion of interaction terms in a modelling approach as necessary in the context of the aims of the paper. In addition, we do not understand the suggestion to use mothers values as outcomes to be predicted or explained by child values.	No change to manuscript

Editor/Reviewer Comments	Author's Response	Reference page
R.4.4. A substantial number of comparisons are being made, and while for some conclusions the differences are clear (eg differences in means - child v adult), there are other analyses where the differences are less pronounced (eg ApoA-1 being lower in girls than boys). Please perform an analysis (ie a multiple testing adjustment or FDR) to show that the less pronounced results that are explicitly reported (eg page 12 lines 5, 8, 28-32) are beyond what would be expected by chance.	We acknowledge the reviewers' suggestion that multiple testing correction be undertaken and therefore we have amended the paper to account for multiple comparisons using Benjamini-Hochberg with a FDR of 10% for (a) mean differences - adult versus child and (b) differences in means by sex in children and adults. We have amended the statistical methods and results sections accordingly. The following text has been added to the methods: "P-values were adjusted using Benjamini-Hochberg (B-H) with a false discovery rate (FDR) of 10% to account for multiple comparisons." (Page 12) We have updated Figure captions accordingly to include the text "Significant associations after p-values adjusted for multiple testing using Benjamini-Hochberg procedure are shown in bold (FDR=0.10)" (Page 23) The overarching aim of the paper (and the special series within which this paper belongs) is to describe the data that is available and is intended to be primarily of a descriptive nature therefore we have not made adjustments for multiple comparisons for the parent-child correlations - instead interpreting with caution; presenting correlations and confidence intervals and focusing on patterns enabling readers to draw their own conclusions. (Page 15, 16)	Pages 12, 23, 15, 16
R.4.5. Minor Issues. Page 7 lines 16-19, Please specify what methods were used to select the 70 metabolites.	We agree that this should be clarified and have amended the text to carefully describe how the subset of metabolites were chosen. We have amended the text in the methods to read: "We eliminated the 5 ratio measures for each of the 14 lipoprotein subclass particles. In addition, the 7 other measures within each of the lipoproteins (esterified cholesterol, free cholesterol, total cholesterol, triglycerides, phospholipids, total lipids and particle concentration) are all highly correlated and therefore we only reported total lipids for each of the lipoprotein subclass particles."	Page 9
R.4.6. Please explain why Glycerol has roughly half the	Small quantities of ethanol can sometimes be introduced in the sample either from disinfectants	Supplementary table 1

Editor/Reviewer Comments	Author's Response	Reference page
sample size of the other factors.	used in the blood collection process or during the sample storage or preparation procedures. For samples where ethanol is detected, glycerol and sometimes b-hydroxybutyrate cannot be quantified. We have put a foot note on supplementary table 1 to provide the reader with this information. The text reads: "Note: The presence of ethanol in the sample can affect quantification of glycerol and on some occasions 3hydroxybutyrate. Ethanol can be introduced in to a sample from disinfectants used during blood collection/processing of sample."	
R.4.7. Page 12 line 21 please use lower case for "correlation coefficient".	Thank you, we have modified the text in the results section	Page 15

References

1. Rueedi R, Ledda M, Nicholls AW, et al. Genome-Wide Association Study of Metabolic Traits Reveals Novel Gene-Metabolite-Disease Links. PLOS Genetics 2014;10(2):e1004132. doi: 10.1371/journal.pgen.1004132

2. Kettunen J, Demirkan A, Würtz P, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nature Communications 2016;7:11122. doi: 10.1038/ncomms11122

https://www.nature.com/articles/ncomms11122#supplementary-information

3. Clifford SA, Davies S, Wake M. Child Health CheckPoint: Cohort summary and methodology of a physical health and biospecimen module for the Longitudinal Study of Australian Children. Submitted to BMJ Open October 2017.

4. Wasserstein RL, Lazar NA. The ASA's Statement on p-Values: Context, Process, and Purpose. The American Statistician 2016;70(2):129-33. doi: 10.1080/00031305.2016.1154108

5. Five ways to fix statistics. Nature 2017;551(7682):557-59. doi: doi: 10.1038/d41586-017-07522-z

6. McShane BB, Gal D. Statistical Significance and the Dichotomization of Evidence. Journal of the American Statistical Association 2017;112(519):885-95. doi: 10.1080/01621459.2017.1289846

7. Elliott P, on behalf of UKB, Peakman TC, et al. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International Journal of Epidemiology 2008;37(2):234-44. doi: 10.1093/ije/dym276

VERSION 2 – REVIEW

REVIEWER	Raphaële Castagné EQUITY research team - Inserm Unit of Epidemiology and Public Health Faculté de Médecine, Université Paul Sabatier 37 Allées Jules Guesde Toulouse 31000 France
REVIEW RETURNED	06-Aug-2018

GENERAL COMMENTS	The authors have done a very nice job of putting together this study where strengths of the work include the good sample size, the study design and quality of the data. While I thank the authors in amending the paper to take into account most of the reviewers comments the results in the way they are presented and discussed are somewhat in contradiction with the “descriptive nature” claimed by the authors in their response. Even if the aim is to be descriptive, it’s probably more relevant to focus on adjusted differences and to be more precise in the results description, and avoid phrasing such as ‘most’, ‘values were similar’, ‘majority of’etc... I am still not fully convinced by the rationale given to take forward only 74 out of the 228 metabolites measured. Redundant metabolites could also be used in a sensitivity analyses. Sentences such as “sex-specific metabolic profiles in children and adults”, “Distinct age- and sex-specific profiles were observed” should be avoid in a ‘description’. Such language appears to me to be much more certain than is warranted by the results. Additionally “Differences in metabolite levels by age (adults compared to children)” is used as a title sub-section: are the authors suggesting that parents and children differs only by their age and nothing else ? I understand that the point of the paper is not to focus on the role of each of the confounders explaining the relationship between children and parents metabolic differences. In that case why is the disadvantage index included to estimate the partial correlation ? The authors may need to consider the residuals concentration of each metabolites after controlling for the main confounders and look at differences/correlation on those residuals. I believe the authors should be much more cautious in their interpretation as this is a descriptive job, and due to the unadjusted nature of their results they should make a point on the potential confounders able to explain the observed differences in the discussion to allow the “readers to draw their own conclusions”. To conclude, the authors should be clearer about the descriptive or analytical approach they want to develop in the paper and correct the paper accordingly.

REVIEWER	Joanne Sordillo Harvard Medical School
REVIEW RETURNED	03-Aug-2018

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.

REVIEWER	Diana L. Santos Ferreira University of Bristol, UK
REVIEW RETURNED	23-Jul-2018

GENERAL COMMENTS	I thank the authors for their replies. I am happy with the current manuscript. I would suggest, however, keeping BMI in Table 2 as it is important information to enable readers to draw their own conclusions. Thank you for the opportunity to read this manuscript.

REVIEWER	Andrew Vincent University of Adelaide
REVIEW RETURNED	25-Jul-2018

GENERAL COMMENTS	Statistical review of “Metabolomics: Population epidemiology and concordance in 11-12 year old Australians and their parents”. This is a very nicely written manuscript presenting a lot of data. The author’s responses and adaptations are appropriate. I have one final very minor issue regarding the wording of their first conclusion. The first sentence of the discussion concludes with “… many metabolite measures have high parent-child concordance.” I believe that the authors are referring to Figure 4, in which the majority of the correlations are between 0.2-0.3. Indeed there is high agreement in the level of concordance across metabolites, but the levels themselves are at best moderate. Please reword this sentence to avoid confusion.

VERSION 2 – AUTHOR RESPONSE

Editor/Reviewer Comments	Author’s Response	Reference page
Reviewer 1 : Raphaële Castagné, EQUITY research team - Inserm Unit of Epidemiology and Public Health, Faculté de Médecine, Université Paul Sabatier, Toulouse, France		

Editor/Reviewer Comments	Author's Response	Reference page
R.1.17. The authors have done a very nice job of putting together this study where strengths of the work include the good sample size, the study design and quality of the data. While I thank the authors in amending the paper to take into account most of the reviewers comments the results in the way they are presented and discussed are somewhat in contradiction with the "descriptive nature" claimed by the authors in their response. Even if the aim is to be descriptive, it's probably more relevant to focus on adjusted differences and to be more precise in the results description, and avoid phrasing such as 'most', 'values were similar', 'majority of' etc...	We thank the reviewer for acknowledging our attempts to amend the paper in order to take in to account the majority of reviewer's comments. In reference to the last sentence, we think focusing on overall patterns and using terms such as "most" "majority" is reasonable, as we are highlighting general patterns of the metabolomic profile rather than drilling down on specific metabolites. This is in keeping with the aims/scope of the series of papers to which this belongs. We note that the other reviewers were generally happy with the changes/updates that had been made to the manuscript; however we have addressed several of the reviewers concerns by amending some text/language in the manuscript to better suit the descriptive nature of the paper (see R.1.3 and R.1.6).	See R.1.3 and R.1.6

Editor/Reviewer Comments	Author's Response	Reference page
R.1.18. I am still not fully convinced by the rationale given to take forward only 74 out of the 228 metabolites measured. Redundant metabolites could also be used in a sensitivity analyses.	Restriction of metabolites to a more manageable number including excluding some that have had their values derived (rather than directly quantified) is a common approach in the literature.[1-4] Our overall goal was to simplify this dense and potentially complex data set such that it was comprehensible to the non-expert reader, without sacrificing key scientific content. As metabolomics is an increasingly important approach in clinical medicine, we feel that the accessibility of the general concepts and appreciation of overall patterns is important in this largely descriptive analysis. We must also consider that other reviewers had no further comments/queries in regards to the rationale for inclusion of metabolites. We do not quite understand the suggestion of including redundant metabolites in sensitivity analyses. In general, sensitivity analyses are undertaken to check the robustness of results/findings; in particular to check for consistency in results when using alternative assumptions or analysis strategies – for example, to check robustness of results obtained from an analysis to possible biases and/or missing data.[5, 6] Therefore, we are not clear how inclusion of the remaining metabolites in the manuscript would be applicable in this context.	No change to manuscript
R.1.19. Sentences such as "sex-specific metabolic profiles in children and adults", "Distinct age- and sex-specific profiles were observed" should be avoid in a 'description'. Such language appears to me to be much more certain than is warranted by the results.	We thank the reviewer for the suggestion and have omitted reference to "sex-specific" and "age-specific" in the text of the manuscript where appropriate; rather refining language used to be more descriptive. E.g. "We identified differences in…..by sex"	Page 2, Line 26/35 Page 12, Line 13 Page 15, Line 11/25 Page 16, Line 7/27 Page 17, Line 19

Editor/Reviewer Comments	Author's Response	Reference page
R.1.20. Additionally "Differences in metabolite levels by age (adults compared to children)" is used as a title sub-section: are the authors suggesting that parents and children differs only by their age and nothing else?	We have changed the title of the subsection from "Differences in metabolite levels by age (adults compared to children)" to "Differences in metabolite levels – adults compared to children".	Page 12, Line 4
R.1.21. I understand that the point of the paper is not to focus on the role of each of the confounders explaining the relationship between children and parents metabolic differences. In that case why is the disadvantage index included to estimate the partial correlation? The authors may need to consider the residuals concentration of each metabolites after controlling for the main confounders and look at the differences/ correlation on those residuals.	We thank the reviewer for these suggestions. However, we do not feel that additional adjustments are warranted, given the descriptive aims of the paper and that the methods were chosen to be consistent with the other papers from the same cohort in this BMJ Open series. We agree that further analyses exploring metabolomic associations (with further adjustment) is also of interest but the intention of the paper is primarily to describe the metabolomic measures available for Child Health CheckPoint. Analyses and manuscripts are in progress exploring many of the suggested additional analyses, but are beyond the scope of this paper.	No change to manuscript

Editor/Reviewer Comments	Author's Response	Reference page
R.1.22. I believe the authors should be much more cautious in their interpretation as this is a descriptive job, and due to the unadjusted nature of their results they should make a point on the potential confounders able to explain the observed differences in the discussion to allow the "readers to draw their own conclusions". To conclude, the authors should be clearer about the descriptive or analytical approach they want to develop in the paper and correct the paper accordingly.	We have added text to the discussion to clarify that potential confounders may possibly explain the observed differences in the paper – and included this as a limitation. E.g. "In addition, given the descriptive aims of the paper, additional factors and potential confounders not considered could explain some of the results observed." We have attempted to address many of the reviewers concerns and tone down some of the language used in the text to better suit the descriptive aims of the paper (also see R.1.3, R.1.4).	Page 14, Line 31 Discussion, Page 14-17
Reviewer 2: Joanne Sordillo, Harvard Medical School, USA		
R.2.10. No additional comments.	No action required	No change to manuscript
Reviewer 3 : Diana L. Santos Ferreira, University of Bristol, UK		
R.3.1 I thank the authors for their replies. I am happy with the current manuscript. I would suggest, however, keeping BMI in Table 2 as it is important information to enable readers to draw their own conclusions. Thank you for the opportunity to read this manuscript.	Thank you for the suggestion and feedback – we have put BMI back in Table 2 as the reviewer has suggested in order for readers to draw their own conclusions. [Page 12 , Table 2]	Page 12 , Table 2
Reviewer 4 : Andrew Vincent, University of Adelaide		

Editor/Reviewer Comments	Author's Response	Reference page
R.4.1 This is a very nicely written manuscript presenting a lot of data. The author's responses and adaptations are appropriate. I have one final very minor issue regarding the wording of their first conclusion. The first sentence of the discussion concludes with "… many metabolite measures have high parent-child concordance." I believe that the authors are referring to Figure 4, in which the majority of the correlations are between 0.2-0.3. Indeed there is high agreement in the level of concordance across metabolites, but the levels themselves are at best moderate. Please reword this sentence to avoid confusion.	We agree that this could be clarified. We have changed the first sentence in the discussion to read: "……many metabolite measures have moderate parent-child concordance and in general there is a high level of agreement in the magnitude of concordance across metabolites. '' [Page 14, Line 5]	Page 14, Line 5

REFERENCES

[1] Würtz P, Wang Q, Kangas AJ, Richmond RC, Skarp J, Tiainen M, et al. Metabolic Signatures of Adiposity in Young Adults: Mendelian Randomization Analysis and Effects of Weight Change. PLOS Medicine. 2014;11:e1001765.

[2] Würtz P, Wang Q, Niironen M, Tynkkynen T, Tiainen M, Drenos F, et al. Metabolic signatures of birthweight in 18 288 adolescents and adults. International Journal of Epidemiology. 2016;45:1539-50.

[3] Wang Q, Ferreira DLS, Nelson SM, Sattar N, Ala-Korpela M, Lawlor DA. Metabolic characterization of menopause: cross-sectional and longitudinal evidence. BMC Medicine. 2018;16:17.

[4] Lehtovirta M, Pahkala K, Niinikoski H, Kangas AJ, Soininen P, Lagström H, et al. Effect of Dietary Counseling on a Comprehensive Metabolic Profile from Childhood to Adulthood. The Journal of Pediatrics. 2018;195:190-8.e3.

[5] Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. PLOS Medicine. 2007;4:e297.

[6] Saltelli A, Ratto M, Andres T, Campolongo F, Cariboni J, Gatelli D, et al. Global sensitivity analysis: The primer. West Sussex, UK: John Wiley & Sons; 2008.