nature portfolio

Peer Review File

Prenatal famine exposure restricts genetic effects on birth weight with implications for metabolic disease risk

Corresponding Author: Ms M. Taeubert

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is an interesting extension of the multiple studies this group has carried out on the basis of the Dutch Hunger Winter Study. The authors have done a good work from which the key message is that the severe famine during pregnancy, in particular during mid-to-late pregnancy, as compared to early pregnancy and no exposure suppresses the known genetic influences (probed by a polygenic score) on birth weight. They draw this conclusion by showing that the correlations and regression coefficients of birth weight on genetically predicted birth weight is weaker when the women had been exposed to famine mid-to-late pregnancy.

My main concern here is whether this is really a suppression of the genetic influence or whether it has the expected influences although within a different distribution of birth weights induced by the famine? In other words, one may question to what extent the weakening of these statistics are dependent on the known reduction in average and dispersion of the birth weight, and on the few extreme values seen in the scatterplots. Perhaps, running the correlations by non-parametric tools such as Spearman, may help confirming the differences.

The authors also show that the famine-induced deviation of actual birth weight from the genetically predicted influenced BMI, waist circumference and fasting p-glucose in a different way from he same deviations in controls not exposed to the famine. Before jumping to an interpretation of this intriguing finding of putative effects of the specific environmental exposure, it would here be good to see that the statistics are robust, and to see if the separate relations to genetically predicted and observed birth weight.

It is a pity to put away the results on BMI and waist circumference in the supplement. I suggest you find a way to add them to the main body of the paper (maybe using common tables). Then it would be necessary to deal more explicitly with these results and discuss them together including the discrepancies, what the links between them might be and what mechanisms are driving them.

Obviously, there appears to be a distinct and highly significant reduction of about 4 days of duration of gestation, a difference that does matter for birth weight. The authors have appropriately adjusted for gestational age in their analyses, however, there may be a complex bidirectional relation between fetal growth and timing of delivery, possibly generating a collider bias when just adjusting for gestational age. Maybe worth exploring what the adjustment do to the associations? Are there a relationship between genetically predicted birth weight and gestational age?

Finally, in terms of the general value of the findings, a discussion is needed about the limitations made by the (fortunately) rather seldom extreme exposures experienced during the Dutch Hunger Winter. Does these findings help us understanding more broadly what effects food restrictions may have, and was it just a question of reduced caloric value of food but also effects of specific malnutrition?

Minor comments:

I suggest that the title is softened and allows for the uncertainty about what is behind these data.

You include a description of sibling controls, who however apparently have not been used for anything in this study, so I

suggest them removed to avoid confusion.

It would be helpful if Table 1 also include stats on birth weight and genetically predicted birth weight, re the arguments above.

The graphs showing associations with genetically predicted minus observed birth weight are slightly confusing by having excess birth weight to the left.

It is not clear why you include the results where there is no distinction between early and late exposure to famine (Supplemental figures 3-4).

Reviewer #2

(Remarks to the Author)
Dear editor,

Thank you for the opportunity to review the manuscript “Nurture over nature: prenatal exposure to famine suppresses genetic influences on birth weight with consequences for metabolic health” by Taubert et al.

The authors study individuals exposed to famine during pregnancy form the Dutch Hunger Winter Families Study. They assess how prenatal famine exposure affects expected birth weight estimated by a polygenic index. They further assess how prenatal famine exposure affects fasting glucose in adulthood. The authors report how prenatal famine exposure, especially in middle-to-late pregnancy, can override the expected birth weight. They also report middle-to-late pregnancy famine exposure being linked to higher fasting glucose levels in adulthood.

The study utilizes a unique cohort from severely adverse conditions. The study is novel and uses sound methods. The current study provides a fascinating example of environmental factors modifying a genetics-driven phenotype. The adult implications are both discussed and studied.

The manuscript is well-written, compelling and promising. I suggest both major and minor revisions based on my observations and comments.

MAJOR COMMENTS:

1. 118-125 Why was the group split into early vs. Mid-late pregnancy? It is a bit unclear whether this represents trimesters or first and second half of pregnancy A more granular approach could be interesting as the authors report differences between groups with a different exposure timing.

2. 143 what about other recognised exposures such as maternal age or twin pregnancy? Is there any data available on maternal smoking from the conditions of the cohort? Reference 40 for example states maternal smoking as an important factor and uses PGI. If no data are available, this should be discussed in the limitations section.

3. 144-145 the authors should further elaborate or speculate on the meaning and implications of differences between the exposure timing groups. Throughout the manuscript, the differences between early and later pregnancy famine exposure are brought up.The implications or background of these differences should be expanded on.

4. 196-197 did you consider adjusting with further adult covariates besides age? BMI or other anthropometric measurements? Do you have data on body composition?

MINOR COMMENTS:
5. 52-54: The sentence is somewhat unclear in this context, maybe rephrase? An example of environmental factors being influenced by genetic make-up could clarify the sentence. The references do not themselves easily answer this.

6. 58-60 Reference 6’s results point to epigenetic differences when exposure occurs periconceptually. No differences in methylation were reported with a later exposure. Consider rephrasing the sentence or expand the commentary on this reference.

7. 64-67 Reference 14 is somewhat niche but illustrates an example of a moderation between exposure (breastfeeding) and outcome (IQ) by genetic factors. If the authors can provide a reference more suited to the current manuscript, it could be included here instead of reference 14.

8. 84-85 What is the rationale or reason behind using both related and unrelated controls? Especially when using a polygenic index, it would seem that using a mixed set of controls could pose an issue. Can the authors comment on whether the results differ when using only siblings or unrelated controls?

9. 90-91 In what capacity were the siblings used as controls if no birth weight data were available? No further mention of siblings can be found in the manuscript, so the sibling controls' role is somewhat unclear.

10. 98 does any information on smoking exist?

11. 100 please define "time controls" here instead of later.

12. 114-115 is this definition of famine widely used or the authors' own definition? If used elsewhere, please include a reference.

13. 125 what is the role of the 51 individuals conceived shortly after famine exposure? How were they includeed in the analysis?

14. 259-260: reference 28 does not exactly describe metabolic outcomes. I recommend rephrasing.

15. 308-310 sentence somewhat self-contradictory, please rephrase

16. 311 ref 29 is interesting and well-suited here. Can the authors provide other examples of factors moderating genetically driven birth weight trajectories?

17. 322 unclear what is referenced here, please rephrase.

18. 325-327 DNA methylation as a mechanism could be expanded, discussed further or omitted. As it is now, it is unclear as to how it links to the current study's main point.

GENERAL COMMENTS:

19. reference 4 is very interesting and noteworthy. Thank you for including this as a reference.

20. The discussion is written very clearly and is both interesting and easy to read.

21. Unfortunately I do not have access to all the references (7,8,15,17).

22. 288: I'm not entirely convinced that using the phrase "adult metabolic health" is justified when the only measured outcome is fasting glucose concentration.

23. 328 the authors use the phrase "disease risk later in life". Could this be used elsewhere in the manuscript to replace "adult metabolic health"?

24. Why were no birth records available for sibling controls?

TABLES AND FIGURES

25. Table 1: please convert GA to weeks instead of days

26. Table 1: I am also interested in seeing data on how many were born preterm or low birth weight (or very preterm / very low birth weight). Also, were any participants born SGA? Could they be outliers in the data?

27. Figure 1: I am confused as to why sibling controls and non-related time controls were both included as controls, and how they were treated in the analyses. Also, what is the role of the post-famine conception group? Where do they fall in the analysis? Please clarify these groups, or if they are not part of this, consider modifying the figure and manuscript for clarity and brevity.

28. Figure 2A is very illustrative and sums up the population nicely. The later figures are demonstrative as well.

SUPPLEMENTS

29. 25-28 Do the authors have any data on macronutrients? Protein deficiency?

30. 46-47: what data were gathered in the structured interview? Was this as a general description of the cohort or something pertinent to this current study?

Reviewer #3

(Remarks to the Author)
This is a very well written paper that delivers on its objectives. It is clear and concise. The data source is perfect for the question being asked. The statistical analytic methods are well described and suitable for these data. I have a few minor concerns.

1) The use of sibling controls is described on line 84-85 (page 4) and later in the paragraph it is noted that birthweight data were not available for the sibling controls. Then in what way did these controls contribute to the analysis? The authors should clarify where the sibling controls contributed. If they were not used in this paper, I would recommend dropping mention of them so as not to confuse the reader.

2) I'm not in agreement with the authors about their conclusions in lines 237-240:
"The birth weight PGI was not different in individuals exposed in early gestation or those exposed in mid-to-late gestation as compared to controls (for early gestation 238 β=-0.18 SD, 95% CI: -0.40–0.04; p=0.10; for mid-to-late gestation β=-0.08 SD, 95% CI: -0.28–0.11; p=0.39)."
The p-value for the early gestation is 0.10 (borderline significant) and the beta is -0.18 in contrast to a -value of 0.39 and beta of -0.08 for mid-to late gestation exposure. These could be interpreted as differing from one another.

3) The Dutch Famine Study created an incredibly unique data set that is continuing to answer important questions about what drives birthweight and fetal growth, among other outcomes. However, there are limitations that should be discussed further in this manuscript and are often invoked in papers using these data.
a) This was a well nourished population prior to the onset of the famine conditions. This both makes the data set highly valuable but also constrains its generalizability. Famine is rarely suffered by such populations and may act very differently in a population experiencing famine in the modern day, such as African countries or even Gaza.
b) Similarly, this is not a diverse population that includes African or Hispanic individuals. Given the different life time and prenatal stressors for those families, these results also might not be generalizable.
c) Younger readers may be unfamiliar with the events that led to the famine. While certainly the reader can look further, I would recommend that the paper conclude (or state in the background) a sentence or two that underscores the tremendous human rights violations that produced these data. Epidemiologists sometimes forget the human cost of what we are analyzing. While we did not cause the conditions that gave rise to these data, we should acknowledge those human costs.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I find the authors response to the critique adequate and have no further comments.

Reviewer #2

(Remarks to the Author)
Dear editor,

Thank you for the opportunity to review the revised version of the manuscript now titled “Prenatal famine exposure restricts genetic effects on birth weight with implications for metabolic disease risk”.

I have carefully read the authors’ response to my previous comments and examined the revised manuscript. I am satisfied that the authors have adequately addressed my concerns, and I find the revisions to be clear and appropriate. In my opinion, the manuscript is now suitable for publication, and I recommend acceptance.

Thank you for the opportunity to review this work.

Reviewer #3

(Remarks to the Author)
Thank you for careful consideration of my review as well as those of the other reviewers.

Response to reviewers

Reviewer 1

This is an interesting extension of the multiple studies this group has carried out on the basis of the Dutch Hunger Winter Study. The authors have done a good work from which the key message is that the severe famine during pregnancy, in particular during mid-to-late pregnancy, as compared to early pregnancy and no exposure suppresses the known genetic influences (probed by a polygenic score) on birth weight. They draw this conclusion by showing that the correlations and regression coefficients of birth weight on genetically predicted birth weight is weaker when the women had been exposed to famine mid-to-late pregnancy.

RESPONSE: We thank the reviewer for carefully reading our manuscript. Our specific responses to the comments are given below.

Comment 1

a) My main concern here is whether this is really a suppression of the genetic influence or whether it has the expected influences although within a different distribution of birth weights induced by the famine? In other words, one may question to what extent the weakening of these statistics are dependent on the known reduction in average and dispersion of the birth weight, and on the few extreme values seen in the scatterplots. Perhaps, running the correlations by non-parametric tools such as Spearmanc, may help confirming the differences.

RESPONSE: We appreciate the reviewer's suggestion. We have now repeated all correlation analyses using Spearman correlation and confirmed the patterns observed with Pearson correlation, indicating that the results are robust to non-parametric methods and not driven by extreme values. For your convenience we have included here a table directly comparing Pearson and Spearman coefficients. Given the greater robustness of the Spearman approach, we have replaced the previously reported Pearson correlations with Spearman correlations throughout the manuscript.

Comparison of Pearson and Spearman coefficients.

	Spearman correlation		**Pearson correlation**	
	Correlation coefficient	**p value**	**Correlation coefficient**	**p value**
Birth weight and birth weight PGI correlations.				
Controls	0.38	5.5×10^{-7}	0.44	4.8×10^{-9}
Early gestational exposure	0.15	0.07	0.16	0.05
Mid-to-late gestational exposure	0.10	0.09	0.09	0.12
Deviation from genetically predicted birth weight and adult fasting glucose.				
Controls	-0.03	0.72	-0.05	0.54
Early gestational exposure	0.05	0.52	-0.002	0.98
Mid-to-late gestational exposure	0.15	0.01	0.17	0.003
Deviation from genetically predicted birth weight and adult waist circumference.				
Controls	-0.26	9.4×10^{-4}	-0.20	0.01
Early gestational exposure	-0.05	0.51	-0.04	0.60

Mid-to-late gestational exposure	0.08	0.16	0.08	0.18
Deviation from genetically predicted birth weight and adult BMI.				
Controls	-0.20	$9.5x10^{-3}$	-0.20	0.01
Early gestational exposure	-0.08	0.33	-0.06	0.46
Mid-to-late gestational exposure	0.10	0.10	0.09	0.14

b) The authors also show that the famine-induced deviation of actual birth weight from the genetically predicted influenced BMI, waist circumference and fasting p-glucose in a different way from the same deviations in controls not exposed to the famine. Before jumping to an interpretation of this intriguing finding of putative effects of the specific environmental exposure, it would here be good to see that the statistics are robust, and to see if the separate relations to genetically predicted and observed birth weight.

RESPONSE: We have added supplementary tables reporting the separate associations of adult outcomes with observed birth weight (**Supplementary Table 6**) and genetically predicted birth weight (**Supplementary Table 7**). We refer to these results where appropriate:

"Lastly, the individual associations between birth weight and the birth weight PGI and these adult outcomes are provided in the **Supplementary Material** (**Supplementary Tables 6 and 7**)." (line 392 to 394)

Supplementary Table 6: Association between birth weight and adult outcomes.

	Effect estimate (95% CI) [SD]*	**p value**
Fasting glucose		
Controls	-0.10 (-0.25, 0.06)	0.22
Early gestational exposure	0.02 (-0.17, 0.20)	0.85
Mid-to-late gestational exposure	-0.27 (-0.42, -0.12)	$6.2x10^{-4}$
Waist circumference		
Controls	0.12 (-0.05, 0.29)	0.16
Early gestational exposure	0.03 (-0.13, 0.18)	0.75
Mid-to-late gestational exposure	-0.01 (-0.16, 0.14)	0.88
BMI		
Controls	0.12 (-0.05, 0.29)	0.18
Early gestational exposure	0.05 (-0.12, 0.23)	0.54
Mid-to-late gestational exposure	-0.11 (-0.27, 0.04)	0.15

*Effect estimates and 95% confidence intervals (CI) for the multivariable linear regression analysis of birth weight and adult outcomes (fasting glucose, waist circumference, and BMI) are reported in standard-deviation (SD) scores of the adult outcomes. The linear regression analyses were adjusted for sex, first-born status, gestational age, and adult age, and are presented separately for individuals exposed in early (n=145) and mid-to-late (n=283) gestation and controls (n=161). Fasting glucose and BMI were log-transformed prior to the calculation of the Z scores.

Supplementary Table 7: Association between birth weight polygenic index (PGI) and adult outcomes.

	Effect estimate (95% CI) [SD]*	**p value**
Fasting glucose		
Controls	-0.11(-0.26, 0.03)	0.11
Early gestational exposure	0.00 (-0.18, 0.19)	0.98
Mid-to-late gestational exposure	0.05 (-0.07, 0.18)	0.41
Waist circumference		
Controls	-0.06 (-0.22, 0.10)	0.48
Early gestational exposure	0.01 (-0.14, 0.14)	0.92
Mid-to-late gestational exposure	0.09 (-0.03, 0.21)	0.15
BMI		
Controls	-0.07 (-0.23, 0.09)	0.37
Early gestational exposure	-0.01 (-0.18, 0.16)	0.92
Mid-to-late gestational exposure	0.04 (-0.08, 0.17)	0.52

*Effect estimates and 95% confidence intervals (CI) for the multivariable linear regression analysis of the birth weight polygenic index (PGI) and adult outcomes (fasting glucose, waist circumference, and BMI) are reported in standard-deviation (SD) scores of the adult outcomes. The linear regression analyses were adjusted for sex, first-born status, gestational age, and adult age, and are presented separately for individuals exposed in early (n=145) and mid-to-late (n=283) gestation and controls (n=161). Fasting glucose and BMI were log-transformed prior to the calculation of the Z scores.

Comment 2

It is a pity to put away the results on BMI and waist circumference in the supplement. I suggest you find a way to add them to the main body of the paper (maybe using common tables). Then it would be necessary to deal more explicitly with these results and discuss them together including the discrepancies, what the links between them might be and what mechanisms are driving them.

RESPONSE: We appreciate the suggestion. We have moved the findings on waist circumference to the main manuscript and discuss them in more detail. We keep BMI in the **Supplementary Material** as the results are virtually the same.

Introduction section:

"Finally, we examined whether deviations from genetically predicted birth weight in famine-exposed individuals have implications for metabolic risk factors in later life, as expressed by fasting glucose and waist circumference." (line 99 to 101)

Results section:

"Similarly, the effect of deviations from genetic birth weight on adult waist circumference was different for mid-to-late gestation exposure to famine as compared with controls ($p_{interaction}$=0.01), whereas no difference was observed for early gestation exposure compared to controls ($p_{interaction}$=0.10) (**Figure 5**). Among controls, being born lighter than genetically predicted was associated with a lower waist circumference, with a 1 SD lower birth weight corresponding to a 0.20 SD decrease in waist circumference (95% CI: -0.39 – -0.004; p=0.048). This effect was absent in famine-exposed individuals, and in those exposed during mid-to-late

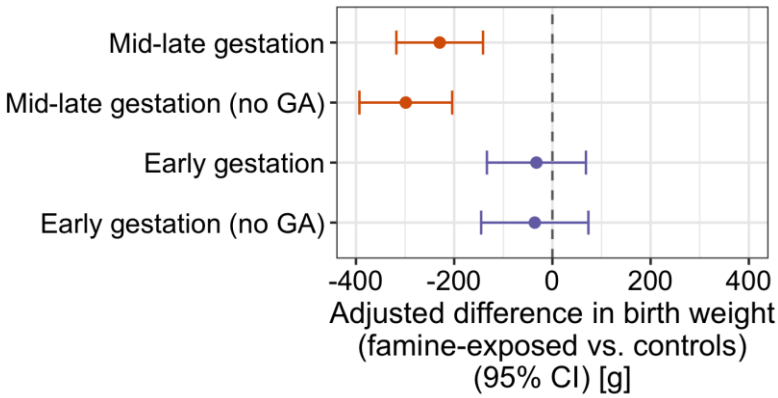
gestation, the effect size shifted in the opposite direction (p>0.05). Findings for BMI were comparable to those for waist circumference (**Supplementary Table 5; Supplementary Figure 5**)." (line 379 to 388)

Discussion section:
"In contrast, among controls, a lower than genetically predicted birth weight was associated with a lower waist circumference, suggesting a potentially protective effect. This pattern was absent in individuals exposed to early gestation and reversed among those exposed in mid-to-late gestation." (line 437 to 440)

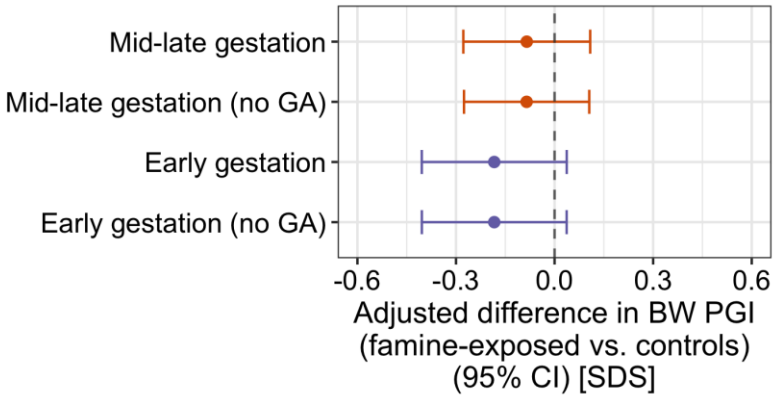
Comment 3
Obviously, there appears to be a distinct and highly significant reduction of about 4 days of duration of gestation, a difference that does matter for birth weight. The authors have appropriately adjusted for gestational age in their analyses, however, there may be a complex bidirectional relation between fetal growth and timing of delivery, possibly generating a collider bias when just adjusting for gestational age. Maybe worth exploring what the adjustment do to the associations? Is there a relationship between genetically predicted birth weight and gestational age?

RESPONSE: We have now performed additional sensitivity analyses to evaluate the impact of adjusting for gestational age on the association between famine exposure and birth weight (both observed birth weight and the birth weight polygenic index). These analyses showed no meaningful differences in the associations, suggesting that collider bias is not driving our results (see figures below).

In addition, we examined the univariable relationship between the birth weight polygenic index and gestational age and found no association (controls b=0.88; early gestation b=0.63; mid-to-late gestation b= -0.56 (p>0.28 for all). Effect estimates are given in days per SD change in the PGI).



Effect of gestational age adjustment on the association between prenatal famine exposure and observed birth weight. Shown are the adjusted differences in birth weight between famine-exposed (mid-to-late and early gestation) and control groups using multivariable linear regression models. The main models are adjusted for sex, firstborn status, and gestational age. For comparison purposes models were also run without adjustment for gestational age (no GA). Effect estimates and 95% confidence intervals are depicted for each model and are reported in grams.



Effect of gestational age adjustment on the association between prenatal famine exposure and birth weight polygenic index. Shown are the adjusted differences in birth weight PGI between famine-exposed (mid-to-late and early gestation) and control groups using multivariable linear regression models. The main models are adjusted for sex, firstborn status, and gestational age. For comparison purposes models were also run without adjustment for gestational age (no GA). Effect estimates and 95% confidence intervals are depicted for each model and are reported in standard-deviation (SD) units of the birth weight PGI.

Comment 4

Finally, in terms of the general value of the findings, a discussion is needed about the limitations made by the (fortunately) rather seldom extreme exposures experienced during the Dutch Hunger Winter. Do these findings help us understanding more broadly what effects food restrictions may have, and was it just a question of reduced caloric value of food but also effects of specific malnutrition?

RESPONSE: Thank you for bringing up this point. We have revised our *discussion* accordingly:

"Finally, the generalisability of our findings warrants consideration. The Dutch Hunger Winter affected a previously well-nourished population of predominantly European ancestry, and the famine was characterised mainly by severe caloric restriction with relatively stable macronutrient composition (5,17). As such, the observed effects are more likely driven by undernutrition rather than specific nutrient deficiencies. However, evidence from more recent analyses suggests that reductions in protein intake during the most extreme phases of the famine, may have contributed to long-term health outcomes (46). These historical and population-specific circumstances may limit direct extrapolation to other famine settings, particularly those involving prolonged malnutrition, different nutritional profiles, or populations with different genetic backgrounds. Nonetheless, our findings may still inform broader understanding of how severe caloric restriction during pregnancy can influence foetal growth, its association with genetics and later-life metabolic risk." (line 469 to 480)

Minor comments:

Comment 5

I suggest that the title is softened and allows for the uncertainty about what is behind these data.

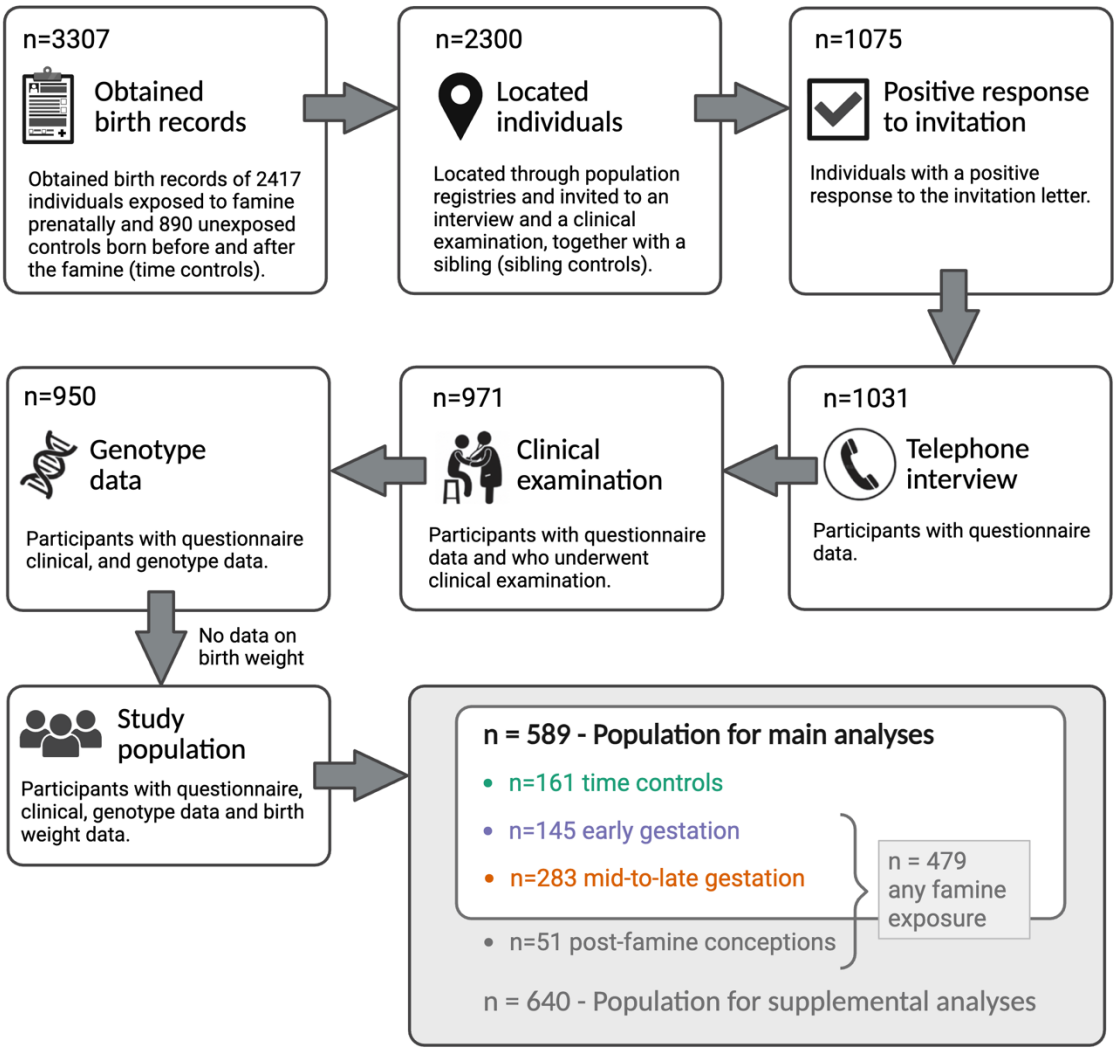
RESPONSE: We have changed the title to "Prenatal famine exposure restricts genetic effects on birth weight with implications for metabolic disease risk" to make it more appropriate in the context of our findings.

Comment 6

You include a description of sibling controls, who however apparently have not been used for anything in this study, so I suggest them removed to avoid confusion.

Response: For clarity we have removed mention of the sibling controls from the *methods* section. We have additionally updated the population flow chart to provide a more comprehensive overview of the process leading to the final study population and have moved it to the **Supplementary Material** (**Supplementary Figure 1**).

"Participants were interviewed, participated in a clinic exam, and DNA was extracted from blood samples and sent for genotyping (13,18). The analysis sample for this study was formed from participants for whom birth weight and genetic data were available, including 161 time controls, 145 individuals exposed to famine in early gestation, and 283 individuals exposed in mid-to-late gestation (n=589). Additional details on the recruitment process and study population are found in **Supplementary Figure 1**." (line 118 to 123)



Supplementary Figure 1. Flow chart of the study population. Historical birth records were retrieved from three institutions in famine-exposed cities, including all singleton births between 1 February 1945 and 31 March 1946 and a systematic sample of births from 1943 and 1947. From these records, we identified 3,307 individuals: 2,417 whose mothers were exposed to famine during or immediately

preceding pregnancy and 890 unexposed time controls born before or after the famine. Of these 3,307 individuals, 2,300 were traced through population registries and invited to participate in a telephone interview and a clinical examination, along with a same-sex sibling not exposed to famine (sibling controls). In total, 1,075 individuals responded positively to the invitation. Between 2003 and 2005, 1,031 interviews and 971 clinical examinations were conducted. DNA extracted from blood samples was available for 950 individuals. Birth weight data were unavailable for all sibling controls (n = 310) because they were not recruited through clinics that maintained obstetric records; these individuals were therefore excluded from the present analysis. The analysis sample comprised participants recruited via obstetric records with complete birth weight and genetic data. The main analysis population included 161 time controls, 145 individuals exposed to famine in early gestation, and 283 individuals exposed in mid-to-late gestation. The supplementary analysis additionally included 51 individuals conceived shortly after the end of the famine, incorporated into the broader "any famine exposure" group that combined all famine-exposed categories.

Comment 7

It would be helpful if Table 1 also include stats on birth weight and genetically predicted birth weight, re the arguments above.

RESPONSE: We have added a supplementary table (**Supplementary Table 3**) reporting the mean and standard deviation of both observed birth weight and the birth weight polygenic index for each group.

Supplementary Table 3. Birth weight and birth weight polygenic score (PGI) distribution among controls, and famine-exposed in early and mid-to-late gestation.

	Controls ***(n = 161)***	**Early gestational exposure** ***(n = 145)***	**Mid-to-late gestational exposure** ***(n = 283)***	**p value**
Birth weight [g], mean ± SD	3498 ± 486	3472 ± 580	3233 ± 459	9.7×10^{-9}
Birth weight PGI [SD], mean ± SD	0.086 ± 0.92	-0.085 ± 0.95	0.001 ± 1.01	0.31

Values are shown as the mean ± standard deviation for famine-exposed (split into exposure in early gestation and mid-to-late gestation) and controls of the study population. Comparing the three categories by ANOVA (two-sided).

Comment 8

The graphs showing associations with genetically predicted minus observed birth weight are slightly confusing by having excess birth weight to the left.

RESPONSE: We considered different approaches to present the association between deviations from genetically predicted birth weight and the adult outcomes when writing the manuscript. We chose to plot genetically predicted minus observed birth weight (resulting in excess birth weight appearing on the left) because this direction makes the association with glucose levels more intuitive: a birth weight lower than genetically predicted corresponds to higher glucose levels. Reversing the direction (observed minus genetically predicted birth weight) inverts the association, such that a birth weight higher than genetically predicted would appear as lower risk. We believe the chosen orientation provides a clearer and more intuitive interpretation of the results.

Comment 9

It is not clear why you include the results where there is no distinction between early and late exposure to famine (Supplemental figures 3-4).

RESPONSE: We have now clarified our reasoning for including the results for the broader "any" famine exposure category:

"Results for the any famine exposure group, which combines the different exposure groups to increase sample size and assess timing-independent effects, can be found in the **Supplementary Material** (**Supplementary Figure 9 and 10**)." (line 395 to 397)

Reviewer 2

Dear editor,
Thank you for the opportunity to review the manuscript "Nurture over nature: prenatal exposure to famine suppresses genetic influences on birth weight with consequences for metabolic health" by Taubert et al.

The authors study individuals exposed to famine during pregnancy form the Dutch Hunger Winter Families Study. They assess how prenatal famine exposure affects expected birth weight estimated by a polygenic index. They further assess how prenatal famine exposure affects fasting glucose in adulthood. The authors report how prenatal famine exposure, especially in middle-to-late pregnancy, can override the expected birth weight. They also report middle-to-late pregnancy famine exposure being linked to higher fasting glucose levels in adulthood.

The study utilizes a unique cohort from severely adverse conditions. The study is novel and uses sound methods. The current study provides a fascinating example of environmental factors modifying a genetics-driven phenotype. The adult implications are both discussed and studied.

The manuscript is well-written, compelling and promising. I suggest both major and minor revisions based on my observations and comments.

Response: We thank the reviewer for the compliments and for carefully reading our manuscript. We give our specific responses to the comments below.

MAJOR COMMENTS:

Comment 1
118-125 Why was the group split into early vs. Mid-late pregnancy? It is a bit unclear whether this represents trimesters or first and second half of pregnancy A more granular approach could be interesting as the authors report differences between groups with a different exposure timing.

RESPONSE: We have now clarified our reasoning for splitting the group into exposed in early and mid-to-late gestation in the *methods* section:

"The effect of prenatal famine exposure on birth weight is largely confined to individuals exposed later in pregnancy and those exposed periconceptionally show little to no effect (7). We therefore defined exposure based on timing of conception: individuals conceived during the famine (maternal last menstrual period date between 26 November 1944 and 12 May 1945) were classified as exposed in early gestation, while those conceived before the famine (maternal last menstrual period date between 20 April and 25 November 1944) were classified as exposed in mid-to-late gestation **(Figure 1A**). This approach allows us to distinguish exposure groups with well-established differences in birth weight outcomes, while maximizing the number of participants within each group." (line 152 to 160)

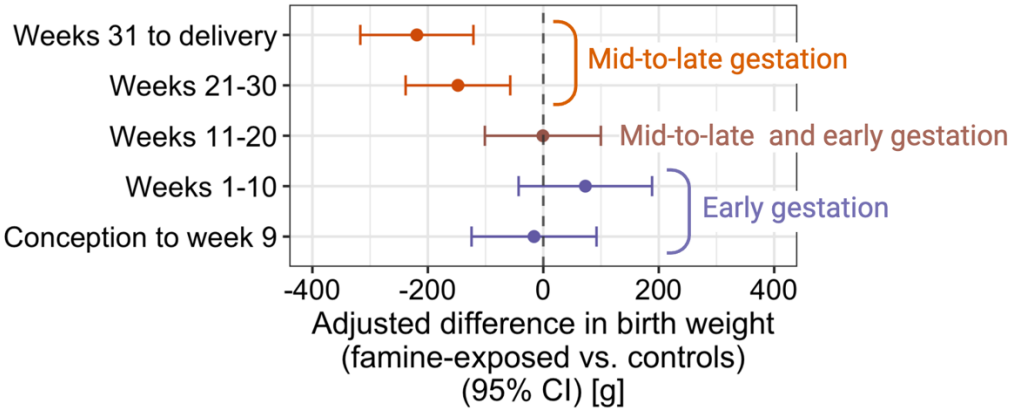
As a sensitivity analysis, we performed the analysis using finer 10-week gestational exposure windows that have previously been used in this cohort (Cheng et al. 2024; Taeubert et al. 2024; Zhou et al. 2024). These analyses confirm the same overall pattern: lower birth weight among individuals exposed later in gestation (weeks 21–30 and weeks 31 to delivery), and no effect among those exposed earlier in gestation (conception to week 9, weeks 1–10). Individuals exposed to during weeks 11-20 of gestation are a mixture between the early and mid-late

exposure groups and similarly do not show an association with birth weight. The exact exposure definitions of these 10-weeks exposure groups can be found in the *methods* section (lines 164 to 176) and the distribution of individuals classified as exposed in early and mid-to-late gestation across the 10-week exposure windows is shown in **Supplementary Table 1.**

We have included these results in the **Supplementary Material** (**Supplementary Table 1** and **Supplementary Figure 4**). The manuscript has been modified where appropriate in the *methods* and *results* sections and **Figure1A** has been updated to show the 10-week gestational windows across the exposure and control groups.

"To obtain more fine-grained effects of exposure timing during gestation, the association between prenatal famine and birth weight was examined using 10-week gestational exposure windows that have previously been used in this cohort (13,19,22). In the regression analysis, the two indicators of famine exposure were replaced with indicator variables identifying exposure within each of the gestational time windows." (line 240 to 244)

"Finally, we repeated this analysis using more finely defined 10-week gestational exposure windows previously applied in this cohort (13,19,22), which confirmed the same overall pattern of associations (**Supplementary Figure 4**)." (line 319 to 321)



Supplementary Figure 4: Association between 10-week prenatal famine exposure groups and observed birth weight. The association between 10-week prenatal famine exposure groups and observed birth weight was assessed using multivariable linear regression models. Shown are the adjusted differences in birth weight between famine-exposed (exposed between conception to week 9 of gestation, as well as exposed in gestation weeks 1-10, 11-20, 21-30, and 31 up until delivery) and control groups, accounting for sex, firstborn status and gestational age. Effect estimates and 95% confidence intervals are depicted for each model and are reported in grams.

Supplementary Table 1. Distribution of individuals classified as exposed in early and mid-to-late gestation across the 10-week exposure windows.

10-week categories	**Early gestational exposure *(n =145)***	**Mid-to-late gestational exposure *(n = 283)***
Conception to week 9	94	0
Weeks 1-10	73	0
Weeks 11-20	24	100
Weeks 21-30	0	144

Weeks 31 to delivery	0	133

Note: participants can belong to up to two of the 10-week gestational exposure windows. Therefore, the total of the 10-week categories add up to more than the total of the early and mid-to-late gestational exposure categories.

Comment 2

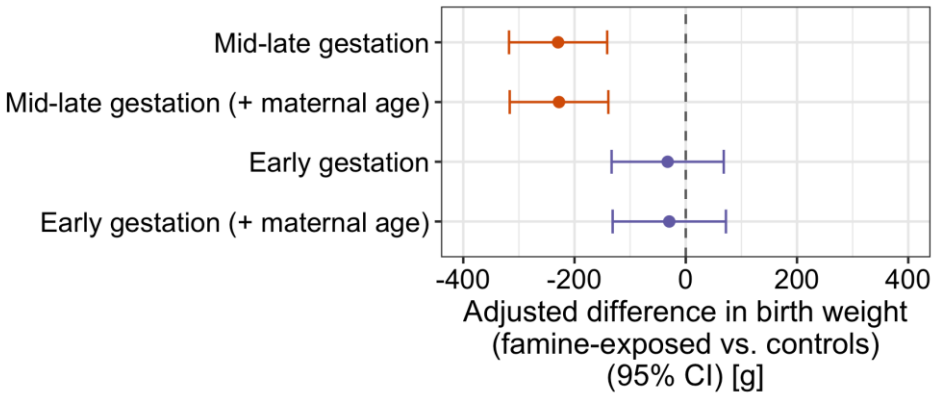
143 what about other recognised exposures such as maternal age or twin pregnancy? Is there any data available on maternal smoking from the conditions of the cohort? Reference 40 for example states maternal smoking as an important factor and uses PGI. If no data are available, this should be discussed in the limitations section.

RESPONSE: Only singleton births were included in the cohort, and therefore twin pregnancies were not relevant for the present analyses. Information on maternal age at delivery was available, and we have now performed an additional sensitivity analysis including maternal age as a covariate in the models assessing the association between prenatal famine exposure and birth weight. The results were unchanged, and we now report this analysis in the **Supplementary Material** (**Supplementary Figure 2**) and refer to the results in the manuscript where appropriate.

With regard to maternal smoking during pregnancy, this information is not available. However, maternal smoking during the famine period is highly unlikely. The famine was marked by severe shortages not only of food but also of non-essential goods, which would have greatly restricted civilian access to cigarettes.

"As a sensitivity analysis, we first re-estimated the association between prenatal famine exposure and birth weight using the aforementioned model with additional adjustment for maternal age at delivery." (line 236 to 238)

"Additional adjustment for maternal age at delivery did not materially change these estimates (**Supplementary Figure 2**)." (line 315 to 316)



Supplementary Figure 2: Effect of additional adjustment for maternal age on the association between prenatal famine exposure and observed birth weight. Shown are the adjusted differences in birth weight between famine-exposed (mid-to-late (n=283) and early (n=145) gestation) and control (n=161) groups using multivariable linear regression models. The main models are adjusted for sex, firstborn status, and gestational age. As a sensitivity analysis, models were also run with additional adjustment for maternal age at time of birth (+ maternal age). Effect estimates (point) and 95% confidence intervals (error bars) are depicted for each model and are reported in grams.

Comment 3

144-145 the authors should further elaborate or speculate on the meaning and implications of differences between the exposure timing groups. Throughout the manuscript, the differences between early and later pregnancy famine exposure are brought up. The implications or background of these differences should be expanded on.

RESPONSE: We have expanded the *discussion* section to clarify the biological relevance of differences by exposure timing:

"Our findings corroborated existing literature reporting that individuals exposed to prenatal famine in the later stages of gestation, but not in early gestation, have lower birth weights compared to controls (6). Notably, this pattern is consistent with the fact that foetal weight gain accelerates rapidly toward the end of gestation, making birth weight particularly sensitive to nutritional constraints during this period (32)." (line 408 to 412)

Comment 4

196-197 did you consider adjusting with further adult covariates besides age? BMI or other anthropometric measurements? Do you have data on body composition?

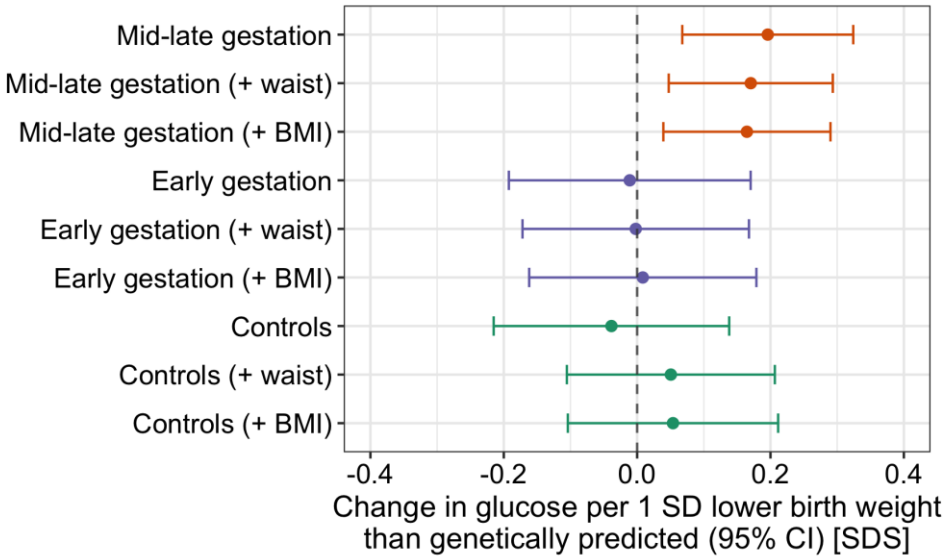
RESPONSE: We appreciate the reviewer's suggestion to consider additional adult covariates beyond age. In the primary analyses, we limited adjustment to age because traits such as fasting glucose, BMI, and waist circumference are highly interrelated and may be part of the same causal pathway. To explore this further we have now conducted additional sensitivity analyses. We examined the association between the deviation from genetically predicted birth weight and adult fasting glucose while additionally adjusting for waist circumference and BMI. The overall pattern of associations remained unchanged.

For completeness, we also examined the association between the deviation from genetically predicted birth weight and waist circumference and BMI while additionally adjusting for fasting glucose. Again, we observed only minimal changes in effect sizes and no change in the overall trend. These results suggest that, although these adult traits are interrelated and may reflect overlapping phenotypes, their associations with the deviation from genetically predicted birth weight likely operate through at least partly distinct pathways.

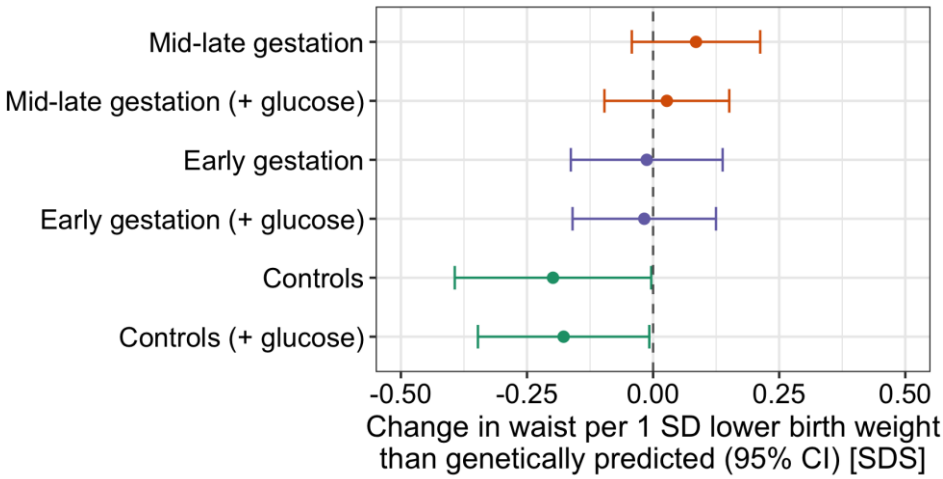
With respect to body composition, no additional measures beyond anthropometric variables and skinfold thickness measurements were available. Among these, we found waist circumference and BMI to be the most relevant measures for the present analyses.

We have included these additional analyses in the **Supplementary Material** (**Supplementary Figures 6-8**) and refer to them where appropriate in the manuscript:

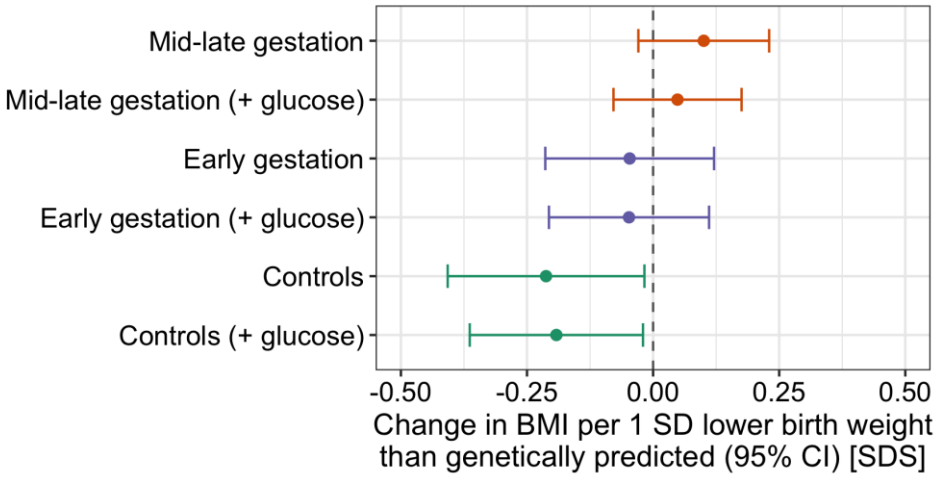
"As a sensitivity analysis, we assessed whether associations between deviations from genetically predicted birth weight and adult outcomes were independent of one another by mutually adjusting models for fasting glucose, BMI, and waist circumference. Although effect estimates differed slightly after mutual adjustment, the overall patterns remained consistent, indicating largely independent associations (**Supplementary Figures 6-8**)." (line 388 to 392)



Supplementary Figure 6. Deviation from genetically predicted birth weight and adult fasting glucose – effect of adjusting for waist circumference and BMI. The association between the deviation from genetically predicted birth weight and fasting glucose was assessed using multivariable linear regression models. Shown are the changes in fasting glucose per 1 SD lower birth weight than genetically predicted, adjusted for sex, first-born status, gestational age, and adult age, and presented separately for individuals exposed in mid-to-late (n=283) and early (n=145) gestation and controls (n=161). These models were additionally adjusted for waist circumference (+ waist) and BMI (+ BMI). Effect estimates (point) and 95% confidence intervals (error bars) are depicted for each model and are reported in standard-deviation (SD) scores of fasting glucose. Fasting glucose was log-transformed prior to the calculation of the Z scores.



Supplementary Figure 7. Deviation from genetically predicted birth weight and waist circumference – effect of adjusting for fasting glucose. The association between the deviation from genetically predicted birth weight and waist circumference was assessed using multivariable linear regression models. Shown are the changes in waist circumference per 1 SD lower birth weight than genetically predicted, adjusted for sex, first-born status, gestational age, and adult age, and presented separately for individuals exposed in mid-to-late (n=283) and early (n=145) gestation and controls (n=161). These models were additionally adjusted for fasting glucose (+ glucose). Effect estimates (point) and 95% confidence intervals (error bars) are depicted for each model and are reported in standard-deviation (SD) scores of waist circumference.



Supplementary Figure 8. Deviation from genetically predicted birth weight and BMI – effect of adjusting for fasting glucose. The association between the deviation from genetically predicted birth weight and BMI was assessed using multivariable linear regression models. Shown are the changes in BMI per 1 SD lower birth weight than genetically predicted, adjusted for sex, first-born status, gestational age, and adult age, and presented separately for individuals exposed in mid-to-late (n=283) and early (n=145) gestation and controls (n=161). These models were additionally adjusted for fasting glucose (+ glucose). Effect estimates (point) and 95% confidence intervals (error bars) are depicted for each model and are reported in standard-deviation (SD) scores of BMI. BMI was log-transformed prior to the calculation of the Z scores.

MINOR COMMENTS:

Comment 5

52-54: The sentence is somewhat unclear in this context, maybe rephrase? An example of environmental factors being influenced by genetic make-up could clarify the sentence. The references do not themselves easily answer this.

RESPONSE: We have revised the sentence in the *introduction* section to clarify its meaning:

"On the other hand, some environmental exposures are partly shaped by an individual's genetic make-up (e.g., genetic influences on diet, socioeconomic status, or smoking behaviour), complicating the identification of environmental exposures that are truly exogenous (1–4)." (line 77 to 80)

Comment 6

58-60 Reference 6's results point to epigenetic differences when exposure occurs periconceptually. No differences in methylation were reported with a later exposure. Consider rephrasing the sentence or expand the commentary on this reference.

Sentence in question: "We, as well as others, have previously reported that this environmental exposure has a marked effect on birth weight, in particular when occurring later in gestation (5–8)."

Reference 6: Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proceedings of the National Academy of Sciences. 2008 Nov 4;105(44):17046–9.

RESPONSE: We appreciate this clarification. While Reference 6 primarily investigates DNA methylation differences associated with periconceptional exposure, it also reports birth weight differences stratified by timing of exposure, including for individuals exposed late in gestation and those exposed periconceptionally. For this reason, we included it among the references supporting the statement.

Comment 7

64-67 Reference 14 is somewhat niche but illustrates an example of a moderation between exposure (breastfeeding) and outcome (IQ) by genetic factors. If the authors can provide a reference more suited to the current manuscript, it could be included here instead of reference 14.

RESPONSE: Thank you for the suggestion. We have now included an additional example of a moderation between an exposure (BMI genetics) and outcome (BMI) by family socioeconomic status:

Ghirardi G. The development of body mass index from adolescence to adulthood: A genotype-family socioeconomic status interaction study. Soc Sci Med. 2025 Nov;384:118539.

Comment 8

84-85 What is the rationale or reason behind using both related and unrelated controls? Especially when using a polygenic index, it would seem that using a mixed set of controls could pose an issue. Can the authors comment on whether the results differ when using only siblings or unrelated controls?

RESPONSE: Thank you for your comment. We clarify this point in the response to Comment 9.

Comment 9

90-91 In what capacity were the siblings used as controls if no birth weight data were available? No further mention of siblings can be found in the manuscript, so the sibling controls' role is somewhat unclear.

RESPONSE: Sibling controls were originally recruited as part of the Dutch Hunger Winter Families Study. However, because they were not recruited through the clinics that maintained obstetric records, birth weight data were not available for these individuals. As a result, they were not included in the present analyses. To avoid confusion, we now do not explicitly mention the sibling controls in the *methods* section. We have additionally updated the flow chart of the study population to make it more comprehensive and have moved it to the **Supplementary Material** (**Supplementary Figure 1**).

"Participants were interviewed, participated in a clinic exam, and DNA was extracted from blood samples and sent for genotyping (13,18). The analysis sample for this study was formed from participants for whom birth weight and genetic data were available, including 161 time controls, 145 individuals exposed to famine in early gestation, and 283 individuals exposed in mid-to-late gestation (n=589). Additional details on the recruitment process and study population are found in **Supplementary Figure 1**." (line 118 to 123)

Comment 10

98 does any information on smoking exist?

Sentence in question: “Birth weight, gestational age, sex and information on whether the participant was the firstborn (firstborn status) were obtained from the birth records of the famine-exposed individuals and the time controls”.

RESPONSE: As the sentence in question refers specifically to variables obtained from birth records, we interpret the reviewer’s comment as asking whether information on maternal smoking was available. Maternal smoking during pregnancy was not recorded in these birth records and is highly unlikely during the period of the famine.

Comment 11
100 please define “time controls” here instead of later.

RESPONSE: We have now defined the time controls when they are first mentioned in the *methods* section:

“Famine-exposed individuals and non-exposed individuals born two years before and after the famine (time controls) were identified from the review of archival obstetric records.” (line 115 to 116)

Comment 12
114-115 is this definition of famine widely used or the authors’ own definition? If used elsewhere, please include a reference.

RESPONSE: This is our own definition for famine exposure, which has been used in previous publications of the Dutch Hunger Winter Families Study. We have now included the relevant references (Cheng et al. 2024; Taeubert et al. 2024; Tobi et al. 2018; Zhou et al. 2024).

Comment 13
125 what is the role of the 51 individuals conceived shortly after famine exposure? How were they included in the analysis?

RESPONSE: In this study, we focus on the early and mid-to-late gestation famine-exposed groups. Individuals conceived shortly after the famine were included only in supplementary analyses, as part of the broader any famine exposure category. We consider these post-famine conceptions as famine-exposed since the mother experienced the famine for the full duration shortly before conception. To clarify which groups were included in the main analysis we have removed this group from **Figure 1A** in the main manuscript. We have additionally updated the flow chart of the main population and have moved it to the **Supplementary Material** (**Supplementary Figure 1**).

Comment 14
259-260: reference 28 does not exactly describe metabolic outcomes. I recommend rephrasing.

RESPONSE: We have rephrased the sentence in the *results* section to improve clarity:

“In adulthood, exposure to prenatal famine was associated with risk factors for metabolic disease in the Dutch Hunger Winter Families Study (23,31).” (line 358 to 359)

Comment 15

308-310 sentence somewhat self-contradictory, please rephrase.

RESPONSE: For clarity we have modified the sentence in the *discussion* section of the manuscript:

"The effect of the PGI on birth weight was also notably attenuated for those exposed in early gestation, although to a lesser degree compared to those exposed in mid-to-late gestation." (line 423 to 425)

Comment 16

311 ref 29 is interesting and well-suited here. Can the authors provide other examples of factors moderating genetically driven birth weight trajectories?

RESPONSE: Thank you for this suggestion. We have now included this additional example (Pereira, Rietveld, and van Kippersluis 2022) in the *discussion* section:

"Few studies have investigated environmental factors that might modulate the link between genetics and foetal growth. One study found that maternal BMI during gestation modestly moderate the PGI–birth weight relationship in offspring (33), whereas another study found no evidence of effect modification by maternal smoking (34). These findings highlight the challenge in detecting strong gene–environment interactions and the need for further research on factors that might modify effect of genetic predisposition on birth weight. Our study provides a valuable example how an environmental influence can override the effect of genetic make-up in humans." (line 425 to 432)

Comment 17

322 unclear what is referenced here, please rephrase.

RESPONSE: We have rephrased the sentence to more clearly specify that the cited references relate to long-term cardiometabolic outcomes associated with birth weight:

"Together, these findings suggest that famine-related reductions in prenatal growth during mid-to-late gestation may be involved in the risk of metabolic disease later in life. They also underscore how prenatal environmental factors may modify the pathways linking birth weight to long-term health outcomes, including cardiovascular disease and type 2 diabetes (35,36)." (line 440 to 444)

Comment 18

325-327 DNA methylation as a mechanism could be expanded, discussed further or omitted. As it is now, it is unclear as to how it links to the current study's main point.

RESPONSE: We have modified the sentence in the *discussion* section to clarify the relevance of DNA methylation changes in this context:

"Additionally, DNA methylation has been proposed as a potential mechanism linking prenatal adversity to later-life health outcomes, and differences in DNA methylation have also been predominantly observed in individuals exposed to famine during early gestation (41-43)." (line 447 to 450)

GENERAL COMMENTS:

Comment 19
Reference 4 is very interesting and noteworthy. Thank you for including this as a reference.

RESPONSE: We are happy to hear that the reviewer found this reference of interest.

Comment 20
The discussion is written very clearly and is both interesting and easy to read.

RESPONSE: We appreciate the positive feedback.

Comment 21.
Unfortunately I do not have access to all the references (7,8,15,17).

RESPONSE: We are sorry to hear that you were unable to access some of the referenced materials. We are happy to provide PDF versions of references 7 and 8 and will contact the editor to explore whether these can be shared through the journal to preserve the anonymity of the peer-review process. References 15 and 17 are books, which we recognize may further limit accessibility. We have therefore replaced these citations with an alternative reference (Burger et al. 1948) that is more readily available and covers the same background information.

Comment 22
288: I'm not entirely convinced that using the phrase "adult metabolic health" is justified when the only measured outcome is fasting glucose concentration.

RESPONSE: We understand the reviewer's point and have revised the sentence to reflect our aim more accurately:

"We investigated the interplay between genetic and environmental factors in shaping the relationship between famine exposure, birth weight, and metabolic risk factors in individuals who were exposed to the Dutch Hunger Winter of 1944-45 in utero." (line 399 to 401)

Additionally, we have also moved waist circumference from the supplement into the main manuscript in order to provide a broader focus on metabolic risk factors beyond fasting glucose.

Comment 23
328 the authors use the phrase "disease risk later in life". Could this be used elsewhere in the manuscript to replace "adult metabolic health"?

RESPONSE: We appreciate the suggestion. We have removed the term "adult metabolic health" from the manuscript and replaced it "metabolic disease risk" and "metabolic risk factors" throughout the manuscript.

Comment 24
Why were no birth records available for sibling controls?

RESPONSE: Birth records were not available for siblings due to the nature of the recruitment process of our study. Birth records from three medical institutions in famine-exposed cities were used to identify singleton births in 1945 and early 1946 (famine-exposed individuals), and in 1943 and 1947 (time controls). These individuals were then traced through population

registries and invited to participate in the study together with a same-sex sibling. Therefore, because the siblings were recruited through the participants themselves rather than through the medical institutions that kept the obstetric records, we were not able to obtain birth records for them.

We have modified the flow chart of the study population in order to make the recruitment design clearer (**Supplementary Figure 1**).

TABLES AND FIGURES

Comment 25
Table 1: please convert GA to weeks instead of days

RESPONSE: We have now converted gestational age to weeks instead of days in **Table 1**.

Comment 26
Table 1: I am also interested in seeing data on how many were born preterm or low birth weight (or very preterm / very low birth weight). Also, were any participants born SGA? Could they be outliers in the data?

RESPONSE: Thank you for this suggestion. Among the 589 individuals included in the main analysis, 11 were born preterm (gestational age <37 weeks) and 22 had low birth weight (<2500 g). Unfortunately, we do not have information on small-for-gestational-age (SGA) status in this cohort.

To assess whether individuals born preterm or with a low birth weight might be driving our findings, we conducted a sensitivity analysis excluding these individuals from the analysis. The associations between famine exposure and birth weight remained virtually unchanged, indicating that our results are not driven by these individuals.

We have included these results in the **Supplementary Material** (**Supplementary Table 4** and **Supplementary Figure 3**) and updated the main text where appropriate in the *methods* and *results* sections:

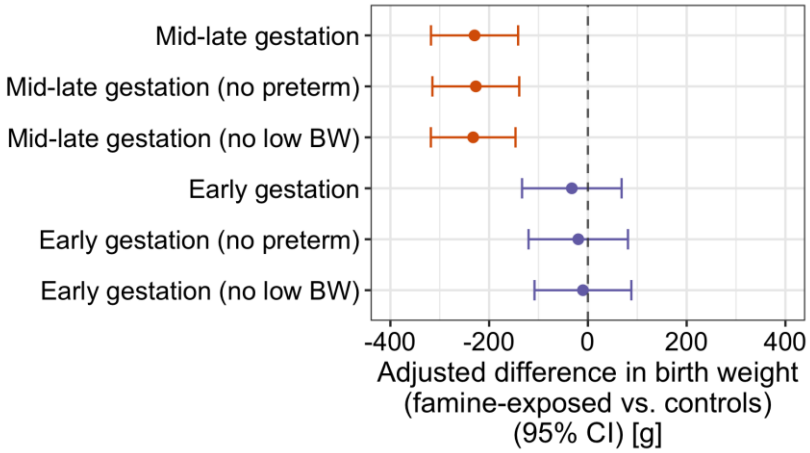
"To further test the robustness of the observed effects, analyses were repeated after excluding individuals who were born preterm (gestational age <37 weeks) or had a low birth weight (<2500 g)." (line 238 to 240)

"These findings were also unchanged after excluding preterm and low birth weight births, indicating that the observed effects are not driven by these extreme cases (**Supplementary Table 4, Supplementary Figure 3**)." (line 316 to 319)

Supplementary Table 4. Number of preterm births and low birth weight individuals across famine-exposed groups and controls.

	Controls ***(n = 161)***	**Early gestational exposure** ***(n = 145)***	**Mid-to-late gestational exposure** ***(n = 283)***
Preterm births (<37 weeks) [yes], n (%)	0 (0%)	2 (1.4%)	9 (3.2%)

Low birth weight (<2500g) [yes], n (%)	3 (1.9%)	5 (3.4%)	14 (4.9%)



Supplementary Figure 3. Effect of excluding preterm and low birth weight births on the association between prenatal famine exposure and observed birth weight. Shown are the adjusted differences in birth weight between famine-exposed (mid-to-late (n=283) and early (n=145) gestation) and control (n=161) groups using multivariable linear regression models. The main models are adjusted for sex, firstborn status, and gestational age. For comparison purposes models were run excluding participants born preterm (n=11, <37 weeks) and those with a low birth weight (n=22, <2500g). Effect estimates (point) and 95% confidence intervals (error bars) are depicted for each model and are reported in grams.

Comment 27

Figure 1: I am confused as to why sibling controls and non-related time controls were both included as controls, and how they were treated in the analyses. Also, what is the role of the post-famine conception group? Where do they fall in the analysis? Please clarify these groups, or if they are not part of this, consider modifying the figure and manuscript for clarity and brevity.

RESPONSE: Sibling controls were originally recruited as part of the Dutch Hunger Winter Families Study. However, because they were not recruited through the clinics that kept obstetric records, birth weight data were unavailable, and they were therefore not included in this study.

Regarding the famine-exposed groups, only the early and mid-to-late gestation groups were included in the main analysis. The post-famine conception group was considered only in supplementary analyses, where it was included as part of the broader any famine exposure category. To improve the clarity of the manuscript, we have revised the *methods* section and updated the population flow chart to include a more comprehensive description of the recruitment process and to clarify which groups are included in the main and supplementary analyses (**Supplementary Figure 1**).

Comment 28

Figure 2A is very illustrative and sums up the population nicely. The later figures are demonstrative as well.

RESPONSE: Thank you for the positive remarks.

SUPPLEMENTS

Comment 29
25-28 Do the authors have any data on macronutrients? Protein deficiency?

RESPONSE: We unfortunately do not have data on the specific macronutrient composition of the rations.

Comment 30
46-47: what data were gathered in the structured interview? Was this as a general description of the cohort or something pertinent to this current study?

RESPONSE: In the current study, we used data obtained from birth records and the clinical examination only; the structured telephone interview is mentioned as part of the general description of the cohort. The interview collected information on sociodemographic characteristics, socioeconomic status, current health status, and lifestyle habits.

Reviewer 3

This is a very well written paper that delivers on its objectives. It is clear and concise. The data source is perfect for the question being asked. The statistical analytic methods are well described and suitable for these data. I have a few minor concerns.

RESPONSE: We thank the reviewer for the positive feedback. Our specific responses to the comments are given below.

Comment 1

The use of sibling controls is described on line 84-85 (page 4) and later in the paragraph it is noted that birthweight data were not available for the sibling controls. Then in what way did these controls contribute to the analysis? The authors should clarify where the sibling controls contributed. If they were not used in this paper, I would recommend dropping mention of them so as not to confuse the reader.

RESPONSE: Indeed, the sibling controls are originally a part of the Dutch Hunger Winter Families Study but could not contribute to this analysis due to lack of birth weight data in this group (unlike the participants identified through institutions with birth records available). We have modified the *methods* section and the flow chart of the study population (**Supplementary Figure 1**) to improve clarity on the composition of the main study population:

"Participants were interviewed, participated in a clinic exam and DNA was extracted from blood samples and sent for genotyping (13,18). The analysis sample for this study was formed from participants for whom birth weight and genetic data were available, including 161 time controls, 145 individuals exposed to famine in early gestation, and 283 individuals exposed in mid-to-late gestation (n=589). Additional details on the recruitment process and study population are found in **Supplementary Figure 1**." (line 118 to 123)

Comment 2

I'm not in agreement with the authors about their conclusions in lines 237-240: "The birth weight PGI was not different in individuals exposed in early gestation or those exposed in mid-to-late gestation as compared to controls (for early gestation 238 β=-0.18 SD, 95% CI: -0.40–0.04; p=0.10; for mid-to-late gestation β=-0.08 SD, 95% CI: -0.28–0.11; p=0.39)."

The p-value for the early gestation is 0.10 (borderline significant) and the beta is -0.18 in contrast to a -value of 0.39 and beta of -0.08 for mid-to late gestation exposure. These could be interpreted as differing from one another.

RESPONSE: Thank you for this comment. We agree that the effect estimates show a different pattern between the early and mid-to-late gestation exposure groups and that this warrants careful wording. We have revised the sentence in the *results* section accordingly to better reflect the observed pattern:

"The birth weight PGI did not differ between famine-exposed individuals and controls. The effect estimates, however, suggested a modest reduction among those exposed in early gestation, with a less pronounced difference among those exposed in mid-to-late gestation (early gestation: β = −0.18 SD, 95% CI −0.40 to 0.04, p = 0.10; mid-to-late gestation: β = −0.08 SD, 95% CI −0.28 to 0.11, p = 0.39)." (line 334 to 339)

Comment 3

The Dutch Famine Study created an incredibly unique data set that is continuing to answer important questions about what drives birthweight and fetal growth, among other outcomes. However, there are limitations that should be discussed further in this manuscript and are often invoked in papers using these data.
a) This was a well-nourished population prior to the onset of the famine conditions. This both makes the data set highly valuable but also constrains its generalizability. Famine is rarely suffered by such populations and may act very differently in a population experiencing famine in the modern day, such as African countries or even Gaza.
b) Similarly, this is not a diverse population that includes African or Hispanic individuals. Given the different life time and prenatal stressors for those families, these results also might not be generalizable.
c) Younger readers may be unfamiliar with the events that led to the famine. While certainly the reader can look further, I would recommend that the paper conclude (or state in the background) a sentence or two that underscores the tremendous human rights violations that produced these data. Epidemiologists sometimes forget the human cost of what we are analyzing. While we did not cause the conditions that gave rise to these data, we should acknowledge those human costs.

RESPONSE: We thank the reviewer for bringing up these important points. We have included these limitations in the *discussion* section of the manuscript:

"Finally, the generalisability of our findings warrants consideration. The Dutch Hunger Winter affected a previously well-nourished population of predominantly European ancestry, and the famine was characterised mainly by severe caloric restriction with relatively stable macronutrient composition (5,17). As such, the observed effects are more likely driven by undernutrition rather than specific nutrient deficiencies. However, evidence from more recent analyses suggests that reductions in protein intake during the most extreme phases of the famine, may have contributed to long-term health outcomes (46). These historical and population-specific circumstances may limit direct extrapolation to other famine settings, particularly those involving prolonged malnutrition, different nutritional profiles, or populations with different genetic backgrounds. Nonetheless, our findings may still inform broader understanding of how severe caloric restriction during pregnancy can influence foetal growth, its association with genetics and later-life metabolic risk." (line 469 to 480)

We have also added additional context on the events that led to the famine in the *methods* section:

"**Historical background: The Dutch Hunger Winter of 1944 to 1945**
The Dutch famine occurred between 26 November 1944 and 5 May 1945 and began as a result of a food-supply embargo by German occupying forces in early October 1944, a consequence of wartime policies that severely restricted civilian access to food. The severity and widespread nature of the famine are well documented (5,17). Prior to the famine, nutrition in the Netherlands had been generally adequate. Official rations fell below 900 kcal/day in late November 1944. The famine ended with liberation 5 May 1945, after which available food supplies rapidly increased by Allied efforts." (line 104 to 111)

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