Transgenerational associations of parental famine exposure in early life with offspring risk of adult obesity in China

Wei-Yuan Yao[1,2], Leah Li[3], Hui-Ru Jiang[1], Yong-Fu Yu[4*], Wang-Hong Xu[1,2*]

Affiliations

[1] Department of Epidemiology, School of Public Health, Fudan University; Key Laboratory of Public Health Safety, Ministry of Education (Fudan University), 138 Yi Xue Yuan Road, Shanghai, 200032, China

[2] Yiwu Research Institite, Fudan University, Building V of Zhongfu Square, Yiwu, Zhejiang Province, 322000, China

[3] Population, Policy and Practice Research and Teaching Department, University College London Great Ormond Street Institute of Child Health, London, United Kingdom

[4] Department of Biostatistics, School of Public Health and The Key Laboratory of Public Health Safety of Ministry of Education, Fudan University, 138 Yi Xue Yuan Road, Shanghai, 200032, China

Key words: famine exposure; transgeneration; DOHaD; body mass index; waist circumference; obesity

Running title: famine exposure and obesity in offspring

Contact Info: Wang-Hong Xu, MD, Ph.D, Professor of Epidemiology, Fudan University School of Public Health, 138 Yi Xue Yuan Road, Shanghai 200032, China, Tel: (+86)-21-54237679, Email: wanghong.xu@fudan.edu.cn; Or Yong-Fu Yu, Department of Biostatistics, School of Public Health and The Key Laboratory of Public Health Safety of Ministry of Education, Fudan University, Shanghai, China, email: yu@fudan.edu.cn

Word Count: 3894

Competing Interests: The authors declared no conflict of interest.

Funding: This work was supported by the National Nature Science Foundation of China (81573221) and the Three-year Action Plan on Public Health, Phase V, Shanghai, China (GWV-10.1-XK16)

The authors' contribution: WHX, LL and YFY designed the study and critically revised the manuscript; WYY analyzed data and wrote the paper; WYY and HRJ contributed to data analysis and critical revision of the manuscript; WHX and YFY had primary responsibility for final content. All authors read and approved the final manuscript.

Study importance

What is already known about this subject?

Emerging evidence suggests the transgenerational association of early-life exposure to famine with obesity.

What are the new findings in your manuscript?

Maternal exposures to famine in utero and early-childhood were associated with elevated levels of body mass index, waist circumference, and higher risks of overweight and central obesity in their offspring, while paternal childhood-exposure was negatively associated with these measurements.

How might your results change the direction of research or the focus of clinical practice?

Our results support transgenerational influence of nutritional conditions in early-life, indicate complex underlying biological mechanisms, and implicate the importance to improve maternal nutrition during gestation.

Abstract

Objective: To investigate the transgenerational associations between exposure to famine in early-life and obesity.

Methods: We used the longitudinal data from the China Health and Nutrition Survey during 1989-2015. We included 1113 fathers and 1207 mothers (946 mother-father pairs) born in 1955-1966, and 1895 adult offspring. Offspring were classified into subgroups according to the famine exposure of their parents (unexposed, maternal exposed, paternal exposed, parental exposed) and exposure timing (during fetal development, childhood).

Results: Maternal exposure to famine in early-life was associated with elevated levels of body mass index (BMI), waist circumference (WC), overweight and central obesity of their offspring, while paternal exposure was inversely associated with these measurements. Compared with offspring of unexposed parents (P0M0), the maternal exposed group (P0M1) had higher mean BMI [by 1.3 kg/m^2 (95% confidence interval: 0.3, 2.4), WC [by 1.5 cm (-1.0, 3.9)], overweight [OR (95%CI):3.1(1.6, 6.1)] and central obesity [OR (95%CI: 1.9 (1.02, 3.7)]. No significant heterogeneity was observed in the associations by sex of offspring.

Conclusions: Fetal and early-childhood exposure to famine in parents may be associated with their offspring's risk of obesity during adulthood. A better understanding of the transgenerational associations is important for developing strategies to reduce obesity risk in future generations.

Introduction

Intrauterine malnutrition has been associated with increased susceptibility of offspring to non-communicable diseases (1). This transgenerational phenomenon of the *developmental origins of health and disease* (DOHaD) is supported by experimental rodent studies. In particular, the associations of maternal prenatal and early postnatal malnutrition with body size and composition of their offspring have been consistently observed (2, 3), as this early-life stage is a critical developmental window during which nutritional deficiency may affect the development of diseases in later life (4), and also in their offspring (5).

Although experimental evidence has suggested that obesity is an intergenerational or even transgenerational condition (6), there is a paucity of epidemiological evidence in humans. For maternal exposure, most related studies observed a significant association of maternal fetal exposure to famine with offspring's birthweight (7, 8) and neonatal adiposity (9). Low birthweight, an indicator of abnormal fetal growth and development (10), has been closely related to survival, health, growth and development of newborns (11), as well as obesity beyond childhood (12). Therefore, the association of maternal fetal exposure to famine with offspring's birthweight can be used as indirect evidence for the transgenerational effect of famine exposure on obesity.

Regarding paternal exposure, on the other hand, evidence is accumulating for its transgenerational influence. A Dutch famine study found that offspring whose fathers (but not mothers) were undernourished prenatally were heavier and more obese than offspring whose fathers and mothers were not (9, 13). Exposure to famine of grandfathers was associated with an increased risk of obesity and cardiovascular disease in offspring and grandchildren (14). Pembrey, *et al* (15) found that paternal grandfather's food supply was only linked to the mortality risk ratios of grandsons, and paternal grandmother's food supply was only associated with the granddaughters' mortality risk ratios, indicating a sex-specific transgenerational effect

of nutrition in early-life. These previous studies indicate complex intergenerational and transgenerational inheritance patterns in humans.

A recent study based on the China Health and Nutrition Survey data found that early-life exposure to the Chinese famine was associated with increased body mass index (BMI) and waist circumference in two consecutive generations (16). This previous study treated the childhood-exposed participants as unexposed, and thus, did not fully evaluate the joint effect of maternal and paternal exposures.

In this study, we took advantage of the "natural experiment" of the Chinese Great Famine during 1959-1961 to evaluate the potential transgenerational effect of malnutrition in prenatal life and early childhood on obesity. Specifically, we estimated the independent and joint associations of maternal and paternal famine exposures with the measures of adiposity and risks of overweight and central obesity in their offspring.

Methods

Study design and participants

The China Health and Nutrition Survey (CHNS) is an ongoing longitudinal household survey. It was initiated in 1989 and had a further nine waves in 1991, 1993, 1997, 2000, 2004, 2006, 2009, 2011, and 2015. Details of the CHNS were described elsewhere (17). In brief, a multistage random cluster sampling design was adopted to ensure adequate representation. At each survey, participants completed a structured questionnaire on socioeconomic characteristics, lifestyle factors, general health, and medical history. Physical measurements and blood samples of participants were taken by trained health workers with a standardized protocol. The survey protocols and the process for obtaining informed consent for CHNS were approved by the institutional review committees of the University of North Carolina at Chaple Hill, the National Institute of Nutrition and Health, and the Chinese Center for Disease Control and Prevention.

The data were downloaded from the website (https://www.cpc.unc.edu/projects/china) (accessed on 21 September 2021).

Unlike the surveys from 1991 onwards, the 1989 survey included only preschool children and adults aged 20-45 years, used different stadiometers or scales for height and weight measurements, and did not collect information on pregnant status of women. In this study, we included adult participants aged ≥18 years in surveys from 1991 to 2015 whose parents participated in any wave of the CHNS survey, and their fathers and/or mothers were born in 1955-66. Nine provinces were included in all waves. Three autonomous cities (Beijing, Chongqing and Shanghai) added since 2011 were excluded from this study. As shown in **Figure S1**, a total of 1 113 fathers, 1 207 mothers and 946 mother-father pairs born in 1955-66 were included in this study as F1 generation, and 1 513, 1 670 and 1 288 adult offspring respectively as F2 generation.

A total of 1 895 adult offspring (3 389 observations) with at least one parent born in 1955-66 were included in the final analyses. Among them, 1 061 had one, 440 had two, 224 had three, 99 had four, and 77 had five or more measurements.

Exposure to famine

The Chinese Great Famine happened during 1959-1961. We first classified the parents into three groups according to the timing of exposure (that is, their birth year): 1) unexposed (born in 1963-66); exposed during 2) fetal development (born in 1959-62); and 3) childhood (born in 1955-58), as in previous studies (18, 19).

We then classified the offspring into subgroups according to the exposure status of their parents. Offspring whose parents were both born in 1955-66 were classified into four groups: (1) neither parent exposed to famine (P0M0); 2) father exposed only (P1M0); 3) mother exposed only (P0M1); and 4) both parents exposed to famine (P1M1). Offspring whose mother

or father was exposed to famine were further classified into eight groups according to exposure timing of their parents, as shown in **Figure S2**. No observation was in the group of unexposed father and childhood-exposed mother (p0m2), and thus the group of unexposed father and fetal-exposed mother (p0m1) was the same as the P0M1group. In all analyses, the P0M0 group was used as the reference group.

Anthropometric measurements

At each survey, height (to the nearest 0.1 cm) and weight (0.1 kg) were measured using a calibrated beam scale and an electronic weight scale respectively in lightweight clothes without shoes. Waist circumference (0.1 cm, WC) was measured at the midpoint between the bottom of the rib cage and the top of the iliac crest at the end of exhalation. Overweight was defined as BMI ≥25 kg/m^2. Central obesity was defined as WC>90 cm in men and >80 cm in women according to the World Health Organization (WHO) criteria (20).

Covariates

The covariates used in the main analyses included sex, age and birth year of offspring, and maternal and paternal BMI. In the sensitivity analyses, we also considered year of education, energy intake, physical activity, residential areas (urban/rural), and severity of famine exposure.

The dietary assessment included three consecutive 24-h diet recalls (21). Total energy intake was calculated based on the amount of food consumed and the Chinese Food Composition Table (2002 and 2004) (22, 23). Physical activities (PA) in domestic, occupational, leisure, and transportation settings during a typical week were collected using a questionnaire (24). The level of PA was expressed as metabolic equivalent tasks (METs) based on the Compendium of Physical Activities. The severity of famine exposure was determined according to the total fertility loss from 1958 to 1965 (18, 25).

Statistical analysis

Characteristics of F1 parents and F2 offspring were compared by famine exposure of F1 parents using ANOVA (for continuous measures) and Chi-square tests (for categorical measures).

We first compared the mean level and 95% confidence interval (CI) of BMI and WC by sex using linear regression models with adjustment for birth year and age at measurement. We tested the interactions of parental famine exposures with sex to assess whether their associations with offspring BMI and WC differed between men and women. As the interaction terms were non-significant, we then evaluated the associations for both sexes combined.

To assess how trajectories of BMI and WC differed by famine exposure group, we applied cubic spline models to capture the non-linear curves of age-related changes in BMI and WC. We adopted three-level models with a random intercept adjusting for sex and birth year of offspring, maternal and paternal BMI, and the birth year of the mother (in analysis of paternal famine exposure) and father (in analysis of maternal exposure). Measurements were level-1 units, clustered within offspring (level-2), which were further clustered within households (level-3, as siblings were correlated). The number of knots (k) was determined according to the observed age - BMI and age - WC relationship and the Akaike's information criterion of the models. For repeated binary outcomes (overweight and central obesity), we applied generalized linear mixed-effects models (GLMM) with a cubic spline function and a random intercept. We estimated the difference in mean BMI and WC levels (95% CIs) between each famine exposure group and the unexposed group. We also estimated the odds ratios (ORs, and 95% CIs) of overweight and central obesity for each parental famine exposure group (vs non-exposed) to evaluate the associations with the risk of overweight and central obesity in offspring.

Sex, birth year, and age at measurement of offspring were adjusted in all models. Maternal

and paternal age and BMI at the interview closest to birth of their offspring, which were defined as those at the most recent interview before birth of their offspring, or at the latest interview before pregnancy for pregnant mothers, or at the first interview for those interviewed after birth of their offspring, were also treated as potential confounders. There were no missing data in these covariates, but 1.1% (38/3389) of offspring had missing physical activity measures and 32.8% (1112/3389) had missing or unavailable (for the 2015 survey) energy intake values. The missing data were handled by generating a multiple randomly imputed dataset based on the characteristics of the participants of each survey. However, additionally adjusting for years of education, energy intake and physical activity of offspring did not change the results and the covariates were not included in the final models.

We repeated main analyses by (1) including only F1 parents from rural areas and their F2 offspring; (2) restricting to F1 parents from areas suffering severe famine and their F2 offspring; and (3) using a lower BMI cutoff (≥24.0 kg/m^2) for overweight in both sexes according to the Expert Consensus on Obesity Prevention and Treatment in China (26); (4) using lower sex-specific cutoffs for overweight (≥22.5 kg/m^2 in men and ≥22.8 kg/m^2 for women) as Chinese (compared to the White population) have greater risks of obesity-related diseases for a given BMI (27); (5) including offspring with at least two repeated physical measurements to assess the impact of sample attrition, and (6) focusing on offspring whose parents were both born in 1955-1966.

All analyses were performed using SAS version 9.4 and R version 4.0. All tests were two-sided. P<0.05 was considered as statistical significance.

Results

Demographics of F1 parents and F2 observations by parental famine exposure status

As shown in **Table S1**, the mean age in unexposed fathers (32.2 ± 6.3 years) and in

unexposed mothers at the interview closest to birth of their offspring (31.8 ± 6.3 years) was significantly lower than those in fetal-exposed (fathers: 35.6 ± 6.1 years, mothers: 34.6 ± 6.0 years) and childhood-exposed groups (fathers: 38.5 ± 5.6 years, mothers: 38.3 ± 5.5 years). BMI, WC, prevalence of overweight and central obesity did not differ by famine exposure status in both fathers and mothers. The differences in characteristics of fathers or mothers by famine exposure did not change substantially among father-mother pairs who both were born in 1955-1966.

Presented in **Table 1** are characteristics of observations for F2 offspring in adulthood classified by parental famine exposure. A significant difference was observed for birth year, age at survey, and years of education among subgroups of offspring (all p values <0.05). PA level was found different by parental famine exposure status in men while energy intake significantly differed among women (most *p values* <0.05).

Trajectories of BMI and WC in F2 offspring by famine exposures of F1 parents

The fitted trajectories for BMI and WC in male and female offspring showed a monotonically increasing pattern with age (**Figure 1** and **2**), and a significant interaction was observed between age of offspring and parental famine exposures (p for interaction <0.05).

Associations of famine exposure of parents with body measurements of offspring

As shown in **Table 2**, the ls-means of BMI and WC significantly differed by parental exposure status in male offspring. After adjusting for potential confounders, male offspring of childhood-exposed fathers had a significantly lower BMI and WC than those of unexposed groups (**Table S2**). Maternal exposure, on the other hand, was associated with a higher body measurements in offspring.

Since no sex heterogeneity was observed in the associations of interest (all p for

interaction >0.05), we conducted the analyses among all subjects. As shown in **Table 2**, offspring of childhood-exposed fathers had a lower BMI [β(95%CI): -0.8 (-1.6, 0.1)] and WC [β(95%CI): -2.1 (-4.1, -0.2)] than those of unexposed groups, while maternal exposure was associated with a higher BMI in offspring, with β (95%CI) of 0.7 (0.2, 1.3) for offspring of fetal-exposed mothers and 1.0 (0.2, 1.8) for offspring of childhood-exposed mothers. Combined analyses of parental exposure demonstrated that the group of P0M1 had a higher mean BMI [by 1.3 kg/m^2 (95% CI: 0.3, 2.4) and WC [by 1.5 cm (95%CI: -1.0, 3.9)] than the P0M0 group. Compared with offspring of unexposed parents (P0M0), the groups of p0m1 and p1m2 had higher BMI and WC, while the group of p2m0 had lower levels of the two measurements.

Associations of famine exposure of F1 parents with obesity in F2 offspring

As presented in **Table 3**, the lower risks of overweight and central obesity in offspring of childhood-exposed fathers and the higher risks in offspring of fetal-exposed fathers or mothers were consistently observed in all multi-variable models. Further analysis by parental exposure status showed 3.1-fold (95%CI: 1.6, 6.1) odds of developing overweight in offspring of the P0M1 group, and 1.9-fold (95%CI:1.02, 3.7) risk of central obesity in offspring of the group than those in the P0M0 group.

Stratified analysis by sex of offspring shows similar association patterns (**Figure S3** and **S4)**, and demonstrates 2.3-fold (95%CI: 1.05, 5.1) odds of developing overweight and 2.5-fold (95%CI:1.1, 5.3) risk of central obesity in male offspring of the P0M1 group, and 5.0-fold (95%CI:1.2, 20.7) risk of overweight in female offspring of the group than those in the P0M0 group. However, no significant interaction was observed with sex of offspring.

When focusing on offspring with both parents born in 1955-1966, we separated the F2 participants by famine exposure timing of fathers, mothers and both parents, respectively. Similarly, the results indicated that F2 offspring of fetal-exposed mothers had higher risks of

overweight and central obesity, while those of childhood-exposed fathers had lower risks, with a significant interaction between maternal and paternal exposures. With regards to famine exposure timing of parents, compared with the P0M0 group, the groups of p0m1 and p1m2 had significantly higher risks of overweight and central obesity, while the group of p2m0 demonstrated lower risks (**Figure 3**).

Sensitivity analyses

Including only F1 parents from rural areas or from areas suffering severe famine, using a lower cutoff for overweight, or restricting to F2 offspring with at least two observations did not qualitatively alter the transgenerational associations of famine exposure of F1 with BMI, WC and risks of obesity of F2 offspring (**Table S3** to **S6)**.

Discussion

In this study exploring the potential influence of parental exposure to the Chinese famine of 1958 to 1961 on body measurements of offspring based on a longitudinal study in Chinese populations, we found that: 1) maternal exposure to famine was associated with elevated levels of BMI, WC, and higher risks of overweight and central obesity in their offspring; 2) paternal childhood-exposure to famine, on the contrary, was found negatively associated with the body measurements; 3) No significant sex heterogeneity was found in transgenerational associations of famine exposure with offspring's obesity.

Our findings of positive associations of maternal exposure to famine with obesity of offspring are consistent with an experimental animal study (28) and a small number of observational studies in humans (9, 29). Using a mouse model of low birthweight produced by maternal caloric undernutrition during late gestation, Jimenez-Chillaron, *et al* (28) demonstrated that adiposity phenotypes could progress to offspring (F2) through the maternal

lineage. Studies based on the Dutch famine showed that maternal prenatal exposure to famine was associated with the low birthweight of F2 offspring (29); the offspring of F1 women who were exposed to famine in utero had poor health in later life than those of unexposed F1 women (9). Using data from the CHNS, an ongoing large-scale longitudinal household survey, Li, et al (16) observed an increased BMI in older (≥25 years) or male offspring of parents exposed to famine. Our findings extend the previous evidence and support the potential transgenerational effect of maternal malnutrition in early life on offspring in the Chinese population.

While few population studies directly investigated the transgenerational association of maternal prenatal malnutrition with the risk of obesity in offspring, numerous animal and human studies indicate that nutritional experience in early life may trigger a sex-specific transgenerational response along the male line (9, 13). Multiple Dutch famine studies found that the adult offspring of prenatal malnourished fathers were more likely to have higher body weights and BMIs (9, 13). Exposure to famine of grandfathers was associated with an increased risk of obesity and cardiovascular disease in offspring and grandchildren (14). The Överkalix cohorts in northern Sweden showed that grandchildren of the grandfathers who experienced shortage of food supply had higher prevalence of diabetes (30). The Uppsala Multi-generation Study suggested that paternal grandfather's food access in pre-puberty was associated with increased mortality risk in his male, but not female, grandchildren (31). On the basis of the animal data and the population studies in humans, the hypothesis of the paternal origins of health and disease (POHaD) was introduced in recent years (32), which proposed the pre-conceptional origins of disease through paternal exposome.

In this study, however, we observed smaller BMI and WC and lower risks of overweight and central obesity in offspring of childhood-exposed fathers after adjusting for birth year of offspring and exposure status of mothers, opposite to most previous studies. Plausible explanations for our findings are not available. It may be partly explained by the son preference

in Chinse culture, which allowed male infants to suffer less food shortage during the severe famine period (33). We also for the first time observed a significant interaction between maternal and paternal exposures to famine on offspring obesity, demonstrating counteracting effect of paternal and maternal exposures on offspring's risk of obesity. Due to the small sample size and limited observations in the P0M1 group, we could not exclude the possibility of chance. Further studies with a larger sample size are warranted to confirm our findings and explore the potential mechanisms.

Indeed, potential physiological and molecular mechanisms have been proposed underlying the transgenerational association patterns (34). The cross-generational phenotype inheritance proposed by Kuzawa(35) is an updated "Thrifty phenotype hypothesis", which points out that the irreversible thrift phenotype of F1 generation caused by fetal malnutrition may cause similar phenotype in F2 and even F3 generations (36). The transgenerational transmission of thrift phenotype has been supposed through programmed changes in nutrient metabolism physiologically (37) and by epigenetic modification in specific genes molecularly (38). Both human and animal studies show that prenatal adverse environments might largely induce alternations in DNA methylation that can be inherited to next generations (39, 40), and thus affecting the development of offspring prenatally (41) and resulting in long-lasting consequence for adult offspring (42). A previous study indicated that persistent changes in DNA methylation by prenatal famine exposure depended on the sex of the exposed individual (43), which may help to explain the opposite associations of maternal and paternal exposure with their offspring's body size.

Regarding the transgenerational influence of adverse early-life exposures by sex of offspring, most previous studies observed significant sex-specific effects (15). Based on the CHNS survey data, Li, et al (16) found a significant association of early-life exposure of mothers and/or fathers with BMIs of male offspring, but not with BMIs of female offspring. In

this study, however, we did not observe significant sex-specific transgenerational associations. The different results of the current study from the previous report derived from a similar survey data may be accounted by the heterogeneous effects of maternal and paternal exposures. In Li's report, famine exposure of F1 participants in early life included both maternal and paternal exposures (16), while in our study the effect of maternal and paternal exposures were evaluated independently and jointly.

The main strength of the study is the large-scale longitudinal survey covering most regions of China, ensuring the representation of our sample. Second, the classification by famine exposure status and timing of mothers, fathers and both provide us an opportunity to evaluate the independent and joint effects of maternal and parental exposures prenatally and early postnatally. Finally, we adjusted multiple confounders including birth year and age at survey of offspring, age and BMI of mothers and fathers at first interview, and other related factors, minimizing their potential confounding effect.

This study has several limitations. First, the famine exposure was defined based on birth year of F1 parents, which may have led to non-differential misclassification bias and thus underestimated associations investigated in this study. Second, the small number of observations of offspring in several subgroups classified by parental famine exposure status and exposure timing limited our ability to evaluate the transgenerational associations more deeply. Third, residual confounding bias could still exist for the complexity of observational study. Nonetheless, we adjusted for birth year of offspring in all models to decrease the secular effects, and for age at survey to reduce the bias that may result from the inconsistencies of age in each group. Considering the statistical power, the unlikeliness of years of education, energy intake and PA level as confounders, and the unchanged results by additionally adjusting for the factors, we reported the results derived from the model not including these covariates. Moreover, the age of F2 offspring included in this study ranged from 18 to 40 years, which are not the peak

ages for obesity. A long-term follow-up is needed to provide enough statistical power and enable us to evaluate the impact of famine exposure on offspring's obesity in later adulthood. Finally, we did not take the weight of sampling of the CHNS into consideration, selection bias is possible. However, the presentation of the samples may only influence the external validity of our results but not the internal validity.

Conclusion

In conclusion, this exploratory analysis demonstrates transgenerational associations of parental famine exposure in early life with offspring's obesity in adulthood. Our findings provide evidence that nutritional conditions in early life in one generation may influence phenotype in subsequent generations, and indicate complex biological mechanisms underlying the phenomenon. The findings that maternal exposure to famine may increase the risk of obesity in offspring suggest that early-life exposure to the Chinese Great Famine may have contributed to the epidemic of obesity in young generations in China, and indicate that improving maternal nutrition during gestation may have a profound impact on their offspring, through which the burden of obesity-related diseases in Chinese populations can be reduced.

Acknowledgments

We are grateful to the participants and staff of the China Health and Nutrition Survey for their contributions to the study.

Data availability: Data can be accessed by the linkage of https://www.cpc.unc.edu/projects/china.

Reference

1. Gluckman PD, Hanson MA, Buklijas T. A conceptual framework for the developmental origins of health and disease. Journal of developmental origins of health and disease. 2010;1(1):6-18. Epub 2010/02/01. doi: 10.1017/S2040174409990171. PubMed PMID: 25142928.
2. Hanafi MY, Saleh MM, Saad MI, Abdelkhalek TM, Kamel MA. Transgenerational effects of obesity and malnourishment on diabetes risk in F2 generation. Molecular and cellular biochemistry. 2016;412(1-2):269-80. Epub 2015/12/29. doi: 10.1007/s11010-015-2633-6. PubMed PMID: 26708218.
3. Kaczmarek MM, Mendoza T, Kozak LP. Lactation undernutrition leads to multigenerational molecular programming of hypothalamic gene networks controlling reproduction. BMC Genomics. 2016;17:333. Epub 2016/05/06. doi: 10.1186/s12864-016-2615-4. PubMed PMID: 27146259; PubMed Central PMCID: PMCPMC4857247.
4. Wang N, Cheng J, Han B, et al. Exposure to severe famine in the prenatal or postnatal period and the development of diabetes in adulthood: an observational study. Diabetologia. 2017;60(2):262-9. Epub 2016/11/04. doi: 10.1007/s00125-016-4148-4. PubMed PMID: 27807599.
5. Wahlqvist ML, Krawetz SA, Rizzo NS, et al. Early-life influences on obesity: from preconception to adolescence. Ann N Y Acad Sci. 2015;1347:1-28. Epub 2015/06/04. doi: 10.1111/nyas.12778. PubMed PMID: 26037603; PubMed Central PMCID: PMCPMC4522218.
6. Bays H, Scinta W. Adiposopathy and epigenetics: an introduction to obesity as a transgenerational disease. Curr Med Res Opin. 2015;31(11):2059-69. Epub 2015/09/04. doi: 10.1185/03007995.2015.1087983. PubMed PMID: 26331354.
7. Lumey LH, Stein AD. Offspring birth weights after maternal intrauterine undernutrition: a comparison within sibships. American journal of epidemiology. 1997;146(10):810-9. Epub 1997/12/31. doi: 10.1093/oxfordjournals.aje.a009198. PubMed PMID: 9384201.
8. Stein AD, Lumey LH. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: the Dutch Famine Birth Cohort Study. Human biology. 2000;72(4):641-54. Epub 2000/10/26. PubMed PMID: 11048791.
9. Painter RC, Osmond C, Gluckman P, et al. Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. BJOG : an international journal of obstetrics and gynaecology. 2008;115(10):1243-9. Epub 2008/08/22. doi: 10.1111/j.1471-0528.2008.01822.x. PubMed PMID: 18715409.
10. Labayen I, Moreno LA, Ruiz JR, et al. Small birth weight and later body composition and fat distribution in adolescents: the Avena study. Obesity (Silver Spring, Md). 2008;16(7):1680-6. Epub 2008/05/10. doi: 10.1038/oby.2008.258. PubMed PMID: 18464751.
11. Fallucca S, Vasta M, Sciullo E, Balducci S, Fallucca F. Birth weight: genetic and intrauterine environment in normal pregnancy. Diabetes care. 2009;32(12):e149. Epub 2009/11/27. doi: 10.2337/dc09-1489. PubMed PMID: 19940212.
12. Tian JY, Cheng O, Song XM, et al. Birth weight and risk of type 2 diabetes, abdominal obesity and hypertension among Chinese adults. Eur J Endocrinol. 2006;155(4):601-7. doi: 10.1530/eje.1.02265. PubMed PMID: WOS:000241498000012.
13. Veenendaal MVE, Painter RC, de Rooij SR, et al. Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine. Bjog-Int J Obstet Gy. 2013;120(5):548-54. doi: 10.1111/1471-0528.12136. PubMed PMID: WOS:000316284300006.
14. Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet. 2002;10(11):682-8. doi: 10.1038/sj.ejhg.5200859. PubMed PMID: WOS:000179860400004.
15. Pembrey ME, Bygren LO, Kaati G, et al. Sex-specific, male-line transgenerational responses in humans. European journal of human genetics : EJHG. 2006;14(2):159-66. Epub 2006/01/05. doi: 10.1038/sj.ejhg.5201538. PubMed PMID: 16391557.
16. Li J, Yang Q, An R, et al. Famine and Trajectories of Body Mass Index, Waist Circumference, and Blood Pressure in Two Generations: Results From the CHNS From 1993-2015. Hypertension. 2021.
17. Popkin BM, Du S, Zhai F, Zhang B. Cohort Profile: The China Health and Nutrition Survey--monitoring and understanding socio-economic and health change in China, 1989-2011. Int J Epidemiol. 2010;39(6):1435-40. Epub 2009/11/06. doi: 10.1093/ije/dyp322. PubMed PMID:

19887509; PubMed Central PMCID: PMCPMC2992625.
18. Jiang H, Yu Y, Li L, Xu W. Exposure to the Great Famine in Early Life and the Risk of Obesity in Adulthood: A Report Based on the China Health and Nutrition Survey. Nutrients. 2021;13(4). Epub 2021/05/01. doi: 10.3390/nu13041285. PubMed PMID: 33919739; PubMed Central PMCID: PMCPMC8070734.
19. Li C, Lumey LH. Exposure to the Chinese famine of 1959-61 in early life and long-term health conditions: a systematic review and meta-analysis. Int J Epidemiol. 2017;46(4):1157-70. Epub 2017/03/25. doi: 10.1093/ije/dyx013. PubMed PMID: 28338900.
20. National Institutes of Health, Managing Overweight and Obesity in Adults. 2013.
21. Xu X, Hall J, Byles J, Shi Z. Dietary Pattern Is Associated with Obesity in Older People in China: Data from China Health and Nutrition Survey (CHNS). Nutrients. 2015;7(9):8170-88. Epub 2015/09/26. doi: 10.3390/nu7095386. PubMed PMID: 26404368; PubMed Central PMCID: PMCPMC4586581.
22. Yang YX, Wang GY, Pan XC. China Food Composition Table 2009. Peking University Medical Press: Beijing, China2009. 4–191 p.
23. Chinese Center for Disease Control and Prevention. China Food Composition Table. Peking University Medical Press Beijing; 2009.
24. Zuo H, Shi Z, Yuan B, et al. Interaction between physical activity and sleep duration in relation to insulin resistance among non-diabetic Chinese adults. BMC public health. 2012;12:247. Epub 2012/03/30. doi: 10.1186/1471-2458-12-247. PubMed PMID: 22455464; PubMed Central PMCID: PMCPMC3342099.
25. Peng XZ. Demographic Consequences of the Great Leap Forward in Chinas Provinces. Popul Dev Rev. 1987;13(4):639-70. doi: Doi 10.2307/1973026. PubMed PMID: WOS:A1987M895600003.
26. Chinese Nutrition Society Obesity Prevention and Control Section, Chinese Nutrition Society Clinical Nutrition Section, Chinese Preventive Medicine Association Behavioral Health Section, Section CPMASaH. Expert Consensus on Obesity Prevention and Treatment in China. Chin Prev Med. 2022;23(5):321. doi: 10.16506/j.1009-6639.2022.05.001.
27. He W, Li Q, Yang M, et al. Lower BMI cutoffs to define overweight and obesity in China. Obesity (Silver Spring, Md). 2015;23(3):684-91. Epub 2015/02/04. doi: 10.1002/oby.20995. PubMed PMID: 25645003.
28. Jimenez-Chillaron JC, Isganaitis E, Charalambous M, et al. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes. 2009;58(2):460-8. Epub 2008/11/20. doi: 10.2337/db08-0490. PubMed PMID: 19017762; PubMed Central PMCID: PMCPMC2628621.
29. Stein AD, Lumey LH. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: The Dutch Famine Birth Cohort Study. Hum Biol. 2000;72(4):641-54. PubMed PMID: WOS:000089847500006.
30. Kaati G, Bygren LO, Pembrey M, Sjostrom M. Transgenerational response to nutrition, early life circumstances and longevity. European Journal of Human Genetics. 2007;15(7):784-90. doi: 10.1038/sj.ejhg.5201832. PubMed PMID: WOS:000247436300012.
31. Vagero D, Pinger PR, Aronsson V, van den Berg GJ. Paternal grandfather's access to food predicts all-cause and cancer mortality in grandsons. Nat Commun. 2018;9(1):5124. Epub 2018/12/13. doi: 10.1038/s41467-018-07617-9. PubMed PMID: 30538239; PubMed Central PMCID: PMCPMC6290014.
32. Soubry A. POHaD: why we should study future fathers. Environ Epigenetics. 2018;4(2). doi: ARTN dvy007
10.1093/eep/dvy007. PubMed PMID: WOS:000449457000005.
33. Mu R, Zhang X. Why does the Great Chinese Famine affect the male and female survivors differently? Mortality selection versus son preference. Economics and human biology. 2011;9(1):92-105. Epub 2010/08/25. doi: 10.1016/j.ehb.2010.07.003. PubMed PMID: 20732838.
34. Gomez-Verjan JC, Barrera-Vázquez OS, García-Velázquez L, Samper-Ternent R, Arroyo P. Epigenetic variations due to nutritional status in early-life and its later impact on aging and disease. Clinical genetics. 2020;98(4):313-21. Epub 2020/04/05. doi: 10.1111/cge.13748. PubMed PMID: 32246454.
35. Wells JC. Environmental quality, developmental plasticity and the thrifty phenotype: a review of evolutionary models. Evol Bioinform Online. 2007;3:109-20. Epub 2007/01/01.

PubMed PMID: 19461971; PubMed Central PMCID: PMCPMC2684123.
36. Kuzawa CW. Fetal origins of developmental plasticity: are fetal cues reliable predictors of future nutritional environments? American journal of human biology : the official journal of the Human Biology Council. 2005;17(1):5-21. Epub 2004/12/22. doi: 10.1002/ajhb.20091. PubMed PMID: 15611967.
37. Drake AJ, Liu L. Intergenerational transmission of programmed effects: public health consequences. Trends in endocrinology and metabolism: TEM. 2010;21(4):206-13. Epub 2009/12/17. doi: 10.1016/j.tem.2009.11.006. PubMed PMID: 20005734.
38. Kaspar D, Hastreiter S, Irmler M, Hrabé de Angelis M, Beckers J. Nutrition and its role in epigenetic inheritance of obesity and diabetes across generations. Mammalian genome : official journal of the International Mammalian Genome Society. 2020;31(5-6):119-33. Epub 2020/05/01. doi: 10.1007/s00335-020-09839-z. PubMed PMID: 32350605; PubMed Central PMCID: PMCPMC7368866 of interest.
39. Aiken CE, Tarry-Adkins JL, Ozanne SE. Transgenerational Developmental Programming of Ovarian Reserve. Scientific reports. 2015;5:16175. Epub 2015/11/04. doi: 10.1038/srep16175. PubMed PMID: 26525600; PubMed Central PMCID: PMCPMC4630792.
40. Holliday R. Epigenetics: a historical overview. Epigenetics. 2006;1(2):76-80. Epub 2007/11/14. doi: 10.4161/epi.1.2.2762. PubMed PMID: 17998809.
41. Portha B, Grandjean V, Movassat J. Mother or Father: Who Is in the Front Line? Mechanisms Underlying the Non-Genomic Transmission of Obesity/Diabetes via the Maternal or the Paternal Line. Nutrients. 2019;11(2). Epub 2019/01/27. doi: 10.3390/nu11020233. PubMed PMID: 30678214; PubMed Central PMCID: PMCPMC6413176.
42. Heijmans BT, Tobi EW, Stein AD, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(44):17046-9. Epub 2008/10/29. doi: 10.1073/pnas.0806560105. PubMed PMID: 18955703; PubMed Central PMCID: PMCPMC2579375.
43. Tobi EW, Lumey LH, Talens RP, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Human molecular genetics. 2009;18(21):4046-53. Epub 2009/08/07. doi: 10.1093/hmg/ddp353. PubMed PMID: 19656776; PubMed Central PMCID: PMCPMC2758137.

Figure Legends

Figure 1 Fitted trajectories for body mass index (BMI) of adult offspring by parental exposure to famine

Offspring more than 30 years excluded from the fitting due to their unbalanced distributions by parental exposure status.

The BMI (kg/m^2) trajectories fitted by a linear mixed effect model with fixed effect given by a cubic spline for age (years), and with participant-specific random intercept. All p values for interaction with age<0.05.

F0: unexposed fathers, F1:fetal-exposed fathers, F2: childhood-exposed fathers; M0: unexposed mothers, M1: fetal-exposed mothers, M2: childhood-exposed mothers; P0M0, neither parents exposed to famine, P0M1, maternal exposed only, P1M0, paternal exposed only, P1M1: both parents exposed to famine.

Figure 2 Fitted trajectories for waist circumference (WC) of adult offspring by parental exposure to famine

Offspring more than 30 years excluded from the fitting due to their unbalanced distributions by parental exposure status.

The WC (cm) trajectories fitted by a linear mixed effect model with fixed effect given by a cubic spline for age (years), and with participant-specific random intercept. All p values for interaction with age<0.05.

F0: unexposed fathers, F1:fetal-exposed fathers, F2: childhood-exposed fathers; M0: unexposed mothers, M1: fetal-exposed mothers, M2: childhood-exposed mothers; P0M0, neither parents exposed to famine, P0M1, maternal exposed only, P1M0, paternal exposed only, P1M1: both parents exposed to famine.

Figure 3 Associations of famine exposures of F1 parents with the risk of overweight and central obesity in F2 offspring whose fathers and mothers were both born in 1955-1966

[a] P0M0, neither parents exposed to famine; P0M1, maternal exposed only; P1M0, paternal exposed only; P1M1: both parents exposed to famine. [b] p0m1: unexposed father and fetal-exposed mother, p1m0: fetal-exposed father and unexposed mother, p1m1: both parents fetal-exposed to famine, p1m2: fetal-exposed father and childhood-exposed mother, p2m0: childhood-exposed father and unexposed mother, p2m1: childhood-exposed father and fetal-exposed mother, p2m2: both parents childhood-exposed to famine.

All OR (95%CI) derived from multivariable-adjusted generalized linear mixed-effects models with a spline for the age-outcome relation adjusted for sex and birth year of offspring, maternal and paternal age and BMI at the interview closest to birth of their F2 offspring, and additionally for birth year of mothers in analysis by paternal exposure and for birth year of fathers in analysis by maternal exposure.

Online Supporting Information

Table S1 Characteristics of F1 parents at the interview closest to birth of their F2 offspring classified by exposure to the Chinese Great Famine.

Table S2 Associations of famine exposure of parents with body measurements of adult offspring by sex

Table S3 Sensitivity analyses examining associations between famine exposure of F1generaion and body measurements of F2 offspring in rural areas or areas suffering severe famine

Table S4 Sensitivity analyses examining associations between famine exposures of F1 parents and risk of obesity in F2 offspring in rural areas or areas suffering severe famine

Table S5 Sensitivity analyses examining associations between famine exposures of F1 parents and the risk of redefined overweight in F2 offspring

Table S6 Sensitivity analyses examining associations of famine exposure of F1 parents with body measurements of F2 offspring surveyed at least twice during adulthood

Figure S1 Flow chart for selection of study subjects

Figure S2 Classification of F2 offspring by famine exposure status and timing of F1 parents

Figure S3 Odds ratios and 95% confidence intervals of parental famine exposures with the risk

of overweight of adult offspring by sex

Figure S4 Odds ratios and 95% confidence intervals of parental famine exposures with the risk of central obesity of adult offspring by sex