The fetal origins of adult disease, the evidence and mechanisms
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Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine

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Peter D Gluckman
Mark A Hanson
Tessa J Roseboom
**ABSTRACT**

**Introduction:** We previously showed that maternal undernutrition during gestation is associated with increased metabolic and cardiovascular disease in the offspring. Also, we found increased neonatal adiposity among offspring of women who themselves had been undernourished prenatally. In the present study we investigated whether these transgenerational effects have led to altered body composition and poorer health in adulthood in the grandchildren.

**Subjects:** The adult offspring (F2) of a cohort of men and women (F1) born around the time of the 1944-45 Dutch famine. We approached the F2 through their parents. Participating F2s (n = 360, mean age 37 yrs) completed an online questionnaire.

**Results:** Adult offspring of prenatally exposed fathers had higher weight and BMI than offspring of prenatally unexposed fathers (+4.9 kg, p = 0.03; + 1.6 kg/m², p = 0.006). No such effect was found for offspring of prenatally exposed mothers. We observed no differences in adult health between the F2 generation groups.

**Conclusions:** Offspring of prenatally undernourished fathers, but not mothers, were heavier and more obese than offspring of fathers and mothers who had not been undernourished prenatally. We found no evidence of transgenerational effects of grandmaternal undernutrition during gestation on health of this relatively young group, but the increased adiposity in the offspring of prenatally undernourished fathers may lead to increased chronic disease rates in the future.
INTRODUCTION

Human and animal studies have shown that the fetal environment can affect the health of an individual throughout the life-course. This is thought to reflect programming, the process by which the same genotype can give rise to several phenotypes depending on the early environment. These programming effects may even be transmitted across generations. Feeding rats a protein restricted diet during gestation not only resulted in higher blood pressure and endothelial dysfunction in the offspring, but also in the grand-offspring\(^1\). Low protein diet during pregnancy led to insulin resistance in the adult male and female F2 offspring\(^2\)\(^-\)\(^4\). There is evidence suggesting that the glucose metabolism of the F3 generation is also affected by F0 undernutrition\(^5\). In mice, maternal general undernutrition during gestation led to reduced birth weight, impaired glucose tolerance and obesity in the F1 and F2 generation in a gender specific manner\(^6\).

Studies reporting on transgenerational effects of prenatal undernutrition in humans are scarce. A historical study of three generations in Overkalix, Sweden, reported that limited food supply of the grandparents influenced grandchildren’s later mortality and disease risk in a sex-specific manner, partly operating exclusively through the paternal line\(^7\). We have previously reported that individuals exposed to the Dutch famine of 1944-45 in utero have increased rates of cardiovascular disease, type 2 diabetes and breast cancer. The first indications of a potential transgenerational effect of prenatal famine exposure described that women prenatally exposed to the Dutch famine had slightly smaller babies than unexposed women\(^8\), but later reports from the same group described that first born babies of women exposed to famine in early gestation were heavier at birth\(^9\).

We have previously reported that prenatal famine exposure affects adult health later in life and that the effects of prenatal famine exposure may not be limited to the immediately succeeding generation. We found increased neonatal adiposity and poorer health among offspring of women who themselves had been exposed to famine prenatally\(^10\). This study, however, was based on parents’ recall of their offspring’s size at birth and later health, which may have led to a level of inaccuracy. In the study reported here we contacted the offspring directly to measure their body composition and investigate their health.

METHODS

Participants and selection
The Dutch Famine Birth Cohort consists of 2414 men and women born as term singletons in the Wilhelmina Gasthuis, a local hospital in Amsterdam, between 1 November 1943 and 28 February 1947. The selection procedures and loss to follow up until 2002 have been described in detail elsewhere\(^11\)\(^-\)\(^12\). Cohort members were eligible for participation in this study if they lived in the Netherlands on September 1\(^{st}\), 2008 and if their address was known to us. From 2002 on,
31 persons had died, 6 had emigrated, 11 had an unknown address and 8 had requested their address to be removed from our database. A total of 1371 eligible individuals were invited to participate. The study was approved by the local Medical Ethics Committee and carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent.

**Exposure to famine**

The official daily food rations for the general population of 21 years and older were used to define exposure to famine\(^\text{13}\). A person was considered to be prenatally exposed to famine if the average daily food-ration of the mother during any 13-week period of gestation contained less than 1000 calories. Based on this definition, babies born between 7 January 1945 and 8 December 1945 had been exposed in utero. People born before 7 January 1945 and conceived and born after 8 December 1945 were considered as unexposed to famine in utero and acted as control groups.

**Generations**

We studied two generations. F1 were the men and women from the Dutch famine birth cohort, born between November 1943 and February 1947. F2 were the offspring of F1 men or women.

**Data collection**

Information about the mother, the course of the pregnancy and the size of the baby at birth (F1) was extracted from medical birth records\(^\text{12}\). Previously, at age 58, F1 participants visited the clinic or were seen at home where F1 weight was measured with Seca scales or Tefal portable scales and height using a fixed or portable stadiometer. We asked them about the birth weight, birth length and gestational age at delivery of their children (F2)\(^\text{10}\).

In the current study, all F2 participants were asked to give information concerning their medical history, lifestyle and children. Data were collected by means of a standardized questionnaire which was filled out at home by the participants either on a paper or using a web-based form. The questionnaire included questions on height, weight, smoking, alcohol consumption and exercise behavior. We obtained information about symptoms or a history of cardiovascular, pulmonary, psychiatric and metabolic disease and medication use. The questions concerning cardiovascular disease included the Rose questionnaire\(^\text{14}\). Questions were combined to achieve categories relating to cardiovascular disease, pulmonary disease, hay fever, eczema, cholesterol, diabetes and hypertension. For each condition, questions were phrased as “has a doctor ever diagnosed (condition)” or “has a doctor ever prescribed medication for (condition)”.

**Statistical methods**

We used linear regression for continuous variables and logistic regression for dichotomous variables to compare (grand) maternal, birth and adult outcomes of those exposed and those unexposed to famine during gestation and also to compare offspring of these groups. To take into account the correlation of characteristics between siblings, we used mixed models to analyze the
association between F1 famine exposure during different stages of gestation and F2 birth and health characteristics. To deal with missing values, we used multiple imputation. We adjusted for possible confounding factors, such as F2 age, sex, birth weight and F1 BMI and weight. We report the F2 characteristics stratified according to F1 sex. We used SPSS 19.0 (Chicago, IL, USA) for all analyses.

RESULTS

In the eligible population of 1371 cohort members (F1), 483 F2s were willing to participate in the current study. Of these, 360 (74.5%) completed the questionnaire. The mean age at participation was 37 years (range 18 to 47 years). In total, 135 males and 225 females participated. Birth weight or gestational age did not differ between F1 participants and non-participants (p > 0.8).

F2 of F1 exposed men were 2.1 years younger than offspring from unexposed F1 men (Table 1). Birth characteristics were not different between F2 offspring of the exposed and unexposed F1 men. F2 of F1 exposed women had a higher ponderal index at birth compared to F2 of unexposed F1 women (Table 1). Exposed F1 men and women were comparable to unexposed F1 men and women with regards to anthropometric measures at age 58.

Table 1 Characteristics of F2 participants according to F1 gender.

<table>
<thead>
<tr>
<th></th>
<th>F1 male</th>
<th>F1 female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
<td>Unexposed</td>
</tr>
<tr>
<td>N</td>
<td>52</td>
<td>99</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>42</td>
<td>35*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.9</td>
<td>36.0*</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3351</td>
<td>3342</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>50.5</td>
<td>49.4</td>
</tr>
<tr>
<td>Ponderal index (kg/m³)</td>
<td>27.6</td>
<td>26.3</td>
</tr>
<tr>
<td>Adult length (m)</td>
<td>1.76</td>
<td>1.74</td>
</tr>
<tr>
<td>Adult weight (kg)</td>
<td>78.8‡</td>
<td>73.5</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>25.2‡</td>
<td>23.8</td>
</tr>
</tbody>
</table>

*p<0.05
‡ p<0.05 after correction for age and sex of F2
*geometric mean and SD
**F2 adult body composition**

**BMI**

Offspring of exposed F1 fathers had a higher BMI than offspring of unexposed F1 fathers (+1.6 kg/m², 95%CI 0.5 to 2.6) after adjusting for F2 sex and age (p 0.006). Adding F2 birth weight and F1 BMI to the model did not change the association (+1.5 kg/m², 95%CI 0.4 to 2.5, p 0.005). There was no effect of maternal F1 exposure to famine on the BMI of the offspring (unadjusted -0.69 kg/m², 95%CI -3.5 to 2.1, p 0.36; adjusted -0.60 kg/m², 95%CI -1.7 to 0.5, p 0.30).

**Weight**

Offspring of exposed F1 fathers were heavier than offspring of unexposed F1 fathers (+4.9 kg 95%CI 0.8 to 9.1, p 0.03) after adjustment for F2 sex and age. This effect remained when adjustments were made for F2 birth weight and F1 weight (+4.5 kg, 95%CI 0.9 to 8.1, p 0.01). Offspring of F1 famine exposed mothers were not heavier than F2 of unexposed mothers (unadjusted -1.7 kg, 95%CI -5.5 to 2.0, p 0.36; adjusted -0.7 kg, 95%CI -4.5 to 3.1, p 0.72).

**F2 self-reported health**

We did not find differences in the prevalence of cardiovascular and pulmonary disease, hay fever, eczema, elevated cholesterol, diabetes or hypertension between offspring of men and women exposed to famine during gestation and offspring of unexposed men and women (all p>0.1) (Table 2).
Table 2 Prevalence of F2 self-reported disease* according to F1 gender

<table>
<thead>
<tr>
<th>F2 of F1 men</th>
<th>F1 exposed</th>
<th>F1 unexposed</th>
<th>all</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>52</td>
<td>99</td>
<td>151</td>
</tr>
<tr>
<td>Cardiovascular %</td>
<td>5.9</td>
<td>2.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Pulmonary %</td>
<td>9.8</td>
<td>8.2</td>
<td>8.7</td>
</tr>
<tr>
<td>Hay fever %</td>
<td>19.6</td>
<td>30.6</td>
<td>26.8</td>
</tr>
<tr>
<td>Eczema %</td>
<td>25.5</td>
<td>24.5</td>
<td>24.8</td>
</tr>
<tr>
<td>Cholesterol %</td>
<td>0.0</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Diabetes %</td>
<td>0.0</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Hypertension %</td>
<td>3.9</td>
<td>2.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F2 of F1 women</th>
<th>exposed</th>
<th>unexposed</th>
<th>all</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>106</td>
<td>103</td>
<td>209</td>
</tr>
<tr>
<td>Cardiovascular %</td>
<td>1.9</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Pulmonary %</td>
<td>4.9</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Hay fever %</td>
<td>20.6</td>
<td>18.0</td>
<td>19.3</td>
</tr>
<tr>
<td>Eczema %</td>
<td>19.4</td>
<td>24.0</td>
<td>21.7</td>
</tr>
<tr>
<td>Cholesterol %</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Diabetes %</td>
<td>1.0</td>
<td>0.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Hypertension %</td>
<td>3.9</td>
<td>5.0</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* defined as answering confirmative to questions phrased as “has a doctor ever diagnosed” or “has a doctor ever prescribed medication for” the different conditions.

**DISCUSSION**

In this study we found that offspring of fathers that had been exposed to famine prenatally were heavier and had a higher BMI than offspring of unexposed fathers. This effect remained after adjustment for birth weight and paternal weight and BMI. We could not demonstrate a transgenerational effect on health among offspring of prenatally exposed men or women.

These findings fit within the growing body of evidence that transgenerational non-genomical inheritance specifically takes place in the paternal line. For instance, embryonic exposure to the endocrine disruptor vinclozolin increased a variety of adult onset diseases in the subsequent generations specifically through the paternal line15,16. Dietary exposures have also been shown to produce transgenerational effects through the male line; in Sprague-Dawley rats, high fat diet consumption of fathers induced impaired glucose tolerance and insulin secretion in their female offspring17. In mice, paternal low protein diet induced altered expression of genes involved in lipid and cholesterol metabolism in liver of the offspring, compared to offspring of control fed
male mice. These expression differences were thought to be the result of alterations of the epigenome\textsuperscript{18}.

There is evidence that prenatal exposure to the Dutch famine induced epigenetic alterations in the F1 generation that persist throughout life\textsuperscript{19,20}. It is unknown whether these epigenetic alterations are transmitted to the next generation, and whether they are sex-specific so that they could explain the transgenerational effects that we found only in the paternal line. Although epigenetics is a very likely mechanism explaining transgenerational effects in model organisms, in humans other mechanisms may also play a role.

The transgenerational effects we found may also have been transmitted through environmental factors such as food preferences and physical activity. Although the transgenerational effects reported here were also seen after adjustment for paternal BMI and weight, parental obesity is known to be associated with the level of overweight and obesity in children\textsuperscript{21} and it significantly alters the risk of obesity in adulthood\textsuperscript{22}. Parents are responsible for the quality and availability of the food in the home, and their food habits will be adopted by their children\textsuperscript{23}. Children from obese or overweight families have been shown to have a higher preference for fatty foods, a lower liking of vegetables and lower physical activity than children from lean families\textsuperscript{24}. We have reported previously that people who were conceived during the Dutch famine were twice as likely to consume a high-fat diet and to have a tendency to be less physically active\textsuperscript{25}. Transgenerational propagation of unhealthy lifestyle patterns may have contributed to the increased weight and BMI of F2 of exposed F1 men.

Previously, we found that participants who were themselves exposed to famine prenatally, rated their offspring’s health more often as poor than did unexposed participants\textsuperscript{10}. We set out this study to investigate the self-reported health of the F2 offspring, but we could not demonstrate any effects of F1 famine exposure in utero on the health of the F2 generation. A number of methodological issues may explain the fact that we did not find an effect on F2 health. The studied group consisted of only 360 people, which limits the power to detect an effect. The statistical power to detect effects was further reduced since the mean age of the F2 participants was 37 years, which is relatively young when studying the prevalence of chronic disease. Also, our analyses are based on self-reported health questionnaires which are a fairly crude measure of health.

In conclusion, we did not find a transgenerational effect of prenatal famine exposure on grand-offspring’s health in this study, but we found increased weight and BMI among F2 of in utero famine exposed men. These results warrant further follow up of the health of the F2 as they age, since their increased adiposity may predispose them to increase disease risk later on. Also, they suggest that transmission through the paternal line may occur, future studies in this cohort will investigate mechanisms that may elucidate the biological processes that underlie these findings.
REFERENCE LIST


2. Benyshek DC, Johnston CS, Martin JF. Post-natal diet determines insulin resistance in fetally malnourished, low birthweight rats (F1) but diet does not modify the insulin resistance of their offspring (F2). Life Sci. 2004;74:3033-3041.


