Prenatal exposure to the Dutch famine and glucose tolerance and obesity at age 50
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Chapter 4

MATERIAL AND METHODS:
THE DUTCH FAMINE BIRTH COHORT STUDY
The Dutch Famine Birth Cohort Study is a study of persons born in the Wilhelmina Gasthuis in Amsterdam shortly before, during and after the Dutch Famine of 1944-45. Data on prenatal visits, size at birth and infant feeding were abstracted from the medical birth records. Information on glucose tolerance, obesity and other cardiovascular risk factors were measured at age 50. In the first section of this chapter the study design is described. The subsequent sections describe the birth records, population register procedure, the home interview and the visit to the Special Research Unit of the Academic Medical Center in Amsterdam. The definition of prenatal exposure to famine has already been discussed in chapter 2.

1. Study design

The possibility of studying long-term effects of prenatal exposure to famine on health and diseases later in life depended on three conditions. Firstly, the availability of birth records of persons born shortly before, during and shortly after the Dutch Famine in Western Holland. Secondly, the possibility to trace people from birth to current address. The third condition was the willingness of the subjects born around the famine period to participate in a study.

Study design

All persons with birth records who were born around the Dutch Famine in 1944-45 in Western Holland were candidates to be included in a new study. For the new Dutch famine study it was decided to continue with the Wilhelmina Gasthuis Famine Birth Cohort Study of dr Lumey. That study had shown that the birth records of the obstetrics department of the Wilhelmina Gasthuis were still kept in the city archives of Amsterdam. The population register in Amsterdam was able to retrieve current addresses of persons born in Amsterdam and women of this cohort were already participating in the study.

For the design of the new study some changes had to be made, which are described in this chapter. The study period was extended with persons born one year before the famine or conceived one year after the famine. The in- and exclusion criteria were changed as premature born children were excluded. The most important difference was that men were included in the cohort.

Study participants

All men and women who were born between November 1, 1943 and February 28, 1947 in the Wilhelmina Gasthuis in Amsterdam were eligible candidates to be included in the study. This population consists of three groups. Group I were all children born between November 1, 1943 and October 31, 1944. Group II were all children born between November 1, 1944 and February 28, 1946. Group III were all children born between March 1, 1946 and February 28, 1947.

Sample size

The power calculations were based on the 120-minute glucose concentrations after a standard oral glucose load as the main outcome variable. Estimates of the variance of the 120-minute glucose levels were derived from the pooled of data the three previous MRC Southampton studies (Hertfordshire, Preston and Sheffield).
It was estimated that a study in about 700 subjects would have a 93% power at a significance level of 5% to detect an increase of 10% in the 120-minute glucose in 150 persons prenatally exposed to famine at some part during gestation compared to 250 unexposed people.

Based on this power calculation we planned to perform an oral glucose tolerance test in 700 men and women, 450 prenatally exposed persons and 250 unexposed persons. With a participation rate of 70% we had to interview 1000 men and women at their homes.

2. The birth records

The Wilhelmina Gasthuis was the main hospital in Amsterdam at the time of the famine. At that time, women either delivered at home or were referred to a maternity ward of a hospital for social or medical reasons. In those years the University Maternity Clinic of the Wilhelmina Gasthuis had an average of 1500 deliveries a year. This was around 10% of the total deliveries in Amsterdam. During the War the Wilhelmina Gasthuis continued to operate. In 1983 the Wilhelmina Gasthuis moved to the Academic Medical Center.

Birth Ledgers
A short record of every delivery at the maternity ward of the Wilhelmina Gasthuis was written down in the birth ledgers (partusboeken) of the University clinic. We used these birth ledgers to identify all eligible persons born in the described period. The birth ledgers contained only very general information on date of birth, name of the parents and identification numbers. Supplementary general information of the mother and child was retrieved from the admission ledgers (opnameboeken) of the maternity ward. The detailed information about mother and child was recorded in the medical records of the mother. Children who were admitted to the hospital after birth at home were not included in this study. We also did not include abortions and immature babies born before 28 weeks. We retrieved the birth ledgers of 5425 men and women born between 1 November 1943 and 28 February 1947.

Singletons and live born
We excluded all multiple pregnancies and stillborn children. Pregnancies and birth weights of twin and triplets are not comparable with singletons. The information from the birth ledgers was used to identify a twin or stillborn baby. In total we excluded 168 twins and triplets and 181 stillborn children (Table 1).

Random Sample
For reasons of study efficiency we took a random sample of persons unexposed to famine in prenatal life. We mainly based the number of the random sample on the power calculations. We took a random sample of 650 live born singletons of group I (born between November 1, 1943 and October, 31 1944) and 650 live born singletons of group III (born between March 1, 1946 and February 28, 1947). Together with the 1380 live born singletons of group II (born between November 1, 1944 and February 28, 1946) the study cohort consisted of a total of 2680 children (Table 1).
Table 1: The Dutch Famine Study cohort born at the Wilhelmina Gasthuis in Amsterdam.

<table>
<thead>
<tr>
<th>Group</th>
<th>Date of birth</th>
<th>Born</th>
<th>Twin</th>
<th>Stillborn</th>
<th>Singleton/Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Nov 1, 1943-Oct 31, 1944</td>
<td>1386</td>
<td>50</td>
<td>31</td>
<td>650</td>
</tr>
<tr>
<td>II</td>
<td>Nov 1, 1944-Feb 28, 1946</td>
<td>1487</td>
<td>49</td>
<td>58</td>
<td>1380</td>
</tr>
<tr>
<td>III</td>
<td>Mar 1, 1946-Feb 28, 1947</td>
<td>2552</td>
<td>69</td>
<td>92</td>
<td>650</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5425</td>
<td>168</td>
<td>181</td>
<td>2680</td>
</tr>
</tbody>
</table>

Medical records
For this group of 2680 children the more detailed medical records retrieved from the city archives (gemeentearchief) of Amsterdam where the obstetric records of the Wilhelmina Gasthuis are currently kept. These medical records provided detailed information on prenatal care, parity, date of last menstrual period and children’s size at birth. Children were excluded from the study if the medical records were missing (n=27, 1%).

Prematurely born children were also excluded from the study because we wanted to exclude the possible effects of short duration of gestation on the studied outcomes. We defined prematurity as gestational age less than 259 days (37 weeks). The gestational age was calculated from the last menstrual period and from the obstetricians’ estimation at the first prenatal visit in combination with the obstetricians’ examination of the child at birth. Exclusion based on last menstruation period only could be incorrect due to the ammenorhea during the famine. We excluded 239 (8.9%) babies because the gestational age at birth was less than 37 weeks, calculated either from the date of the last menstrual period (n=206) or by the obstetrician’s estimations (n=33). In total, the study sample consisted of 2414 babies born at term.

Measurements of the medical records
The medical records contained general information of the mother, including age and marital status. At the first prenatal visit in the hospital, which mostly took place at the end of the second trimester of gestation, information was collected on reproductive and medical history. At the first prenatal visit, the date of birth was estimated from the last menstrual period and the height of the fundus. Several pelvic measurements were taken. During the following prenatal visits, every 2 weeks in the last trimester, blood pressure and maternal weight were recorded.

We abstracted mother’s weight measured at the last prenatal visit (considered to be missing if not measured within two weeks before birth). We also recorded mother’s weight measured as close as possible to the start of the third trimester (considered to be missing if not measured between 36 and 160 days before birth). Maternal weight gain in the third trimester of gestation was estimated as the difference between these weights multiplied by the ratio of trimester duration (13 weeks) and the time interval between the weight measurements. Maternal body height was not measured, and therefore we abstracted the mother’s interspinous distance (distance between the anterior superior iliac spines) as a proxy for skeletal size. A report was made of the delivery and maternal diseases after delivery were described.

The information of the child consisted of the date of birth, gender, birth weight, crown-to-heel length, three head circumferences and six head diameters. The ponderal
index was calculated as the birth weight divided by the cube of the crown-to-heel length. In our analyses we did not use the head circumference because there was a sudden increase of on average 0.5 cm in all head circumference measurements in the second half of 1946 that did not paralleled by increased head diameters. Head circumference was subsequently estimated by: $\pi \times (\text{biparietal diameter} + \text{occipitofrontal diameter}) \times 0.5$. Placental weight was not measured but the placental length, width and thickness were recorded. Placental area was estimated as $\pi \times \text{length} \times \text{width} \times 0.25$. Placental length refers to the longest placental diameter and placental width to the longest perpendicular diameter. Congenital disorders and other child abnormalities were recorded.

After a mean stay of 12 days after birth of the child the mother and child were discharged from hospital. At discharge type of infant feeding and weight of the child were recorded. Type of feeding was classified as exclusive breastfeeding, breast and bottle-feeding or exclusive bottle-feeding.

**Selective Fertility**

The number of births in Amsterdam in the last three months of 1945 was 50% (2024 versus 4043) lower than in the last three months of 1944 (Table 2). After September 1944, many women in the reproductive age did not have reproductive hormone cycles. Decline in libido, oligospermy and impotence were reported in men.\textsuperscript{25} Choices of couples not to conceive can also explain the reduced birth rates.\textsuperscript{6} It is possible that the women who became pregnant were a selective portion of each social class.\textsuperscript{7} Generally it was estimated that during the famine period fertility decreased by 50% compared to pre-famine levels.\textsuperscript{28}

**Social economic status**

Shortly after liberation it was assessed that in Amsterdam 57% of the population were of poor social economic class: 36% of the population were of middle social economic class and only 7% of the population were of high social economic class.\textsuperscript{2} The proportion of mothers from lower and middle social economic background was also relatively high in the Wilhelmina Gasthuis.\textsuperscript{1} Hospital deliveries for patients living under poor housing and/or social conditions and for unmarried mothers were encouraged. However little is known about differences in selection patterns for deliveries in this hospital during the famine period.

Information about the occupation of the head of the family was abstracted from the Admission card (Cribkaart) of the central admission records of the Wilhelmina Gasthuis. These admission cards are currently kept at the medical archive of the Academic Medical Center. The cards contain information on marital status and occupation of the head of the family which was in most cases the occupation of the husband. The occupation of the head of the family was later dichotomized into a manual or non-manual profession as had been done in the study by Stein and Susser.\textsuperscript{6}
### Table 2: Live born deliveries in Amsterdam and in the Wilhelmina Gasthuis during the period 1944-1946.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Total born</th>
<th>W.G. born</th>
</tr>
</thead>
<tbody>
<tr>
<td>1944:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-Mar 1944</td>
<td>1976</td>
<td>1811</td>
<td>3787</td>
<td>347</td>
</tr>
<tr>
<td>April-June 1944</td>
<td>1952</td>
<td>1854</td>
<td>3806</td>
<td>359</td>
</tr>
<tr>
<td>July-Sep 1944</td>
<td>2060</td>
<td>1961</td>
<td>4021</td>
<td>387</td>
</tr>
<tr>
<td>Oct-Dec 1944</td>
<td>2072</td>
<td>1971</td>
<td>4043</td>
<td>368</td>
</tr>
<tr>
<td>Total 1944</td>
<td>8094</td>
<td>7626</td>
<td>15720</td>
<td>1332</td>
</tr>
<tr>
<td>1945:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-Mar 1945</td>
<td>2126</td>
<td>2052</td>
<td>4178</td>
<td>295</td>
</tr>
<tr>
<td>April-June 1945</td>
<td>2067</td>
<td>1951</td>
<td>4018</td>
<td>286</td>
</tr>
<tr>
<td>July-Sep 1945</td>
<td>1552</td>
<td>1514</td>
<td>3066</td>
<td>280</td>
</tr>
<tr>
<td>Oct-Dec 1945</td>
<td>1076</td>
<td>948</td>
<td>2024</td>
<td>194</td>
</tr>
<tr>
<td>Total 1945</td>
<td>6821</td>
<td>6465</td>
<td>13286</td>
<td>1055</td>
</tr>
<tr>
<td>1946:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-Mar 1946</td>
<td>2270</td>
<td>2154</td>
<td>4424</td>
<td>499</td>
</tr>
<tr>
<td>April-June 1946</td>
<td>3514</td>
<td>3321</td>
<td>6835</td>
<td>745</td>
</tr>
<tr>
<td>July-Sep 1946</td>
<td>3176</td>
<td>2931</td>
<td>6107</td>
<td>613</td>
</tr>
<tr>
<td>Oct-Dec 1946</td>
<td>2641</td>
<td>2558</td>
<td>5199</td>
<td>546</td>
</tr>
<tr>
<td>Total 1946</td>
<td>11601</td>
<td>10964</td>
<td>22265</td>
<td>2403</td>
</tr>
</tbody>
</table>

Sources: Statistic Bureau of the Amsterdam 1944-1946. Total number of births from the records of the Wilhelmina Gasthuis Amsterdam.

### 3. Population register

In the Netherlands all residents have to be registered in the town were they are currently living. An almost complete registration of the Dutch population is ensured because a number of official documents, like passports and driving licenses, can only be obtained if one is registered.

The current addresses of persons born in the Wilhelmina Gasthuis were traced by the employees of the population register of Amsterdam. They started with a computerized search on the tapes of persons living in Amsterdam since the eighties. The result was that one quarter of our study sample was found on tape. For the persons who were not found on tape a hand search for the personal registration card (persoonskaart) in the population register archives was done.

When the personal registration card was found in the archives its was checked if the person was still living in Amsterdam. When the person was not living in Amsterdam anymore a request was sent to the city where the person had moved to. This was repeated until the current address was found. Not all new cities were...
registered on the personal registration cards. In case of death the date and place of death were recorded. In case of emigration date and country of emigration were recorded and checked for remigration. When a personal card was not found in the archives further follow-up was not possible.

When the current address was found the population register of Amsterdam sent an information letter about the research project to the person. If the person refused to cooperate with the study, by sending back a refusal card to the population register, we only received the current city of residence. If the person did not send back the refusal card we received the complete address from the population register.

The population register traced 2155 (89%) of the 2414 persons of our study group. Among the 259 who could not be traced were 130 without person cards and 129 who had moved away from Amsterdam without leaving the name of the new city of residence. Of the 2155 persons who were found, 265 (12 %) had died, 199 (9%) had emigrated from the Netherlands and 164 (8%) refused cooperation. The population register provided us with the current addresses of the remaining 1527 persons of which 566 (37%) were still living in Amsterdam.

4. Interview

Subjects were contacted to participate in the study by mail. After agreement an appointment for a home visit was made by a research nurse. The home visit lasted on average one hour and a half and consisted of an interview and blood pressure, weight and height measurements. During the interview information was collected on four subjects: general information, medical information, life style factors and reproductive history. The interview was, where possible, based on validated questionnaires used in other epidemiological studies in the Netherlands or the UK. Non responders received a second invitation for participation after three months. Seven trained research nurses carried out the interviews, which took place between January 1995 and July 1996.

Mean travel distance was 35 kilometer with a maximum of 80 kilometers from the Academic Medical Center in Amsterdam. We asked 1018 subjects to be interviewed. Of these 912 (90%) people (424 men and 488 women) agreed. Mean birth weights of the 912 interviewed and 106 non-responders did not differ from the rest of the 2414 subjects of the total study group.

Measurements during interview

Blood pressure was measured at home twice before and twice after the interview with a Profilomat automatic device. Weight and height were measured twice with a SECA weight scale and with a portable stadiometer.

General information

Information was recorded about marital status, number of children and number of persons currently living in the house. To measure the socio-economic status the education and occupational history of the subject and of the partner were collected. One research nurse coded all occupations with the 5 digital occupation classification scheme of the Central Bureau of Statistics of 1992. Current socioeconomic status was determined from the person’s or partner’s occupation code, whichever was higher, according to the ISEI-92 index. This index reflects the education needed for an occupation and the income generated by it, on a scale ranging from 16 for the lowest to
Information on the education and profession of the father and mother of the participant was obtained using the questions from the Dutch GLOBE study. A relatively high number of participants did not have information about their father. Therefore social economic status at birth was estimated for most of the participants from the admission cards, as described before.

Medical Information
Subjects were asked if a doctor had ever told them that they had - or still have - a myocardial infarction, stroke, diabetes, hypertension, hypercholesterolemia, hyperthyroidism, lung diseases or cancer. If one of these questions was positively answered then the age at diagnosis and current treatment were recorded. The MRC Hertfordshire questionnaire and the EPIC questionnaire were used as sources. Angina pectoris was assessed with the Dutch translation of the Rose questionnaire, which has been used in the Zutphen Study. Family history of heart disease, stroke, diabetes mellitus and hypertension were recorded including cause and age of death of the parents of the participants. Complaints of the joints or back pain were collected. Psychological treatment, if any, was recorded. Currently prescription medication was recorded including dose and usage. The prescriptions were coded by category of medication and grouped as effecting blood glucose, blood pressure or lipid profile. Current general health and the comparison of current health to last year health were measured by the self rated health question. Subjective health was measured with six COOP-WONCA cards.

Life style factors
Smoking, alcohol consumption, physical activity and weight history questions were based on the questions used in the EPIC study. Current or past smoking, number of cigarettes and number of years one was smoking cigarettes were recorded. Alcohol consumption was measured by number of glasses a week. Alcohol consumption was separately asked for beer, wine, soft alcohol and strong alcohol beverages. Physical activities during work and leisure time were recorded in terms of type of activities, duration and months per year, including activities like gardening and walking. Also the daily number of climbed stairs was recorded. Weight history, number of attempts to loose more than 5 kg, current slimming activities and the recalled weight around age 20, before childbearing, were also recorded.

Reproductive history
For women age at menarche and month and year of last menstruation were recorded. Detailed information was collected on the reproductive history of women, including number of pregnancies, children, miscarriages and abortions. For each of their children, information was collected of birth weight and length, pregnancy duration and current health and level of education. For the mother information was recorded, if applicable, about years of birth control pills, and whether the uterus and/or ovaries had been extirpated.

5. Visit to the special research unit
During the home visits all participants were invited to the Special Research Unit of the Academic Medial Center in Amsterdam for more detailed research. All participants who visited the special research unit signed an informed consent form. During the visit
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several detailed measurements were done after an overnight fast. Fasting blood samples were taken, an oral glucose tolerance test (OGTT) was performed, blood pressure and antropometric indicators were measured. Also an ECG and lung function tests were performed. The participants took an overnight urine collection with them to the visit. The Dutch EPIC food questionnaire, which was filled in at home, was also checked on completeness. The visits were always scheduled in the morning, 5 days a week including a Saturday. Six trained research nurses did the measurements and the nurses of the Special Research Unit performed the OGGT. The visits took place between March 1995 and August 1996.

After the clinical visit the participants and their general physicians received a letter with the main results on diabetes mellitus, blood pressure, cholesterol levels and ECG abnormalities.

In total 741 (81%) of the interviewed participants visited the Academic Medical Center, 356 males and 385 females. In 718 participants fasting blood samples were taken. In the remaining 23 persons only non-fasting blood samples were taken because they were treated for diabetes mellitus.

The oral glucose tolerance test and lipid profile
The overnight fast started at 22,00 hour the previous night. In the morning between 8,00 and 10,00 fasting blood samples were taken for plasma glucose, insulin, proinsulin, 32-33-split proinsulin, lipid and lipoprotein concentrations and other measurements. After the fasting blood samples had been taken the prepared glucose drink, 75 glucose in water solution, was given to the participants who had to drink it within 5 minutes. After a 5-minute rest the blood pressure was taken twice. Blood samples were taken for plasma glucose and insulin concentrations at 30 min, 60 min and 120 minutes after the start of the glucose load. Between the blood samplings lung function, ECG and antropometry were measured. After the 120-minute blood samples blood pressure was measured and participants could eat and return home.

The OGGT was performed following WHO protocols. Blood samples were taken by venapunction. After each blood collection samples were centrifuged within 30 minutes and pipetted in 2ml aliquots tubes. The blood and urine samples were brought to the general lab at the AMC for analysis or frozen minus 80 degree Celsius for later analyses.

The OGGT test had to be stopped because of extreme nausea in 2 cases and because of vasovagal reaction in 2 cases. In another 10 subjects the 120-minute blood sample could not be obtained because venapunction failed. In total for 702 participants fasting and 120-minute glucose blood samples could be obtained.

All glucose samples were analysed at the Department of General Clinical Chemistry laboratory of the Academic Medical Center. Plasma glucose was measured by the glucose dehydrogenase method (H747, Merk 12194, The Netherlands). HbA1c was measured with HPLC method Biorad. Insulin, proinsulin and 32-33 plasma split proinsulin were measured at the Department of Clinical Biochemistry at Addenbrooks Hospital in Cambridge UK. Plasma insulin was measured by the immunoenzymoetric assay (Medgenix Diagnostics SA, B2-6220 Fleuris, Belgium). Intact proinsulin by two-side microtitre plate based time resolved fluorescence assay (Defia) and 32-33 split proinsulin by a two-side immunometric assay. Total cholesterol, LDL-cholesterol, Triglyceride, Apolipoprotein A and apolipoprotein B were analyzed at the Special Laboratory of Endocrinology and Radiochemy “ENDO laboratory” of the Academic Medical Center. Cholesterol was measured with the cholesterol oxidase method
Triglycerides was measured full enzymatic (H747 BM 195797 + BM 1127764).

In the analyses the WHO definitions for defining non-insulin-dependent diabetes mellitus and impaired glucose tolerance were used. The fasting insulin and 32-33 split proinsulin concentrations were used as measures of insulin resistance because they are thought to reflect the degree of exposure of the pancreatic β cell to glucose and, therefore, to increased demands for insulin. We have used the 30 minute relative insulin increment log ([30 minute glucose-fasting insulin]/ 30 minute glucose) as a measure of insulin deficiency.

**Antropometric measurements**

Weight was measured in light clothes without shoes to the nearest 100g with a SECA scale. Height was measured with the head in the horizontal Frankfort plane using a fixed stadiometer to the nearest 0.5 cm. Circumferences were measured with a flexible tape to the nearest 0.1 cm. Waist circumference was measured midway between the lateral iliac crest and the lowest rib. Hip circumference was measured at the widest part of the hips, usually at the level of the great trochanter. The maximum head circumference was measured on the most anterior protuberance of the forehead and the most posterior protuberance of the occiput. All antropometric measurements were done twice and the mean was used in the analyses. We calculated adult body mass index as the weight divided by the square of height. During the study time measuring instruments were calibrated monthly. The nurses were trained on the antropometric measuring techniques before and during the study period.

**References**

Material and Methods


