Metabolic consequences of prenatal exposure to the Dutch famine

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Metabolic consequences of prenatal exposure to the Dutch famine

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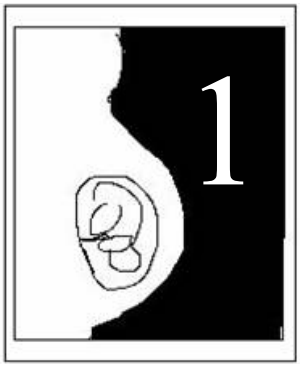

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Introduction

Susanne de Rooij

Introduction

Being too fat is rapidly on the way of becoming a bigger threat to public health worldwide than hunger and underfeeding. While it is estimated that 815 million people in our world suffer from undernourishment, over 1 billion people are estimated to be overweight.[1,2]

One of the major health problems related to being overweight is type 2 diabetes. The number of people suffering from type 2 diabetes is increasing very fast, especially in developing countries. In the year 2000 there were 171 million people with diabetes and it is predicted that there will be 366 million by the year 2030, of whom three quarters will be living in developing countries.[3]

It is obvious that the expansion in the prevalence of obesity is related to the enormous increase in the occurrence of diabetes. However, the exact underlying cause and the fact that especially developed countries are touched by the raising diabetes epidemic remain subject of debate in the scientific community.

One of the first hypotheses trying to explain the upcoming diabetes epidemic is the '*thrifty genotype*' hypothesis.[4] In the prehistoric times of hunting and gathering, people who possessed genes that promoted efficient deposition of body fat in times of food abundance had a better chance of surviving in times of poor food availability. The selective survival of these 'thrifty genes' is suggested to have led to the expansion of obesity and diabetes in our current time of plentiful food supply and a sedentary life style, where there is no need to store fat so efficiently but the thrifty genes prescribe to do so. The drawbacks of this theory are that the responsible genes remain largely elusive and that a purely genetic mechanism cannot explain why the dramatic increase in diabetes occurred within a generation or two.

The discovery of a very strong association between low birth weight and type 2 diabetes in a population of men and women born in Hertfordshire, England, led Barker and Hales to postulate the '*thrifty phenotype*' hypothesis as an alternative explanation for the rapid expansion of diabetes worldwide.[5] The '*thrifty phenotype*' hypothesis postulates that type 2 diabetes results from undernutrition during fetal life. The fetus is suggested to adapt its structure and physiology in response to poor nutritional circumstances, resulting in a diminished capacity to produce insulin and in insulin resistance. These properties offer a survival benefit in a postnatal environment with little food, but predispose to the development of glucose intolerance and type 2 diabetes in a postnatal environment where there is plenty food. The '*fetal origins of disease*' concept can explain why diabetes is so rapidly spreading over the world, especially in developing countries. Our diet is changing fast from a low calorie, low fat diet to a high calorie, high fat diet.

Fetal nutrition, however, remains limited due to intergenerational constraints on placental growth. The apparent contradiction between undernutrition during fetal life followed by overnutrition in adult life may be leading to diabetes in later life. This contrast is most prominently seen in developing countries, which undergo a transition from chronic malnutrition to adequate nutrition. Countries such as India, which is predicted to have the most inhabitants with diabetes worldwide by the year 2030, exemplify this.[3]

The '*fetal origins*' hypothesis has gained quite some ground during the last decade. A problem is that it is hard to obtain scientific evidence in humans to substantiate the theory. It is obviously not ethical to undernourish pregnant mothers and see what happens to the health of their offspring. Therefore, birth weight, which is a proxy for the fetal environment, has been the main object of study in the expanding field of the fetal origins of adult disease. This is not exactly ideal, because birth weight is the result of a range of different factors, including maternal, placental and nutritional ones. An alternative way to study the effects of prenatal undernutrition is to look at people who were in utero during periods of famine.

The Dutch famine

The Dutch Hungerwinter was a five month period at the end of World War II during which the urban western part of the Netherlands was struck by a severe famine. The Dutch famine was a consequence of a number of cascading events. While the southern part of the Netherlands was already liberated by the allied forces, liberation of the northern part came to a halt when Operation Market Garden, aimed at gaining control of the bridge across the Rhine at Arnhem, failed. In order to support the Allied offensive, the Dutch government in exile arranged a strike of the national railways to hamper movement of German troops. In turn, as a reprisal, the German administration put an embargo on all food transports. Early November 1944 this embargo was partially lifted by allowing transport of food across water. At that time, however, an unusually early and severe winter had set in and all canals were frozen. Food stocks in the western cities of the Netherlands ran out rapidly and rations soon fell below 1000 calories per person a day in late November 1944. At the height of the famine from December 1944 to April 1945 the official daily rations varied between 400 and 800 calories. Children younger than one year were relatively protected, as their official daily rations never fell below 1000 calories.

The undernutrition during the winter of 1944-1945 was accompanied by severe cold, a shortage in fuel and a lack of personal items such as soap, clothing and shoes. Approximately 30,000 people starved to death. In May 1945 the allied forces

succeeded in liberating the western part of the Netherlands and the famine finally came to an end.

The Dutch famine birth cohort study

The Dutch famine was of course a humanitarian disaster, but a number of characteristics of the famine make it a unique opportunity to study the consequences of prenatal undernutrition for health in later life. Usually, studying effects of prenatal famine in humans is difficult, because the period of undernutrition lasts longer than gestation. The Dutch famine, however, struck a population that was well nourished before the famine and the food situation improved swiftly after the liberation. The period of undernutrition was thus restricted to five months, which also allows us to study the gestation specific effects of undernutrition. Furthermore, detailed information on the weekly rations of people living in Amsterdam is available. Birth records were kept on babies born in the Wilhelmina Gasthuis in Amsterdam and these men and women could be retrieved by the Dutch population registry. A total of 2,414 of them comprises the Dutch famine birth cohort.

A first round of data collection in the Dutch famine birth cohort at the age of 50 years yielded some remarkable results. Exposure to famine in early gestation was associated with an excess in dyslipidemia,[6] more obesity in women,[7] a higher prevalence of coronary heart disease[8] and breast cancer,[9] and an increased negative self perception of health.[10] Exposure to famine in mid gestation was associated with more obstructive airways disease[11] and microalbuminuria.[12] Exposure to famine during late and mid gestation was associated with higher 2h glucose concentrations, while 2h insulin concentrations were raised in all exposure groups.[13] All of these findings were independent of size at birth.

Aim and outline of this thesis

A second round of data collection at the age of 58 years was aimed at establishing the previously reported associations between prenatal exposure to famine and various clinical outcomes and at elucidating the pathophysiology of these associations. This thesis is an attempt to meet these goals focusing on the altered glucose and insulin metabolism in men and women exposed to famine in utero.

In ***chapter 2*** the association between prenatal exposure to the Dutch famine and glucose/insulin homeostasis at age 58 is described. To assess whether exposure to famine during gestation leads to a more rapid progression of glucose intolerance, a comparison is made between glucose tolerance at ages 50 and 58.

Recently, evidence has been found suggesting that the fetal environment may alter the effects of specific genes. In ***chapter 3*** a possible interaction between prenatal famine exposure and the Pro12Ala polymorphism of the *PPAR-γ2* gene on glucose and insulin metabolism is investigated.

The next three chapters address possible mechanisms mediating the association between prenatal exposure to famine and impaired glucose tolerance: impaired insulin secretion, insulin resistance and increased activity of the hypothalamic-pituitary-adrenal (HPA) axis. In ***chapter 4*** results of an intravenous glucose tolerance test (IVGTT), which was performed in a subsample of the Dutch famine birth cohort, are presented. The IVGTT allows estimation of the relative roles of insulin secretion and insulin resistance in impaired glucose tolerance.

Results of a physiological stress test (i.e. a dexamethasone suppression/$ACTH_{1-24}$ stimulation test), again performed in the subsample, are described in ***chapter 5*** and results of a psychological stress protocol, performed in the whole study group, are described in ***chapter 6***.

The metabolic syndrome is a constellation of metabolic risk factors and has often been suggested to have its origins in utero. In ***chapter 7*** the relationship between famine exposure in utero and the prevalence of the metabolic syndrome is evaluated.

Chapter 8 contains a review bringing together all of the findings presented in this thesis.

Finally, in ***chapter 9*** the findings from the the Dutch famine birth cohort study are put into an evolutionary perspective and consequences of this for future research are discussed.

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Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine

Susanne de Rooij, Rebecca Painter, Tessa Roseboom, David Phillips, Clive Osmond, David Barker, Michael Tanck, Robert Michels, Patrick Bossuyt, Otto Bleker

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Abstract

Aims/hypothesis

People who were small at birth have an increased risk of type 2 diabetes in later life. People who were in utero during the Dutch famine had decreased glucose tolerance and raised insulin concentrations at age 50. We aimed to evaluate whether prenatal famine exposure leads to more rapid progression of impaired glucose/insulin homeostasis with increasing age.

Methods

We performed an OGTT in 702 men and women at age 50 and in 699 men and women at age 58, all born as term singletons immediately before, during or after the 1944-1945 Dutch famine.

Results

People who had been exposed to famine in utero had significantly higher 120-min glucose concentrations at age 58 compared with people who had not been exposed to famine (difference=0.4 mmol/l, 95% CI 0.1 to 0.7, adjusted for sex and BMI). Glucose tolerance deteriorated between the age of 50 and 58. The unadjusted 120-min glucose concentrations rose by 0.2 mmol/l (95% CI 0.0 to 0.4), while 120-min insulin concentrations had increased by 64 pmol/l (95% CI 48 to 82). There were no differences in the rates of glucose and insulin level increase between the famine-exposed group and the unexposed group (p = 0.28 for the difference in increase in glucose concentrations and p = 0.09 for insulin concentrations).

Conclusions/interpretation

Although we confirmed that undernutrition during gestation is linked to decreased glucose tolerance, the effect does not seem to become more pronounced at age 58 as compared with age 50.

Introduction

People who were small at birth have an increased risk of type 2 diabetes in later life.[1-7] One interpretation of this association is that an adverse intrauterine environment permanently impairs glucose homeostasis, primarily by inducing insulin resistance.[8,9] This interpretation is strongly supported by animal studies, which have consistently shown that experimental undernutrition during gestation leads to impaired glucose homeostasis.[10] The Dutch famine was a period of extreme food shortage in the west of the Netherlands that occurred during the last 5-6 months of World War II. The famine offers a unique opportunity to study the effects of prenatal undernutrition on health in later life. In 1998, we found the first direct evidence in humans that exposure to undernutrition during gestation reduced glucose tolerance in later life.[11]

The prevalence of type 2 diabetes and IGT increases with age, mainly as a result of the increase in BMI, insulin resistance and a fall in insulin secretion.[12-14] It is not yet known whether the age-related decline in glucose tolerance is increased in people who experienced an adverse intrauterine environment. In animal experiments the effects of prenatal undernutrition on glucose tolerance have been shown to increase with age. Rats that were undernourished during gestation showed a decrease in glucose tolerance between 3 and both 12 and 15 months of age.[15,16] In humans the prenatal influences on blood pressure have been shown to increase with age.[17,18] The present study was designed to assess glucose tolerance in the famine cohort at age 58 and to evaluate whether the previously demonstrated effect of prenatal famine exposure on glucose tolerance in the Dutch famine birth cohort progresses with aging.

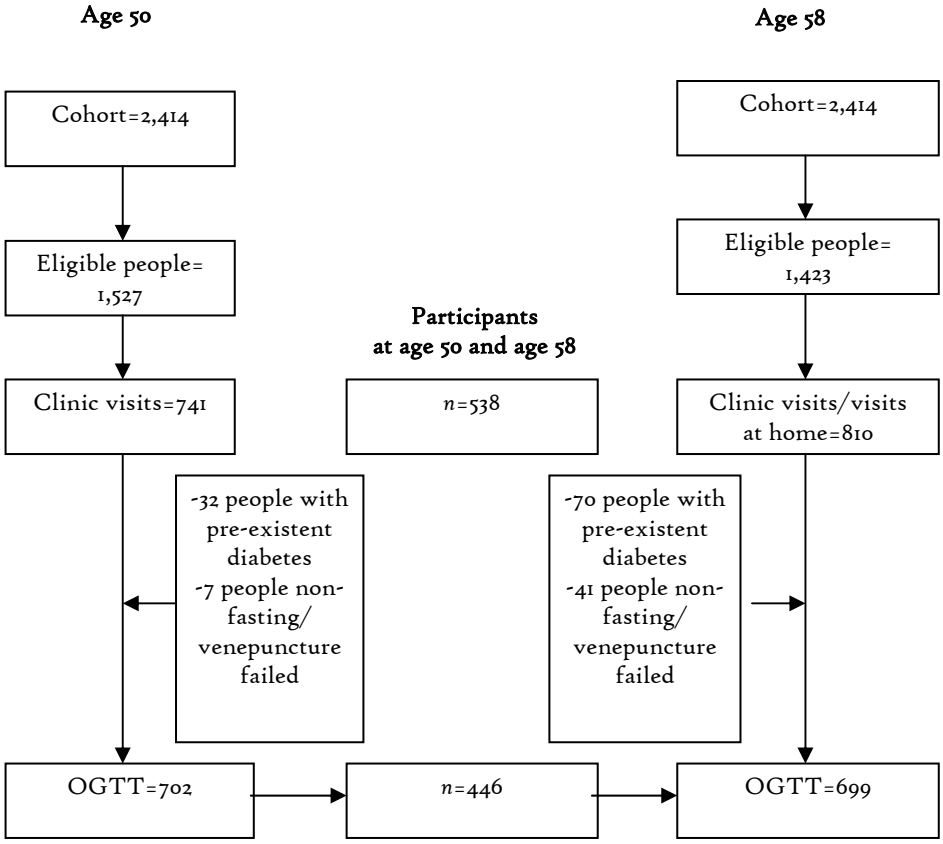
Subjects and methods

Participants

The participants were selected from the Dutch famine birth cohort (see Fig. 1). This cohort consists of 2,414 men and women born as term singletons between 1 November 1943 and 28 February 1947 in the Wilhelmina Gasthuis in Amsterdam. The selection procedures of the cohort have been described in detail elsewhere.[11] Of the cohort, 1,423 (59%) were still living in the Netherlands and their current address was known. A total of 810 people (57%) agreed to participate and underwent medical examination and a standardised interview. Two-thirds of this group (538 people, 66%) had also participated in the previous study at age 50.

People who agreed to participate had mean birthweights similar to those of eligible people who did not participate (3,357g vs. 3,349g, p = 0.78). Informed consent was obtained from all participants. The local medical ethics committee had approved the study. The study was carried out in accordance with the Declaration of Helsinki.

Figure 1 Participants selected from the Dutch famine birth cohort



Exposure to famine

We defined the famine period based on the official daily rations for the general population older than 21 years. These rations were about 7,560 kJ/day in December 1943 and gradually decreased to about 5,880 kJ in October 1944. On 26 November 1944 the rations fell below 4,200 kJ and after 12 May 1945 they rose above 4,200 kJ again. In June 1945 rations were over 8,400 kJ. The rations during the famine did not apply to children younger than 1 year, who were relatively protected. Their official daily rations were always higher than 4,200 kJ, which is adequate according to The Oxford Nutrition Survey.[19]

We considered fetuses to be exposed to famine if the average daily rations of the mother during any 13-week period of gestation were below 4,200 kJ. Babies born between 7 January 1945 and 8 December 1945 were thus exposed. We defined periods of 16 weeks each to differentiate between those who had been exposed in late gestation (born between 7 January and 28 April 1945), in mid-gestation (born between 29 April and 18 August 1945) and in early gestation (born between 19 August and 8 December 1945). Babies born before 7 January 1945 and babies conceived and born after 8 December 1945 were considered as unexposed to famine in utero and acted as control group.

Study parameters

Birth measurements and information about the health and status of the mother were taken from the medical birth records.[11] Trained nurses carried out the medical examinations and the interview. Methods for OGTT and anthropometry at age 50 have been described in detail elsewhere.[11] At age 58, a standard 75-g OGTT was performed after an overnight fast. People with pre-existent diabetes, defined as taking oral or injected glucose-lowering medication, were excluded from the test. Plasma glucose concentrations were measured by standardised enzymatic photometric assay on a Modular P Analyzer (Roche, Basel, Switzerland) and plasma insulin concentrations by immunoluminometric assay on an Immulite 2000 Analyzer (Diagnostic Product Corporation, Los Angeles, CA, USA). We measured height with a fixed or portable stadiometer, weight with SECA and portable Tefal scales and waist and hip circumferences with a flexible tape measure. We interviewed all participants and asked them about their socio-economic status, their medical history, lifestyle and use of medication, using standardized questions. Current socio-economic status was coded according to ISEI-92, which is based on the person's, or their partner's occupation, whichever status was highest.

Statistical methods

In order to make the results comparable to those of our previous study, we defined IGT as a 120-min glucose concentration of 7.8-11.0 mmol/l and type 2 diabetes as a 120-min glucose concentration of >11.0 mmol/l. Logarithmic transformations were applied to glucose, insulin and BMI values, because they had skewed distributions. We used linear regression analysis to determine the effect of prenatal exposure to famine at different stages of gestation on glucose tolerance at age 58. To detect a possible age-related deterioration in glucose tolerance, we only included data of subjects participating at age 50 as well as at age 58, using repeated-measures analysis. We first calculated differences between the exposed and unexposed groups.

Then differences were calculated between unexposed subjects and subjects prenatally exposed to famine in late, mid- or early gestation. We adjusted for maternal and birth characteristics, sex, BMI, smoking and socio-economic status. For the last three variables, measurements at age 50 and age 58 were both included in the repeated-measures analysis.

Results

Study group characteristics

We were able to perform a standard OGTT on 699 of the 810 people (86%) asked to participate in the study at age 58. Seventy of the 810 people had to be excluded from the OGTT, because they had pre-existing diabetes (see Table 1 for distribution over study groups). The test was not performed on another 41 people due to the fact that they had not adhered to fasting instructions (n = 7) or because of difficulties in venepuncture (n = 34). For 446 of these 699 people, data on 120-min glucose and insulin values at age 50 were available. Of the 699 participants, 291 (42%) had been exposed to famine in utero. The response rates were similar in the famine-exposed and control groups. Table 1 shows that mothers exposed in late and mid-gestation weighed significantly less at the last prenatal visit than mothers who were unexposed to famine. Mothers exposed during late gestation gained almost no weight in the last trimester of pregnancy. Babies exposed to famine in late and mid-gestation were lighter and shorter than unexposed babies. They also had smaller heads and placentas.

Famine exposure and plasma glucose and insulin concentrations at age 58

People who had been exposed to famine in utero had significantly higher plasma glucose concentrations 120 min after administration of the standard oral glucose load than unexposed people (Table 2). After adjusting for sex and BMI, 120-min glucose concentrations were 0.4 mmol/l (95% CI 0.1 to 0.7) higher among the exposed people than among unexposed people. For people exposed during late gestation the difference compared with unexposed people was 0.3 mmol/l (95% CI -0.1 to 0.8), for people exposed during mid-gestation 0.4 mmol/l (95% CI 0.0 to 0.9) and for people exposed during early gestation 0.4 mmol/l (95% CI −0.1 to 1.0). After adjustment for sex and BMI, 120-min insulin concentrations were 27 pmol/l (95% CI 0 to 58) higher among exposed than among unexposed people. The difference for people exposed during late gestation compared with unexposed people was 25 pmol/l (95% CI −12 to

68). The difference for people exposed during mid-gestation was 22 pmol/l (95% CI -18 to 68) and for people exposed during early gestation 37 pmol/l (95% CI −12 to 97).

Table 1 Maternal, birth and adult characteristics according to timing of prenatal exposure to the Dutch famine

	Exposure to famine						
	Born before	In late gestation	In mid gestation	In early gestation	Conceived after	All (±SD)	*n*
General							
Number	215	122	104	65	193	699	-
Proportion of men	.48	.43	.39	.40	.51	.46	699
Maternal characteristics							
Age at delivery (years)	29	31 [b]	29	27	29	29 (±6)	699
Proportion of primiparous women	.37	.21 [b]	.32	.43	.35	.33	699
Proportion doing manual labour	.82	.71	.71	.61 [b]	.70	.73	566
Weight gain 3rd trimester (kg)	2.8	0.1 [b]	4.4 [b]	5.0 [b]	3.5	2.9 (±2.9)	486
Weight at last antenatal visit (kg)	66.4	62.8 [b]	63.6 [b]	69.2	69.2	66.4 (±8.7)	613
Birth outcomes							
Gestational age (days)	284	283	286	288	286	285 (±11)	598
Birth weight (g)	3387	3206 [b]	3181 [b]	3470	3497	3363 (±474)	699
Birth length (cm)	50.5	49.6 [b]	49.6 [b]	50.8	50.8	50.3 (±2.1)	691
Head circumference (cm)	32.8	32.5 [b]	32.2 [b]	32.9	33.3	32.8 (±1.6)	690
Ponderal index (kg/m³)	26.2	26.1	26.0	26.4	26.7	26.3 (±2.4)	691
Placental area (cm²)	297	269 [b]	269	276	275	280 (±68)	594
Adult characteristics							
Body mass index (kg/m²)[a]	27.6	27.8	27.5	27.3	28.5	27.9 (±1.2)	698
Current smoker (%)	22	25	26	32	24	25	696
Current SES (ISEI-92)	46	50 [b]	50 [b]	45	47	47 (±1)	689
Proportion of diabetic patients excluded from OGTT	.10	.09	.10	.08	.07	.09	810

[a]Geometric means and SD

[b]$p < 0.05$ for difference from people who were not prenatally exposed to famine

SES Socio-economic status

Effects of mother's weight and birthweight

The weight of the mother at the last prenatal visit, birthweight, birth length and head circumference were all inversely related to 120-min glucose and insulin concentrations.

Adjusted for sex and BMI the 120-min glucose concentrations increased by 0.8% (95% CI 0.5 to 1.1) per kilogram decrease in mother's last weight, 13.2% (95% CI 7.4 to 19.2) with each kilogram decrease in birthweight, 2.6% (95% CI 1.4 to 3.9) with each centimetre decrease in birth length and 1.9% (95% CI 0.3 to 3.7) with each centimetre decrease in head circumference, all adjusted for sex and adult BMI. The 120-min insulin concentrations increased by 17 pmol/l (95% CI 10 to 23) per kilogram decrease in mother's last weight. Additional adjusting for birthweight showed that the effect of prenatal exposure to famine on 120-min glucose concentrations was larger than could be explained by the famine-related differences in birthweight. After adjustment for sex, BMI and birthweight, 120-min glucose concentrations were 0.2 mmol/l (95% CI -0.1 to 0.6) higher among the exposed than among unexposed people. Adjusting for other possible confounding variables including maternal age at delivery, current smoking status, current socio-economic status or alcohol consumption had little effect on the association between famine exposure and 120-min glucose concentrations. The prevalence of IGT and type 2 diabetes based on the OGTT did not differ significantly between the exposed and the unexposed people.

Table 2 Means of plasma glucose and insulin concentrations and prevalences of IGT and type 2 diabetes according to timing of prenatal exposure to the Dutch famine

	Exposure to famine							
	Born before	In late gestation	In mid-gestation	In early gestation	Conceived after	All (±SD)	*n*	*p*
Fasting								
Glucose (mmol/l)[a]	5.6	5.5	5.5	5.6	5.6	5.6 (±1.1)	697	0.99
Insulin (pmol/l)[a]	57	59	55	58	57	57 (±1.8)	692	0.21
HbA1C (%)[a]	5.5	5.5	5.6	5.6	5.6	5.6 (±1.1)	692	0.81
120-minute								
Glucose (mmol/l)[a]	5.8	6.2	6.2	6.2	5.9	6.0 (±1.4)	678	0.02
Insulin (pmol/l)[a]	242	263	254	269	240	249 (±2.1)	672	0.06
Prevalence of diabetes based on OGTT (%)	5	5	5	6	4	5	678	0.43
Prevalence of IGT based on OGTT (%)	16	16	15	14	14	15	678	0.76

[a]Geometric means and SD

p values are for differences between exposed and non-exposed groups adjusted for sex and BMI

Effects of age

Table 3 shows the effects of age on progression of 120-min glucose and insulin concentrations in people who participated at age 50 as well as at age 58. Mean glucose concentrations after 120 min had increased by 0.2 mmol/l (95% CI 0.0 to 0.4). Mean insulin concentrations after 120 min had increased by 64 pmol/l (95% CI 48 to 82). A large part of the increase in 120-min glucose and insulin concentrations could be attributed to an increase in BMI. Mean BMI had increased by 1.1 units (95% CI 0.9 to 1.3). Adjusted for sex and BMI at both ages, the 120-min glucose concentrations between age 50 and 58 had increased non-significantly by 0.1 mmol/l (95% CI -0.1 to 0.2), while the adjusted 120-min insulin concentrations had increased by 51 pmol/l (95% CI 38 to 64). Exposure to famine in utero was not associated with an excess in age-related glucose tolerance deterioration (0.0 vs. 0.1 mmol/l for exposed vs. unexposed, p = 0.28), nor with an excess of age-related rise in insulin (39 vs. 58 pmol/l for exposed vs. unexposed, p = 0.09), nor an excess of age-related rise in BMI (1.0 vs. 1.2 unit for exposed vs. unexposed, p = 0.48) (all adjusted for sex and BMI).

Table 3 Progression of 120-min glucose and insulin levels and BMI between age 50 and age 58, according to timing of prenatal exposure to the Dutch famine

	Exposure to famine							
	Born before (CI)[c]	In late gestation (CI)[c]	In mid-gestation (CI)[c]	In early gestation (CI)[c]	Conceived after (CI)[c]	All (CI)[c]	*n*	*p*
n	135	80	67	43	121	446	-	-
Proportion of men (%)	52	47	42	43	49	48	446	-
120-min glucose (mmol/l)[a]	0.3 (0.0 to 0.6)	-0.1 (-0.6 to 0.2)	-0.1 (-0.5 to 0.3)	0.2 (-0.4 to 0.8)	-0.1 (-0.4 to 0.3)	0.1 (-0.1 to 0.2)	446	0.28
120-min insulin (pmol/l)[a]	71 (48 to 92)	35 (-1 to 66)	33 (-9 to 7)	54 (-2 to 98)	47 (21 to 70)	51 (38 to 64)	446	0.09
BMI (kg/m²)[b]	1.1 (0.5 to 1.6)	0.8 (-3.0 to 4.1)	1.3 (-0.3 to 2.8)	0.9 (-0.5 to 2.6)	1.3 (0.7 to 1.9)	1.1 (0.9 to 1.3)	446	0.48

[a]Estimated marginal means (adjusted for sex and BMI) for differences
[b]Estimated marginal means (adjusted for sex) for differences
[c]95% CI's of estimated marginal means for differences
p values are for differences between exposed and unexposed groups adjusted for sex and BMI

Table 3 shows that the relatively largest increases in plasma glucose and insulin concentrations occurred among people who were born before the famine compared with people conceived after the famine. The mean plasma glucose was 0.4 mmol/l higher (p = 0.04) and the mean plasma insulin was 24 pmol/l higher (p = 0.08). There was a small effect of birthweight on the increase in 120-min glucose concentrations between the age of 50 and 58.

For each kilogram decrease in birthweight, there was a 6% (95% CI 0 to 12) larger increase in 120-min glucose concentrations, adjusted for sex and BMI at both ages. Birthweight did not affect the increase in 120-min insulin concentrations.

Discussion

In this unique cohort study of subjects born before, during and after the Dutch famine, we found that undernutrition during gestation was associated with reduced glucose tolerance and raised insulin concentrations at age 58. This confirms our previous findings at age 50.[11] We found that both 120-min glucose and insulin concentrations were higher in people exposed to famine at any stage of fetal development than in unexposed people. Importantly, this effect was larger than could be explained by the lower birthweight of babies born during the famine and by the low weight gain of their mothers. As with our previous study, this association was independent of people's current BMI.[11]

This is a population-based study of men and women recruited from the original famine cohort and still living in the Netherlands. At this age a significant number of individuals have overt type 2 diabetes and related diseases, especially cardiovascular disease. Selective participation of the more healthy subjects in our cohort could have influenced our results. Although we maximised response rates by visiting and studying subjects in their homes if necessary, people with type 2 diabetes, cardiovascular disease, high cholesterol levels and high blood pressure were less likely to attend the survey clinic (data not shown). However, there was no evidence of a difference in response rates between those who were famine-exposed or non-exposed. Consequently, we do not think that selective participation has affected the relative differences in glucose and insulin levels among the exposed and the unexposed groups. It could, however, have affected our power to detect effects of famine on type 2 diabetes.

Glucose tolerance deteriorated between the age of 50 and 58. A large part of this decline could be attributed to an increase in BMI. The decline in glucose tolerance was not more marked in the famine-exposed groups than in the control groups (Table 3). People who were born before the famine had the relatively largest increase in 120-min glucose and insulin concentrations. A possible explanation is that these people experienced the famine during their first year of life, which may have affected their postnatal growth pattern. Reduced early growth has been shown to be associated with later IGT and type 2 diabetes.[20,21] Although infants were relatively protected during the famine, their official daily rations always being higher than

4200 kJ, we do not know whether the famine affected their growth.[19] Early mortality rates were highest for people born before the famine and the deaths were mainly related to undernutrition or infections, indicating that growth was probably also affected.[22]

We found that people with low birthweight had a greater age-related progression of glucose intolerance. A larger age-related progression of raised blood pressure has already been demonstrated for people with low birthweight, but to the best of our knowledge this is the first direct evidence of an age-related amplification of the effect of reduced fetal growth on glucose tolerance in humans.[17,18]

The effect of prenatal exposure to famine on glucose tolerance could be mediated through a number of mechanisms. Animal experiments indicate that undernutrition during gestation affects the development of the pancreas, which leads to an impaired function of the beta cell and consequently insulin deficiency.[23] In contrast, most human evidence points to the importance of insulin resistance. People who were thin at birth or had low birth weights were shown to be more insulin resistant as adults.[24,25] The raised insulin concentrations that we found in famine-exposed individuals (Table 2) are consistent with insulin resistance acting as a mediator of the effect of famine exposure on glucose tolerance. Increasing evidence suggests that skeletal muscle is a key site for programming of insulin resistance. Muscle is a major site of glucose uptake and associations between a low ponderal index and altered metabolism of adult skeletal muscle have been found.[26,27] Recently, an association between low birthweight and specific changes in muscle insulin-signalling protein expression was found.[28] Another mechanism that could explain the association between prenatal exposure to famine and later glucose intolerance involves the hypothalamic-pituitary-adrenal (HPA) axis. Maternal sheep and guinea pigs that were undernourished in late gestation gave birth to offspring in which HPA function was altered in adult life.[29,30] In humans, low birthweight is associated with elevated basal plasma cortisol concentrations and increased adrenocortical responsiveness to adrenocorticotrophin at adult age.[31,32] It has been hypothesized that undernutrition during gestation alters the setpoint of the HPA axis resulting in an increased activity and consequently an increased secretion of glucocorticoids, which is associated with glucose intolerance and insulin resistance .[33,34]

In summary, although we confirmed that poor nutrition in utero is related to decreased glucose tolerance in later life, the effect of prenatal exposure to the Dutch famine does not seem to become more pronounced at age 58 as compared with age 50. Progression of glucose intolerance was found to be related to famine exposure during the first year of life and to low birthweight.

This suggests that prenatal famine exposure, famine exposure during the first year of life and low birthweight may contribute to glucose intolerance by different mediating mechanisms.

Acknowledgements

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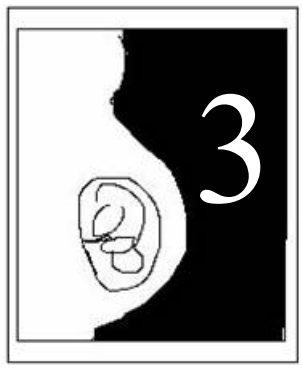

The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 gene on glucose/insulin metabolism interact with prenatal exposure to famine

Susanne de Rooij, Rebecca Painter, David Phillips, Clive Osmond,
Michael Tanck, Joep Defesche , Patrick Bossuyt,
Robert Michels, Otto Bleker , Tessa Roseboom

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Abstract

Objective

An adverse fetal environment may permanently modify the effects of specific genes on glucose tolerance, insulin secretion and insulin sensitivity. In the present study, we assessed a possible interaction of the peroxisome proliferator-activated receptor (PPAR)-γ2 Pro12Ala polymorphism with prenatal exposure to famine on glucose and insulin metabolism.

Research Design and Methods

We measured plasma glucose and insulin concentrations after an oral glucose tolerance test and determined the *PPAR-γ2* genotype among 675 term singletons born around the time of the 1944-1945 Dutch famine.

Results

A significant interaction effect between exposure to famine during mid-gestation and the *PPAR-γ2* Pro12Ala polymorphism was found on the prevalence of impaired glucose tolerance and type 2 diabetes. The Ala allele of the *PPAR-γ2* gene was associated with a higher prevalence of impaired glucose tolerance and type 2 diabetes but only in participants who had been prenatally exposed to famine during mid-gestation. Similar interactions were found for area under the curve for insulin and insulin increment ratio, which were lower for Ala carriers exposed to famine during mid-gestation.

Conclusions

The effects of the *PPAR-γ2* Pro12Ala polymorphism on glucose and insulin metabolism may be modified by prenatal exposure to famine during mid-gestation. This is possibly due to a combined deficit in insulin secretion, as conferred by pancreatic beta cell maldevelopment and carrier type of the Ala allele in the *PPAR-γ2* gene.

Introduction

The fetal origins hypothesis proposes that metabolic and cardiovascular disease originates through adaptations made by the fetus in response to an adverse fetal environment.[1] An adverse fetal environment may alter gene expression and lead to physiological or morphological phenotypes associated with disease.[2] Based on this hypothesis, one can expect the effects of polymorphisms associated with specific diseases to depend on the type of fetal environment. Size at birth, a marker of the fetal environment, has been shown to modulate the effects of a number of genetic polymorphisms.[3-6] In particular, there is accumulating evidence for an interaction between size at birth and the effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (PPAR)-γ2 gene.

PPAR-γ2 is one of three *PPAR-γ* isoforms and is a member of the nuclear hormone receptor subfamily of transcription factors, which regulate transcription of various genes.[7] *PPAR-γ* is implicated in adipocyte differentiation, regulating glucose and lipid homeostasis.[7] The *PPAR-γ* gene contains nine exons and spans >100 kb of genomic DNA on chromosome 3p25.[8,9] Of several mutations identified on the *PPAR-γ2* gene, the proline to alanine change in codon 12 of exon B is one.[10] The Ala allele reduces the transcriptional activity of *PPAR-γ* and may protect against type 2 diabetes compared with the more common Pro/Pro genotype.[11,12] In people with low birth weight, the Pro/Pro genotype has been shown to be associated with raised systolic blood pressure, increased insulin resistance, and elevated plasma insulin concentrations.[13,14] Because this association was not observed in people with normal birth weights, it may be concluded that the fetal environment interacts with the effects of the *PPAR-γ2* Pro12Ala polymorphism. Birth weight is, however, a summary measure of the fetal environment. The Dutch famine birth cohort study provides the opportunity to study the direct effects of a specific adverse fetal environment, namely maternal undernutrition. The Dutch famine birth cohort consists of people born as term singletons in the Wilhelmina Gasthuis in Amsterdam around the time of the Dutch famine. People who were exposed to famine during gestation had higher 120-min plasma glucose and insulin concentrations at age 50 compared with people prenatally unexposed to famine.[15] The purpose of the current study was to determine whether famine exposure during gestation interacts with the effects of the *PPAR-γ2* Pro12Ala polymorphism on glucose and insulin metabolism.

Research Design and Methods

Selection procedures

The Dutch famine birth cohort consists of 2,414 men and women born as term singletons in the Wilhelmina Gasthuis in Amsterdam between 1 November 1943 and 28 February 1947.[15] All 1,423 members of the cohort who lived in the Netherlands on 1 September 2002 and whose current address was available were invited to the hospital. Of the cohort of 1,423 eligible people, a total of 810 people agreed to participate in the study. The local medical ethics committee had approved the study, which was also carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Exposure to famine

Exposure to famine was defined according to the official daily food rations for the general population aged ≥21 years.[16] An individual 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 <1,000 calories. Based on this definition, babies born between 7 January 1945 and 8 December 1945 were exposed in utero. We delineated periods of 16 weeks each to differentiate between those who were exposed in late gestation (born between 7 January and 28 April 1945), in mid-gestation (born between 29 April and 18 August 1945), and in early gestation (born between 19 August and 8 December 1945). People born before 7 January 1945 and conceived and born after 8 December 1945 were considered as unexposed to famine in utero.

Study parameters

The medical birth records provided information about the mother, the course of the pregnancy, and the size of the baby at birth.[15] Trained nurses took all measurements and conducted a standardized interview. We measured height using a fixed or portable stadiometer and weight with Seca and portable Tefal scales. Information about socio-economic status, medical history, lifestyle, and use of medication was obtained in a standardized interview. We defined current socioeconomic status according to International Socioeconomic Index of Occupational Status 92, which is based on the participant's, or their partner's occupation, whichever status was highest.[17] After an overnight fast, we performed an oral glucose tolerance test (OGTT) with a standard load of 75 g. Venepuncture was performed at 0, 30, 60 and 120 min after the administration of the oral glucose load to assess plasma glucose and insulin concentrations. DNA material was extracted from the fasting blood sample. Participants with preexistent diabetes, defined as taking oral or injected antidiabetic medication, were excluded from the OGTT. Plasma glucose concentrations were

measured by standardizd enzymatic photometric assay on a Modular P analyzer (Roche, Basel, Switzerland) and plasma insulin concentrations by immunoluminometric assay on an Immulite 2000 analyzer (Diagnostic Product, Los Angeles, CA). The insulin assay sensitivity was 15 pmol/l. The Pro12Ala polymorphism of the *PPAR-γ2* gene was determined by the PCR restriction fragment-length polymorphism method. The following primers were used: forward primer 5'-CAAGCCCAGTCCTTTCTGTG-3' and reverse primer 5'-AGTGAAGGAATCGCTTTCCG-3'.

Statistical methods

Impaired glucose tolerance was defined as a 120-min glucose level between 7.8 and 11.0 mmol/l. Type 2 diabetes was defined as a 120-min glucose level of >11.0 mmol/l.[18] The ratio of the 30-min increment in insulin to 30-min increment in glucose concentrations was used as an index of insulin secretion.[19] Insulin resistance was estimated by homeostasis model assessment (HOMA-IR).[20] The area under the curve (AUC) was calculated for insulin, according to the following formula: [15*log (insomin)] + [30*log (ins30min)] + [45*log (ins60min)] + [30*log (ins120min)]/120. Logarithmic transformations were applied to glucose, insulin, insulin increment ratio, HOMA-IR, and BMI values because they had skewed distributions. Allele frequencies were estimated by gene counting, and departure from Hardy-Weinberg equilibrium was tested using a χ^2 test with one degree of freedom. Genotypes were treated as categorical variables. We used linear regression analysis to compare glucose and insulin concentrations and logistic regression to compare the prevalence of impaired glucose tolerance and type 2 diabetes between genotype groups and exposure groups. Possible interactions between the effects of prenatal famine exposure and the Pro12Ala polymorphism of the *PPAR-γ2* gene on glucose/insulin metabolism were assessed by adding an interaction term (genotype*exposure) to the regression equation. We adjusted for sex and BMI in all analyses. Additional adjustment was done for maternal and birth characteristics, smoking, and current socioeconomic status. We considered differences to be statistically significant if *P* values were ≤ 0.05.

Results

Genotypes of the *PPAR-γ2* Pro12Ala polymorphism were available for 772 of 810 participants. Sixty-two of 772 participants had to be excluded from participating in the OGTT because they had preexisting diabetes.

The test was not performed on another 35 participants due to the fact that they had not adhered to fasting instructions (n = 7) or due to difficulties in venepuncture (n = 28). Of 675 participants who underwent an OGTT and for whom a genotype was available, 280 (42%) had been exposed to famine in utero (Table 1). People with type 2 diabetes and people with impaired glucose tolerance were grouped together for analysis, because of the relative small number of people with type 2 diabetes based on the OGTT (31 cases).

Table 1 Maternal, birth and adult characteristics according to timing of prenatal exposure to the Dutch famine

	Exposure to famine						
	Born before	In late gestation	In mid-gestation	In early gestation	Conceived after	All	*n*
General							
n	208	116	103	61	187	675	-
Proportion of men (%)	47	41	40	40	52	46	675
Age	59	59	58	58	57	58 ± 1	675
Number of participants with Pro/Ala genotype	57	33	25	12	43	170	-
Number of participants with Ala/Ala genotype	3	1	4	2	5	15	-
Maternal characteristics							
Age at delivery (years)	29	31*	29	27	29	29 ± 6	675
Primiparous (%)	36	22*	21	43	35	33	675
Manual labour (%)	82	73	71	59*	69	73	549
Weight gain 3rd trimester (kg)	2.8	0.1*	4.4*	4.9*	3.5	2.9 ± 2.9	470
Weight at last antenatal visit (kg)	66.4	63.1*	63.6*	69.3	69.4	66.5 ± 8.6	593
Birth outcomes							
Gestational age (days)	285	284	286	288*	286	285 ± 11	580
Birth weight (g)	3393	3208*	3189*	3486	3494	3366 ± 474	675
Adult characteristics at age 58 years							
BMI (kg/m²)†	27.7	27.7	27.4	27.4	28.6	27.9 ± 1.2	675
Current smoking (%)	22	26	25	33*	23	24	673
Current socio economic status	48	52*	52*	47	49	50 ± 14	675

Data are means ± SD, except where given as numbers and percentages.
*Statistically significant difference ($P \leq 0.05$) compared with participants unexposed to famine in utero.
†Geometric means ± SD.

The prevalences of the Pro/Ala and the Ala/Ala genotypes were 25 and 2% respectively (Table 1). The frequency of the Ala allele was 0.148. The observed genotype frequencies were in Hardy-Weinberg equilibrium for all study groups. The Ala carriers were grouped together for analysis because of the low frequency of the Ala/Ala genotype. Excluding participants with the Ala/Ala genotype from analyses did not change the results (data not shown). The proportions of Ala carriers

did not significantly differ between the unexposed group (27%) and the groups exposed in late (29%), mid- (28%), and early gestation (23%).

Table 2 shows that there were no significant differences between participants carrying the Pro/Pro genotype and those carrying the Ala allele in terms of BMI, plasma glucose and insulin concentrations, the prevalence of impaired glucose tolerance and type 2 diabetes, AUC for insulin, insulin increment ratio, and insulin resistance. Participants who were prenatally exposed to famine had 120-min glucose concentrations that were 0.4 mmol/l (95% CI 0.1 to 0.7) higher and 120-min insulin concentrations that were 27 pmol/l (95 % CI 0 to 57) higher than the glucose and insulin concentrations of unexposed participants. The prevalence of impaired glucose tolerance and type 2 diabetes, AUC for insulin, insulin increment ratio, and HOMA-IR did not differ between participants exposed and participants unexposed to famine in utero.

Table 2 Geometric means ± SD for BMI, plasma glucose and insulin concentrations, and prevalence of impaired glucose tolerance and type 2 diabetes according to *PPAR-γ2* gene polymorphism

	Pro/Pro	**Ala**	*P*
n	490	185	
BMI (kg/m²)	27.9 ± 1.2	28.0 ± 1.2	0.64
OGTT			
Glucose 0 min (mmol/l)	5.6 ± 1.7	5.5 ± 1.7	0.19
Glucose 30 min (mmol/l)	8.6 ± 2.2	8.6 ± 2.2	0.74
Glucose 60 min (mmol/l)	8.2 ± 2.1	8.1 ± 2.1	0.43
Glucose 120 min (mmol/l)	6.0 ± 1.8	5.8 ± 1.8	0.11
Insulin 0 min (pmol/l)	57 ± 4.0	55 ± 4.0	0.29
Insulin 30 min (pmol/l)	297 ± 1.8	299 ± 1.9	0.95
Insulin 60 min (pmol/l)	386 ± 1.8	394 ± 1.8	0.74
Insulin 120 min (pmol/l)	254 ± 2.1	232 ± 2.2	0.12
Prevalence of impaired glucose tolerance and diabetes (%)	20.6	18.6	0.54
Area under curve insulin (pmol/l)	252 ± 1.7	252 ± 1.7	0.85
Insulin increment ratio	80 ± 2.4	84 ± 2.4	0.55
HOMA-IR index (mmol*pmol/l²)	14.2 ± 2.7	13.5 ± 2.6	0.21

P values for differences adjusted for sex, BMI, and prenatal exposure to famine during late, mid- and early gestation.

Table 3 shows that carriers of the Ala allele in the group exposed to famine during midgestation had a higher prevalence of impaired glucose tolerance and type 2 diabetes than carriers of the Pro/Pro genotype (odds ratio adjusted for sex and BMI 2.7 [95% CI 0.9 to 7.8]). Conversely, in the group unexposed to famine during gestation, carriers of the Ala allele had a lower prevalence of impaired glucose tolerance and type 2 diabetes compared with carriers of the Pro/Pro genotype (OR 0.6 [95% CI 0.3 to 1.2]).

Table 3 Prevalence of impaired glucose tolerance and type 2 diabetes and geometric means for insulin-associated variables according to timing of prenatal exposure to the Dutch famine and Pro12Ala polymorphism

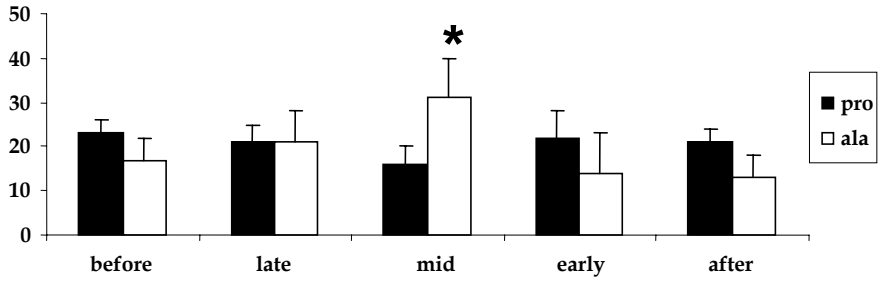
	Exposure to famine				
	Born before (148/60)	In late gestation (82/34)	In mid-gestation (74/29)	In early gestation (47/14)	Conceived after (139/48)
Prevalence of impaired glucose tolerance/diabetes (%)					
Pro/Pro	22.5	20.5	15.9	21.7	20.6
Ala	16.9	20.6	31.0	14.3	12.8
All	20.4	20.5	20.4	20.0	18.6
P value*	-	0.46	0.03	0.84	-
AUC of insulin (pmol/l)					
Pro/Pro	248	245	268	274	247
Ala	270	270	221	218	249
All	255	252	253	260	247
P value*	-	0.80	0.03	0.09	-
Insulin increment ratio					
Pro/Pro	81	79	91	78	73
Ala	87	86	69	60	95
All	82	81	84	74	79
P value*	-	0.65	0.05	0.17	-
HOMA-IR (mmol x pmol/l^2)					
Pro/Pro	14.1	13.4	13.9	15.0	14.6
Ala	14.1	15.1	12.1	12.7	12.6
All	14.1	13.9	13.4	14.4	14.0
P value*	-	0.19	0.56	0.49	-

Frequencies of Pro/Pro/Ala carriers in parentheses. *P values for interaction genotype times famine exposure during late, mid- and early gestation, adjusted for sex and BMI.

The interaction between famine exposure in midgestation and carrying the Ala allele on the prevalence of impaired glucose tolerance and type 2 diabetes was statistically significant (P = 0.03, Fig. 1). Inclusion of the 62 participants with known type 2 diabetes who were excluded from the OGTT yielded the same results (P = 0.02 for interaction, data not shown). Carriers of the Ala allele exposed in midgestation had a lower AUC for insulin (-54 pmol/l [95% CI −117 to -2]) and a lower insulin increment ratio (-22 [95% CI −42 to 7]) than midexposed participants with the Pro/Pro genotype. Carriers of the Ala allele unexposed to famine, on the other hand, had a higher AUC for insulin (12 pmol/l [95% CI −15 to 44]) and a higher insulin increment ratio (14 [95% CI −3 to 35]) than unexposed participants with the Pro/Pro genotype. The interactions between famine exposure in midgestation and carriership of the Ala allele were both significant on AUC for insulin and insulin increment ratio (P = 0.03 and 0.05, respectively). The participants exposed to famine in midgestation who carried the Ala allele also had a lower HOMA-IR compared with carriers of the Pro/Pro genotype, but this interaction was not statistically significant. Additional adjustment for confounding variables other than sex and BMI (including maternal characteristics, birth weight, gestational age, current smoking, and current socioeconomic status) did not significantly alter the results.

There were no significant interactions between the Pro12Ala polymorphism and birth weight in terms of plasma glucose and insulin concentrations, prevalence of impaired glucose tolerance and type 2 diabetes, AUC for insulin, insulin increment ratio, and insulin resistance.

Figure 1 - Prevalence of impaired glucose tolerance and type 2 diabetes (± SE) for participants carrying either the Pro/Pro genotype or the Ala allele according to timing of prenatal exposure to the Dutch famine. *Indicates a statistically significant interaction between genotype and famine exposure during midgestation.



Conclusions

Our findings show that prenatal exposure to famine during midgestation interacts with the Pro12Ala polymorphism of the *PPAR-γ2* gene in influencing the prevalence of impaired glucose tolerance and type 2 diabetes. The Ala allele of the *PPAR-γ2* gene was associated with a higher prevalence of impaired glucose tolerance and type 2 diabetes but only in participants who had been prenatally exposed to famine during midgestation. The Ala allele was also associated with lower insulin concentrations but, again, only in participants who had been exposed to famine during midgestation.

Although this study is the first to demonstrate that genetic influences can be modified by prenatal nutrition, gene-nutrient interaction regarding the Pro12Ala polymorphism has been shown before. Women who carried the Ala allele and who were given a hypocaloric diet for 6 months had a greater increase in insulin sensitivity and fasting carbohydrate oxidation and a greater decrease in fasting lipid oxidation compared with women on the diet who carried the Pro/Pro genotype.[21]

Our findings differ from the study of Eriksson et al., who reported that the effects of low birth weight on raised fasting insulin concentrations and insulin resistance were confined to carriers of the Pro/Pro genotype.[13] We did not find evidence that the effect of the Pro/Pro genotype on fasting insulin concentrations or insulin resistance is modified by famine exposure or size at birth.

Rather, we found the highest prevalence of impaired glucose tolerance and type 2 diabetes in carriers of the Ala allele who were exposed to famine during midgestation. The fact that our results diverge from the results of the study of Eriksson et al. may lie in the difference between the use of size at birth and maternal undernutrition as markers of fetal environment. Size at birth is a summary measure of fetal growth and a result of a wide range of maternal, placental, and fetal factors, of which maternal nutrition is just one. It has been shown in animals as well as in humans that restricted maternal nutrition can produce permanent effects on adult health without affecting size at birth.[22-24] Our inability to show associations with birth weight may have resulted from differences in population characteristics such as the older age and the surplus of women in the Helsinki cohort used in Eriksson et al.'s study and the interference of famine exposure in our cohort. Alternatively, the fact that, compared with Eriksson et al.'s study, in our study maternal nutrition may have had a relatively large contribution to birth weight compared with placental, fetal and other maternal characteristics may have contributed to the discrepancy.

The combination of carrying the Ala allele and prenatal exposure to famine during midgestation did not only affect the prevalence of impaired glucose tolerance and type 2 diabetes but also resulted in lower insulin concentrations and a lower insulin increment ratio. These findings suggest that this group of people is insulin deficient. There is evidence that carrying the Ala allele leads to impaired insulin secretion of the pancreatic beta cell.[25] On the other hand, the Ala allele exhibits a reduced ability for transcriptional activity of *PPAR-γ*, leading to improved insulin sensitivity because lower levels of adipose tissue mass are accumulated.[11] Fetal undernutrition has been shown to decrease the number and function of beta cells in rats as well as in humans.[26,27] In humans, beta cells are found at about 10-11 weeks of gestation and develop for a large part during midgestation.[28] It is possible that prenatal famine exposure in midgestation impairs the development of the beta cells, leading to impaired insulin secretion. Carrying the Ala allele may further reduce insulin secretion. We speculate that the combination of impaired beta cell development and Ala allele carriership produces a degree of insulin deficiency that can no longer be compensated for by improved insulin sensitivity related to carrying the Ala allele.

The interaction between effects of the Pro12Ala polymorphism and prenatal exposure to famine seemed to involve exposure to famine during midgestation only. Although participants who were exposed to famine during late and early gestation also had higher 120-min glucose concentrations predisposing to type 2 diabetes, we did not find interactions with the Pro12Ala polymorphism for these groups. This might indicate that, depending on trimester of exposure to famine, different mechanisms relating to impaired glucose tolerance may be affected. As mentioned above, the Pro12Ala polymorphism may have interacted with famine during

midgestation because midgestation is an important period for the development of the beta cells.[28]

A limitation of the study is the relatively small sample size. In our cohort, only 29 participants who carried the Ala allele had been exposed to famine during mid-gestation. Our findings should therefore be confirmed by future studies. Another limitation is that our finding is a post hoc finding. We aimed to look for an interaction between prenatal exposure to famine and the Pro12Ala polymorphism on glucose/insulin metabolism but did not have clear hypotheses about the direction of the interaction and investigated several glucose/insulin-associated variables.

In conclusion, our study provides the first evidence that genetic influences can be modified by nutrition of the human fetus in utero. The effects of the *PPAR-γ2* Pro12Ala polymorphism on the glucose/insulin metabolism are modified by prenatal exposure to famine during midgestation. This is possibly due to a combined deficit in insulin secretion, as conferred by pancreatic beta cell maldevelopment and carrier type of the Ala allele in the *PPAR-γ2* gene.

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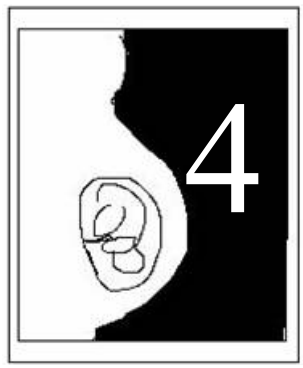

Impaired insulin secretion after prenatal exposure to the Dutch famine

Susanne de Rooij, Rebecca Painter, David Phillips,
Clive Osmond, Robert Michels, Ian Godsland,
Patrick Bossuyt, Otto Bleker, Tessa Roseboom

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Abstract

Objective

We previously reported that people prenatally exposed to famine during the Dutch Hunger Winter of 1944-1945 have higher 2-h glucose concentrations after an oral glucose tolerance test in later life. We aimed to determine whether this association is mediated through alterations in insulin secretion, insulin sensitivity, or a combination of both.

Research Design and Methods

We performed a 15-sample intravenous glucose tolerance test in a subsample of 94 normoglycemic men and women from the Dutch Famine Birth Cohort. We used the disposition index, derived as the product of insulin sensitivity and the first-phase insulin response to glucose as a measure of the activity of the β-cells adjusted for insulin resistance. In all analyses, we adjusted for sex and BMI.

Results

Glucose tolerance was impaired in people who had been prenatally exposed to famine compared with people unexposed to famine (difference in intravenous glucose tolerance test K_g value -21% [95% CI: -41 to -4]). People exposed to famine during midgestation had a significantly lower disposition index (-53% [95% CI: −126 to −3]) compared with people unexposed to famine. Prenatal exposure to famine during early gestation was also associated with a lower disposition index, but this difference did not reach statistical significance.

Conclusions

Impaired glucose tolerance after exposure to famine during midgestation and early gestation seems to be mediated through an insulin secretion defect.

Introduction

There is increasing interest in the effects of early nutrition on the predisposition to glucose intolerance and type 2 diabetes. In several animal models, a restricted diet during gestation has been shown to impair glucose tolerance in later life.[1-4]

A number of animal studies suggest that impairment of glucose tolerance is caused by an insulin secretion defect due to permanent alterations in the structure and function of the pancreatic β-cell made by the fetus when nutrient supplies failed to meet demand.[5-9] However, other animal studies suggest that insulin resistance and hyperinsulinemia cause the impaired glucose tolerance after prenatal undernutrition.[1,10,11]

Evidence for a direct link between prenatal nutrition and glucose and insulin metabolism in humans is scarce. Research in recent years has focused on the long-term consequences of variations in birth weight and of gestational diabetes. Small babies have been found to develop more impaired glucose tolerance and type 2 diabetes in later life.[12-16] Above-average birth weight babies and babies exposed to maternal gestational diabetes are also at increased risk for type 2 diabetes.[17,18] The results of most low-birth weight studies imply that the impaired glucose tolerance and type 2 diabetes are caused by hyperinsulinemia and insulin resistance.[15,16,19-22]

In contrast, a study that introduced the disposition index as a measure of insulin secretion in low-birth weight people found impaired insulin secretion in subjects who were small at birth.[23] However, birth weight is a summary variable of fetal growth that only indirectly relates to maternal nutrition.

The Dutch Famine Birth Cohort Study provides a unique opportunity to evaluate the effects of maternal undernutrition on the predisposition to glucose intolerance and diabetes and its mediating mechanisms. In 1944-1945, severe famine affected the western part of the Netherlands. This famine lasted 5 months and was clearly delineated in time, which enables us to study effects of exposure to famine during specific periods of gestation. Previously, we reported an association between prenatal exposure to famine and impaired glucose tolerance at age 50 years as well as at age 58 years.[24,25] In this study, we aim to determine whether prenatal exposure to famine resulted in defective insulin secretion, an increase in insulin resistance, or a combination of both factors.

Research Design and Methods

The Dutch Famine Birth Cohort

All singletons born alive between 1 November 1943 and 28 February 1947 in the Wilhelmina Gasthuis, Amsterdam, were eligible for the Dutch Famine Birth Cohort. The selection procedures for this cohort have been described elsewhere.[24] A total of 2,414 babies were included, of whom 1,423 (58%) were living in the Netherlands and whose current address was known to the investigators.

Exposure to famine

We defined the famine period according to the daily official food rations for the general population aged >21 years. The official rations accurately reflect the variation over time in the total amount of food available in the west of the Netherlands.[26] We considered fetuses to have been exposed to famine if the average daily rations during any 13-week period of gestation were <1,000 calories. Therefore, babies born between 7 January 1945 and 8 December 1945 were considered exposed. We used periods of 16 weeks each to differentiate between people who had been exposed in late gestation (born between 7 January 1945 and 28 April 1945), in midgestation (born between 29 April and 18 August 1945), and in early gestation (born between 19 August and 8 December 1945). People born before 7 January 1945 and conceived after 8 December 1945 were considered unexposed.

Participants

In the Dutch Famine Birth Cohort, 810 people (57%) agreed to participate at age 58 years. We were able to perform a standard 75-g oral glucose tolerance test on 699 subjects (86%). Results of the oral glucose tolerance test are described elsewhere.[25] Based on the results, we randomly selected 100 normoglycemic individuals (10 men and 10 women from each of the five study groups) for participation in the intravenous glucose tolerance test. We excluded men and women with glucose intolerance or overt diabetes because these conditions are known to affect insulin secretion and insulin action. We defined normoglycemia as 120-min glucose concentrations <7.8 mmol/l in accordance with the definition in our previous study and the 1999 World Health Organization recommendations.[24,27]

Study parameters

The medical birth records provided information about the mother, the course of the pregnancy, and the size of the baby at birth.[24] After an overnight fast, participants underwent a 15-sample intravenous glucose tolerance test performed by trained nurses.[20] Each participant received a glucose dose of 0.5 g/kg body wt as 50% wt/vol dextrose. Insulin sensitivity measured by this protocol has been validated against the reference euglycemic clamp technique ($r = 0.92$).[28] Blood was sampled from the

opposite arm at the following time points: -30, -5, 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, and 180 minutes. Plasma glucose concentrations were measured by standardized enzymatic photometric assay on a Modular P analyzer (Roche, Basel, Switzerland), and plasma insulin concentrations were measured by immuno-luminometric assay on an Immulite 2000 analyzer (Diagnostic Products, Los Angeles, CA). We measured height with a portable stadiometer and weight with a portable Tefal scale. We asked participants about their use of medication. Information on socioeconomic status, medical history, and lifestyle was retrieved from a standardized interview. Current socioeconomic status was coded according to International Socio-Economic Index 92, which is a numeric scale based on the person's or his or her partner's occupation, whichever status is highest.[29]

Statistical analysis

We calculated basal glucose and insulin as the mean of the two fasting samples. We used the intravenous glucose tolerance test glucose elimination index (K_g) as a measure of overall glucose tolerance and calculated it as the least square slope of the log of the glucose concentrations between 20 and 60 minutes after the glucose load (i.e., the regression slope of the decay line). A low value of K_g indicates poor glucose tolerance. We used the trapezoidal rule (base x average height under insulin curve) to determine the first-phase or acute insulin response to glucose (AIR_G) as area under the curve for insulin from 0 to 10 minutes and the second-phase insulin response to glucose as area under the curve for insulin from 10 to 180 minutes. Insulin sensitivity (S_i) and glucose effectiveness (S_g) were determined using the minimal model of glucose disappearance,[30] with model identification by non-linear regression using the MLAB mathematical modelling package (Civilized Software, Bethesda, MD). S_i quantifies insulin sensitivity as the fractional rate of clearance of the glucose distribution space per unit plasma insulin concentration. S_g represents the fraction of the glucose distribution space cleared per minute solely as a result of the ability of elevated glucose levels to stimulate their own normalization. We derived the disposition index as the product of S_i and AIR_G. Disposition index is a measure of the activity of the β-cells, adjusted for the level of insulin resistance. A low disposition index indicates impaired insulin secretion. The variables basal glucose, insulin, K_g, AIR_G, second-phase insulin response, S_i, S_g, disposition index, BMI, and current socioeconomic status had skewed distributions and were logarithmically transformed to normality. We used linear regression analysis to compare the metabolic variables among people exposed in early gestation, midgestation, and late gestation and people unexposed to famine. We adjusted for sex and BMI in all analyses. Additional adjustment was done for maternal and birth characteristics, smoking, levels of physical exercise, and socioeconomic status.

Results

Of the 100 selected subjects, three individuals were unable to participate. The test was terminated in a further three individuals due to a vasovagal reaction or difficulties with venepuncture. The group of 94 participants thus consisted of 47 women and 47 men. They were aged 58 years (SD 1 yr). A total of 54 people (57%) had been prenatally exposed to famine. Table 1 shows that mothers of people exposed to famine in early gestation gained more weight during the last trimester than unexposed mothers. Mothers exposed in late gestation gained almost no weight in the last trimester. Mothers exposed in midgestation weighed less at the last antenatal visit. Babies exposed to famine during midgestation were lighter than unexposed babies. At adult age, socioeconomic status was lower in participants exposed to famine in early gestation compared with unexposed participants.

Table 1 Maternal, birth, and adult characteristics according to timing of prenatal exposure to the Dutch famine

	Exposure to famine						
	Born before	In late gestation	In mid gestation	In early gestation	Conceiv-ed after	All	*n*
General							
n	19	18	18	18	21	94	
Proportion of men	.47	.56	.50	.44	.52	.50	94
Age (years)	59	58	58	58	57	58 ± 1	94
Maternal characteristics							
Proportion of primiparous women	.32	.22	.39	.50	.22	.34	94
Weight gain 3rd trimester (kg)	1.9	-0.1*	4.5	5.8*	3.1	2.8 ± 3.2	63
Weight at last antenatal visit (kg)	68.8	65.4	64.0*	70.0	69.5	67.7 ± 7.6	84
Birth outcomes							
Gestational age (days)	284	285	285	291	289	286 ± 10	82
Birth weight (g)	3311	3361	3155*	3497	3698	3413± 458	94
Ponderal index (kg/m³)	26.2	27.9	25.8	25.6	27.2	26.6 ± 2.5	92
Adult characteristics							
BMI (kg/m²) †	27.4	27.1	29.0	28.2	27.9	28.1 ± 1.1	94
Current smoking	0	22	22	28	15	17	93
Current socioeconomic status (ISEI) †	48	53	47	40*	49	47 ± 1	92

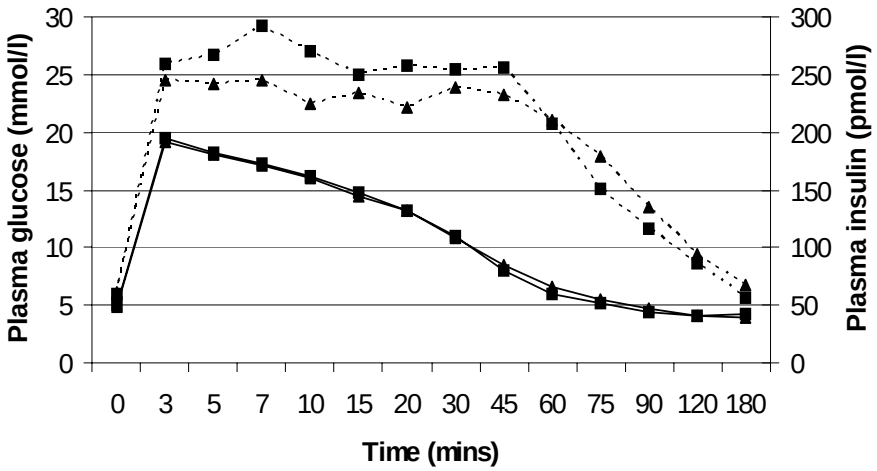
Data are means ± SD unless otherwise indicated. *Statistically significant difference ($P < 0.05$) compared with people unexposed to famine in utero. †Geometric means ± SD. ISEI, International Socio-Economic Index.

Famine exposure

Figure 1 shows the plasma glucose and insulin concentrations during the intravenous glucose tolerance test for participants who had been exposed to famine in utero compared with unexposed participants. There were no differences in basal glucose and insulin concentrations between the exposed and unexposed groups (Table 2). After the glucose load, participants prenatally exposed to famine had lower glucose tolerance compared with unexposed participants, as indicated by a lower glucose

tolerance index (K_g) (-21% [95% CI -41 to -4]). The reduction in K_g was most marked in participants exposed in midgestation and early gestation (late gestation: -4% [-29 to 19], midgestation: -24% [-52 to -1], and early gestation: -37% [-68 to -12]). There was a small nonsignificant decrease in first-phase insulin response (AIR_G) in participants exposed to famine in midgestation and early gestation.

Figure 1 Geometric means of plasma glucose (solid lines) and insulin (dotted lines) concentrations during the intravenous glucose test for people who were exposed (▲) or unexposed (■) to famine in utero.



The second-phase insulin response did not differ between exposed and unexposed groups. S_i and S_g were both lower in participants who had been exposed during midgestation and early gestation, but these differences did not reach statistical significance. The disposition index was significantly lower in participants exposed in midgestation (-53% [-126 to −3]) and tended to be lower in participants exposed in early gestation (-30% [-90 to 12]). Additional adjustment for smoking, levels of physical exercise, and socioeconomic status did not alter the results.

Maternal and birth characteristics

Basal glucose and insulin concentrations, second-phase insulin response, and S_g were not associated with maternal characteristics or with any birth outcome. Primiparity was associated with K_g, AIR_G, and disposition index. Compared with people with a multiparous mother, K_g of people with a primiparous mother decreased by 22% [95% CI -42 to −4], AIR_G decreased by 38% [-78 to 7], and disposition index decreased by 65% [-119 to -24]. S_i was associated with maternal weight gain during the third trimester. S_i decreased by 5% [-11 to 0] with each gained kilogram.

Table 2 Glucose and insulin variables derived from the intravenous glucose tolerance test, according to timing of prenatal exposure to the Dutch famine

	Exposure to famine						
	Born before	In late gestation	In mid gestation	In early gestation	Conceived after	All	*n*
Basal glucose (mmol/l)	5.1	4.8	5.0	5.0	5.0	5.0 ± 1.1	93
Basal insulin (pmol/l)	55	60	56	71	63	61 ± 1.6	93
Glucose tolerance index (K_g) (10^{-2} • min^{-1})	1.96	1.82	1.55*	1.41*	1.87	1.72 ± 1.4	90
First-phase insulin response (AIR_G) (min • pmol • l^{-1})	2343	2354	2095	2118	2648	2311± 1.8	88
Second-phase insulin response (min • pmol • l^{-1})	20308	21299	26515	24680	24212	23211± 1.9	85
S_i (10^{-4} • min^{-1} per pmol/l)	0.67	0.64	0.54	0.59	0.61	0.61 ± 1.8	94
S_g (10^{-2} • min^{-1})	1.81	1.91	1.69	1.65	2.00	1.81 ± 1.5	94
Disposition index (S_i x AIR_G)	1576	1539	1059*	1252	1576	1392 ± 2	88

Data are geometric means ± SD unless otherwise indicated. *Statistically significant difference (P < 0.05, adjusted for sex and BMI) compared with people unexposed to famine in utero.

Birth weight was associated with AIR_G. Per kilogram decrease in birth weight, AIR_G decreased by 35% [-80 to -2].

Additional adjustment for parity did not greatly attenuate the effect of famine exposure on K_g (exposure in midgestation: -21% [-47 to 1], exposure in early gestation: −32% [-61 to -8] and disposition index (exposure in midgestation: -36% [-99 to 7]). There was a trend towards a significant interaction between exposure to famine in early gestation and weight of the mother at the last antenatal visit. Additional adjustment for other maternal and birth characteristics did not change results on prenatal famine exposure.

Conclusions

We found that prenatal exposure to famine, especially during midgestation or early gestation was related to impaired glucose tolerance as measured by an intravenous glucose tolerance test and that this is likely to be caused by an insulin secretion defect.

The results of the intravenous glucose tolerance test indicated that people who were exposed to famine in utero had decreased glucose tolerance compared with people unexposed to famine in utero. These results match the results of the oral glucose tolerance tests we performed in this cohort at age 50 and 58 years.[24,25]

People who were exposed in midgestation had a lower disposition index than unexposed people. People exposed in early gestation also had a lower disposition

index, but the difference from unexposed people did not reach statistical significance.

In contrast to the evidence of many animal studies, we found no association between prenatal undernutrition and insulin resistance.[1,10,11] This may be related to the type of exposure in utero. Animals, in which insulin resistance was found, were protein restricted during gestation, while the participants in our study were prenatally exposed to a hypocaloric diet and were deprived of protein, carbohydrate, and fat.[1,10,11]

The disposition index evaluates insulin secretion, while at the same time taking insulin sensitivity in account, and can therefore provide an index of how effective insulin secretion is in compensating for insulin resistance.[31] A low disposition index indicates that β-cell functioning is inadequate for the degree of insulin resistance. Defective β-cell functioning may thus mediate the association between prenatal famine exposure in midgestation and early gestation and impaired glucose tolerance in later life. Our results confirm the results of many experimental animal studies in which the offspring of mothers exposed to a low-protein diet during gestation showed reduced β-cell mass and impaired β-cell function.[5-9] Our results also confirm the findings of Jensen et al. who found that men who had low birth weight, which is a proxy for a poor fetal environment, had a lower disposition index.[23]

We can only speculate about the mechanism underlying the link between fetal undernutrition and impaired functioning of the β-cell. Rats exposed to a low-protein diet during pregnancy and lactation had reduced β-cell mass caused by decreased rates of β-cell proliferation and increased rates of β-cell death.[32] Bilateral uterine artery ligation in the rat, which mimics placental insufficiency, caused an insulin secretion defect that was specific for glucose stimulation. This suggests a possible impairment in glucose sensing or a defect in the signalling pathway that elicits insulin secretion by the β-cell.[33]

We studied a cohort of people who were born immediately before, during, or after the 1944-1945 Dutch famine. A limitation of our study is that the famine had a profound effect on early mortality and fertility, which may have introduced selection bias in our study.[34] Early mortality rates were highest among people born before the famine and lowest among people conceived after the famine. These two groups, however, were similar in terms of the parameters we investigated. Also, adjusting for maternal characteristics, which may be proxies for determinants of fertility, did not greatly attenuate the effects we found of prenatal famine exposure. We therefore think selective early mortality and fertility can have had only a limited influence.

Other limitations of our study were the small sample number and the possible bias introduced by the study design. We excluded all people with impaired glucose tolerance and type 2 diabetes as indicated by an oral glucose tolerance test.

We chose this design because if we had included people with impaired glucose tolerance and type 2 diabetes, we probably would have found insulin deficiency as a consequence of the present pathology and not necessarily because of the prenatal exposure to famine. Excluding these people could, however, have affected our results.

Although based on a small study sample, this is the first direct evidence suggesting that an insulin secretion defect mediates the association between fetal undernutrition and the development of impaired glucose tolerance in humans.

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Hypothalamic-Pituitary-Adrenal-axis activity in adults who were prenatally exposed to the Dutch famine

Susanne de Rooij, Rebecca Painter, David Phillips,
Clive Osmond, Robert Michels, Patrick Bossuyt,
Otto Bleker, Tessa Roseboom

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Abstract

Objective

The hypothalamic-pituitary-adrenal (HPA) axis has been proposed to be susceptible to fetal programming, the process by which an adverse fetal environment elicits permanent physiological and metabolic alterations predisposing to disease in later life. It is hypothesized that fetal exposure to poor circumstances alters the set point of the HPA axis leading to increased HPA axis activity and subsequent increased cortisol concentrations. In this study, we tested the hypothesis that prenatal exposure to famine during different periods of gestation is associated with increased activity of the HPA axis.

Design and Methods

We assessed plasma cortisol concentrations after a dexamethasone suppression and an $ACTH_{1-24}$-stimulation test in a group of 98 men and women randomly sampled from the Dutch famine birth cohort. Cohort members were born as term singletons around the 1944-1945 Dutch famine.

Results

Cortisol profiles after dexamethasone suppression and $ACTH_{1-24}$-stimulation were similar for participants exposed to famine during late, mid- or early gestation (P = 0.78). Cortisol concentrations after dexamethasone suppression test did not differ between those exposed and those unexposed to famine in utero (mean difference −2% [95% CI -27 to 23]). Neither peak cortisol concentration (20 nmol/l [95% CI -27 to 66]), cortisol increment (-5 nmol/l [95% CI −56 to 47) or cortisol area under curve post-ACTH1-24 injection (4% [95% CI -4 to 12]) differed between exposed and unexposed participants.

Conclusions

Prenatal famine exposure does not seem to affect HPA axis activity at adult age, at least not at the adrenal level. This does not exclude altered HPA axis activity at the levels of the hippocampus and hypothalamus.

Introduction

The fetal origins hypothesis states that an adverse fetal environment may elicit permanent physiological and metabolic alterations predisposing to disease in later life, a process called fetal programming.[1] The hypothalamic-pituitary-adrenal (HPA) axis has been proposed to be susceptible to fetal programming. Prenatal undernutrition may program the HPA axis by permanently altering the set point of the HPA axis in utero, leading to increased activity and subsequent increased concentrations of cortisol that predispose to cardiovascular disease and type 2 diabetes.[2-4] In utero resetting of the HPA axis may thus be an important link between early life events and these chronic adult diseases.

Animal studies have established that prenatal nutrition can affect the functioning of the HPA axis in adult life. The newborn progeny of rats exposed to a low protein diet during late gestation, show increased HPA axis activity as indicated by elevated levels of basal plasma corticotropin (ACTH) and corticosterone.[5] Modest as well as severe prenatal undernutrition during early and late gestation in sheep produces increased post-natal activity of the HPA axis after a corticotropin releasing hormone plus arginine vasopressin challenge.[6,7] Studies in guinea pigs that were undernourished during a short period in late gestation have shown clear sex-specific alterations in HPA axis activity at adult age.[8] Basal ACTH and cortisol responses are reduced in male guinea pigs, while in female guinea pigs basal ACTH responses remain unchanged and cortisol responses are elevated compared with normally fed animals.

Fetal programming of the HPA axis has been identified in humans who were small at birth. Small size at birth is considered to reflect a poor fetal environment. Low birth weight was found to be associated with elevated fasting plasma cortisol concentrations in a variety of populations of men and women.[9,10] People who were small at birth also show increased responsiveness to dexamethasone suppression and increased responsiveness to corticotropin releasing hormone and ACTH stimulation, although some studies failed to find evidence for these associations.[11-14]

In previous studies, we have shown that prenatal exposure to undernutrition during different periods of gestation in the Dutch famine was associated with a range of risk factors for, as well as an increased prevalence of, coronary heart disease (CHD) and type 2 diabetes at adult age.[15-21] The Dutch famine was a period of 5 months of severe undernutrition at the end of World War II. Although it was a historical disaster, it provides us with the opportunity to study the long-term health effects of undernutrition during different periods in pregnancy. Programming of the HPA axis may explain the association between famine exposure in utero and the increased risk of CHD and type 2 diabetes in later life.

In a new study, we tested the hypothesis that prenatal exposure to famine during late, mid- and/or early gestation is associated with increased activity of the HPA axis.

Materials and methods

The Dutch famine birth cohort

The Dutch famine birth cohort members were born as term singletons between 1 November 1943 and 28 February 1947 at the Wilhelmina Gasthuis in Amsterdam, the Netherlands. The selection procedure is described in detail elsewhere.[15] At age 58, 1423 of the 2414 original cohort members (58%) were still alive, living in the Netherlands and their address was known to the investigators. These people were eligible for our study.

Exposure to famine

We defined the famine period according to the official daily food-rations for the general population older than 21 years. The official rations accurately reflect the variation over time in the total amount of food available in the west of the Netherlands.[22] We considered fetuses to have been exposed to famine in utero if the average daily rations for those older than 21 years during any 13-week period of gestation were less than 1000 calories. According to this definition, people born between 7 January 1945 and 8 December 1945 were considered to be exposed to famine in utero. We defined periods of 16 weeks each to differentiate between those who were exposed to famine in late gestation (born between 7 January 1945 and 28 April 1945), in mid gestation (born between 29 April and 18 August 1945) and in early gestation (born between 19 August and 8 December 1945). People born before 7 January 1945 and conceived and born after 8 December 1945 were considered to be unexposed to famine in utero.

Participants

All 1423 eligible Dutch famine birth cohort members were invited to attend the clinic for a data collection protocol, which included a 75g oral glucose tolerance test.[23] We were able to complete this test for 699 of the 810 study participants. Based on the results of the oral glucose tolerance test, we selected 100 normoglycaemic men and women for participation in a dexamethasone suppression and an $ACTH_{1-24}$-stimulation test using the same protocol as described by Reynolds et al.[13] For this purpose, 10 men and 10 women were randomly selected from each of the following study groups: people born before the famine, people exposed to famine in late gestation, people exposed in mid gestation, people exposed in early gestation and people conceived after the famine. We defined normoglycaemia as 120-min plasma

glucose concentrations below 7.8 mmol/l in accordance with the definition in our previous study and the 1999 WHO recommendations.[15,24] Participants taking oral glucocorticoids were excluded from selection. The local medical ethics committee approved the study, which was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Dexamethasone suppression and $ACTH_{1-24}$-stimulation test

The participants were asked to take 0.25 mg dexamethasone at 2200 h and to fast overnight. The following morning they attended the clinic where a cannula was placed in the antecubital vein. After a 30-min period of resting, a baseline blood sample was taken and 1 μg of $ACTH_{1-24}$ (Tetracosactid, Synacthen, Novartis Pharma, Arnhem, the Netherlands) was injected intravenously as a bolus. Venous blood samples were taken 20, 30, 40 and 60 min after injection of $ACTH_{1-24}$. Plasma cortisol concentrations were measured by enzyme immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) with a detection limit of 50 nmol/l. Cortisol concentrations in the baseline sample were used to indicate dexamethasone suppression. We used low doses of dexamethasone (0.25 mg) and $ACTH_{1-24}$ (1 μg) instead of conventional doses (e.g. 1 mg dexamethasone and 250 μg $ACTH_{1-24}$) to be able to detect the subtle alterations in cortisol responses after suppression and stimulation to be expected in healthy participants. Conventional doses would induce maximal effects in all participants and minimise the possibility of detecting differences between participants exposed and unexposed to famine in utero.

Interview and anthropometric measurements

We measured height with a portable stadiometer and weight with a portable Tefal scale. We asked participants about their use of medication. All other data used in this study were collected on the day the oral glucose tolerance test had been performed.[23] Waist circumference was measured with a flexible tape measure. Information on socioeconomic status, medical history and lifestyle was retrieved from a standardized interview. Current socioeconomic status was coded according to ISEI-92, a numeric scale based on the person's, or their partner's occupation, whichever status is highest.[25]

Clinical measurements

Plasma glucose concentrations obtained during the earlier performed oral glucose tolerance test were measured by a standardized enzymatic photometric assay on a Modular P analyser (Roche, Basel, Switzerland). Plasma insulin concentrations were measured by an immuno-luminometric assay on an Immulite 2000 analyser (Diagnostic Products Corporation). Blood was drawn for analysis of low- and high-density lipoprotein cholesterol.

Blood pressure was measured in duplo on two occasions (morning and afternoon) using an automated device (Omron 705CP/IT, Omron Healthcare UK, West Sussex, UK). Mean systolic and diastolic blood pressure was calculated using all available measurements. Standard 12-lead electrocardiograms (ECG) were taken from the participants. The presence of CHD was defined as the presence of one or more of the following: angina pectoris according to the Rose/WHO questionnaire; Q waves on the ECG (Minnesota codes 1-1 or 1-2) or a history of coronary revascularisation (angioplasty or bypass surgery). We defined the metabolic syndrome according to the recommendations of the National Cholesterol Education Program (NCEP), modified in 2004 in accordance with the ADA.[26,27]

Statistical methods

We took the highest concentration of cortisol in the line of 20, 30, 40 and 60-min cortisol concentrations of each individual as the peak cortisol level. We subtracted the baseline cortisol concentration from the peak cortisol concentration to obtain cortisol increment. We calculated the area under the curve (AUC) for cortisol from 0 to 60 minutes post-$ACTH_{1-24}$ following the trapezoidal rule (base x average height under cortisol curve). To obtain normality in case of a skewed distribution, we applied logarithmic transformations to the variables: body mass index (BMI), socioeconomic status, 2-h glucose, 2-h insulin, systolic blood pressure, high density lipoprotein (HDL) cholesterol, post-dexamethasone cortisol and AUC cortisol. Spearman's rank correlations were calculated between maternal, birth, adult and cortisol outcomes. Linear and logistic regression analyses were used to detect possible differences in cortisol outcomes between the groups of people exposed and unexposed to famine in utero. Effect sizes and confidence intervals (CI) are given as percentage difference between groups for logarithmically transformed outcome variables. We used a general linear mixed model analysis to investigate differences between the exposed and unexposed groups in the course of cortisol concentrations over time after injection of $ACTH_{1-24}$. In all our regression analyses we adjusted for gender and BMI. Additional adjustment was performed for a range of variables including maternal and birth characteristics, smoking, socioeconomic status, treatment with estrogen, treatment with anti-depressive medication and use of inhaled corticosteroid medication. We considered differences to be statistically significant if *P*-values were smaller than 0.05.

Results

Three people who were exposed to famine during early gestation were unable to participate. Inadvertently, in the group of participants born before the famine, one

person too many was included. The group of 98 participants with dexamethasone suppression/ $ACTH_{1-24}$-stimulation tests consisted of 47 men and 51 women with a mean age of 58 years (S.D. 1 yr), of which 57 (58%) had been exposed to famine during gestation. Table 1 shows that mothers of participants exposed to famine in early gestation were younger and gained more weight during the third trimester of pregnancy compared to mothers of unexposed participants. Mothers exposed in mid gestation also gained more weight, but weighed less at the end of pregnancy. Mothers exposed in late gestation gained no weight and weighed less at the end of pregnancy. Babies exposed to famine in mid gestation were smaller at birth compared to unexposed babies.

Table 1 General, maternal and birth characteristics according to timing of prenatal exposure to the Dutch famine. Results are means and S.D., except when indicated differently.

	Exposure to famine						
	Born before	In late gestation	In mid-gestation	In early gestation	Conceived after	All (S.D.)	*n*
n	21	20	20	17	20		98
General characteristics							
Proportion of men	.48	.50	.50	.41	.50	.48	98
Age	58.9	58.4	58.0	57.8	57.3	58.1 (0.7)	98
Maternal characteristics							
Age at delivery (years)	30	32	29	25*	29	26 (6)	98
Weight gain 3rd trimester (kg)	1.6	0.0*	4.8*	5.7*	3.2	2.8 (3.2)	66
Weight at last antenatal visit (kg)	67.7	62.9*	63.8*	68.9	70.2	66.7 (8.2)	88
Birth outcomes							
Gestational age (days)	285	283	285	290	288	286 (11)	84
Birth weight (g)	3318	3323	3086*	3464	3727	3380 (471)	98

*Statistically significant difference ($P < 0.05$) compared with people unexposed to famine in utero.

Table 2 shows that at age 58, those exposed to famine in early gestation had a lower socioeconomic status compared to those unexposed. They had higher 2-h glucose and 2-h insulin concentrations compared with unexposed participants. The 2-h insulin concentrations were also higher for those exposed to famine during mid gestation.

Cortisol concentrations and maternal and birth characteristics

Table 3 shows correlations between maternal, birth and adult characteristics and cortisol outcomes. Post-dexamethasone and peak cortisol concentrations post-$ACTH_{1-24}$ injection were higher if the mother was older at delivery. With every year increase in maternal age, post-dexamethasone concentrations in the offspring increased by 2% (95% CI 0 to 4) and post-$ACTH_{1-24}$ peak cortisol concentrations increased by 4 nmol/l (95% CI 0 to 7 nmol/l). Plasma cortisol concentrations after dexamethasone suppression were lower in those with low birth weight. Post-dexamethasone concentrations decreased by 23% (95% CI 7 to 39) per kilogram decrease in birth weight.

Table 2 Adult characteristics according to timing of prenatal exposure to the Dutch famine. Results are means and S.D., except when indicated differently.

	Exposure to famine					
	Born before	In late gestation	In mid-gestation	In early gestation	Conceived after	All (S.D.)
n	21	20	20	17	20	98
Proportion of current smokers	.10	.15	.15	.24	.15	.15
Current socio-economic status #	51	52	46	40*	49	47 (1.3)
Body mass index (kg/m²) #	26.0	27.3	27.0	27.9	27.7	27.2 (1.1)
Waist:hip ratio	91.1	91.9	91.5	93.1	90.0	91.5 (7.8)
Systolic blood pressure (mmHg) #	135	132	139	132	132	134 (1.2)
Diastolic blood pressure (mmHg)	80	78	84	82	82	81 (11)
Total cholesterol (mmol/l)	5.8	6.1	6.1	5.8	5.9	5.9 (1.0)
HDL cholesterol (mmol/l) #	1.54	1.43	1.41	1.50	1.37	1.45 (1.3)
2-h glucose (mmol/l) #	5.3	5.2	5.6	6.1**	5.1	5.4 (1.2)
2-h insulin (pmol/l) #	167	197	256**	294**	187	213 (1.2)
CHD	.05	.00	.10	.13	.05	.06
Metabolic syndrome prevalence †	.24	.15	.45	.18	.25	.26

CHD, coronary heart disease; HDL cholesterol, high density lipoprotein cholesterol; #Geometric means and S.D.; *statistically significant difference (P < 0.05) compared with people unexposed to famine in utero; **statistically significant difference (P < 0.05, adjusted for gender and BMI) compared with people unexposed to famine in utero; †according to the recommendations of the NCEP, modified in 2004 in accordance with the ADA.[26,27]

Table 3 Associations between maternal, birth and adult characteristics and plasma cortisol concentrations.

	Maternal age	Birth weight	Gender (men=1, women=2)	BMI	2-h glucose	2-h insulin
Post-dexamethasone cortisol	.15	.23*	-.34*	-.18	-.03	-.22*
Peak cortisol post-ACTH	.23*	.05	.08	-.12	.23*	.04
Cortisol increment post-ACTH	.00	-.07	.36*	.18	.28*	.36*
Cortisol area under curve (AUC) post-ACTH	.18	.17	-.05	-.12	.23*	.08

Spearman's rank correlations; *statistically significant correlation (P < 0.05).

Analysis of men and women separately showed that the association between low birth weight and low post-dexamethasone cortisol concentrations was statistically significant in women only (36% decrease per kilogram decrease in birth weight [95% CI 5 to 57] vs. 9% decrease in men [95% CI −22 to 32]).

Cortisol concentrations and adult characteristics

Men had higher cortisol concentrations after dexamethasone suppression (47% [95% CI 18 to 82]) and a lower cortisol increment after $ACTH_{1-24}$ injection (-75 nmol/l [95% CI −125 to −25]) compared with women (see also Table 3). Peak cortisol concentrations and cortisol AUC post-$ACTH_{1-24}$ injection were higher with higher 2-h glucose concentrations. Peak cortisol concentrations rose by 25 nmol/l (95% CI 3 to 47) and cortisol AUC by 4% (95% CI 0 to 8) per mmol/l increase in 2-h glucose

concentrations. Cortisol increment post-$ACTH_{1-24}$ injection was higher with higher 2-h insulin concentrations. Cortisol increment increased by 0.23 nmol/l (95% CI 0.08 to 0.38) per pmol/l increase in 2-h insulin concentrations. Repeated measures analyses of the cortisol profiles showed that there were positive associations between the cortisol profile and waist:hip ratio ($P < 0.01$), 2-h glucose ($P = 0.03$) and 2-h insulin concentrations ($P = 0.04$) and the prevalence of the metabolic syndrome according to the recommendations of the NCEP ($P = 0.03$). Gender, BMI, age, lifestyle variables (smoking, alcohol), socioeconomic status and other clinical outcomes (systolic and diastolic blood pressure, total and HDL cholesterol and the prevalence of CHD) were not associated with the cortisol profile.

Cortisol concentrations and prenatal famine exposure

Cortisol profiles after suppression with dexamethasone and stimulation with $ACTH_{1-24}$ for participants exposed to famine during late, mid- or early gestation did not significantly differ from each other ($P = 0.78$) and were, therefore, grouped together for comparison with participants unexposed to famine in utero. The cortisol profiles after suppression with dexamethasone and stimulation with $ACTH_{1-24}$ are shown in Figure 1.

Figure 1 Cortisol profiles plotted as means (± S.E.M.) after dexamethasone suppression (0) and $ACTH_{1-24}$-stimulation (from 0 to 60 min) for participants exposed and unexposed to famine in utero. $P = 0.62$ for difference between the exposed and the unexposed group based on regression analysis, adjusted for gender and BMI.

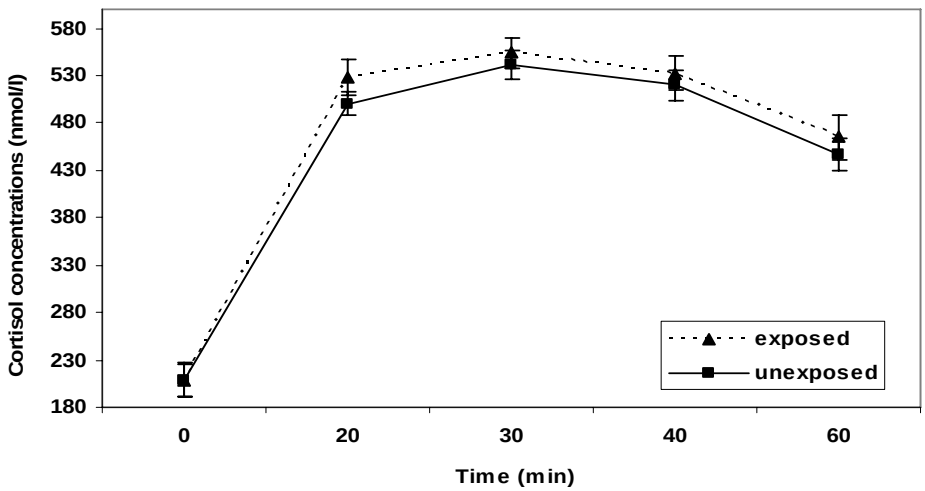

Table 4 shows that cortisol concentrations after suppression with dexamethasone did not differ between those exposed and unexposed to famine in utero (mean difference −2% [95% CI -27 to 23]). Peak cortisol concentrations after injection with $ACTH_{1-24}$ did not differ between prenatally famine exposed and unexposed (20 nmol/l [95% CI -27 to 66]) subjects. There were also no differences between the exposed and unexposed groups in cortisol increment post-$ACTH_{1-24}$ (-5 nmol/l [95% CI −56 to 47) and cortisol AUC post-$ACTH_{1-24}$ (4% [95% CI -4 to 12]). Repeated measures analysis of the cortisol profiles showed that profiles of exposed and unexposed participants did not significantly differ from each other (P = 0.62). There was no interaction between sex and famine exposure (P = 0.94). Adjusting for maternal age, maternal weight gain, weight at the last antenatal visit, birth weight, smoking, socioeconomic status, treatment with estrogen, treatment with anti-depressive medication, and use of inhaled corticosteroid medication also did not alter the results.

Table 4 Plasma cortisol concentrations according to timing of prenatal exposure to the Dutch famine. Results are means and S.D., except when indicated differently.

	Exposure to famine						
	Born before	In late gestation	In mid-gestation	In early gestation	Conceived after	All (S.D.)	*n*
Post-dexamethasone cortisol (nmol/l)#	177	205	166	158	186	178 (1.8)	96
Peak cortisol post-ACTH (nmol/l)	608	600	590	584	539	584 (110)	89
Cortisol increment post-ACTH (nmol/l)	402	351	389	399	358	380 (123)	87
Cortisol AUC post-ACTH (nmol/l·min)#	27623	27496	27101	27581	26008	27369 (1.2)	84

#Geometric means and S.D.

Discussion

In the present study, we aimed to investigate whether prenatal exposure to famine is associated with increased activity of the HPA axis at age 58. We performed a dexamethasone suppression and an $ACTH_{1-24}$-stimulation test, but found no differences in suppressed and stimulated cortisol concentrations between participants exposed and unexposed to famine in utero. These results suggest that prenatal famine exposure does not program HPA axis activity, at least not at the adrenal level.

This study is a unique population-based study of men and women recruited from the original Dutch famine birth cohort still living in the Netherlands.[15] The famine had a sudden beginning and end, and lasted for 5 months. This enables us to study the consequences of undernutrition during different stages of gestation. Although we found dexamethasone suppressed and $ACTH_{1-24}$-stimulated cortisol concentrations in those prenatally exposed to famine in late, mid- or early gestation to be similar to

cortisol concentrations of those unexposed, we think it is too early to conclude that famine exposure in utero does not affect functioning of the HPA axis at all. There are some methodological drawbacks and issues that have to be taken into account.

Our study sample was relatively small and the considerable fluctuations in cortisol concentrations make it difficult to detect differences between groups. Also, participants were sampled from a normoglycaemic group. HPA programming by famine exposure may be more pronounced among those with impaired glucose tolerance and prenatal famine related differences in normoglycaemic participants might be too subtle to detect. With a significance level of $\alpha = 0.05$, our study had 80% power to detect a minimal difference of 1 S.D. (about 100 nmol/l) in peak cortisol level between participants unexposed to famine and participants exposed to famine in late, mid- or early gestation.

Another issue concerns the fact that the dexamethasone suppression / $ACTH_{1-24}$-activation test primarily acts at the adrenal level of the HPA axis, while higher levels of the HPA axis remain mostly unmeasured. The results of the present study suggest that prenatal famine exposure does not affect functioning of the HPA axis at the adrenal level, but it may affect functioning at a higher level. Experiments in which rats were pre- or perinatally undernourished showed physiological and functional changes at the levels of the hippocampus and hypothalamus.[5,28] Higher levels of the HPA axis can be tested by means of psychological stressors.[29] We are currently investigating whether psychological stress leads to different HPA axis responses in humans exposed to famine in utero.

A final issue concerns the impact of increasing age. Various cohort studies have shown associations between low birth weight and cortisol concentrations across different age groups, implying there is no effect of age on the fetal programming of the HPA axis.[10] Evidence form animal studies, however, seems to dispute this. Programming of the HPA axis by prenatal exposure to glucocorticoids was shown to be dependent on increasing age in studies of male guinea pigs.[30,31] Glucocorticoids-exposed 80-day-old guinea pigs showed a reduction in basal and activated pituitary-adrenal function compared with unexposed animals, while this effect was no longer observed at the age of 150 days. Programming effects on the activity of the HPA axis were still present, but persisted only on the level of the hippocampus. These age-dependent effects on the HPA axis are possibly modulated by changes in mineralcorticoid receptor levels. Effects of prenatal famine exposure on HPA axis activity may also have changed with increasing age. Unfortunately, we were not able to test possible effects of increasing age, because all participants are of about the same age.

We found elevated dexamethasone suppressed and $ACTH_{1-24}$-stimulated cortisol concentrations in participants born to mothers who were older at delivery. This

result agrees with the result of a study that investigated HPA axis function in a cohort of children born in Jamaica.[32]

Some studies have shown associations between HPA axis responsiveness to $ACTH_{1-24}$-stimulation and low birth weight.[13,14] We found no evidence for such an association in our study, but we found that low birth weight was associated with lower cortisol concentrations following dexamethasone suppression, although in women only. While most studies report that low birth weight is linked with high morning cortisol concentrations which are suppressed normally following dexamethasone,[10,13,14] associations with greater suppressibility following dexamethasone have been reported in some populations of men and women.[11,12]

To our knowledge, gender differences in the association between low birth weight and responsiveness of the HPA axis have not been reported before. Studies of Reynolds et al. showed gender differences in the cortisol responses to HPA manipulation, as did our results, but found no interaction effect of gender and low birth weight on HPA responsiveness.[13,14] Animal experiments have shown gender-specific HPA reactions as a consequence of prenatal undernutrition, which points to an increase in adrenal sensitivity in females.[8] However, the link between small size at birth and greater suppressibility of the HPA axis in women in our study does not seem to be caused by undernutrition in utero. Size at birth is a summary measure of fetal growth, which only indirectly relates to maternal nutrition. It is a result of a wide range of maternal, placental and fetal factors. It has been shown that size at birth can produce permanent effects on adult health independently from prenatal undernutrition; it has also been found that prenatal undernutrition can have profound effects in later life without affecting size at birth.[18,33,34]

In summary, we found no evidence in support of the hypothesis that prenatal famine exposure programs response to dexamethasone suppression and $ACTH_{1-24}$-stimulation at the adrenal level of the HPA axis. At the levels of the hippocampus and hypothalamus there may be programming by famine exposure in utero. We are currently testing this assumption by performing psychological stress tests in our cohort.

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Cortisol responses to psychological stress in adults after prenatal exposure to the Dutch famine

Susanne de Rooij, Rebecca Painter, David Phillips, Clive Osmond
Michael Tanck, Patrick Bossuyt, Tessa Roseboom

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Abstract

Objective

Experimental studies in animals show that prenatal undernutrition leads to lifelong alterations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Some studies have shown associations between low birth weight and an increased HPA response to psychological stress. We tested the hypothesis that prenatal exposure to the Dutch 1944-1945 famine leads to an elevated HPA response to psychological stress in adult life.

Methods

We measured salivary cortisol responses to a psychological stress protocol among 694 adults who were born as term singletons in Amsterdam, the Netherlands, around the time of the 1944-1945 Dutch famine. We compared cortisol profiles of participants exposed to famine during late (n = 120), mid (n = 100), or early gestation (n = 62) to profiles of participants unexposed to famine during gestation (n = 412).

Results

The mean increase in cortisol concentrations from baseline was 30% (95% CI 23 to 37). There were no statistically significant differences in the mean profile of cortisol response to the psychological stress protocol between participants exposed and unexposed to famine in utero. The mean sex and BMI adjusted difference in cortisol response for those exposed compared to those unexposed was -6% (95% CI: -15 to 2). The cortisol profiles of those exposed in late (-4% [95% CI: -16 to 7]), mid (-9% [95% CI: -22 to 3]) or early gestation (-4% [95% CI: -20 to 10]) did not differ from the profile of those unexposed to famine.

Conclusion

We conclude that prenatal exposure to famine does not seem to be associated with the response of the HPA axis to psychological stress. However, the stress protocol we have used may have been unsuccessful in inducing a strong enough HPA axis activation to be able to detect famine related differences.

Introduction

A large body of epidemiological data has demonstrated an association between an adverse fetal environment, as indicated by small size at birth, and various diseases in later life, including hypertension, type 2 diabetes and cardiovascular disease. It has been suggested that this phenomenon results from permanent adaptations in structure or physiology of the fetus in response to its poor environment. This process is called fetal programming.[1] While these adaptations may be beneficial for the short-term survival of the fetus, they may predispose to disease in adult life.

In utero programming of the magnitude of the stress response has been proposed as one of the mechanisms linking adverse prenatal circumstances to the development of cardiovascular and metabolic disease in later life.[2] It is proposed that changes in the set-point of the hypothalamic-pituitary-adrenal (HPA) axis during fetal life could result in long-term changes in secretion of key neuroendocrine mediators of the stress response, which in turn predispose to cardiovascular and metabolic disease in later life.

Animal experiments in a variety of species have shown that programming of the HPA axis can be induced prenatally by nutrient restriction.[3-7] For example, sheep that were nutrient restricted during early gestation showed increased ACTH and cortisol responses to a corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) challenge in later life.[3] The offspring of rats fed a 50% food restricted diet had reduced body and adrenal weights, glucocorticoid and mineralocorticoid receptor expressions in the hippocampus, CRH expression in the hypothalamus and plasma ACTH concentrations and elevated concentrations of corticosterone at birth.[4]

The evidence in humans is conflicting. A number of studies failed to find an association between low birth weight and 24-h cortisol concentrations.[8-10] However, associations between low birth weight and fasting and ACTH stimulated cortisol concentrations have been reported.[11-14] People with low birth weight show increased cortisol responses to awakening and to ACTH stimulation. These findings suggest that HPA axis responsivity to stress is susceptible to fetal programming, whereas unstressed HPA function is not.[15] Recent studies, which demonstrated that men with low birth weight have elevated cortisol concentrations in response to psychological stress, confirm this hypothesis.[16,17]

The use of birth weight as a marker of the fetal environment has limitations, because birth weight is the result of a wide range of maternal, placental and fetal factors. The Dutch famine birth cohort consists of men and women born around the time of the Dutch famine and offers a unique opportunity to study the direct effects of maternal famine on adult outcomes.

The Dutch famine lasted five months, beginning in late November 1944 and ending in early May 1945. Previously, we have shown that famine exposure during early gestation is associated with an increase in coronary heart disease in later life.[18,19] Exposure in late, mid and early gestation is associated with impaired glucose tolerance.[20,21] Fetal programming of the HPA response to stress may contribute to the increased prevalence of metabolic and heart disease in adults exposed to famine in utero. We therefore wanted to test the hypothesis that men and women exposed to famine during gestation have increased HPA responses to psychological stress.

Methods

Selection Procedures

The Dutch famine birth cohort consists of 2414 men and women who were born as term singletons in the Wilhelmina Gasthuis in Amsterdam between 1 November 1943 and 28 February 1947. The selection procedure and subsequent loss to follow up have been described in detail elsewhere.[20,22] Cohort members who were living in the Netherlands on 1st September 2002 and whose address was known to the investigators were eligible for participation. Of the original cohort of 2414, 160 babies had not been registered in Amsterdam at birth and were lost to follow up. A further 328 people had died, 213 people had emigrated, 157 people refused permission for the study to record their address, and 125 people were not traceable to a current address by the municipalities. Eight people requested their address be removed from the study's database. At the start of the study there were 1423 eligible men and women who 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 procedures were carried out with the adequate understanding and written consent of the participants.

Exposure to famine

We defined the famine period according to the daily official food-rations for the general population older than 21 years. The official rations accurately reflect the variation over time in the total amount of food available in the west of the Netherlands.[23] A person was considered to be prenatally exposed to famine if the average daily ration during any thirteen-week period of gestation contained less than 1000 calories. Therefore, people born between 7 January 1945 and 8 December 1945 were considered as exposed in utero. We delineated periods of 16 weeks each to differentiate between those who had been exposed in late gestation (born between 7 January and 28 April 1945), in mid gestation (born between 29 April and 18 August 1945) and in early gestation (born between 19 August and 8 December 1945).

Cohort members born before 7 January 1945 and conceived after 8 December 1945 were considered unexposed to famine in utero and acted as control group.

Study parameters

The medical birth records provided information about the mother, the course of pregnancy and the size of the baby at birth.[20] Participants visited the clinic for a data collection protocol which included anthropometry, a standard 75g oral glucose tolerance test, a standard 12 lead electrocardiogram, measurement of blood pressure and lipid profile and a standardised interview which yielded information on the participant's socio-economic status, medical history, use of medication and lifestyle. Methods of the data collection protocol have been described in detail elsewhere.[19,21] We defined the metabolic syndrome according to the definition of the NCEP.[24]

Stress test protocol

The stress protocol was performed in the afternoon (between 12 and 2 pm), about an hour after participants had eaten a light lunch. The protocol started with a 20-min baseline period, followed by three 5-min psychological stress tests (Stroop test, mirror-tracing test and speech test). The Stroop test and the mirror-tracing test were each followed by a 6-min recovery period. Following the speech test, the participant was allowed to recover for 30 min.

The Stroop test is a computerised colour-word conflict challenge. After a short introduction, participants were allowed to practise until they grasped the meaning of the test. A mistake or exceeding the response time limit of five seconds was automatically followed by a short beep. In the mirror-tracing test a star had to be traced that could only be seen in mirror image (Lafayette Instruments Corp, Lafayette, IN, USA). Every divergence from the line of the star induced a short beep. The participants were allowed to practise one circuit of tracing. Participants were instructed to give priority to accuracy over speed and were told that most people could perform five circuits of the star without divergence from the line.

Prior to the speech test, participants listened to an audiotaped instruction in which they were told to imagine a situation in which they were falsely accused of pick pocketing. They were given 2 min to prepare a 3 min speech in which they had to respond to the accusation and which had to be performed in front of a videocamera. Participants were told the number of repetitions, eloquence and persuasiveness of their performance would be marked by a team of communication-experts and psychologists.

Saliva samples were collected using Salivettes (Sarstedt, Rommelsdorf, Germany) at seven time points during the protocol: at 5 and 20 min in the baseline period; at 6 min after completion of the Stroop test and the mirror-drawing test; at 10, 20 and 30 min after completion of the speech test.

Saliva was extracted by centrifuging the Salivettes and stored at -80°C until analysis. Salivary cortisol concentrations were measured using a time-resolved immunofluorescent assay (DELFIA).[25] This assay had a lower detection limit of 0.4 nmol/liter, an inter-assay variance of 9–11% and an intra-assay variance of less than 10%.

Statistical methods

Baseline cortisol was calculated as the mean of the first and second cortisol concentration measured during the baseline period. We took the highest concentration of all cortisol concentrations as the peak cortisol level. We calculated the area under the curve (AUC) for cortisol from the first baseline measurement to the second recovery period following the trapezoidal rule (base x average height under cortisol curve). We applied logarithmic transformations to normalise skewed distributions. Variables with skewed distributions are reported as geometric means and standard deviations; effect sizes are given in percentages difference between groups. We used linear and logistic regression analyses to compare maternal, birth, adult and cortisol outcomes of those exposed in late, mid or early gestation and those unexposed to famine during gestation. In order to assess whether the profile of cortisol concentrations during the stress protocol (including seven measurements) depended on famine exposure in late, mid or early gestation we applied a generalized linear model procedure (Proc Mixed, SAS 9, Cary, NC, USA) using an unstructured covariance matrix. In all our analyses we adjusted for gender and BMI. In our analyses of peak cortisol and cortisol AUC we adjusted for baseline cortisol concentrations. Additional adjustment was done for maternal characteristics, birth outcomes, smoking, socio-economic status, treatment with anti-depressive medication and season in which the test protocol was performed. We considered differences to be statistically significant if *p*-values were smaller than 0.05.

Results

Characteristics of the study population

Of the 1423 eligible Dutch famine birth cohort members invited to participate in the study, 740 people (52%) visited the clinic. People who agreed to participate had mean birth weights similar to those of eligible people who did not participate (3363g vs. 3343g, p = 0.41) and similar to the mean birth weight of the original cohort of 2412 people (3363g vs. 3346g, p = 0.38). A total of 725 participants completed the stress protocol. Fifteen men and women were unable to participate in the stress protocol due to logistical problems (n = 5) or because they were feeling unwell (n = 10). A total of 31 participants had no cortisol result on any of the seven saliva samples, due to the

fact that there was insufficient saliva to assess the cortisol concentration in the sample. The persons with all data missing were proportionally distributed among the five famine exposure groups (data not shown). They were excluded from further data-analysis. The group of 694 participants of whom the data could be used consisted of 333 men and 361 women, with a mean age of 58 years (SD 1 yr), of which 282 (41%) had been exposed to famine in utero. The mean number of missing cortisol samples per person was 1.0 sample (SD 1.6).

Table 1 Maternal, birth and adult characteristics according to timing of prenatal exposure to the Dutch famine

	Exposure to famine						
	Born before	In late gestation	In mid gestation	In early gestation	Conceiv-ed after	All (SD)	*n*
Number	215	120	100	62	197	694	
Proportion of men	.51	.44	.40	.48	.51	.48	694
Age	59.2	58.4	58.1	57.8	57.3	58.3 (0.9)	694
Maternal characteristics							
Age at delivery (years)	28.7	31.2 [a]	29.5	26.9[a]	28.5	29.0 (6.4)	694
Weight gain third trimester (kg)	2.8	0.0 [a]	4.4[a]	4.9[a]	3.5	2.9 (2.9)	492
Weight at last antenatal visit (kg)	66.6	62.9[a]	63.7[a]	68.8	69.6	66.5 (8.6)	617
Birth outcomes							
Gestational age (days)	284	283	285	288[b]	285	285 (11)	597
Birth weight (g)	3394	3199[b]	3202[b]	3461	3490	3366 (470)	694
Ponderal index (kg/m³)	26.2	26.1	25.9	26.1	26.6	26.2 (2.4)	688
Adult characteristics							
Body mass index (kg/m²)[c]	28.3	28.1	27.7	28.0	28.7	28.3 (1.2)	693
Proportion of current smokers	.22	.27	.27	.29	.22	.24	693
Current socio-economic status[c]	46	50	50	45	48	48 (1.4)	686
Waist/hip ratio	93.8	92.8	90.9	92.6	93.1	92.9 (9.0)	690
2-hr glucose concentration (mmol/l)[c]	5.7	6.0	6.2[e]	6.2	5.9	5.9 (1.4)	610
2-hr insulin concentration (pmol/l)[c]	241	241	248	269	235	242 (2.1)	606
Systolic blood pressure (mmHg)[c]	137	135	135	134	135	135 (1.1)	692
Diastolic blood pressure (mmHg)	81	81	80	82	82	81 (10)	692
Coronary heart disease prevalence	.07	.06	.04	.08	.07	.06	686
Metabolic syndrome prevalence[d]	.30	.33	.28	.36	.30	.31	688

Data are given as means and standard deviations, except where given as numbers and percentages.
[a]Statistically significant difference ($p < 0.05$) compared to people unexposed to famine.
[b]Adjusted for gender.
[c]Geometric means and standard deviation.
[d]According to the definition of the NCEP.
[e]Adjusted for gender and BMI.

Table 1 shows that mothers of babies who had been exposed to famine during late and mid gestation weighed less at the last antenatal visit compared to unexposed mothers. Babies exposed to famine during late and mid gestation were lighter compared to unexposed babies. At adult age, participants exposed to famine during mid gestation had higher 2-hr plasma glucose concentrations compared to those unexposed to famine during gestation. The groups of participants exposed to famine during gestation did not differ from the groups of participants unexposed to famine in terms of smoking behaviour, socio-economic status, treatment with oestrogen, anti-depressive medication or corticosteroid medication, prevalence of diabetes, the metabolic syndrome or coronary heart disease.

Cortisol at baseline, peak cortisol and cortisol AUC

Table 2 shows the mean baseline cortisol concentrations, peak cortisol concentrations and cortisol area under the curve from baseline to the second recovery period according to timing of prenatal exposure to famine. Men had higher baseline cortisol concentrations than women (30% [95% CI: 20 to 42]) (Fig.1). Men also had higher peak cortisol concentrations (24% [95% CI: 14 to 34]) and a higher cortisol AUC (25% [95% CI: 16 to 35]) compared to women. BMI was not associated with baseline cortisol (p = 0.41), but participants with a higher BMI had lower peak cortisol and cortisol AUC values. Peak cortisol concentrations were 1.3% lower (95% CI: 0.05 to 2.2) and cortisol AUC 1.3% lower (95% CI: 0.05 to 2.1) per unit increase in BMI. There were no associations between baseline cortisol, peak cortisol and cortisol AUC and maternal characteristics (maternal age at delivery, weight gain during the third trimester and weight at the last antenatal visit), nor were there any associations with birth outcomes (gestational age, ponderal index). Birth weight was also not associated with baseline cortisol (-2% [95% CI: -12 to 8] per kilogram increase in birth weight), peak cortisol (-4% [95% CI: -15 to 7]) and cortisol AUC (-1% [95% CI: -13 to 10]) (Fig.2).

Table 2 Baseline saliva cortisol concentrations and increase from baseline concentrations according to timing of prenatal exposure to the Dutch famine

	Exposure to famine						
	Born before	In late gestation	In mid gestation	In early gestation	Conceived after	All (SD)	*n*
Baseline cortisol (nmol/l)	4.0	3.7	3.4[a]	3.8	3.9	3.8 (1.8)	646
Peak cortisol (nmol/l)	7.1	5.6	6.9	5.8	6.8	6.6 (1.8)	456
Area under curve cortisol (nmol/l·min)	457	360	410	366	422	413 (1.8)	427

Data are given as geometric means and standard deviations.
[a]Statistically significant difference (p < 0.05, adjusted for gender and BMI) compared to people unexposed to famine.

Figure 1 Cortisol concentrations (given as geometric means) during the stress protocol (baseline 1 and 2, stroop task, mirror drawing task, speech task, recovery 1 and 2) according to gender.

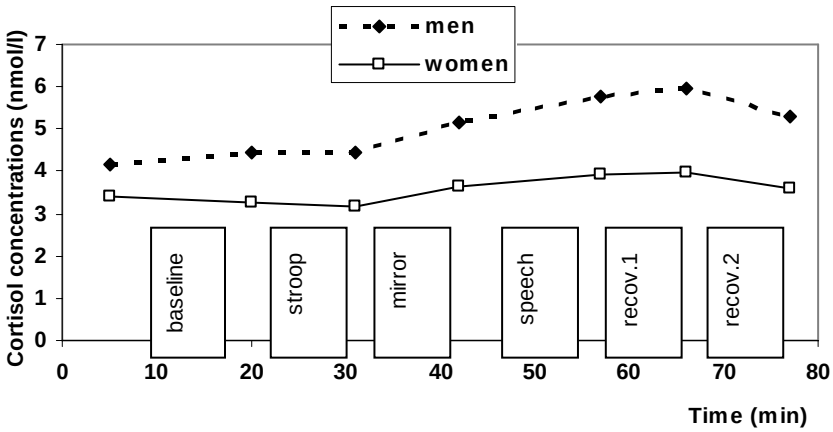
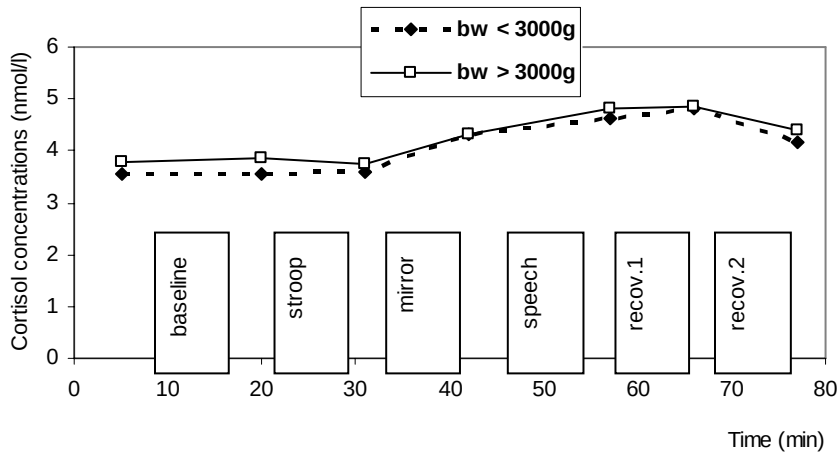


Figure 2 Cortisol concentrations (given as geometric means) during the stress protocol (baseline 1 and 2, stroop task, mirror drawing task, speech task, recovery 1 and 2) according to low or normal birth weight class.



Baseline cortisol (p = 0.50), peak cortisol (p = 0.58) and cortisol AUC (p = 0.46) did not differ among participants born before the famine and participants conceived after the famine (i.e. the unexposed control groups). Participants exposed to famine during mid gestation had baseline cortisol concentrations that were 15% (95% CI: 1 to 30) lower compared to unexposed participants. Further analysis showed that the effect of exposure to famine during mid gestation on baseline cortisol was only present in women (-27% compared to unexposed [95% CI: -51 to -8]) and not in men (-2% compared to unexposed [95% CI: -21 to 20]).

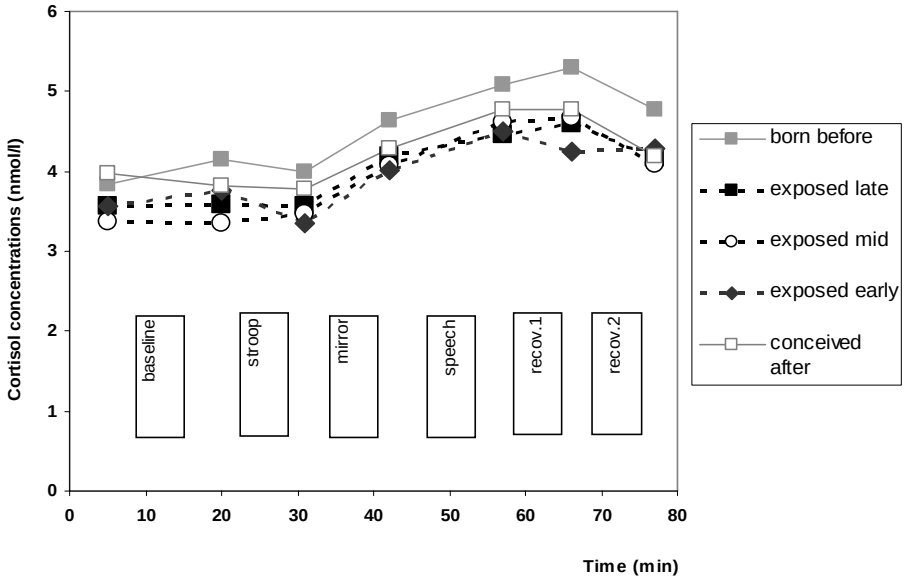
Peak cortisol (-6% [95% CI: -15 to 2]) and cortisol AUC (-6% [95% CI: -14 to 2] did not differ between exposed and unexposed. In a multivariable model adjusting for gender, BMI, season, maternal and birth characteristics, smoking, socio-economic status and treatment with anti-depressive medication, oestrogen or corticosteroids, we found that the effect of exposure to famine during mid gestation on the basal cortisol concentration was attenuated (-8% [95% CI: -33 to 13]).

Cortisol profile during the stress protocol

The average cortisol profile peaked during the first recovery period after the speech test. The mean increase from baseline was 30% (95% CI 23 to 37). Men had higher cortisol concentrations compared to women (Fig.1). Repeated measures analysis showed that the average cortisol profile of men was 32% higher than the average profile of women (95% CI 22 to 41). The men's cortisol response to stress seemed to increase more than the women's response, but this difference did not reach statistical significance (p = 0.07). A higher BMI was associated with a lower cortisol profile. Per unit increase in BMI the mean cortisol profile decreased by 1% (95% CI 0 to 2).
Both in men and in women, there were no associations between the cortisol profile and maternal characteristics, nor were there associations between the cortisol profile and birth outcomes, including birth weight. Fig. 2 shows the average cortisol profile for those with a birth weight below 3000g and for those with a birth weight above 3000g. The decrease in cortisol profile per kilogram increase in birth weight was 1% (95% CI -10 to 7).

Fig. 3 shows the cortisol profile for participants exposed to famine during late, mid or early gestation and participants born before and conceived after the famine. The cortisol profile of participants born before the famine did not differ significantly from the profile of participants conceived after the famine (p = 0.46). There were no significant differences between the average cortisol profiles of those exposed to famine at any time of gestation and those unexposed to famine during gestation. The mean sex and BMI adjusted difference was -6% (95% CI: -15 to 2) for the exposed compared to the unexposed participants. For participants exposed to famine during late gestation the difference compared to unexposed participants was -4% (95% CI: -16 to 7), versus -9% (95% CI: -22 to 3) for participants exposed in mid gestation and -4% (95% CI: -20 to 10) for participants exposed during early gestation. This was similar for men and women (p for interaction = 0.48). Additional adjustment for maternal and birth characteristics, smoking, socio-economic status, treatment with anti-depressive medication, oestrogen or corticosteroids and season did not change our findings regarding prenatal exposure to famine.

Figure 3 Cortisol concentrations (given as geometric means) during the stress protocol (baseline 1 and 2, stroop task, mirror drawing task, speech task, recovery 1 and 2) according to timing of prenatal exposure to famine.



Discussion

In this cohort study of men and women born around the time of the 1944-1945 Dutch famine, we could not demonstrate an effect of prenatal undernutrition on HPA-axis responses to psychological stress at age 58. We did find that women exposed to famine in midgestation had lower baseline cortisol concentrations, but this result was post-hoc and unexpected and may be a spurious finding.

Although evidence from animal studies show an effect of prenatal nutrient restriction on the HPA-axis response to stress in later life,[3,5-7] we were unable to demonstrate altered HPA responses to a psychological stress protocol in people who had been prenatally exposed to the Dutch famine. There could be a number of explanations for this discrepancy.

First, the cortisol responses we obtained may have been inadequate to demonstrate an effect of prenatal exposure to famine. Peak salivary cortisol concentrations were between 4.5 and 5.0 nmol/l, considerably less than levels of 12.0 nmol/l achieved, for example, by a number of studies that used the Trier Social Stress Test.[26] This may be due to the fact that we videotaped the speech test instead of using an audience to induce social-evaluative threat. In a meta-analysis of 208 psychological stress tests, Dickerson and Kemeny showed that the presence of evaluating individuals results in a robust HPA axis activation.[27]

The cortisol responses that were induced by our stress protocol may not have been robust enough to detect any differences between participants exposed and unexposed to famine during gestation, especially since variability in our data was large.
Secondly, the use of cortisol concentrations obtained at the beginning of the stress protocol as baseline may be inadequate as it is unable to account for the effects of anticipation of the stress protocol. Recent work suggests that this effect may be important as the association between birth weight and the cortisol response to stress was reported to be weak when compared with clinical pretest baseline measurements, while it was reported to be strong when compared to measurements obtained at home.[17]
The stress protocol that we have used may thus have been unsuitable to detect any famine related differences. However, there is also the possibility that prenatal exposure to the Dutch famine did not program the HPA response to stress. Recently, we found no evidence for an association between prenatal exposure to famine and HPA-axis responsiveness to an $ACTH_{1-24}$ stimulation test, which mainly measures HPA function at the adrenal level.[28] We now report evidence that HPA-axis functioning at the higher levels of the hippocampus and hypothalamus, as measured by the stress protocol, also does not seem to be associated with prenatal famine exposure. However, we did show that people exposed in early gestation were more stress responsive in terms of systolic blood pressure compared to people unexposed to famine.[29] These findings may indicate that fetal programming of the autonomic nervous system is more important than the HPA-axis in linking prenatal exposure to famine to the increased susceptibility to cardiovascular disease.
We could not replicate recent findings of an association between low birth weight and cortisol responses to psychological stress.[16,17] This could have to do with the above mentioned methodological limitations, but there may be a biological explanation for this phenomenon. Low birth weight in our study is most likely induced by maternal undernutrition, whereas low birth weight in the other studies is likely to be caused by placental problems, especially so because one of these studies concerned twins.[16] Consequences for the HPA response to stress of low birth weight induced by maternal undernutrition may differ from consequences of low birth weight induced by placental insufficiency.[30]
We found that men had higher salivary cortisol responses to stress than women. Although evidence on the association between gender and HPA axis responses to stress is contradictory, our study results confirm findings from a study of Kudielka et al. which showed free salivary cortisol response to the Trier Social Stress Test to be elevated in elderly men.[26] The authors hypothesized that lower levels of corticosteroid binding globulin (CBG) in elderly men compared to elderly women may underlie the association between gender and cortisol responses to stress.

In summary, we found no evidence for a programming effect of prenatal exposure to famine on stress response of the HPA-axis. However, the stress protocol we have used may have been unsuccessful in inducing a strong enough HPA axis activation to be able to detect famine related differences.

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The metabolic syndrome in adults prenatally exposed to the Dutch famine

Susanne de Rooij, Rebecca Painter, Frits Holleman,
Patrick Bossuyt, Tessa Roseboom

Submitted

Abstract

Background

Epidemiological studies have shown that the metabolic syndrome might originate in utero.

Objective

We aimed to determine whether exposure to prenatal famine is associated with an increased prevalence of the metabolic syndrome.

Design

We assessed the prevalence of the metabolic syndrome according to the NCEP definition in 783 Dutch famine birth cohort members. Participants were born as term singletons around the time of the 1944-1945 Dutch famine.

Results

Exposure to famine during gestation was not associated with the metabolic syndrome (OR 1.2 [95% CI: 0.9 to 1.7]). Low birth weight was also not significantly associated with the metabolic syndrome (OR 1.3 [0.9 to 1.8] per kilogram decrease in birth weight). Exposure to famine during gestation was associated with higher 2h glucose (0.4 mmol/l [0.1 to 0.7]) and insulin concentrations (29 pmol/l [1 to 61]) and higher triacylglycerol concentrations (0.1 g/l [0.0 to 0.2]). Participants exposed to famine in early gestation had higher fasting glucose concentrations (0.2 mmol/l [0.0 to 0.4]) and a higher LDL/HDL ratio (0.5 units [0.1 to 0.9]) compared with unexposed participants. Men exposed in early gestation had lower HDL-cholesterol (-0.1 mmol/l [-0.2 to 0.0]) than unexposed men.

Conclusions

Prenatal exposure to famine or low birth weight is not associated with a higher prevalence of the metabolic syndrome. Our findings suggest that although elements of the metabolic syndrome may be programmed by fetal undernutrition, the syndrome does not appear to have a single underlying origin in utero and may even not be a syndrome at all.

Introduction

Epidemiological studies have shown that several metabolic abnormalities may have their origin in fetal life. Men and women who were small at birth, a proxy for a poor fetal environment, develop more impaired glucose tolerance, insulin resistance, dyslipidemia, hypertension and obesity in later life.[1-6] These associations are hypothesized to result from permanent structural and physiological adaptations made by the fetus in response to a poor environment in utero.[7]

The metabolic syndrome is known by several names: Syndrome X, the Deadly Quartet and the Insulin Resistance Syndrome.[8-10] It is a constellation of interrelated metabolic risk factors that predisposes to the development of type 2 diabetes and cardiovascular disease.[11,12] Clinical manifestations of the syndrome include glucose intolerance, insulin resistance, central obesity, dyslipidemia and hypertension. A number of different definitions are being used to diagnose the metabolic syndrome.[13-16]

Several studies have shown that small size at birth is associated with an increased risk of the metabolic syndrome, and it has even been suggested that the syndrome should be renamed the small baby syndrome.[17-24] Results of these studies should, however, be interpreted with caution. Half of these studies only found associations between low birth weight and the metabolic syndrome if low birth weight was combined with high adult BMI,[19] catch-up growth,[21,22] or lifestyle factors such as smoking and low physical activity.[23]

The effects of low birth weight on health in adult life have been hypothesized to result from poor maternal nutrition.[25] The Dutch famine birth cohort provides the opportunity to study the consequences of maternal undernutrition in later life. Cohort members were born around the time of the Dutch famine which occurred at the end of World War II, between November 1944 and May 1945. The mean caloric rations during the famine were as low as 400-800 calories a day. Previous findings in this unique cohort study have demonstrated that exposure to famine during any stage of gestation was associated with impaired glucose tolerance,[26,27] exposure to famine during midgestation was related to more microalbuminuria,[28] and exposure during early gestation to an excess in dyslipidemia,[29] obesity in women,[30] altered blood clotting,[31] and a higher prevalence of coronary heart disease.[32,33] The effects were independent of size at birth as well as adult risk factors.

Based on the associations between prenatal exposure to famine and various metabolic outcomes, we hypothesized that exposure to the Dutch famine in utero may lead to an increased prevalence of the metabolic syndrome.

Subjects and Methods

Population

All participants were members of the Dutch famine birth cohort. This cohort consists of 2414 men and women who were born between 1 November 1943 and 28 February 1947 as term singletons in the Wilhelmina Gasthuis, a hospital in Amsterdam, the Netherlands. The selection procedure and subsequent loss to follow up have been described in detail elsewhere.[26,34] Cohort members who were still living in the Netherlands and whose address was known to the investigators were invited to participate in the study. Of the group of 1423 eligible people, 810 agreed 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.[35] 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. We delineated periods of 16 weeks each to differentiate between those exposed in late gestation (born between 7 January and 28 April 1945), in midgestation (born between 29 April and 18 August 1945) and in early gestation (born between 19 August and 8 December 1945). 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.

Study parameters

Information about the mother, the course of the pregnancy and the size of the baby at birth was extracted from medical birth records.[36] We measured height using a fixed or portable stadiometer, weight with Seca and portable Tefal scales. We measured waist circumference with a flexible tape measure midway between the costal margin and the iliac crest. Blood pressure was measured in duplo on two occasions (morning and afternoon) using an automated device (Omron 705 CP/IT; Omron Healthcare UK, West Sussex, UK) and appropriate cuff sizes. Mean systolic and diastolic blood pressure were calculated using all available measurements.

After an overnight fast, we performed an oral glucose tolerance test (OGTT) with a standard load of 75 grams. Participants with pre-existing diabetes (defined as taking glucose-lowering medication) were excluded from the OGTT. Plasma glucose

concentrations were measured by standardised enzymatic photometric assay on a Modular P analyzer (Roche, Basel, Switzerland) and plasma insulin concentrations by immuno-luminometric assay on an Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles, USA). Type 2 diabetes was defined as a fasting glucose level of >7.0 mmol/l and/or a 2hr glucose level of >11.0 mmol/l or taking anti-diabetic medication. Blood was drawn for analysis of Low Density Lipoprotein (LDL)-cholesterol, High Density Lipoprotein (HDL)-cholesterol and triacylglycerol. HDL-cholesterol and triacylglycerol were measured using an enzymatic colorimetric reagent (Roche) on a P-800 Modular (Roche). LDL-cholesterol was calculated using the Friedewald formula. Standard 12-lead electrocardiograms (ECG) were made of all participants. Coronary heart disease was defined as the presence of one or more of the following: angina pectoris according to the Rose/WHO questionnaire; Q_waves on the ECG (Minnesota codes 1-1 or 1-2) or a history of coronary revascularization (angioplasty or bypass surgery). Information about socio-economic status, medical history, lifestyle (smoking, sports participation) and use of medication was obtained in a standardised interview. We defined current socio-economic status according to ISEI-92, which is based on the participant's, or their partner's occupation, whichever status is highest.[36]

Definition of the metabolic syndrome

We used the widely applied National Cholesterol Education Program (NCEP) definition of the metabolic syndrome.[15] NCEP defines the metabolic syndrome as clustering of three or more of the following characteristics: waist circumference ≥ 102 cm in men and ≥ 88 cm in women, triacylglycerol ≥ 1.7 mmol/l, blood pressure ≥ 130/85 mmHg or taking anti-hypertensive medication, HDL cholesterol < 1.03 mmol/l in men and < 1.3 mmol/l in women and fasting glucose ≥ 6.1 mmol/l or taking anti-diabetic medication. In addition, we applied the recently developed definition of the International Diabetes Federation (IDF).[16] The IDF has lower thresholds for two components of the syndrome: waist circumference ≥ 94 cm in men and ≥ 80 cm in women and fasting glucose ≥ 5.6 mmol/l.

Statistical analyses

Logarithmic transformations were applied to variables with skewed distributions. We used linear and logistic regression analysis to compare maternal, birth and adult characteristics between famine exposed and unexposed groups. In all analyses we first compared prenatally famine exposed participants to unexposed participants and then those exposed in late, mid and early gestation to those unexposed in gestation. We also used linear and logistic regression analyses to explore associations between birth weight and adult characteristics.

We adjusted for sex and BMI in all analyses, except the analyses of the prevalence of components of the metabolic syndrome and of the syndrome itself, in which we adjusted for sex only. Additional adjustment was done for maternal and birth characteristics, smoking, practising of sports, and current socio-economic status. We considered differences to be statistically significant if *p*-values were smaller than 0.05.

Results

Population characteristics

A total of 810 men and women participated, of which 27 participants had to be excluded from the analysis because of missing data due to failure of venepuncture or non adherence to fasting instructions. Of the group of 783 participants for whom we had complete data for all components of the syndrome, 359 were men (46%) and 424 were women (54%) with a mean age of 58 years (SD 1). A total of 452 (58%) were unexposed to famine during gestation and 331 participants (42%) were exposed during gestation. Table 1 shows that babies exposed to famine during late and midgestation had lower birth weights and that their mothers weighed less at the end of pregnancy compared to unexposed babies. At age 58, there were no significant differences between exposed and unexposed groups in smoking pattern, sports participation and socio-economic status.

Table 1 Maternal, birth and adult characteristics according to timing of prenatal exposure to the Dutch famine.

	Exposure to famine						
	Born before	In late gestation	In mid gestation	In early gestation	Conceiv-ed after	All (SD)	*n*
n	238	141	116	74	214	783	-
Age (years)	59.2	58.5	58.2	58.0	57.4	58.3 (1.0)	783
Proportion of men	.48	.43	.39	.43	.51	.46	783
Maternal and birth characteristics							
Weight gain 3rd trimester (kg)	2.8	0.0 [a]	4.5 [a]	5.0 [a]	3.5	2.9 (3.0)	549
Weight at last antenatal visit (kg)	66.6	62.5 [a]	63.7 [a]	69.8	69.3	66.5 (8.7)	691
Gestational age (days)	284	283	286	289 [a]	285	285 (11)	671
Birth weight (g)	3392	3195 [a]	3201 [a]	3503	3467	3359 (472)	783
Adult characteristics							
Proportion of current smokers	.22	.27	.26	.31	.23	.25	781
Proportion of people that practice sports	.55	.61	.58	.61	.51	.56	781
Current socio-economic status	48	52	51	47	50	50 (14)	773

Data are given as means (SD), except where given as numbers and percentages.
[a]Statistically significant difference ($p < 0.05$, adjusted for gender) compared to participants unexposed to famine during gestation.

In Table 2 anthropometric and metabolic characteristics at age 58 are given per exposure group. Exposure to famine during gestation was associated with higher 2h glucose (0.4 mmol/l [95% CI 0.1 to 0.7]) and insulin concentrations (29 pmol/l [1 to 61]) and higher triacylglycerol concentrations (0.1 g/l [0.0 to 0.2]). Participants exposed to famine in early gestation had higher fasting glucose concentrations (0.2 mmol/l [0.0 to 0.4]) and a higher LDL/HDL ratio (0.5 units [0.1 to 0.9]) compared with unexposed participants. Men who were exposed to famine during early gestation had lower HDL-cholesterol (-0.1 mmol/l [-0.2 to 0.0]) than unexposed men.

Table 2 Anthropometric and metabolic characteristics according to timing of prenatal exposure to the Dutch famine.

	Exposure to famine					
	Born before	In late gestation	In mid gestation	In early gestation	Conceiv-ed after	All (SD)
n	238	141	116	74	214	783
Anthropometric characteristics						
BMI (kg/m2)[a]	28.0	28.0	27.8	27.5	28.7	28.1 (1.2)
Waist circumference (cm)	96.8	96.2	94.7	95.2	97.6	96.4 (13.1)
Metabolic characteristics						
Fasting glucose (mmol/l)[a]	5.6	5.5	5.5	5.7 [2]	5.5	5.6 (1.1)
2hr glucose (mmol/l)[a]	5.8	6.2	6.2	6.2	5.9	6.0 (1.4)
Fasting insulin (pmol/l)[a]	56	58	56	59	57	57 (1.8)
2hr insulin (pmol/l)[a]	238	263	254	269	240	248 (2.1)
LDL cholesterol (mmol/l)	3.7	3.7	3.5	3.8	3.6	3.6 (1.0)
HDL cholesterol (mmol/l)[a]	1.5	1.5	1.5	1.5	1.5	1.5 (1.3)
LDL/HDL ratio[a]	2.3	2.5	2.3	2.6 [2]	2.4	2.4 (1.4)
Triglycerides (g/l)[a]	1.2	1.3	1.3	1.3	1.3	1.3 (1.8)
Systolic blood pressure (mmHg)[a]	137	135	136	135	135	136 (1.1)
Diastolic blood pressure (mmHg)	81	81	80	82	82	81 (10)
Prevalence coronary heart diasese (%)	6.3	5.7	3.9	8.2	6.9	6.2
Prevalence type 2 diabetes (%)	15.1	14.2	13.8	18.9	12.6	14.4

Data are given as means (SD). [a]Geometric means and standard deviation. [2]Statistically significant difference ($p < 0.05$, adjusted for sex and BMI) compared to participants unexposed to famine during gestation.

The metabolic syndrome and famine exposure

Table 3 shows the prevalence of components of the metabolic syndrome and of the syndrome itself according to definitions of NCEP per exposure group. Exposure to famine during gestation was not associated with the metabolic syndrome (OR 1.2 [95% CI: 0.9 to 1.7]). Although the prevalence of the syndrome was higher in participants exposed in late (1.4 [0.9 to 2.1]) and early gestation (1.4 [0.6 to 1.5]), the difference compared to those unexposed was not statistically significant.

Table 3 Prevalences of components of the metabolic syndrome and the metabolic syndrome itself following the NCEP definition according to timing of prenatal exposure to the Dutch famine.

	Exposure to famine					
	Born before	In late gestation	In mid gestation	In early gestation	Conceiv-ed after	All
n	238	141	116	74	214	783
Components						
Waist circumference ≥ 102 men, ≥ 88 women	.50	.48	.53	.51	.54	.52
Fasting glucose ≥ 6.1 mmol/l or treatment	.29	.28	.22	.31	.24	.27
Triglycerides ≥ 1.7 mmol/l	.27	.31	.32	.37	.32	.31
HDL cholesterol < 1.03 men, < 1.3 women	.11	.23 [a]	.18	.24 [a]	.15	.17
Blood pressure ≥ 130/85 mmHg or treatment	.69	.67	.69	.62	.67	.67
Metabolic syndrome	.30	.38	.29	.38	.30	.32
Metabolic syndrome according to IDF definition	.48	.51	.47	.51	.47	.49

[a]Statistically significant difference ($p < 0.05$, adjusted for gender) compared to participants unexposed to famine during gestation.

Participants prenatally exposed to famine more often had an HDL-concentration below the metabolic syndrome threshold of 1.03 mmol/l for men and 1.3 mmol/l for women compared to unexposed participants (OR 1.8 [1.2 to 2.6]). The difference was most pronounced in those exposed in late (2.0 [1.2 to 3.2] and early gestation (2.1 [1.2 to 3.8]). Additional adjustment for maternal age, maternal weight gain during the third trimester, maternal weight at the end of pregnancy, gestational age, birth weight, smoking and sports participation did not change the results.

IDF definition of the metabolic syndrome

When we defined the metabolic syndrome according to the definition of the IDF (Table 3) the mean prevalence was higher than when the NCEP definition was used, but the difference in prevalence between those exposed to famine during late (OR 1.2 [95% CI: 0.8 to 1.7]) and early gestation (OR 1.2 [0.7 to 1.9]) and those unexposed to famine was much smaller.

The metabolic syndrome and size at birth

Table 4 shows that although more people with low birth weight had the metabolic syndrome (NCEP definition), the association was not statistically significant (OR 1.3 [95% CI: 0.9 to 1.8] per kilo decrease in birth weight). Men and women with low birth weight less often had a waist circumference above the metabolic syndrome threshold of 102 cm for men and 88 cm for women (OR 0.7 [0.5 to 1.0] per kilo decrease in birth weight). People who were small at birth more often had a blood pressure above the metabolic syndrome threshold of 130/85 (OR 1.7 [1.2 to 2.4] per kilo decrease in birth weight).

Table 4 Prevalences of components of the metabolic syndrome and the metabolic syndrome itself following the NCEP definition according to birth weight groups.

Birth weight (grams)	<3000	3000-3500	>3500	*P*
n	179	325	279	
Components				
Waist circumference ≥ 102 men, ≥ 88 women	.48	.51	.55	.04
Fasting glucose ≥ 6.1 mmol/l or treatment	.30	.25	.26	.21
Triglycerides ≥ 1.7 mmol/l	.35	.30	.30	.15
HDL cholesterol < 1.03 men, < 1.3 women	.20	.15	.18	.74
Blood pressure ≥ 130/85 mmHg or treatment	.77	.64	.65	.01
Metabolic syndrome	.37	.30	.32	.31
Metabolic syndrome according to IDF definition	.55	.45	.48	.13

P values for differences between birth weight groups, adjusted for sex.

Discussion

Although prenatal exposure to famine is associated with elements of the metabolic syndrome, we could not demonstrate an association between prenatal famine exposure and the metabolic syndrome, nor did we find an association between birth weight and the syndrome.

A number of methodological issues must be raised. The occurrence of the metabolic syndrome is highly dependent on the definition used to diagnose the syndrome. The mean prevalence in our cohort ranged from 32% according to the widely applied NCEP definition to 49% according to the recently developed IDF definition.[15,16] Differences between famine exposed groups became much smaller when using the IDF definition compared with using the NCEP definition. This discrepancy is due to the fact that the NCEP definition applies higher cut off points for waist circumference and fasting glucose concentrations than the IDF definition.

The use of different definitions of the metabolic syndrome may explain why we could not confirm associations between the metabolic syndrome and low birth weight. Studies that reported such an association used a wide range of definitions.[17-24]

The metabolic syndrome has recently been criticized for its value as a cardiovascular disease risk marker.[37] The criticism centers around the notion that risk is a progressive function of, for example, hyperglycemia and hypertension and cannot simply be regarded as present or absent, depending on whether thresholds are exceeded or not.

We may have underestimated the prevalence of the metabolic syndrome in participants who were exposed to famine during gestation as a consequence of selective participation. At age 50 we found an increase in the occurrence of type 2 diabetes and coronary heart disease among those exposed to famine in utero.[26,32] This may have led to excess mortality and disability among those exposed to prenatal famine, resulting in selective participation of people that were fit enough to attend the clinic at age 58.

In a recent follow-up study of adult survival, however, we found no evidence for excess mortality among subjects exposed to famine in utero.[34] Nonetheless, selective participation may have had an influence on our findings.

Ever since the introduction of the metabolic syndrome, doubts have been raised concerning the integrity of the syndrome as a constellation of metabolic risk factors caused by a unifying underlying pathology, which confers a risk for cardiovascular disease greater than the sum of its parts (see Kahn et al for a review of the literature[37]). Prenatal famine exposure was associated with several metabolic outcomes: participants exposed during gestation had higher 2h glucose, 2h insulin and triacylglycerol concentrations; participants exposed during early gestation had higher fasting glucose and a higher LDL/HDL ratio; men exposed in early gestation also had lower HDL concentrations. We could, however, not demonstrate any effects on blood pressure or waist circumference. Our data suggest that the metabolic syndrome as a clustering of risk factors does not have a single underlying origin in utero and may thus, from an etiologic perspective, not be a syndrome at all, but merely a co-existence of several risk factors for type 2 diabetes and cardiovascular disease. Some of these unfavourable metabolic outcomes may in themselves be the result of adverse circumstances during fetal life, also depending on nature of the circumstances and timing during gestation. Organs and tissues are more vulnerable during periods of rapid growth and development, the so-called 'critical periods'. In this way, exposure to famine during a specific period of gestation may lead to problems associated with the organs or physiological systems being developed at that particular moment, while exposure during another period of gestation may lead to problems associated with other organs and systems. We have shown this previously for microalbuminuria which is related to exposure in midgestation,[28] and for dyslipidemia,[29] obesity in women,[30] fibrinogen,[31] and coronary heart disease,[32,33] all of which are associated with exposure in early gestation.

In conclusion, prenatal exposure to famine or low birth weight is not associated with a higher prevalence of the metabolic syndrome. We suggest that although elements of the metabolic syndrome are programmed during fetal life, the syndrome does not have a single underlying origin in utero and may not even be a syndrome at all.

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The effects of prenatal exposure to undernutrition on glucose and insulin metabolism in later life – Review

Susanne de Rooij, Rebecca Painter, Tessa Roseboom

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Abstract

Purpose of review

Evidence from experimental and epidemiologic studies suggests that early nutrition may play an important role in the pathogenesis of type 2 diabetes. It is hypothesized that the fetus adapts its structure and physiology in response to an adverse environment in utero, which predisposes to chronic disease in later life. We review results from the Dutch famine birth cohort study, in which the effects of prenatal exposure to famine on health in later life are investigated. We focus on the consequences for glucose and insulin metabolism.

Recent findings

People exposed to famine during gestation show impaired glucose tolerance at ages 50 and 58 years. The aetiology of this association seems to lie, at least partly, in programming of the pancreatic beta cell, resulting in an impaired insulin response that is already present in the normoglycaemic state. We found no evidence indicating that the hypothalamic-pituitary-adrenal axis plays an intermediate role in the association between prenatal undernutrition and glucose intolerance.

Summary

Although the exact pathophysiology of the association between exposure to famine in utero and glucose intolerance is not clear, our findings stress the importance of maternal nutrition during gestation for the offspring's glucose metabolism in later life.

Introduction

Early nutrition may play an important role in the pathogenesis of type 2 diabetes. The intermediate pathway suggested is that of 'fetal programming'.[1] In 1992, Hales and Barker proposed that poor nutrition in fetal and early infant life leads to permanent adaptation of the pancreas and metabolic functioning, predisposing to the development of impaired glucose tolerance and type 2 diabetes in later life.[2] The Dutch famine birth cohort enables us to study the effects of prenatal exposure to famine on health in later life. This paper reviews findings from the Dutch famine birth cohort study on glucose and insulin metabolism.

Fetal origins

The fetal programming hypothesis of type 2 diabetes emerged from the observation that among men born in Hertfordshire, England, small size at birth was strongly associated with the development of impaired glucose tolerance and type 2 diabetes in later life.[3] This association has since been established in a variety of different populations including populations from England,[4] Sweden,[5] India,[6] and the US.[7,8] In a systematic review, Newsome et al. included 48 papers and showed a consistent relationship between low birth weight and an adverse profile of later glucose and insulin metabolism.[9] Birth weight, however, is a summary measure of the fetal environment. Experimental studies in animals have shown that poor maternal nutrition can substantially increase the risk of glucose intolerance and type 2 diabetes, without necessarily affecting birth weight.[10-12,13•,14] A unique equivalent of these animal models in humans are follow-up studies of people who were exposed to famine in utero. By investigating the effects of prenatal famine on glucose and insulin metabolism in later life, we can gain insight into the role of early nutrition in programming type 2 diabetes.

The Dutch famine

The Dutch famine happened at the end of World War II. Over a 5-month period the population of the urban west of the Netherlands was severely undernourished. The famine was a consequence of an embargo on the food transports imposed by the German occupying forces as a retaliation for a strike of the Dutch railways, which was aimed at hampering transport of German troops. At the height of the famine from December 1944 to April 1945, the official daily rations for the general population of 21 years and older varied between 400 and 800 calories. Children younger than 1 year were relatively protected; their daily rations never fell below 1000 calories and the specific nutrient components were always above the standards used by the Oxford Nutritional Survey.[15]

Although food also came from other sources, the official rations are believed to adequately reflect the variation of food availability over time during the famine .[16]
Despite the harsh circumstances, women still conceived and gave birth to their babies. The famine was a humanitarian disaster, but it has left us the opportunity to study the effects of maternal malnutrition on health in later life.

The Dutch famine birth cohort study

The Dutch famine birth cohort consists of 2414 men and women who were born as term singletons in the Wilhelmina Gasthuis in Amsterdam between 1 November 1943 and 28 February 1947. The selection procedure and subsequent loss to follow-up have been described in detail elsewhere.[17,18] Detailed birth records were kept during the famine, which provided information about the mother, the course of pregnancy, and the size of the baby at birth.[17]
We defined the famine period according to the daily official food rations for the general population older than 21 years. A person was considered to be prenatally exposed to famine if the average daily ration during any 13-week period of gestation contained fewer than 1000 calories. Therefore, people born between 7 January 1945 and 8 December 1945 were considered exposed in utero. We delineated periods of 16 weeks each to differentiate between those who had been exposed in late gestation (born between 7 January and 28 April 1945), in midgestation (born between 29 April and 18 August 1945), and in early gestation (born between 19 August and 8 December 1945). Cohort members born before 7 January 1945 and conceived after 8 December 1945 were considered unexposed to famine in utero and acted as control group.
Mothers who had been exposed to famine during late and midgestation weighed less at the last antenatal visit than unexposed mothers.[17] Late-exposed mothers gained almost no weight during the last trimester of pregnancy. Babies exposed in late and mid gestation were lighter and shorter than unexposed babies. They also had smaller heads and placentas.
At age 50 (range 48-53 years) and at age 58 (range 56-61 years) we invited all eligible members of the cohort to participate in a data collection protocol. At age 50, a total of 741 cohort members attended the clinic. At age 58, a total of 810 cohort members, of which 538 subjects had also participated at age 50, visited the clinic or were visited at home. A detailed description of the data collection protocols is given elsewhere.[18,19••]
We have shown previously that prenatal exposure to famine during midgestation is associated with a greater prevalence of obstructive airways disease[20] and microalbuminuria,[21] and exposure during early gestation with an excess in dyslipidemia,[22] obesity in women,[23] higher concentrations of fibrinogen,[24] a higher prevalence of coronary heart disease[18,25] and breast cancer,[26] and an increased negative

self-perception of health.[27] We focus here on the consequences of prenatal exposure to famine on glucose and insulin metabolism.

Prenatal exposure to the Dutch famine and glucose tolerance

In line with evidence from experimental animal studies and studies of size at birth, the Dutch famine birth cohort study showed for the first time in humans that at age 50 as well as at age 58 years, glucose tolerance was impaired after exposure to famine during gestation.[17,19••] Men and women exposed to famine in utero had higher 2-hour glucose (0.4 mmol/l [0.1 to 0.7] at ages 50 and 58) and 2-hour insulin concentrations (25 pmol/l [2 to 52] at age 50; 27 pmol/l [0 to 58] at age 58) after an oral glucose tolerance test (OGTT). At age 50, the impaired glucose tolerance was most pronounced in those exposed in late and midgestation. At age 58, however, glucose tolerance was impaired among all famine-exposed groups, regardless of the timing of the exposure during gestation. These associations were independent of adult body mass index and larger than could be explained by famine-related differences in birth weight.

Our findings do not match those of the Leningrad siege study.[28] Men and women who were in utero exposed to malnutrition during the Leningrad siege (1941-1944) did not have significantly altered glucose tolerance compared with those unexposed to the siege. The Leningrad siege, however, occurred over a long period in a previously malnourished population that remained badly nourished after the siege ended. In contrast, the Dutch famine lasted a relatively short time (5 months) and struck a population that had access to adequate nutrition before and after the famine. The transition from a poor nutritional environment to an environment where nutrition was adequate may have played an important role in the later development of impaired glucose tolerance. Studies have shown that prenatally food-restricted rats that received an abundant diet postnatally had a worse glucose profile than rats that received abundant diets both pre- and postnatally.[29]

Mechanisms of impaired glucose tolerance after prenatal exposure to famine

In order to gain insight into the pathophysiology of impaired glucose tolerance in adults who were exposed to famine during gestation, we investigated the relative contributions of increasing age, insulin deficiency, insulin resistance, and altered hypothalamic-pituitary-adrenal (HPA) axis function.

Effects of increasing age

We investigated the progression of glucose intolerance between the ages of 50 and 58 years in our cohort but found that glucose intolerance did not progress more rapidly among people exposed to famine during gestation.[19••]

Studies in prenatally undernourished rats showed that glucose tolerance decreased further between 3 and both 12 and 15 months of age compared with glucose tolerance in normally fed rats.[11,30] The fact that we could not show such an age-related deterioration in glucose tolerance in the prenatally famine-exposed participants in our study may be due to selective participation of the more healthy subjects in our cohort at age 58 compared with age 50. There was no evidence, however, of a difference in response rates between those exposed and those unexposed to famine. Therefore, selective participation probably had limited effects on our results. An alternative explanation may be that 8 years of follow-up is too short a period to notice a prenatal famine-related deterioration.

Insulin metabolism

In order to assess whether the reduced glucose tolerance after prenatal exposure to famine was due to insulin resistance or deficiency, we performed an intravenous glucose tolerance test (IVGTT) in a normoglycaemic sub sample (n = 94) of the Dutch famine birth cohort. Participants exposed to famine in midgestation and early gestation had a lower insulin response relative to insulin sensitivity as indicated by a lower disposition index.[31••] The difference compared with unexposed participants reached statistical significance in participants exposed in midgestation. Exposure to famine during midgestation and possibly early gestation may thus lead to beta cell dysfunction. Midgestation is a critical period in the development of the beta cells.[32]

Our data are in line with the findings of various experimental studies in rats. These studies showed that prenatal undernutrition induced programming of the pancreas by affecting neogenesis, proliferation, and apoptosis of the beta cells, resulting in a reduced beta cell mass and subsequent impaired insulin secretion.[10,33-36]

Most studies in humans suggest that insulin resistance and not insulin deficiency is the mechanism that underlies fetal programming of glucose intolerance,[37-40] but few studies have looked at the insulin response relative to insulin sensitivity. One study in humans that used the disposition index, found that small size at birth was associated with a lower disposition index, confirming our results.[41] We therefore think that beta cell dysfunction may play a larger role in fetal programming of type 2 diabetes than was suggested by studies of size at birth.

Despite the fact that there is strong evidence suggesting that insulin resistance is the link between poor maternal nutrition,[14,42-44] poor fetal growth,[37-40] and impaired glucose tolerance, we were unable to show an association between prenatal exposure to famine and insulin resistance in our cohort. The higher 2-hour insulin concentrations following the OGTT suggest some degree of insulin resistance, but we could not demonstrate this with an IVGTT. Insulin sensitivity was somewhat lower in mid and early exposed participants, but the difference was not statistically significant. We performed the IVGTT in a subsample of normoglycaemic

participants. It may be that beta cell dysfunction precedes insulin resistance and that we will be able to show insulin resistance in the famine-exposed participants of the IVGTT with increasing age.

Our results are supported, however, by results from a study in which mice were fed a 50% calorie-restricted diet during gestation.[45••] Offspring of these mice showed impaired glucose tolerance combined with increased fed insulin levels, suggestive of insulin resistance, but further testing showed that they had normal insulin sensitivity and that beta cell function was impaired. The authors suggested that the hyperinsulinemia was caused by an impairment of insulin clearance, due to a reduction in carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) expression, which is a regulator of hepatic insulin clearance.

The hypothalamic-pituitary-adrenal-axis

Prenatal undernutrition may lead to changes in the setpoint of the HPA axis during fetal life that could result in a long-term increase in secretion of key neuroendocrine mediators of the stress response.[46] Elevated concentrations of glucocorticoids are associated with glucose intolerance and insulin resistance.[47] We therefore performed a psychological stress test consisting of a battery of three stressful tasks (n = 694), and additionally a dexamethasone suppression and corticotropin ($ACTH_{1-24}$) stimulation test in a normoglycaemic subsample of our cohort (n = 97). Salivary cortisol responses following the psychological stress protocol did not differ between those exposed and those unexposed to famine in utero, nor did plasma cortisol concentrations following dexamethasone suppression and $ACTH_{1-24}$ stimulation.[48,49•] We did find an increased response to the psychological stress protocol in terms of systolic blood pressure in those exposed to famine in early gestation.[50]

The fact that we did not find associations between prenatal exposure to famine and HPA axis activity was unexpected, because animal models have provided a large body of evidence showing programming of the HPA axis by prenatal undernutrition.[51,52] Rats as well as sheep that were protein or nutrient restricted during late gestation showed increased postnatal activity of the HPA axis indicated by heightened levels of glucocortocoids. An explanation for the absence of an association between prenatal exposure to famine and altered HPA function may be that the cortisol responses we obtained were inadequate to demonstrate an effect of prenatal exposure to famine. The responses to the psychological stress protocol, for example, were much lower than those obtained in an equivalent protocol.[53]

We feel, however, that the absence of altered HPA activity in response to stress in participants in utero exposed to famine may be real. Results from studies of the association between size at birth and HPA activity are conflicting.

Some studies found associations between low birth weight and fasting cortisol and increased responsiveness to dexamethasone suppression and $ACTH_{1-24}$ stimulation, but others failed to find such associations.[54-58]

Alternative mechanisms

The aetiology of the association between prenatal exposure and decreased glucose tolerance seems to lie, at least partly, in the programming of the pancreatic beta cell, resulting in an impaired insulin response. Programming of other organs and systems may add to the development of impaired glucose tolerance in people who were exposed to famine. Besides beta cells, muscle, liver, and adipose tissue and the neuroendocrine axes have been shown to be susceptible to fetal programming.

Prenatal undernutrition may affect morphologic or physiological development of these organs. Animal experiments have shown that undernutrition during gestation affected morphologic development in terms of reduced skeletal muscle mass,[59] altered liver structure,[42] and increased adipose tissue mass.[60] Physiologically, prenatal undernutrition affected expression of glucose and insulin transporter- and signalling molecules in muscle,[43] liver,[42] and adipose tissue.[13]•

Prenatal exposure to famine may also be related to programming of the adipoinsular axis, which is involved in energy balance. Prenatal food restriction in rats led to permanent dysregulation of the adipoinsular feedback system, inducing increased adiposity, hyperinsulinemia and hyperleptinemia predisposing to the development of type 2 diabetes.[61]

Gene-environment interactions

The fetal origins hypothesis proposes that type 2 diabetes originates through adaptations made by the fetus in response to an adverse intrauterine environment. This environment changes gene expression and leads to physiological or morphologic phenotypes associated with disease.[62] On this basis, one would predict that genes associated with glucose/insulin metabolism will have different effects in people who had different fetal environments.

To test this hypothesis, we investigated whether prenatal exposure to undernutrition interacts with the effects of the Pro12Ala polymorphism of the *PPAR-γ2* gene (peroxisome proliferator activated receptor-γ2), which is involved in adipocyte differentiation, regulating glucose and lipid homeostasis.[63] We showed that the effects of prenatal exposure to famine on glucose and insulin metabolism modulate the effects of the Pro12Ala polymorphism.[64••] People exposed to famine in midgestation more often had type 2 diabetes when they were carriers of the mutant Ala allele. We hypothesized that this gene-early environment interaction is due to a combined deficit in insulin secretion, caused by a famine-induced maldevelopment of the beta cell in combination with carriership of the Ala allele. Our results provide

the first direct evidence in humans in support of the notion that early environment can affect gene expression. We were unable, however, to show interactions between prenatal famine exposure and effects of glucocorticoid receptor polymorphisms on metabolic and cardiovascular outcomes (unpublished).

Conclusion

Although the exact pathophysiology of the association between exposure to famine in utero and glucose intolerance is not yet clear, our results stress the importance of maternal nutrition for the offspring's glucose metabolism in later life. Adequate dietary advice to women before and during pregnancy therefore seems a promising strategy in preventing the development of type 2 diabetes in later life. Little is known, however, about the precise composition of an adequate diet during gestation. Future research may bring us closer to a maternal dietary composition that will provide the offspring with the optimal basis for a healthy glucose/insulin metabolism in later life.

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This study provides the first evidence that genetic influences can be modified by nutrition of the human fetus in utero.

The Dutch famine birth cohort study from an evolutionary perspective and consequences for future research

Susanne de Rooij

Introduction – Predictive adaptive responses

Recently, a theory has been developed that is based on the 'fetal origins of disease' concept and that is gaining popularity in the fetal origins field of research. This theory puts fetal origins into a broader biological perspective and implies that there are both genetic and early environmental factors that determine the level of risk for adult chronic disease. The theory centers around adaptive responses that are made by the developing organism in response to its environment. These responses have no obvious immediate adaptive value but are made in expectation of the future environment.[1]

Consider the coat thickness of a meadow vole, a North American field mouse. The thickness of the coat of the vole at birth is determined by whether it is born in spring or in autumn.[2] Information about the current season is communicated from the mother to the fetus by the maternal melatonin cycle that indicates the length of day.[3] There is no value in this adaptation on a short term basis, because the intrauterine and nest temperature are the same in both spring and autumn. However, it is of great value to the reproductive fitness of the vole to have a thin coat being born in spring with summer near and to have a thick coat being born in autumn with winter coming up. These adaptations during development that are made not for current but for future advantage have been termed predictive adaptive responses (PARs).

Mismatch

PARs are suggested to have evolutionary significance. They are a genetically determined set of responses to the immediate environment, which maximize the chances for reproductive fitness in postnatal life and consequently the survival of the species. However, the adaptations that are made as a result of an early gene-environment interaction may turn out to be inappropriate in later life. It is hypothesized that many diseases have their origin, at least partly, in the prediction of the future environment going the wrong way.[4] This 'mismatch' can occur when the egg/embryo/fetus perceives its future to be in a certain range but is born in a different environmental range. The greater the degree of mismatch, the higher the risk of disease will be.

Mismatch and the Dutch famine birth cohort study

The consequences of the Dutch famine may be a good example of a mismatch between the prenatal prediction of the future environment and the actual environment after birth. Through the mother and the placenta, the fetus in utero during the famine perceived an environment of very low nutritional value and

adjusted its physiology accordingly. The reality of the environment after birth, however, was one of normal or even abundant nutritional value.

This mismatch may have caused the impaired glucose tolerance that we have observed in men and women who had been prenatally exposed to famine. One of the predictive adaptive responses these people could have made when they were exposed in mid gestation was altering the development of their beta cells resulting in reduced insulin secretion. This would have been appropriate in a postnatal environment with little food available but in the actual postnatal environment food was plenty and the adapted beta cells are now unable to provide enough insulin to handle the high levels of glucose.

Implications for future research

The mismatch hypothesis raises a number of questions with important implications for public health. Although a situation such as the Dutch famine is very unlikely to happen again, mismatches between a prenatal environment of low nutritional value and a postnatal environment of excess can still occur. Placental insufficiency, a dieting mother, or a mother suffering from hyperemisus gravidarum are all examples of conditions that can signal poor future surroundings to the fetus. If the actual environment is rich in food, the mismatch theory predicts that the risk of chronic disease is increased.

However, nowadays, the likelihood of an abundunt prenatal situation is probably much higher. The fetus could predict its future environment to be nutritionally rich when maternal glucose levels are high, which is likely to be the case when the mother is obese. The enormous increase in the prevalence of obesity referred to in the Introduction to this thesis also concerns pregnant women. A study published in 2001 of pregnant women in London found that more than a quarter of these women were overweight and one out of ten women were obese.[5] Maternal obesity affects the health of the baby at short term. Risks of maternal obesity for the fetus are: miscarriage, birth defects, even stillbirth and neonatal death.[6-8]

Maternal glucose levels are also high in case the mother suffers from gestational diabetes mellitus (GDM). While GDM used to be a rare phenomenon, it is now occurring more and more often. In an American population, it was found that the prevalence of GDM doubled between 1994 and 2002.[9] As with maternal obesity, there are several short term risks of fetal exposure to GDM, including congenital malformations, caesarian section and macrosomia.[10,11]

Although maternal constraint will in principle always restrict fetal growth to a certain extent, the fetus can still receive signals from the mother that the outside environment is a plentiful one and adjust its organs or hormonal systems to prepare for such an environment.[4] Based on the mismatch theory, one could predict that the

risk of disease will be higher in a poor postnatal environment, as a consequence of the resulting disparity.

Hypothetically, it would be better for those prenatally exposed to a rich diet to continue feeding on a rich diet postnatally. Does this mean that children of obese mothers and children of mothers with diabetes could best consume a high fat/high sugar diet in order to stay healthy? This seems absurd. The fact is, however, that we know very little about the long term consequences of prenatal exposure to maternal obesity and GDM for the child, and are unable to specify the ideal content of the diet for such a child.

The small amount of evidence that is available suggests that prenatal exposure to maternal obesity or GDM has negative consequences for health in later life. Maternal obesity and GDM are both associated with macrosomia and large size at birth has been found to be associated with type 2 diabetes in later life. [12-14] A small study that did a follow up of children of mothers with GDM found increased weight at the age of 8 years and worse neuropsychological development.[15] A study of the Pima Indians found that offspring of mothers with GDM were more obese and had a higher prevalence of type 2 diabetes in later life.[16]

It is predicted that the prevalence of obesity and diabetes will increase with staggering numbers in the near future. It will be of value to public health when we find out what the long term effects of exposure to these abnormalities during gestation are. It must also be investigated what would be an optimal diet for children from obese mothers or mothers with GDM.

Conclusion

Results from the Dutch famine birth cohort study show us that prenatal diet can have metabolic consequences in later life. The 'mismatch' theory suggests that the negative effects on health are caused by a disparity between the poor prenatal environment and the rich postnatal environment. A mismatch in the opposite direction, from rich to poor, is supposed to lead to disease also. Situations that could be interpreted as rich in nutritional value are when the mother is obese or when she suffers from GDM. We feel that besides studying the effects of prenatal undernutrition on health in later life, the increasing prevalence of maternal obesity and GDM makes it inevitable and highly pertinent to study the effects of prenatal exposure to 'overnutrition' for the offspring's health in later life also.

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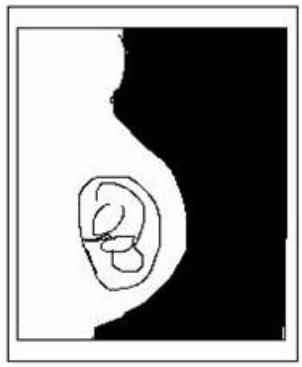

Summary

Type 2 diabetes is rapidly on its way to becoming a worldwide epidemic. In the year 2000 there were 171 million people with diabetes and it is predicted that these numbers will be doubled by the year 2030.[1]
Being small at birth is associated with the risk for developing diabetes.[2] It is hypothesized that this association originates from adaptations that the fetus makes in its structure and physiology in response to a poor environment *in utero*, resulting in insulin resistance and a diminished capacity to produce insulin.[3] These changes improve chances of short term survival for the fetus but may lead to impaired glucose tolerance and diabetes later in life. It is highly likely that undernutrition plays a significant role in this process. Experiments in animals have shown that restricting the diet of pregnant animals induces decreased glucose tolerance and diabetes in the offspring.[4] The Dutch famine offers a unique opportunity to study the effects of prenatal undernutrition in humans.
The Dutch famine was a period of five months at the end of World War II in which the population of the western part of the Netherlands was severely undernourished. The famine struck a population that was adequately nourished before and after the period of famine. Also, the period of famine was relatively short which enables us to study the consequences of undernutrition during specific periods of gestation. Furthermore, detailed information on the weekly rations of people living in Amsterdam is available. The Dutch famine birth cohort consists of 2414 men and women who were born as term singletons around the time of the famine in the Wilhelmina Gasthuis, a hospital in Amsterdam. At the mean age of 50 years, cohort members were invited to participate in a study of the effects of prenatal exposure to the Dutch famine on health in later life. Results of this study showed that exposure to famine during mid and late gestation is associated with higher 2h glucose concentrations, while 2h insulin concentrations are raised in all exposure groups.[5]

In a second round of data collection we aimed to establish the previously reported association between prenatal famine exposure and elevated glucose concentrations and to elucidate the pathophysiology of this association (***chapter 1***).
Chapter 2 describes the results of an oral glucose tolerance test we performed at the mean age of 58 years. We found that exposure to famine in any period of gestation is associated with increased 2h glucose and insulin concentrations. We did not find that prenatal famine exposure leads to a more rapid progression of impaired glucose/insulin homeostasis with increasing age.

The fetal origins hypothesis proposes that an adverse fetal environment may permanently alter the effects of specific genes. In ***chapter 3*** we tested a possible interaction between the Pro12Ala polymorphism of the *PPAR-γ2* gene and prenatal famine exposure on glucose and insulin metabolism. We found that carriers of the Ala allele show a higher prevalence of impaired glucose tolerance and type 2 diabetes but only when they had been exposed to famine during mid gestation. We hypothesized that this is possibly due to a combined deficit in insulin secretion, as conferred by pancreatic beta cell maldevelopment and carrier type of the Ala allele, which is also associated with decreased insulin secretion.

Chapters 4 to 6 contain the results of a number of studies we performed to investigate possible pathways between exposure to famine in utero and decreased glucose tolerance at adult age. ***Chapter 4*** describes the findings of an intravenous glucose tolerance test (IVGTT). With the IVGTT we aimed to estimate the relative contributions of decreased insulin secretion and insulin resistance to the impaired glucose tolerance in those prenatally exposed to famine. Results showed that participants exposed to famine in early and mid gestation have a lower disposition index, which suggests that their beta cells are less capable of producing enough insulin to compensate for insulin resistance.

In chapters 5 and 6 we tested the hypothesis that prenatal exposure to famine during different periods of gestation is associated with increased activity of the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased concentrations of cortisol which predispose to cardiovascular disease and type 2 diabetes. ***Chapter 5*** shows the results of a combined dexamethasone suppression/$ACTH_{1-24}$ activation test. We found no differences in either the dexamethasone suppressed or the $ACTH_{1-24}$ activated cortisol concentrations between those exposed and those unexposed to famine during gestation. In ***chapter 6*** we report that we also did not find any differences between exposed and unexposed participants in the cortisol responses to a psychological stress protocol. The results of these two studies suggest that prenatal exposure to famine does not program the HPA axis.

Based on the associations between prenatal exposure to famine and various metabolic outcomes and the fact that several studies have shown an association between low birth weight and the metabolic syndrome, we suggested that exposure to the Dutch famine *in utero* may lead to an increased prevalence of the metabolic syndrome. In ***chapter 7*** we investigated this hypothesis. We found no statistically significant association between exposure to famine *in utero* and the metabolic syndrome, although we did find that elements of the syndrome are associated with prenatal famine exposure. We therefore proposed that the metabolic syndrome does not have a single underlying origin in utero.

Chapter 8 reviews the results from the studies that we performed within the scope of this thesis. We concluded that the aetiology of the association between prenatal exposure to famine and decreased glucose tolerance seems to lie, at least partly, in programming of the pancreatic beta cell, resulting in an impaired insulin response that is already present in the normoglycaemic state. Finally, in ***chapter 9,*** we tried to place the findings regarding the decreased glucose tolerance in those exposed to famine *in utero* into a broader evolutionary perspective. This evolutionary perspective predicts that overnutrition during gestation followed by normal or poor nutrition in postnatal life will also have consequences for the development of disease in later life. We therefore plead that the effects of exposure to gestational diabetes and maternal obesity *in utero* on health in later life have to be studied, especially because these prenatal situations are becoming more and more common.

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Samenvatting

Type 2 diabetes is hard op weg om een wereldwijde epidemie te worden. In het jaar 2000 waren er 171 miljoen mensen met diabetes en de voorspelling is dat dit aantal in het jaar 2030 verdubbeld zal zijn.[1]
Mensen die klein waren bij de geboorte hebben een hoger risico op het ontwikkelen van diabetes.[2] Verondersteld wordt dat deze associatie zijn oorsprong heeft in aanpassingen van de foetus in structuur en fysiologie aan ongunstige omstandigheden *in utero*, resulterend in insuline resistentie en een verminderde capaciteit om insuline te produceren.[3] Deze aanpassingen vergroten de kans op overleving van de foetus op korte termijn, maar zouden op latere leeftijd tot een verstoorde glucose tolerantie en diabetes kunnen leiden. Ondervoeding speelt waarschijnlijk een belangrijke rol in dit proces. Proefdiermodellen hebben laten zien dat het ondervoeden van zwangere dieren tot verminderde glucose tolerantie en diabetes bij het nageslacht leidt.[4] De Hongerwinter biedt een unieke mogelijkheid om de gevolgen van prenatale ondervoeding bij mensen te onderzoeken.
De Hongerwinter was een periode van vijf maanden aan het einde van de Tweede Wereldoorlog waarin de populatie in het westen van Nederland zeer zwaar ondervoed was. De Hongerwinter trof een populatie die zowel voor als na deze periode adequaat gevoed was. Daarnaast duurde de Hongerwinter relatief kort, waardoor we in staat zijn de gevolgen van ondervoeding tijdens specifieke stadia van de zwangerschap te bestuderen. Verder is er gedetailleerde informatie beschikbaar over de rantsoenen die de mensen in Amsterdam kregen. Het Hongerwinter onderzoekscohort bestaat uit 2414 mannen en vrouwen die rond de Hongerwinter als eenling a term geboren werden in het Wilhelmina Gasthuis in Amsterdam. Op de gemiddelde leeftijd van 50 jaar werden de cohort leden uitgenodigd om mee te doen aan een onderzoek naar de effecten van prenatale blootstelling aan de Hongerwinter op de gezondheid in het latere leven. De resultaten van dit onderzoek lieten zien dat blootstelling aan ondervoeding tijdens het midden en einde van de zwangerschap samenhangt met hogere 2 uurs glucose concentraties, terwijl 2 uurs insuline concentraties in alle blootgestelde groepen verhoogd zijn.[5]

In een tweede ronde van dataverzameling stelden we ons ten doel om de eerder gerapporteerde associatie tussen prenatale Hongerwinter blootstelling en verhoogde glucose concentraties te bevestigen en om de pathofysiologie van deze associatie te onderzoeken (***hoofdstuk 1***).
Hoofdstuk 2 beschrijft de resultaten van een orale glucose tolerantie test die we uitvoerden op de gemiddelde leeftijd van 58 jaar. We vonden dat blootstelling aan de Hongerwinter tijdens elke periode van de zwangerschap geassocieerd is met

verhoogde 2 uurs glucose en insuline concentraties. We vonden echter niet dat prenatale Hongerwinter blootstelling met toenemende leeftijd tot een snellere progressie van een verstoorde glucose/insuline homeostase leidt.

De 'fetal origins' hypothese veronderstelt dat een ongunstige foetale omgeving permanent de effecten van bepaalde genen zou kunnen veranderen. In ***hoofdstuk 3*** onderzochten we een mogelijke interactie tussen het Pro12Ala polymorfisme van het *PPAR-γ2* gen en prenatale bloostelling aan ondervoeding op glucose en insuline metabolisme. We vonden dat de prevalentie van gestoorde glucose tolerantie en type 2 diabetes hoger is onder dragers van het Ala allel, maar alleen wanneer deze blootgesteld zijn geweest aan ondervoeding tijdens het midden van de zwangerschap. We veronderstellen dat dit zou kunnen komen door een defect in insuline secretie dat veroorzaakt wordt door een combinatie van een gestoorde ontwikkeling van de beta cellen in de pancreas en het dragen van het Ala allel, dat op zichzelf ook geassocieerd is met een verminderde insuline secretie.

De hoofdstukken 4 tot en met 6 bevatten de resultaten van een aantal studies die we uitgevoerd hebben om de mogelijke paden tussen blootstelling aan ondervoeding in de baarmoeder en verminderde glucose tolerantie op volwassen leeftijd te onderzoeken. ***Hoofdstuk 4*** beschrijft de uitkomsten van een intraveneuze glucose tolerantie test (IVGTT). Met behulp van de IVGTT wilden we een schatting maken van de relatieve bijdragen van verminderde insuline secretie en insuline resistentie aan de verstoorde glucose tolerantie in mensen die prenataal aan ondervoeding blootgesteld zijn geweest. De resultaten lieten zien dat deelnemers die vroeg of in het midden van de zwangerschap blootgesteld waren een lagere dispositie index hebben, wat doet vermoeden dat hun beta cellen minder goed in staat zijn genoeg insuline te produceren om voor insuline resistentie te compenseren.

In de hoofdstukken 5 en 6 testten we de hypothese dat prenatale blootstelling aan de Hongerwinter gedurende verschillende perioden in de zwangerschap geassocieerd is met een toegenomen activiteit van de hypothalame-pijnappelklier-bijnierschors (HPA) as, wat resulteert in toegenomen concentraties cortisol die het risico op hart - en vaatziekten en type 2 diabetes verhogen. ***Hoofdstuk 5*** laat de resultaten van een gecombineerde dexamethason suppressie / $ACTH_{1-24}$ activatie test zien. We vonden geen verschillen tussen hen die wel en niet tijdens de zwangerschap aan ondervoeding blootgesteld zijn geweest in zowel de door dexamethason onderdrukte als de door $ACTH_{1-24}$ geactiveerde cortisol concentraties. In ***hoofdstuk 6*** rapporteren we dat we ook geen verschillen vonden tussen blootgestelde en niet blootgestelde deelnemers in de cortisol respons tijdens een psychologisch stress protocol. De resultaten van deze beide studies wekken de suggestie dat prenatale blootstelling aan ondervoeding de HPA as niet programmeert.

Gebaseerd op de associaties tussen prenatale blootstelling aan ondervoeding en verschillende metabole uitkomsten en verscheidene studies die een verband tussen laag geboortegewicht en het metabool syndroom laten zien, veronderstelden we dat blootstelling aan de Hongerwinter in de baarmoeder zou kunnen leiden tot een verhoogde prevalentie van het metabool syndroom. In ***hoofdstuk 7*** onderzochten we deze hypothese. We vonden geen statistisch significant verband tussen blootstelling aan ondervoeding in de baarmoeder en het metabool syndroom. We vonden echter wel dat elementen van het syndroom geassocieerd zijn met prenatale blootstelling aan ondervoeding. Op basis hiervan denken we dat het metabool syndroom geen enkelvoudig onderliggende oorsprong *in utero* heeft.

Hoofdstuk 8 beschouwt de resultaten van al de studies die in het kader van dit proefschrift uitgevoerd zijn. We concludeerden dat de etiologie van het verband tussen prenatale blootstelling aan ondervoeding en verminderde glucose tolerantie in ieder geval voor een deel lijkt te liggen in het programmeren van de beta cel in de pancreas, resulterend in een gestoorde insuline respons die reeds in de normoglycaemische staat aanwezig is.

Ten slotte hebben we in ***hoofdstuk 9*** geprobeerd onze bevindingen van de verminderde glucose tolerantie in mensen die *in utero* blootgesteld zijn aan ondervoeding in een breder evolutionair perspectief te plaatsen. Dit evolutionaire perspectief voorspelt dat overvoeding gedurende de zwangerschap gevolgd door een normaal of beperkt dieet in het postnatale leven ook gevolgen heeft voor de ontwikkeling van ziektes later in het leven. Daarom pleiten wij ervoor dat de effecten van blootstelling aan zwangerschapsdiabetes en maternale obesitas in de baarmoeder op de gezondheid later in het leven bestudeerd moeten worden, in het bijzonder omdat deze prenatale omstandigheden steeds vaker voorkomen.

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Curriculum Vitae

Susanne Rosalie de Rooij werd op 10 november 1976 geboren in het RKZ in Hilversum. Haar ouders waren zeer verheugd met de 3030 gram en 50 cm die de verpleegkundige op het geboortedossier van Susanne noteerde. Wat minder blij waren zij met de bedroevend slechte eetlust die hun eerstegeboren spruit tijdens haar vroege levensjaren ten toon spreidde. Misschien dat Susanne daarom altijd wat aan de kleine kant gebleven is...

Op 1-jarige leeftijd verhuisde zij naar het landelijke Loosdrecht, waar zij de kleuter- en lagere school met veel plezier bezocht. Voor de middelbare fase van haar opleiding keerde zij weer terug naar Hilversum om daar na zes jaar het Gymnasium diploma in ontvangst te mogen nemen. Na veel getwijfel en gepieker besloot zij vervolgens psychologie in Amsterdam te gaan studeren. Susanne kwam er tijdens haar studie al snel achter dat zij geen therapeut wilde worden en zag meer in het onderzoek. Daarom koos zij na haar tweede jaar voor de richting Biologische Psychologie. Aan de desbetreffende vakgroep van de Vrije Universiteit te Amsterdam schreef zij een afstudeerscriptie over de relatie tussen depressie en hart- en vaatziekten. Overigens deed zij dit alleen in naam aan de Vu, fysiek deed zij dit tijdens een half jarig verblijf op het altijd zonnige Aruba (al kan het er ook heel hard regenen!). Tijdens deze tropische periode bedacht zij dat het misschien geen kwaad zou kunnen om alvorens definitief voor de onderzoekswereld te kiezen eerst nog eens een kijkje te nemen in het bedrijfsleven. Zij begon aan een tweede studierichting, Arbeids- en Organisatie psychologie, en liep stage bij een werving- en selectiebureau in Den Haag. Zij rondde het geheel af met een scriptie over het meten van potentieel bij mensen en besloot dat onderzoek doen meer was wat bij haar paste. Susanne vond een promotieplaats in het AMC op de afdeling Klinische Epidemiologie en Biostatistiek waar zij het in het huidige proefschrift beschreven onderzoek verrichte. Na haar promotie zal zij aan de slag gaan als onderzoeker op een diabetes project aan het EMGO van de Vrije Universiteit te Amsterdam. Overigens is het met Susannes eetlust uiteindelijk helemaal goedgekomen!