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Contents

The Wilhelmina Gasthuis, Amsterdam. All babies in the Dutch Famine Birth Cohort were delivered at the Wilhelmina Gasthuis

chapter

1

Introduction

Long, long ago, in the beginning, before time began, the Dreamtime animals roamed the land. The tracks left by these ancient creatures; the places where the emu, the kangaroo, and the dingo walked, ate, and rested in the Dreamtime, shape the landscape as we know it today. The stories describing these ancient journeys have been passed down through the generations of Australian Aboriginals. They are part of the kinship tradition in which every individual belongs to a skin group as well as a family. Each skin group is related to a specific Dreamtime creature, and has its own story, its own journey. These stories provide a set of rules governing and explaining behaviour and relationships amongst people and animals [1]. The skin name which a baby will receive is determined before birth. It is based on the place where the baby's mother was at the time when she first felt the baby stir in her womb, and which Dreamtime creature traveled along that same spot in ancient times. At that point, the course of the journey along which that baby will travel throughout its life is plotted.

The fact that events that take place during early life in the womb may shape the course of life has been long acknowledged in the folklore of various cultures around the world. This notion has, however, only gained a scientific basis in the last few decades. We now realize that, depending on the prevailing conditions during development, a given genotype may give rise to a variety of phenotypes- a concept termed developmental plasticity. Irrespective of the environment in which they are raised themselves, water fleas are equipped with a protective 'helmet' if their mothers experience high predatory pressure before and while the clutch is inside their brood pouch [2]. In terms of behavior, the shy, nocturnal phenotype of the desert locust barely resembles its conspicuous swarming phenotype. High population density, however, gives rise to alterations in the egg pod environment which lead to this phenotypic change [3]. Plasticity during early development may thus provide a means of fast response to environmental changes, where evolutionary genetic selection may take many generations to achieve such adaptations [4, 5].

Not all effects of the environment on the developing organism are adaptive. An adverse environment may disrupt developmental processes, leading to abnormalities. When faced with a lack of resources, the fetus may need to make trade-offs in order to survive. By sacrificing the growth of less essential organs, more energy becomes available for essential processes. Interestingly, this appears to hold even if it the period of withdrawal of resources is only short. This suggests the existence of critical windows during development. For example, fewer nephrons are laid down in the fetal kidneys of the offspring of rats that are malnourished during one week in mid gestation, but nephron number is unaffected if food is restricted in early gestation only [6].

Prenatal factors play an important role in the origins of cardiovascular disease and its biological risk factors. In several large epidemiological studies, hypertension [7], type 2 diabetes mellitus [8] and cardiovascular disease [9, 10] have been shown to be more common among people who were small at birth. Small birth size is a composite of maternal,

fetal and placental factors, which makes it difficult to pinpoint to which extent each of these factors contribute to disease in later life. Animal experiments have demonstrated that maternal nutrition plays a key role in the early origins of adult disease [11-13]. The effects of maternal nutrition on cardiovascular and metabolic disease are of particular clinical interest; unlike placental and genetic factors, maternal nutrition presents a possible target for interventions aimed at the primary prevention of cardiovascular disease. However, experimental studies describing the effects of maternal dietary manipulation in humans on the health of the offspring are scarce. In order to develop dietary advice for (pre) pregnant mothers that will benefit the cardiovascular health of their offspring, we first need to investigate if, and how, maternal nutrition affects the offspring's health in humans.

The Dutch famine or Hungerwinter was a period of 5 months in 1944-1945, during the last winter of World War II. After the south of the Netherlands had been liberated by the Allied forces in September 1944, the Dutch government in exile called for a railway strike in order to aid the liberation of the provinces still under German occupation. However, despite the railway strike, the Allies were not able to advance past the river Rhine. The German occupying force retaliated for the railway worker's cooperation in the strike by placing an embargo on all food transports. Food stocks in the large cities in the western Netherlands ran out within a matter of weeks. The Dutch famine began in November 1944. Adult rations were to drop to as low as 400-800 calories per day: less than a quarter of the pre-famine rations. The famine was to continue until the rest of the Netherlands was liberated in early May 1945. Amid the atrocious conditions of war, food shortage and incredible hardship, women continued to have their babies. We have been able to trace a cohort of 2245 of these babies, all born as term singletons in the Wilhelmina Gasthuis in Amsterdam around the time of the Dutch famine. This cohort provides us with the unique opportunity to study the sequelae of a discrete period of extreme maternal malnutrition during gestation on the offspring's health.

The work presented in this thesis explores the effects of famine exposure during specific periods of gestation on adult mortality and morbidity, as well as investigating the associated pathophysiological mechanisms. *Chapter 2* provides a summary of the findings from the Dutch famine birth cohort prior to the start of the work presented in this thesis, which led to the hypotheses tested in this thesis. *Chapter 3* presents the effects of prenatal famine exposure on the timing of onset of coronary heart disease symptoms. The possible mechanisms of pathophysiology of cardiovascular disease after maternal undernutrition are explored in *Chapters 4, 5, 6 and 7*. We investigate whether prenatal exposure to famine programs stress response *(Chapter 4)*, vessel stiffness *(Chapter 5)* and intima media thickness *(Chapter 6)*. In *Chapter 7*, the effects of prenatal exposure to famine on markers of renal disease are presented. *Chapter 8* reports on the incidence of breast cancer among women who experienced the Dutch famine while in utero, as well as exploring markers of fertility and reproductive success among these women. The

effects of maternal famine exposure during gestation on all-cause and cause-specific adult mortality in this cohort is discussed in *Chapter 9*. *Chapter 10* discusses the implications of the findings presented in this thesis for future research.

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Girl scrapes the remains from the bottom of a Central Kitchen messtin during the Dutch Famine (Still from the film 'Honger' by Rudi Hornecker)

chapter

2

Prenatal exposure to the Dutch famine and disease in later life: An overview

Rebecca C. Painter , Tessa J. Roseboom, Otto P. Bleker

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Abstract

Low birth weight is associated with cardiovascular disease in adulthood. Poor maternal nutrition during gestation contributes to low birth weight.

In this paper, we review the findings from a cohort of 2414 people, aged 50 years, born as term singletons around the time of the 1944–1945 Dutch famine, of which 912 people participated in an interview and 741 subjects were also available for hospital examination. We found more coronary heart disease, raised lipids, altered clotting and more obesity after exposure to famine in early gestation compared to those not exposed to the famine. Exposure in mid gestation was associated with obstructive airways disease and microalbuminuria. We found decreased glucose tolerance in people exposed to famine in late gestation.

These findings show that maternal undernutrition during gestation has important effects on health in later life, but that the timing of the nutritional insult determines which organ system is affected. Future research should shed more light upon the underlying pathophysiology of the far-reaching effects of prenatal exposure to famine.

Introduction

Cardiovascular disease remains a major burden to health in the western world, and is beginning to take on epidemic proportions in the developing world. Increases in cardiovascular disease, type II diabetes and hypertension [1] can only partly be explained by parallel increases in smoking and sedentary lifestyle [2,3]. An increasing proportion of low birth weight infants is surviving to adulthood.

Low birth weight has been shown in a large number of studies all over the world to be consistently linked to cardiovascular disease and its biological risk factors [4–7]. The fact that birth size has only a small genetic component and primarily reflects the quality of the intrauterine environment, suggests the origin of the association lies in restricted intrauterine growth [8].

Maternal dietary manipulation in animal models produces small offspring that display shortened life-span [9,10], obesity [11,12], hypertension [13,14], diabetes [14] and alterations in the hypothalamic–pituitary–adrenal axis [15,16], lending support to the notion that the origin of disease in later life lies in impaired development in the womb.
The fetus' developing tissues may be permanently altered by a sub-optimal availability of nutrients, possibly providing a survival advantage in the short term. These adaptations, however, may prove to be detrimental for health in later life. Rapidly growing organs are more vulnerable to reduced availability of nutrients. There is evidence from animal experiments that effects differ depending on which organ is growing rapidly – the so-called critical period – at the time the growth restriction is imposed. While in rats, maternal protein restriction in early gestation did not affect kidney formation or blood pressure in adulthood, rats exposed in mid–late gestation had impaired nephrogenesis and hypertension [17]. Maternal diet during gestation can have profound effects on health in later life, even when the dietary insult was of short duration and had no effect on birth weight [17]. This suggests that reduced birth weight is not a necessary prerequisite for programming of tissues during fetal life.

Although there is strong evidence from animal models that maternal nutritional status during pregnancy can induce permanent changes in the fetus, it is less clear how this might apply to human populations. The 1944–1945 Dutch famine was a 5-month period of extreme food shortage. The famine struck in a previously and subsequently well nourished population. Though disastrous in humanitarian terms, these characteristics provide us with a unique opportunity to study the effects of a short but severe period of maternal undernutrition during different stages of gestation on the offspring. This paper reviews findings on the effects of maternal undernutrition during gestation on health in later life from the Dutch famine birth cohort.

Subjects and methods

The Dutch famine 1944–1945

After 4 years of German occupation of the Netherlands, liberation seemed imminent after the Allies had landed in Normandy on June 6th 1944. Paris, Belgium and the southern parts of the Netherlands had been liberated by the end of summer. The Allied forces had made such rapid progress that the German surrender seemed just a matter of time. The advance of the Allies to the north of the Netherlands, however, came to a halt when attempts to gain control of the bridge over the river Rhine at Arnhem (operation 'Market Garden') failed. The Dutch government-in-exile called a general railway strike in order to disrupt the transport of German reinforcements and troops, and so help the Allied liberation efforts. As a reprisal, the Germans banned all food and fuel transports. By the time this embargo was lifted from transports over water in early November 1944, an unusually early and severe winter had frozen over the canals and barges were unable to relieve the food shortage in the cities in the western part of the Netherlands. The famine had begun.

Rations

The official daily rations for the general adult population, having decreased gradually from about 1800 calories in December 1943 to 1400 calories in October 1944, fell abruptly to below 1000 calories in late November 1944. At the height of the famine between December 1944 and April 1945 the official daily rations in Amsterdam were between 400 and 800 calories. Children under the age of one were relatively protected because their official daily rations never fell below 1000 calories, and the specific nutrient components were always above the standards used by the Oxford Nutritional Survey [18]. Pregnant and lactating women were entitled to supplementary rations. At the peak of the famine, these extra rations could however no longer be provided. Although the black market, soup kitchens, church organizations and foraging trips around the countryside provided additional food, and indeed total food intake may have been up to twice as high as the official rations indicate, the official rations do adequately reflect the variation over time of total food availability throughout the famine [19]. After the liberation of the Netherlands on May 5th 1945, the food situation improved very rapidly. By June 1945, the rations had risen to over 2000 calories a day [18].

The famine had a profound effect on the general health of the population living in cities in the western part of the Netherlands. Mortality among the population of Amsterdam in 1945 was more than double the 1939 mortality rate. Most of the excess mortality is likely to have been due to starvation [20]. Despite the disastrous famine, women were still conceiving and giving birth to babies. It is in these babies that we can study the effects of maternal malnutrition on health in adult life.

The Dutch famine birth cohort study

The effects of exposure to the Dutch famine both during gestation and childhood on health in later life have been investigated using different sources such as military induction records [21–23], psychiatric registries [24,25] and population-based cohorts [26]. Some used self-reported famine exposure [26], others made use of the geographic limitations of the famine to urban western Netherlands [22], in defining famine exposure. The scope of this review however, is limited to the findings on the effects of prenatal exposure to the Dutch famine on health in later life from the Dutch famine birth cohort study.

All term (born after 259 days gestation) singletons born at the Wilhelmina Gasthuis between November 1st 1943 and February 28th 1947 were eligible for inclusion in the Dutch famine birth cohort. After exclusion of babies for whom the medical records could not be traced, 2414 babies were left. The council registry was able to identify 2155 (89%), after subtracting those who were unwilling to participate (164), had emigrated (199) or were deceased (265), 1527 people could be located to a current address. We approached 1018 people living in or close to Amsterdam to participate in the study. Nine hundred and twelve people agreed to an interview in which socio-economic factors, lifestyle and medical history were ascertained. Seven hundred and forty one of the interviewed subjects came to the clinic to undergo oral glucose testing, analysis of plasma lipids and clotting parameters, an electrocardiogram, anthropometry and a blood pressure measurement. The mean age at clinic visit was 50 years (range 48–53 years).

Antenatal and birth records were meticulously kept despite the wartime circumstances, and provided us with information on the mother's weight and health throughout pregnancy as well as the size of the baby and the placenta.

Exposure groups and control group

We considered a baby to be exposed to famine in utero if the average daily ration during any 13-week period of gestation was below 1000 calories. We used three periods of 16 weeks to distinguish between babies exposed in late gestation (born between January 7th and April 28th 1945), mid gestation (born between April 29th and August 18th 1945) and early gestation (born between August 19th and December 8th 1945) (Figure 1).

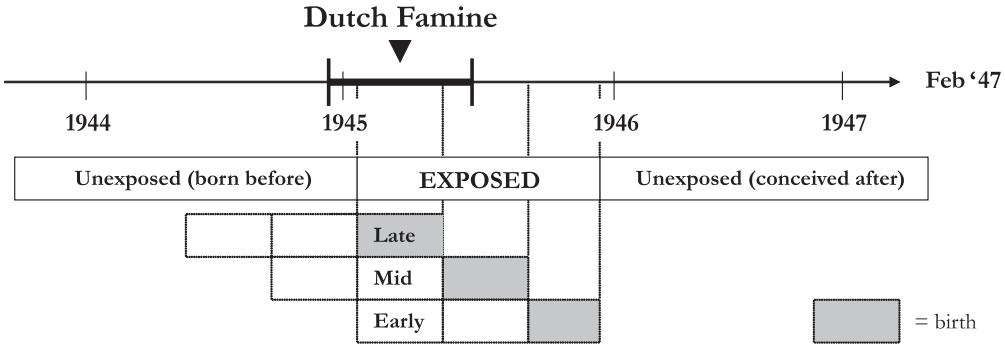
The control group consisted of subjects born before the famine (born between November 1st 1943 and January 6th 1945) and subjects conceived after the famine (born between December 9th 1945 and February 28th 1947).

Statistical methods

The findings are reported in means and standard deviations. Variables with a skewed distribution are reported using geometric means and standard deviations.

Linear regression was used to assess the association between continuous variables and famine exposure. Dichotomous outcome variables were analysed using logistic

Figure 1. The Dutch famine birth cohort: famine exposure and birth in relation to the timing of the Dutch famine.



regression and are reported in odds ratios with 95% confidence intervals. We always adjusted for gender and where appropriate we corrected for adult characteristics (e.g. socio-economic status, smoking and BMI). We did not correct the significance values for multiple testing.

Findings

Women who were exposed to famine in late gestation gained no weight in the third trimester (Table 1). Babies exposed to maternal famine in late or mid gestation were lighter, shorter, thinner and had a smaller head circumference than babies that had not been exposed to famine. Gestation was slightly shorter for babies exposed in late gestation ($p = 0.003$). However, correcting for gestational age did not diminish the association between small size at birth and famine exposure in late or mid gestation. Exposure to

Table 1 Maternal and infant characteristics according to timing of prenatal exposure to the Dutch famine. Mean and standard deviation.

	Exposure to famine						
	Born before	Late gestation	Mid gestation	Early gestation	Conceived after	All (SD)	n
Number	764	307	297	217	829	2414	
Proportion of men	53%	49%	49%	50%	53%	52%	2414
Maternal characteristics							
Age (years)	29	30	28	28	28	28 (6.4)	2414
Primiparous	40%	30%	37%	39%	39%	38%	2414
Not married	13.2 %	9.8%	20.2%	25.8%	16.3%	15.8%	2414
Weight at last antenatal visit (kg)	66.7	61.8*	63.5*	67.9	69.1	66.6 (8.7)	2133
Weight gain 3rd trimester (kg)	3.2	0.0*	4.9*	5.7*	4.3	3.5 (3.2)	1682
Infant characteristics							
Birth weight (g)	3373	3133*	3217*	3470*	3413	3346 (477)	2414
Birth length (cm)	50.5	49.4*	49.8*	50.9*	50.5	50.3 (2.2)	2382
Head circumference (cm)	32.9	32.3*	32.1*	32.8	33.2	32.8 (1.6)	2397
Ponderal index (kg/m^3)	26.1	25.8*	26.0*	26.2	26.5	26.2 (2.4)	2382
Placental area (cm^2)	370	339*	346	340*	350	353 (83)	2056
Gestational age (days)	285	283*	285	287	286	285 (12)	2044

* p corrected for gender <0.05 compared to unexposed (born before and conceived after the famine).

Table 2 Adult characteristics according to timing of prenatal exposure to the Dutch famine. Mean and standard deviation.

	Exposure to famine						
	Born before	**Late gestation**	**Mid gestation**	**Early gestation**	**Conceived after**	**All (SD)**	**n**
Adult characteristics							
Number	264	140	137	87	284		912
Proportion of men	49%	47%	39%	41%	50%	47%	
Plasma glucose 120 min* (mmol/l)	5.7	6.3§	6.1	6.1	5.9	6.0 (1.4)	702
Plasma insulin 120 min* (pmol/l)	160	200§	190	207	181	181 (2.4)	694
LDL/HDL cholesterol *	2.91	2.82	2.69	3.26§	2.94	2.90 (1.53)	704
Fibrinogen (g/l)	3.02	3.05	3.05	3.21	3.10	3.07 (0.6)	725
Factor VII * (% of standard)	128	131	133	117§	133	129 (1.4)	725
BMI (kg/m^2)	26.7	26.7	26.6	28.1	27.2	27.0 (1.2)	741
Coronary heart disease	3.8%	2.5%	0.9%	8.8%§	2.6%	3.3%	736
Microalbuminuria (ACR ≥ 2.5)	8%	7%	12%§	9%	4%	7%	724
Systolic blood pressure (mmHg)	126.0	127.4	124.8	123.4	125.1	125.5 (15.5)	739
Diastolic blood pressure (mmHg)	86.2	86.4	84.4	84.8	85.2	85.6 (9.9)	739
Obstructive airways disease	15.5%	15.0%	24.8%§	23.0%	17.3%	18.1%	733
General health poor	4.5%	6.4%	3.7%	10.3%§	5.3%	5.5%	912

*geometric mean.

§ p corrected for gender <0.05 compared to unexposed (born before and conceived after the famine)

famine in any trimester was associated with smaller placental area. Fewer boys were born after prenatal exposure to famine, though the difference was not statistically significant ($p = 0.08$). Babies exposed to famine in early gestation were slightly heavier and larger than babies that had not been exposed to famine ($p = 0.02$).

People exposed to famine in late gestation had impaired glucose tolerance: they had higher 2-h glucose and insulin values compared to unexposed subjects (Table 2) [27]. Although light babies were less glucose tolerant, confirming the findings in other studies [5], this association did not account for the effect of famine exposure on glucose tolerance.

Exposure to famine in mid gestation was linked to an increase in obstructive airways disease (odds ratio 1.7, 95% confidence interval 1.1–2.6) [28]. This increase was not paralleled by decreased lung function or abnormal serum IgE concentrations. Mid gestational exposure to famine was also associated with an increased prevalence of microalbuminuria (odds ratio 2.1, 95% confidence interval 1.0–4.3) [29]. Again, both findings were independent of size at birth.

Although babies exposed in early gestation were not smaller or lighter than non-exposed babies, this group shows the most striking effects of maternal famine exposure during gestation. A three-fold increase in coronary heart disease (odds ratio 3.0, 95% confidence interval 1.1–8.1) [30], a more atherogenic lipid profile [31], more obesity [32], raised levels of plasma fibrinogen and decreased levels of factor VII [33] were found among people whose mothers had been undernourished in early gestation compared to

the offspring of mothers that had not been exposed to famine. The proportion of people reporting poor self perceived health was significantly higher among those exposed in early gestation compared to people who had not been exposed to famine [34].

The link between smallness at birth and raised blood pressure in later life described in the literature [35] was also found in this cohort [36]: for every kilogram decrease in birth weight we found a systolic blood pressure increase of 2.7 mmHg. Blood pressure and famine exposure were however not associated directly. A decreased protein/carbohydrate ratio during the third trimester was however shown to be associated with increased blood pressure in later life, regardless of famine exposure [37].

People conceived after the famine had the lowest mortality up to the age of 50 (7.2%). Mortality rose from 11.5% among people exposed in early gestation and 11.2% in people exposed in mid gestation, to 14.6% among people exposed in late gestation, and 15.2% among those born before the famine [38]. The excess mortality was mainly due to excess infant mortality. Despite our findings on excess morbidity after exposure to famine in utero, neither all-cause nor cause-specific adult mortality were increased in the exposed groups, compared to the group who had not been exposed to famine [38].

The associations between famine exposure during different stages of gestation were not altered by correcting for relevant adult characteristics.

Discussion

We found that maternal famine exposure during gestation is associated with chronic disease in later life in the offspring. Moreover, the effects in later life varied according to the timing in gestation of exposure to famine. This may reflect the critical periods of rapid development of the organs involved in these effects. These conclusions broadly support the fetal origins hypothesis. Our findings were however, to a large extent, independent of size at birth, underlining the fact that programming may take place even without effects on gross body size at birth.

A number of things should be considered in interpreting these results. Women were significantly less fertile during the famine [39], as is reflected in the small number of people conceived during the famine (exposed in early gestation). The women who were able to conceive in the midst of the famine may have differed from the general population. Possibly only more robust women, or women who had access to additional food, were able to conceive. We cannot estimate to what extent these factors may confound the increase in cardiovascular disease and its biological risk factors among those exposed in early gestation. Adjusting for maternal characteristics such as age, parity, weight and weight gain, however, did not alter the associations, suggesting the findings are independent of measures of maternal fertility and robustness.

The high rates of infant mortality during the famine affected the groups born before the famine and exposed in late gestation most [38]. The two groups with the largest

contrast in infant mortality – those born before the famine versus conceived after the famine – are homogeneous in terms of adult health outcome, indicating that selective survival cannot have had a large confounding effect on outcome in later life.

There are limitations in pinpointing the exact timing of famine exposure during gestation and associated outcomes in later life, due to the relatively small sample size on one hand, and partial overlap between the three famine exposed groups on the other. However, the famine exposure periods do give an estimate of the timing of the focus of effect.

The Dutch famine happened in a previously and subsequently well nourished population. Possibly not the famine exposure alone, but the transition from nutritional deprivation during pregnancy to adequate nutrition later on may have given rise to metabolic conflicts, resulting in disease in later life.

As discussed in a recent review by Gluckman and Hanson [40], plasticity during early development may provide a means of fast response to environmental changes, whereas evolutionary genetic selection can take many generations to achieve adaptations. Prenatal day length determines the coat thickness in meadow vole offspring, providing them with a coat suitable for the season they are born in [41]. Maternal predator exposure induces helmet formation – a means of defense against predators – in water flea offspring [42]. Both examples illustrate that information relevant to the prevailing environment can be communicated to and can illicit permanent changes in the fetus. The mother is thus providing her baby with a forecast of the environment into which it will be born. Situations where the forecast does not match the postnatal situation may result in metabolic conflict and lead to disease in later life.

Other studies using the Dutch famine as a means of assessing the effects of maternal nutrition during gestation on adult health, found more schizophrenia [24], schizophrenia spectrum disorders, congenital anomalies of the central nervous system [23] and antisocial personality disorder [22] among people exposed to famine in the first half of gestation. The authors have suggested stunted brain development underlies these associations [43]. Affective disorder occurred more among people exposed in mid or late gestation [25], possibly reflecting HPA-axis programming. Obesity was more common among 19-year old conscripts exposed to famine in early gestation [21]. Although these studies were done in different populations and the applied methods differed from the ones used in the Dutch famine birth cohort study, the findings confirm maternal undernutrition during gestation can have profound effects not only on adult cardiovascular health, but also on mental health.

Future research

To fully understand the implications of the findings of the Dutch famine birth cohort studies, we need to unravel the responsible mechanisms.

Impaired glucose tolerance (IGT) was more prevalent among those exposed to famine in late gestation [27]. Late gestation may be the critical window for the development of the endocrine pancreas, which undergoes rapid proliferation in mid and late gestation [44]. Growth restriction during this period may lead to a permanent reduction in insulin production capacity, an idea encouraged by a study in rats, which showed a low protein diet throughout gestation produced offspring with impaired pancreatic function [45]. However, in thin babies, IGT is due to insulin resistance [46]. The same mechanism may be responsible for the excess in IGT among those exposed in late gestation. Target tissues for insulin, such as adipose tissue, which also undergoes rapid growth in late gestation [47], may become insulin resistant if faced with nutrient restriction [48]. There is also evidence for downregulation of glucocorticoid receptors in the hippocampus in animals that were exposed to glucocorticoids in the last week of gestation [49]. During maternal undernutrition placental 11-β-hydroxysteroid dehydrogenase, an enzyme that usually protects the fetus from maternal glucocorticoids, is significantly down regulated [50]. The effects of maternal undernutrition and steroid administration may therefore be similar. Hence, the hypothalamic–pituitary–adrenal (HPA)[15,16] or IGF [51] axes may be permanently altered by exposure to maternal undernutrition, and may thus contribute to insulin resistance.

We aim to resolve whether insulin deficiency or insulin resistance is involved in impaired glucose tolerance after prenatal exposure to famine by carrying out intravenous glucose testing in the Dutch famine birth cohort. Physiological stress tests and low dose dexamethasone synacthen tests will be carried out to assess HPA axis programming after prenatal exposure to famine during specific periods of gestation.

Besides HPA based stress response, sympathico-adrenal response to stress is tied into the pathophysiology of coronary heart disease (CHD), and had been implicated in the prenatal programming of CHD [52]. In order to assess the role of sympathico-adrenal programming in the pathophysiology of CHD after prenatal exposure to famine, we plan to measure blood pressure response to stress. The three-fold increase in CHD described in people exposed to famine in early gestation [30] was accompanied by a more atherogenic lipid profile [31], altered clotting [33] and more obesity in women [32]. The CHD increase was however independent of the LDL/HDL ratio. The more atherogenic lipid profile and altered clotting may be the result of altered blood flow to the fetal liver, such has been described in fetuses of women that were thin before conception [53]. There was no evidence for an excess of hypertension among those exposed in early gestation [36]. An increase in atherosclerosis is likely to underlie the increased susceptibility to CHD, though some animal data implicate increased left ventricular mass in the prenatal programming of CHD susceptibility [54]. We are currently studying whether atherosclerosis underlies the associations found, by assessing intima-media thickness in carotid and femoral arteries. We also will be reporting on the progression

of cardiovascular disease and its biological risk markers at more advanced age within the Dutch famine birth cohort.

Microalbuminuria [29] and obstructive airways disease [28] were more common in people exposed to famine in mid gestation. Mid gestation is a period of rapid branching of the bronchial tree, and nephron number increases rapidly in mid gestation [55]. These findings suggest that famine exposure during periods of rapid organ growth permanently affects organ structure, such as nephron number. Due to the fact that the study in a cohort of healthy volunteers does not allow invasive diagnostics, such as tissue biopsies, we are limited to basing our investigations on non-invasive data. Studying bronchial reactivity and renal functional reserve after exposure to famine in utero in mid gestation could shed light upon the underlying mechanisms of pathophysiology.

Although cardiovascular disease has a well-known genetic basis, the Dutch famine birth cohort studies show that fetal environment may be an important factor in determining cardiovascular risk in later life. Possibly people with a certain genetic make-up may be more sensitive to the effects of prenatal famine on health in later life. A number of epidemiological studies have reported gene-environment interactions for genes involved in lipid [56] and glucose [57] metabolism and osteoporosis [58] and early growth. We aim to determine whether the actions of these and other genes are modulated by prenatal famine exposure.

It seems plausible that the excess in CHD and its risk factors should be reflected in excess mortality among the famine-exposed members of the cohort, particularly those exposed in early gestation. At age 50 years, there was no appreciable excess mortality among those who were exposed to famine before birth [38]. Future follow up of mortality should resolve whether the effects on morbidity eventually have repercussions for mortality in the Dutch famine birth cohort.

Our findings identify the early stages of pregnancy as important in determining the offspring's health in later life [30,31,33]. This confirms evidence from animal research that pre-implantation and pre-conceptional factors play an important role in adult health [59]. Possibly epigenetic changes, such as gene imprinting, which takes place before conception, and DNA methylation, may be involved [60,61]. In both animal husbandry and human medicine, the rapid developments in assisted reproduction in the past decades have surpassed the available knowledge about the potential long-term repercussions.

These fields could benefit from more knowledge about the mechanisms of pathophysiology discussed above. The Dutch famine studies may contribute to the identification of such mechanisms. Besides providing an insight into the role of prenatal factors in the origins of chronic disease, this information may also help identify susceptible patient groups and be useful in the development of more appropriate therapies for common chronic diseases in the future. Most importantly, it will contribute to the prevention of chronic disease through the development of adequate dietary advice to women before and during pregnancy.

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Famine in Holland ('Hongersnood in Holland'); war-time poster (collection NIOD)

chapter

3

Early onset of coronary artery disease after prenatal exposure to the Dutch famine

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Abstract

Background Limited evidence suggests that maternal undernutrition at the time of conception is associated with increased cardiovascular disease risk in adult offspring.
Objective We investigated whether persons conceived during the Dutch famine of World War II had an early onset of coronary artery disease (CAD).
Design We compared the age at onset and cumulative incidence of CAD between persons born as term singletons who were exposed to the 1944–1945 Dutch famine during late (n=160), mid- (n=138), or early (n=87) gestation and 590 unexposed subjects at age 50 or 58 y. Age at CAD onset was defined as the age at which angina pectoris was identified (according to the Rose questionnaire), Q waves were observed on an electrocardiogram (Minnesota codes 1–1 or 1–2), or coronary revascularization was performed (by angioplasty or bypass surgery).
Results Of the 83 CAD cases identified, persons conceived during the famine were 3 y younger than the unexposed persons at the time of CAD diagnosis (47 y compared with 50 y) and had a higher cumulative incidence of CAD [13%; hazard ratio (HR) adjusted for sex: 1.9; 95% CI: 1.0, 3.8] than did the unexposed persons. The HR changed little after adjustment for smoking (HR: 1.8), social class (HR: 2.0), or size at birth (HR: 2.0).
Conclusions We found an earlier onset of CAD among persons conceived during the famine, which suggests that maternal nutrition in early gestation may play a role in the onset of CAD. This finding agrees with evidence from animal experiments that identify periconceptional maternal diet as important in the offspring's adult health.

Introduction

Restricted intrauterine growth has been proposed as an important contributor to later coronary artery disease (CAD) and its biological risk factors [1]. Developing organ systems respond negatively to the reduced availability of nutrients, particularly during periods of rapid development—so-called critical periods [2].

Most studies in humans have access only to indirect measures of intrauterine nutrition, such as birth weight. Substantial changes in cardiovascular function can result from maternal or fetal undernutrition without affecting birth weight [3]. To gain more insight into the mechanisms of disease in later life in humans after restricted prenatal nutrition, the sequelae of restricted maternal nutrition during gestation have been studied in the Leningrad Siege Study [4] and the Dutch Famine Birth Cohort Study. The Dutch famine was a 5-mo period of extreme food shortage during the winter of 1944–1945 in World War II. The Leningrad Study reported no effect of maternal malnutrition on the adult offspring's CAD prevalence. The Dutch famine, however, was relatively short compared with the Leningrad Siege Study, which allowed the effects to be studied by trimester of prenatal famine exposure. The previous findings from the Dutch Famine Birth Cohort Study support the hypothesis that the timing of the nutritional insult is important in determining its effect in later life; exposure to the Dutch famine in late gestation was associated with decreased glucose tolerance [5], whereas more microalbuminuria [6] was present among subjects exposed during midgestation. The most marked effects were described in the group of subjects conceived during the famine and include a more atherogenic lipid profile [7], altered clotting [8], more obesity [9], and a tripling of CAD prevalence at age 50 y [10].

The cluster of cardiovascular disease risk factors previously described in persons conceived in famine is in line with studies in animals, which have highlighted the importance of periconceptional maternal nutrition in programming cardiovascular disease risk [11–14]. The effects of maternal periconceptional diet on the course of adult disease have not been investigated. We hypothesized that CAD manifests at an earlier age in persons exposed to famine during early gestation. We reexamined the findings of a study conducted at age 50 y and included information from a subsequent study 8 y later.

Subjects and methods

Selection procedure

The Dutch Famine Birth Cohort consists of 2414 live-born term singletons born in the Wilhelmina Gasthuis in Amsterdam, Netherlands. All infants were born between 1 November 1943 and 28 February 1947. The selection procedure for the study conducted at age 50 y was described in detail elsewhere [5], as was loss to follow-up because of

mortality, emigration, and other reasons [15, 16]. In short, cohort members were eligible for participation if they were living in the Netherlands at the start of the study (January 1995 and September 2002), and their address was known to the Dutch Famine Birth Cohort Study researchers. All eligible subjects were asked to participate at ages 50 and 58 y. Council registries helped trace people who had had a change of address since they were last traced at age 50 y. All participants provided written informed consent. The local Medical Ethics Committee approved the study. The study conformed to the Declaration of Helsinki.

Exposure to famine

We defined famine exposure according to the daily official food rations for adults. In addition to the official rations, food from other sources, such as church organizations, central kitchens, and the "black market," was also available and the people may have had access to up to double the rationed amount at the peak of the famine. The rations do, however, adequately reflect the fluctuation of food availability during the famine [17]. A person was considered prenatally exposed to famine if the average daily rations for adults during any 13-wk period of gestation were <1000 kcal. Therefore, persons born between 7 January 1945 and 8 December 1945 were considered exposed prenatally to famine. Cohort members born between 1 November 1943 and 6 January 1945 (born before the famine) and between 9 December 1945 and 28 February 1947 (conceived after the famine) were unexposed to famine. We defined periods of 16 wk each to differentiate between those who were exposed in late gestation (born between 7 January and 28 April 1945), midgestation (born between 29 April and 18 August 1945), and early gestation (born between 19 August and 8 December 1945), in correspondence with previous publications on this cohort [5, 10]. Persons exposed in early gestation were conceived during the famine. The famine ended in May 1945, with the advance of the allied armies into Holland. Food supplies were rapidly restored, and the average caloric intake in June 1945 was >2000 kcal.

Data collection

Medical birth records provided information about the mother, the course of gestation, and the size of the infant and the placenta at birth [5]. Socioeconomic status (SES) at birth as defined according to the occupation of the head of the family and was classified as either manual or nonmanual on the basis of the information provided by the birth records.

Consenting cohort members came to the hospital. We measured height using a fixed or a portable stadiometer, weight with Seca scales (Hamburg, Germany) or Tefal portable scales (Groupe SEB Nederland BV, Veenendaal, Netherlands). Body mass index was calculated by dividing weight in kilograms by the square of height in meters. Blood pressure was measured twice on 2 occasions (morning and afternoon) with an automated device: a Profilomat (Disentronic Medical Systems AG, Burgdorf, Switzerland)

at age 50 y and an Omron 705CP/IT (Omron Healthcare United Kingdom, West Sussex, United Kingdom) at age 58 y. Mean blood pressure was calculated from both the morning and afternoon measurements. Standard 12-lead electrocardiograms (ECGs) were used for all participants. Trained technicians blinded to the clinical data scored the ECGs according to the Minnesota criteria. Nondiabetic participants underwent standard 75-g oral glucose tolerance testing. Blood was drawn for the measurement of LDL, HDL, and triacylglycerol concentrations. Total cholesterol, HDL, and triacylglycerol concentrations were measured with the use of an enzymatic colorimetric reagent (Roche Diagnostics, Switzerland) on a P-800 Modular (Roche, Switzerland). LDL was calculated by using the Friedewald formula.

Participants were interviewed to obtain information about their medical history, including operations, lifestyle, and use of medication. We defined current SES according to the participant's or their partner's occupation, whichever was highest, using the ISEI-92 [18]. The ISEI-92 scale ranges from 16 (minimumscore; lowest status) to 87 (maximum score; highest status). Trained nurses carried out all measurements and interviews.

The presence of CAD was defined as the presence of one or more of the following: angina pectoris according to the Rose/World Health Organization questionnaire, Q waves on the ECG (Minnesota codes 1–1 or 1–2), or history of coronary revascularization (angioplasty or bypass surgery).

Statistical methods

For the investigation of age at onset of CAD, all subjects that had participated at age 50 or 58 y were included. To study associations between the progression of CAD and the timing of famine exposure during gestation and size at birth, we used the Cox regression model of the cumulative incidence and age of manifestation of CAD and calculated hazard ratios (HRs) and 95% CIs for subjects exposed in late, mid-, and early gestation and compared them with unexposed subjects. We constructed a Kaplan-Meier curve showing the cumulative incidence of CAD as a function of age per famine exposure group.

The time of event was defined as the age at onset of angina pectoris according to the Rose/World Health Organization questionnaire. If no age at onset of angina pectoris was stated, the age at the time of the first coronary revascularization procedure was used, and, in cases where both ages were missing, the age at the time of registration of Q waves on the ECG was used. Subjects who had only participated at age 50 y were censored at the age at that visit. When adjusting for covariates in the Cox model, we used the most recently collected available measurement before the event. If the event had occurred between the time points of participation, an estimation of the covariate at the time of the event was made with the use of linear interpolation.

Table 1 Maternal, birth and coronary artery disease characteristics for men and women who participated in the Dutch famine birth cohort study at age 50 or 58 y

	Time of exposure to famine						
	Born before famine	**Late gestation**	**Mid-gestation**	**Early gestation**	**Conceived after famine**	**All subjects (SD)**	**Total n**
General							
number	289	160	138	87	301		975
men (%)	48	44	39	44	53	47	975
Maternal characteristics							
maternal age (years)	29	31[1]	29	27[1]	28	29 (6)[2]	975
weight at the end of gestation (kg)	67	62[1]	63[1]	68	69	66 (8.7)	854
weight gain in the last trimester (kg)	3.2	0.0[1]	5.0[1]	5.5[1]	4.3	3.4 (3.2)	682
occupation head of family manual (%)	83	71	70	62[1]	69	73	809
primiparous (%)	35	24[1]	34	39	39	34	975
Birth characteristics							
birth weight (g)	3396	3183[3]	3195[3]	3437	3449	3353 (467)	975
head circumference (cm)	32.8	32.4[3]	32.1[3]	32.8	33.2	32.8 (1.6)	965
ponderal index (kg/m^3)	26.2	26.0[3]	25.7[3]	26.0	26.7	26.2 (2.4)	966
Coronary artery disease							
No. of cases	24	12	11	11	25	83	
Cumulative incidence (%)	8	8	8	13[4]	8	9	975
Age of onset (y) [5]	51	50	50	47[4]	49	49 (45-56)	81

[1] Significantly different from those born before or conceived after the famine. p <0.05 (linear or logistic regression).
[2] mean ± SD (all such values).
[3,4] Significantly different from those born before or conceived after the famine after adjustment for sex: [3] p <0.05 (linear or logistic regression) [4] p <0.05 (Cox regression)
[5] mean after quadratic transformation and interquartile range in parentheses.

We used logistic regression analysis to compare the characteristics of persons with and without CAD. Because of the left skewed distribution of age at first occurrence of CAD, this variable is reported in means after we applied a quadratic transformation. Body mass index, SES, the ratio of LDL to HDL, and glucose were log transformed because of their skewed distributions. These variables are reported as geometric means ± SDs; all other variables are reported as means ± SDs. All statistical analyses were performed by using SPPS 12.0.2 (SPSS Inc, Chicago, IL). We considered differences to be statistically significant if *P* values were <0.05.

Results

Study population

The cohort contained 2414 members. Loss to follow-up was described previously [15, 16]. At age 50 y, 1527 (63%) persons were eligible for participation. At age 58 y, 1423 (59%) persons were eligible for participation. A total of 975 subjects participated in this study. At age 50 y (range: 48–53 y), 736 persons participated, of whom 491 participated again at age 58 y. At age 58 y (range: 56–61 y), 732 persons participated in the study, 239

of whom had not participated at age 50 y. The participation rates among those exposed to famine (49%) and among those not exposed to famine (40%) in utero did not differ significantly (P=0.7). The birth weights of persons included in the study (3353 g) did not differ significantly from the birth weights of those not included in the study (3341 g; P= 0.6). Infants born after exposure to famine in late and midgestation were lighter and smaller than the unexposed infants, and their mothers weighed less at the end of gestation (Table 1).

Age at onset of coronary artery disease

A total of 83 subjects had developed CAD by the end of follow-up. We found an overall cumulative incidence of CAD of 9% in men and 8% in women. Persons exposed to famine in early gestation had the highest cumulative incidence of CAD (13%; sex-adjusted HR compared with unexposed persons: 1.9; 95% CI: 1.0, 3.8) (Table 1). The cumulative incidence of CAD in those exposed to famine in late gestation (sex-adjusted HR: 0.8; 95% CI 0.4 to 1.6) and midgestation (sex-adjusted HR: 1.1; 95% CI: 0.6, 2.1) did not differ significantly from that of those unexposed to famine (8% after exposure in mid- and late gestation compared with 8% in unexposed persons). On average, CAD manifested 3 y earlier in those exposed to famine in early gestation (mean age: 47 y; interquartile range: 45–51 y) than in those unexposed to famine (mean age: 50 y; interquartile range: 45–57 y) (Figure 1).

Men and women with CAD were lighter (3275 compared with 3360 g) and thinner (25.9 compared with 26.3 kg/m^3) at birth and had a smaller head circumference (32.5 compared with 32.8 cm) at birth, although none of these differences was significant. After size at birth was adjusted for, the association between famine exposure in early gestation and CAD was not attenuated (HR: 2.0; 95% CI: 1.0, 3.8).

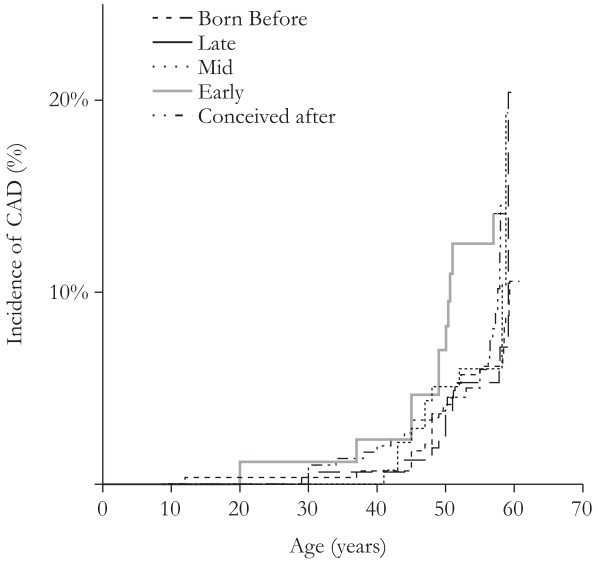

Figure 1. Kaplan-Meier curve of the cumulative incidence of coronary artery disease (CAD) in persons born before the famine (n=24); exposed to famine in late (n=12), mid- (n=11), or early (n=11) gestation; or conceived after the famine (n=25). The cumulative incidence of CAD was significantly greater in persons exposed to famine in early gestation than in those born before or conceived after the famine, P< 0.05 (Cox

Table 2 Characteristics of men and women who participated in the Dutch famine birth cohort study at age 58 y[1]

	Time of exposure to famine						
	Born before famine	Late gestation	Midgestation	Early gestation	Conceived after famine	All subjects	Total n
glucose 120 mins (mmol/l)	5.8	6.0	6.1	6.2	5.8	5.9 (2.4)	632
insulin 120 mins (pmol/l)	243	247	251	264	236	245 (294)	627
triacylglycerol (g/l)	1.2	1.3	1.3	1.3	1.3	1.3 (1.0)	724
LDL:HDL ratio	2.3	2.5[2]	2.3	2.6[2]	2.4	2.4 (1.0)	720
BMI (kg/m^2)	28.4	28.1	27.9	28.0	28.6	28.3 (4.8)	726
ever smoked (%)	64	62	66	76[2]	59	63	727
SES [3]	46	50[2]	49	45	48	48 (14)	721

Means and standard deviations (SD), except where given as percentages.

[1] All values, except for 'ever smoking' are geometric mean or geometric mean ± SD.

[2] Significantly different from those born before or conceived after the famine after adjustment for sex, $p<0.05$ (linear or logistic regression)

[3] Socioeconomic status, determined by using the ISEI-92 [18]

Coronary artery disease risk factors

The distribution of cardiovascular disease risk factors according to famine exposure during various stages of gestation among subjects at age 58 y is shown in Table 2.

In addition to the results shown in Table 2, famine exposure during any period of gestation was associated with elevated glucose concentrations at 120 min ($P=0.04$; adjusted for sex and body mass index) and an elevated ratio of LDL to HDL ($P=0.03$; adjusted for sex). Adjustment for the 2 social risk factors in Table 2, smoking (adjusted HR: 1.8; 95% CI: 0.9, 3.5) and low SES (adjusted HR: 2.0; 95% CI: 1.0, 3.8), had little effect on the association between famine exposure in early gestation and CAD.

Maternal constitution and fertility

There were no significant differences in maternal weight, age, parity, or SES at birth between persons with or without CAD. When these variables were entered into a multivariable Cox model, the association between exposure to famine in early gestation and CAD was little changed (multivariable-adjusted HR: 1.8; 95% CI: 0.9, 3.6).

Discussion

We found that the risk of CAD before the age of 61 y in persons conceived during the Dutch famine was double that of unexposed persons. This association was independent of size at birth and of smoking and low SES. Of the 83 persons with CAD, those who were conceived during the famine were 3 y younger at diagnosis. Ours was the first study to describe the course of CAD in the offspring of mothers nutritionally deprived during early gestation.

Women were less fertile during the famine [19]. Those who did conceive may have been of a different constitution. However, the correction for markers of maternal constitution or fertility, including maternal weight, age, parity, and SES, did not change the association of prenatal famine exposure with CAD.

Selective participation of persons who were fit enough to attend the clinic and prior excess mortality among the most seriously affected persons may have led to an underestimation of the effect of prenatal famine on subsequent CAD progression. However, we believe that the estimate reported in this article is relatively accurate, because analyses of the prevalence of angina pectoris and history of coronary revascularization surgery among persons who were not able to visit the clinic, but who agreed to a home or telephone interview, yielded results in the same direction (RC Painter, SR de Rooij, and TJ Roseboom, unpublished observations, 2005). Moreover, there was no excess all cause or CAD mortality among people conceived in the famine [16].

Although not statistically significant, persons with CAD were also lighter at birth than were persons without CAD. This finding agreed with results from other studies [1, 20]. Suboptimal intrauterine growth has been described to have programming effects on many cardiovascular disease risk factors, including hypertension [21], impaired glucose tolerance [22, 23], and lipid metabolism [24]. Consistent with our previous study of the Dutch Famine Birth Cohort, persons conceived during the famine had higher plasma glucose concentration at 120 min and higher ratios of LDL to HDL cholesterol than did persons who had not been exposed to famine in utero. It is possible that the effects of famine on CAD are mediated through these 2 biological risk factors. It was not possible for us to explore the effect of these risk factors on CAD incidence because, for many subjects, we did not have measurements from before the onset of disease. Moreover, many of the subjects were being treated for type 2 diabetes or hypercholesterolemia.

There are many possible processes by which persons conceived in famine could have increased rates of CAD. Slow intrauterine growth has been shown to be associated with hormonal axis programming [25, 26], alterations in cardiovascular control mechanisms [11, 12, 27], altered myocardial structure [28], endothelial dysfunction [29], and accelerated atherogenesis [30]. In future studies we hope to elucidate the role of these factors in the pathophysiology of coronary artery disease after prenatal famine exposure.

Persons conceived in famine not only had a higher cumulative incidence of CAD, but the disease occurred at an earlier age. Models in which animals were prenatally nutrient restricted had premature aging [31] and more rapid age-related progression of the biological risk factors of CAD [32, 33]. There is some evidence of an association between low birth weight and increased aging rates in human studies too [34, 35]. Although little research has been carried out elucidating the underlying mechanisms,

Jennings et al [36] suggest that telomere shortening induced by prenatal undernutrition may be responsible for the premature senescence of tissues such as the liver and kidney. These studies also pointed out that catch-up growth, such as that which may

have occurred in fetuses conceived during famine but exposed to adequate nutrition during the remainder of gestation, could result in further telomere shortening.

In summary, our findings suggest that maternal nutrition in early gestation may play an important role in the course of CAD. This suggestion is in line with evidence from animal experiments that identified preconceptional and preimplantation maternal diet as important for the offspring's adult health [11–14].

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Two boys in Amsterdam's inner city area 'the Jordaan' during the Dutch Famine (photo NIOD)

chapter

4

Blood pressure response to psychological stressors in adults after prenatal exposure to the Dutch famine

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Abstract

Objective There is increasing evidence that restricted prenatal growth is associated with exaggerated blood pressure responses to stress. We investigated the effect of maternal undernutrition on the adult offspring's stress response.
Design A historical cohort study.
Methods We performed continuous blood pressure and heart rate measurements during a battery of three 5-min physiological stress tests (Stroop test, mirror-drawing test and a public speech task) in 721 men and women, aged 58 years, born as term singletons in Amsterdam at about the time of the Dutch 1944–1945 famine.
Results During the stress tests, the systolic blood pressure (SBP) rose from baseline by 20 mmHg during the Stroop test, by 30 mmHg during the mirror-drawing test and by 47 mmHg during the public speech task. The SBP and diastolic blood pressure increase during stress was highest among individuals exposed to famine in early gestation compared with unexposed subjects (4 mmHg extra systolic increase, P=0.04; 1 mmHg diastolic increase, P= 0.1, both adjusted for sex). Exposure during mid and late gestation was not associated with a stress-related increment of blood pressure (P adjusted for sex > 0.6). Correcting for confounders in a multivariable model did not attenuate the association between famine exposure in early gestation and the SBP increment. The heart rate increment was not related to famine exposure during any part of gestation.
Conclusion We found a greater blood pressure increase during stress among individuals exposed to famine in early gestation. Increased stress responsiveness may underlie the known association between coronary heart disease and exposure to famine in early gestation.

Introduction

Low birth weight is associated with an increase in cardiovascular disease [1] and stroke in adult life [2]. These findings led to the formulation of the fetal origins hypothesis, which states that a suboptimal intrauterine environment may elicit permanent changes in the developing fetus [3]. Such changes may have short-term advantages for the survival of the fetus, but can prove detrimental for health in adult life. Although low birth weight is also associated with a number of cardiovascular risk factors including hypertension [4], the mechanisms responsible for the increase in cardiovascular disease among individuals who were small at birth are not well understood [5]. Heightened blood pressure reactions to psychological stressors have been implicated as risk factors that act independently from resting blood pressure in the development of cardiovascular disease [6–9]. There is growing evidence that restricted growth in prenatal life permanently alters the biological responses to stress in adulthood. Young women with low birth weight have an increased blood pressure response to psychological stressors [10]. Rats that were malnourished in utero had an exaggerated blood pressure response to an olfactory stressor [11]. Cardiovascular control mechanisms were altered in lambs that had been subjected to undernutrition in early gestation, but this only became evident during stimulation with angiotensin II or norepinephrine [12,13].

Programming of the stress response during fetal life may contribute to the increase in cardiovascular disease and its biological risk factors among individuals who were exposed to suboptimal intrauterine conditions. In many studies in humans, birth size, a summary measure of fetal growth, is the only available measure of intrauterine environment. However, in humans and animals, restricted maternal nutrition has been shown to produce permanent effects on adult health, without affecting birth size [14,15]. Whether maternal undernutrition during gestation may programme the stress response in humans is unknown.

The Dutch famine was a 5-month period of severe food shortage during the last winter of World War II, which affected the cities in the western Netherlands worst. Although it was a humanitarian disaster, the Dutch famine presents a unique opportunity to examine the effects of a short but severe period of maternal undernutrition on offspring in later life. Previously, Dutch famine studies have shown that exposure to famine in late gestation is associated with an increase in impaired glucose tolerance and type II diabetes [16], whereas exposure in mid-gestation is linked to microalbuminuria [17]. Individuals exposed in early gestation had a threefold increase in coronary heart disease [18], a more atherogenic lipid profile [19] and altered clotting [20], were more obese [21] and felt less healthy as adults [22]. Famine exposure was not associated with resting blood pressure, but the composition of the maternal diet in late gestation was [23,24]. These findings demonstrate that prenatal famine can have a profound effect

on health in later life, but also that the timing of famine during gestation is crucial in determining the type of effect.

In the current study we investigate whether prenatal exposure to famine during different stages of fetal life programmes stress responsiveness.

Subjects and methods

Study population

The Dutch Famine Birth Cohort consists of 2414 liveborn, term singletons, born in the Wilhelmina Gasthuis in Amsterdam, the Netherlands. All babies were born between 1 November 1943 and 28 February 1947. The selection procedure has been described in detail elsewhere [16], as have loss to follow-up as a result of mortality, emigration and other reasons [25,26]. Cohort members were eligible for participation if they lived in the Netherlands and their address was known to the investigators at the time the study commenced. Council registries helped trace individuals whose current address was initially not available to the investigators. At the start of the study, 1423 individuals (59%) were eligible for participation.

Exposure to famine

We defined famine exposure according to the daily official food rations for adults. In addition to the official rations, food from other sources including church organizations, central kitchens and the 'black market' was also available. Individuals may have had access to up to double the rationed amount at the peak of the famine. The rations do, however, adequately reflect the fluctuation of food availability during the famine [27]. An individual was considered prenatally exposed to famine if the average daily ration for adults during any 13-week period of gestation was less than 1000 calories. Therefore, individuals born between 7 January and 8 December 1945 were exposed prenatally. We defined periods of 16 weeks each to differentiate between those who were exposed in late gestation (born between 7 January and 28 April 1945), mid-gestation (born between 29 April and 18 August 1945) and in early gestation (born between 19 August and 8 December 1945), in correspondence with previous publications on this cohort [18,28]. We compared individuals exposed to famine in utero during different periods of gestation with unexposed individuals, defined as individuals born before and individuals conceived after the famine.

Study parameters

The medical birth records provided information about the mother, the course of the pregnancy and the size of the baby at birth [28]. Socioeconomic status at birth was defined as either manual or non-manual, using the information provided by the birth records.

During a clinic visit, trained study nurses carried out all measurements and conducted the interviews. We performed a standard oral glucose tolerance test (75 g glucose) after an overnight fast. Individuals taking prescription oral or injected antidiabetic medication were excluded from oral glucose tolerance testing, but participated in the remainder of the measurements. A standard 12-lead electrocardiogram was performed. Trained technicians blinded to clinical data scored the electrocardiograms according to the Minnesota criteria. We measured height using a fixed or a portable stadiometer and weight using Seca scales or portable Tefal scales. Body mass index was calculated by dividing weight in kilograms by the square of height in meters. Blood pressure was measured in rest in duplo on two occasions (morning and afternoon) using an oscillometric device (Omron 705CP/IT; Omron Healthcare UK, West Sussex, UK) and appropriate cuff sizes on the non-dominant side. Mean blood pressure was defined as the average of all available measurements from the morning and afternoon. The participants were asked about their own and their partner's occupation and level of training, their smoking history, history of hypertension and cardiovascular disease. All current medication was recorded. Antihypertensive medication use was defined as the current use of a prescription medication that belonged to any of the classes of antihypertensive drugs, regardless of the reason it had been prescribed. The presence of coronary heart disease as defined as the presence of one or more of the following: angina pectoris according to the Rose/WHO questionnaire; Q waves on the electrocardiogram (Minnesota codes 1-1 or 1-2); or a history of coronary revascularization (angioplasty or bypass surgery).

We defined current socioeconomic status according to the participant's occupation or their partner's; whichever was highest, using the International Socio-Economic Index of occupational status (ISEI-92). Values in the ISEI-92 scale range from 16 (low status) to 87 [29].

The local Medical Ethics Committee approved the study. All participants gave written informed consent.

Stress protocol

The stress protocol started in the afternoon (between 12:00 and 14:00 hours), an hour after the participants had eaten a light lunch. Continuous blood pressure recordings were performed using a Finometer or a Portapres Model-2 (Finapres Medical Systems, Amsterdam, the Netherlands).

The protocol started with a 20-min baseline period. The baseline period was followed by a computerized Stroop (colour–word conflict) test, a mirror-tracing test (Lafayette Instruments Corp, Lafayette, Indiana, USA), and a public speech task. The stress tests took 5 min each. The Stroop and mirror-tracing test were followed by a 6-min recovery period. The speech task was followed by a 30-min recovery period. Participants practised the Stroop test until the nurse was confident that they grasped the aim of the test, and were comfortable with the keyboard. During the Stroop test there was a 5-s

response time limit per colour–word matching task. The computer automatically emitted a beep if the response time lapsed, or if an incorrect answer was given. Before the mirror-tracing task, participants completed one practice circuit before starting the test. Participants were instructed to give accuracy priority over speed, and were informed about the average number of circuits usually completed within 5 min by other participants. Before the public speech task, participants listened to a prerecorded scenario in which they were falsely accused of pickpocketing. Participants were instructed to give a 3-min response to the accusations and were given 2 min to prepare. The response was recorded on video. Subjects had been instructed that their response would be judged for eloquence, persuasiveness and the number of repetitions.

Five periods of 5 min each were designated as measuring periods. During these periods the automatic calibration on the Finometer or Portapress was turned off. The periods were defined as follows: baseline (15 min into the baseline period), Stroop, mirror-tracing, speech task (including preparation time), recovery 1 (5 min after completing the speech task), recovery 2 (25 min after completing the speech task). We calculated mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) and heart rate and their standard deviations for each measuring period.

Statistical methods

We calculated stress response by subtracting the mean baseline value from the mean values during the three stress periods. We did so for both SBP and DBP as well as heart rate. We used repeated measures analyses (linear mixed models, SPSS 12.0.2; SPSS Inc., Chicago, Illinois, USA), using an unstructured variance covariance matrix, to analyse how blood pressure and heart rate response during the three 5-min stress test periods depended on famine exposure during different periods of gestation as well as birth size and cardiovascular risk factors. We used linear and logistic regression to compare the maternal, birth and adult characteristics of individuals exposed to famine during different periods of gestation with unexposed individuals. We log-transformed variables with skewed distributions. These variables are reported as geometric means and interquartile ranges.

Results

Study population

At the age of 58 years (range 56–61) all 1423 eligible individuals were invited to participate in the study. A total of 740 individuals (52%) visited the clinic, of which 721 completed the stress protocol. As a result of technical and logistical problems 19 participants did not complete the stress protocol. The birth weights of individuals participating in the study (3363 g) did not differ significantly from the birth weights of eligible indi-

Table 1. Maternal, birth and adult characteristics [a] of study participants

	Exposure to famine during gestation						
	Born before	Late	Mid	Early	Conceived after	All (SD)	N
N	228	125	105	61	202		721
Men (%)	48	44	42	49	51	47	721
Maternal & birth characteristics							
Maternal weight at the end of pregnancy (kg)	67	63[b]	63[b]	69	70	66 (9)	638
Primiparous (%)	36	21[b]	31	41	37	33	721
Maternal age (years)	29	31[b]	29	27	28	29 (6)	721
Birth weight (g)	3391	3206[c]	3201[c]	3462	3485	3363 (465)	721
Birth length (cm)	51	50[c]	50[c]	51	51	50 (2.1)	715
Ponderal index (kg/m^3)	26.2	26.1	25.9[c]	26.1	26.6	26.3 (2.3)	715
Adult characteristics							
BMI (kg/m^2) [d]	28.4	28.1	27.9	27.9	28.8	28.3 (25.3-31.2)	720
SBP (mmHg) [e]	138	137	136	135	136	137 (18)	718
DBP (mmHg) [e]	81	81	80	82	82	81 (10)	718
Antihypertensive medication (%)	27	20	28	13[c]	23	24	721
SES (ISEI-92) [d]	46	50[c]	49	45	48	47 (40-59)	713
Current smoker (%)	22	26	28	28	21	23	720
2 hour insulin (pmol/l) [d]	243	247	251	271	234	244 (152-395)	623
Baseline stress test characteristics							
SBP (mmHg) [f]	129	128	127	125	128	128 (21)	713
DBP (mmHg) [f]	66	66	63[c]	67	68	66 (12)	713
Heart rate (bpm) [f]	75	73	74	76	74	74(11)	712

BMI, Body mass index; DBP, diastolic blood pressure; ISEI-92, International Socio-Economic Index of Occupational Status; SBP, systolic blood pressure; SES, socio-economic status.

[a] means and standard deviation, except where given as percentages.

[b] $p<0.05$ compared to unexposed, linear or logistic regression.

[c] $p<0.05$ adjusted for sex compared to unexposed, linear or logistic regression.

[d] geometric mean and interquartile range

[e] brachial blood pressure outside the stress protocol

[f] mean of continuous measurements from baseline period in stress protocol

viduals not included (3343 g, P=0.4). A total of 291 of the 721 individuals (40%) who completed the stress protocol had been exposed to famine in utero.

Table 1 shows that famine exposure in mid or late gestation was associated with lower maternal weight and birth size. Mean adult blood pressure (measured in the morning and afternoon, outside the stress protocol) did not differ between famine exposed and unexposed individuals, nor did baseline values for SBP and heart rate during the stress test. Compared with unexposed individuals, baseline DBP was 3 mmHg lower among individuals exposed in mid-gestation (P=0.02). Fewer individuals exposed to famine in early gestation were using prescription antihypertensive medication, compared with unexposed individuals.

Famine exposure and stress response

Table 2 shows that individuals exposed to famine in early gestation had the highest mean stress response in terms of SBP and DBP increase during all three stress tests.

Table 2. Systolic and diastolic blood pressure and heart rate increase from baseline during the stress tests according to the timing of famine exposure during gestation.

	ΔSBP[a]			ΔDBP[a]			ΔHR[a]		
Famine exposure	Stroop	Mirror	Speech	Stroop	Mirror	Speech	Stroop	Mirror	Speech
Born before	19	29	46	9	16	20	4	5	10
Late	20	30	45	9	16	20	4	5	13
Mid	19	29	48	9	15	21	4	4	10
Early	23	34	49	11	17	21	4	5	11
Conceived after	19	31	47	9	16	21	4	4	15
All	20 (16)	30 (18)	47 (22)	9 (6)	16 (9)	20 (10)	4 (5)	4 (6)	12 (16)
p[b]	0.04			0.1			0.9		
p[c]	0.05			-			-		

[a] ΔSBP, ΔDBP, Mean systolic and diastolic blood pressure increase (SD) from baseline (mmHg). ΔHR Heart rate increase from baseline (bpm)

[b] *p* for difference early exposed vs unexposed derived from repeated measures analysis using all three stress tests, corrected for sex

[c] *p* for difference early exposed vs unexposed corrected for sex, BMI, systolic brachial blood pressure outside the stress protocol, smoking and 2 hr insulin

The average SBP response to the three stress tests was 4 mmHg higher compared with that of unexposed participants (P=0.04 adjusted for sex, estimate and P derived from repeated measures analysis; Fig. 1). The DBP response to stress among those exposed in early gestation was 1 mmHg higher than in unexposed individuals, but the difference did not achieve statistical significance (P=0.1 adjusted for sex). Individuals who were unexposed had a similar blood pressure response to those exposed in mid or late gestation (P>0.6 adjusted for sex for mid and late gestation compared with unexposed). There were no differences in the heart rate stress response between the three famine-exposed groups and the unexposed group (P>0.4 adjusted for sex).

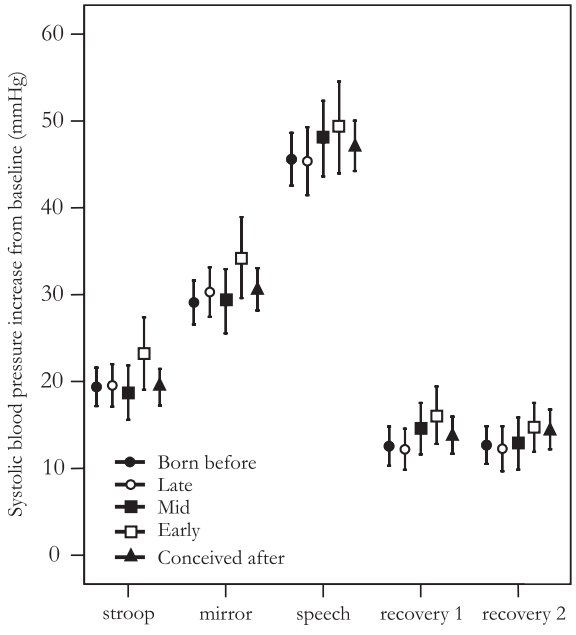

Figure 1 Increase in systolic blood pressure (mmHg, mean and 95% confidence interval) from baseline during stress protocol according to timing of famine exposure in utero.

The effects of exposure to famine in early gestation were present in both men and women (6 and 2 mmHg extra systolic stress response, respectively, compared with unexposed, *P*=0.2 for interaction famine exposure*sex).

Stress response and cardiovascular risk factors

Table 3 shows the relationship between cardiovascular risk factors and the SBP response to the three stress tests. In a multivariate model we found that the effect of famine exposure in early gestation was not attenuated by adjusting for cardiovascular risk factors, including sex, body mass index, mean blood pressure outside the stress protocol, smoking and 2 h insulin concentrations (adjusted extra SBP increase 4 mmHg, *P*=0.05 compared with unexposed). Additional adjustment for the use of antihypertensive medication or maternal age, parity or socioeconomic status at birth or adulthood did not alter the association.

Table 3 The effect of cardiovascular risk factors on systolic blood pressure increase from baseline over all three stress tests in univariate and multivariate analysis

	ΔSBP[a] (univariate)		ΔSBP[a] (multivariate)	
	β[b]	*p*[b]	β[b]	*p*[b]
Male sex	5	<0.0001	4	0.001
Body mass index (kg/m^2)	-1	0.05	-2	0.002
Smoking	-7	<0.0001	-6	<0.0001
Systolic blood pressure (mmHg) [c]	3	<0.0001	4	<0.0001
2 hour insulin (pmol/l)	1	0.05	1	0.06
Socio-economic status (ISEI-92)	1	0.08	-	-
Anti-hypertensive medication	-1	0.5	-	-
Famine exposure in early gestation [d]	4	0.04	4	0.05

ISEI-92, International Socio-Economic Index of Occupational Status

[a] ΔSBP (mmHg): systolic blood pressure increase from baseline

[b] β (estimates) given per SD increase for continuous variables and p values derived from repeated measures analysis, adjusted for sex in univariate analyses

[c] brachial systolic blood pressure measured outside the stress protocol

[d] in comparison to unexposed

Size at birth and stress response

Birth weight was strongly inversely associated with brachial blood pressure measured outside the stress protocol (5 mmHg systolic/3 mmHg diastolic increase in blood pressure per kilogram decrease in birth weight, $P < 0.001$). Baseline values measured during the stress protocol were not associated with birth weight, although they showed a non-significant inverse trend.

Table 4 shows that birth weight (5 mmHg extra increase in SBP per kilogram decrease, *P*=0.002) was associated with a higher SBP response to stress in women, but not in men (*P*=0.006 for interaction birth weight and sex). Similarly, birth length (1 mmHg extra increase in SBP per centimetre decrease, $P = 0.02$) and head circumference (1 mmHg extra increase in SBP per centimetre decrease, $P = 0.04$) were associated with a higher

Table 4 Systolic and diastolic blood pressure and heart rate increase from baseline during the stress tests across birth weight tertiles according to sex

	Men				Women			
Birth weight tertile[a]	n	ΔSBP[b]	ΔDBP[b]	ΔHR[b]	n	ΔSBP[b]	ΔDBP[b]	ΔHR[b]
Low	113	32 (30-35)	15 (14-16)	8.1 (7.0-9.1)	125	33 (30-35)	15 (14-16)	6.4 (5.4-7.4)
Mid	114	36 (33-39)	15 (14-17)	8.0 (6.9-9.1)	122	32 (29-35)	16 (14-17)	6.2 (5.1-7.2)
High	112	33 (30-36)	15 (14-16)	7.0 (5.9-8.1)	127	27 (24-30)	14 (13-15)	5.5 (4.5-6.5)
p[c]		0.32[d]	0.54	0.36		0.002[d]	0.19	0.21

[a] Birth weight tertiles in men: - 3240 g, -3610 g, >3610 g; tertiles in women: -3130 g, -3466 g, >3466 g

[b] ΔSBP, ΔDBP: systolic and diastolic blood pressure increase from baseline (mmHg). ΔHR Heart rate increase from baseline (bpm). Estimated marginal mean increase from baseline over all three stress tests, derived from repeated measures analysis.

[c] *p* value based on analysis of birth weight as a continuous variable

[d] *p* for interaction birth weight*sex=0.006

SBP response to stress in women, but not in men. There was no such association with the ponderal index or with DBP or the heart rate response to stress. Adjusting for body mass index, mean blood pressure outside the stress protocol, smoking and 2 h insulin concentrations did not alter the association between the high stress response in women and low birth weight (4 mmHg adjusted extra SBP increase per kilogram decrease, *P*=0.02). In men, on the other hand, after adjusting for these factors, high birth weight was significantly associated with a higher stress response (4 mmHg adjusted extra SBP increase per kilogram increase, *P*=0.02).

The increase in the systolic stress response after famine exposure in early gestation was not attenuated when we adjusted for size at birth (adjusted for birth weight 4 mmHg extra increase in SBP, *P*=0.04).

Discussion

This study examined the relationship between maternal famine exposure and cardiovascular responses to psychological stress in the offspring. We found that, at the age of 58 years, individuals conceived in famine were more stress responsive in terms of SBP compared with individuals who were unexposed to famine in utero. The association was independent of potential confounders including cardiovascular risk factors and birth size. Famine exposure was not associated with high blood pressure outside the stress protocol.

Fertility was reduced during the famine. Possibly only mothers of a particular constitution may have been able to conceive in the midst of famine. However, correcting for parameters associated with fertility, including socioeconomic status at birth and parity did not attenuate our findings. We conclude that selective fertility can have had only a limited influence.

Previous studies in the Dutch famine birth cohort found an excess of coronary heart disease at the age of 50 years among those conceived in the famine [18]. Our study at 58 years of age may thus have been subject to the selective participation of healthier

individuals among those conceived during the famine. We recently found that mortality among individuals conceived during the famine was not increased at the age of 57 years, suggesting that the effects of selective mortality are likely to have been limited [26]. Any residual effect of selective participation as a result of increased coronary heart disease disability may, however, have diluted our findings.

The Dutch famine birth cohort study is a unique human counterpart for animal models that study the effects of restricted maternal nutrition during different stages of gestation. Although the association between exposure to famine in early gestation and exaggerated stress response in our study was only modestly statistically significant, it is in line with animal models that have identified early gestation as a critical period for cardiovascular control mechanism programming by maternal undernutrition [12,13].
Our finding that the blood pressure response during stress is enhanced after maternal exposure to famine in early gestation may be the result of programmed alterations in autonomic control mechanisms [13], including sympathetic and baroreflex function. The offspring of rat dams on a low protein diet had higher circulating catecholamine concentrations and an increase in catecholamine receptors [30]. Restricted maternal nutrition in early gestation led to altered baroreflex function in lambs [12,31]. In the future, we plan to investigate whether our present finding of an exaggerated stress induced SBP increase after prenatal exposure to famine is caused by the programming of cardiovascular control mechanisms.

There is growing evidence that the hypothalamic pituitary adrenal axis may undergo permanent alterations as a result of a suboptimal intra uterine environment [32–34]. Cortisol may play a permissive role in blood pressure response to stress [35]. Alterations in hypothalamic pituitary adrenal axis activity may have contributed to the exaggerated stress response in individuals conceived in famine.

Age increases the blood pressure stress response independently of the age-related increase in hypertension [36]. Our finding that the stress response was enhanced among individuals who were conceived during the famine may reflect more rapid ageing among this group of individuals. Prenatally nutrient-restricted animals demonstrated premature ageing [37,38] and a more rapid age-related progression of the biological risk factors of coronary heart disease [39–41]. There is also some evidence for an association between a suboptimal intrauterine environment and increased ageing rates in human studies [42,43]. Although little research has been carried out elucidating the underlying mechanisms, Jennings et al. [44] suggested that telomere shortening induced by prenatal undernutrition may be responsible for premature senescence. Such studies also pointed out that catch-up growth, such as may have occurred in fetuses conceived during famine but exposed to adequate nutrition during the remainder of gestation, could result in further telomere shortening.

We found that women who had been small at birth had a higher SBP stress response. Our findings are in line with a study among young adults, which used the same stress

protocol [10]. The effect of famine exposure in early gestation on stress response was not limited to women, and it was not attenuated by adjusting for birth size. This may suggest that small size at birth programmes stress reactivity by affecting different developing organ systems in comparison with those affected by maternal famine in early gestation. Possibly, the timing of the suboptimal fetal environment accounts for the differences in developing organ systems that are affected. Alternatively, the fact that a wide range of maternal, placental and fetal factors contribute to small size at birth and may each exhibit specific effects on developing organs, may underlie the difference in effects in comparison with maternal famine in early gestation. This is supported by evidence from animal studies, which showed that programming of the sympathetic nervous system is limited to female rats in a placental insufficiency model [45], whereas males are also affected in maternal undernutrition models [11,30]. Heightened blood pressure reactions to psychological stressors may act independently from resting blood pressure and hypertension in the development of cardiovascular disease [6–9], possibly by inducing endothelial dysfunction [46]. Coronary heart disease is more prevalent [18] and manifests at a younger age [47] among individuals conceived in famine. Increased stress responsiveness may be one of the mechanisms that contributes to the increase in coronary heart disease after exposure to famine in early gestation.

In summary, we found that individuals exposed to famine in early gestation have an enhanced blood pressure response to psychological stress at the age of 58 years. Increased stress responsiveness may underlie the known association between coronary heart disease and exposure to famine in early gestation. In future studies we aim to determine the mechanisms responsible for increased stress response after prenatal exposure to famine.

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Children chase a bread truck, hoping for an extra scrap (Photo NIOD)

chapter

5

Maternal nutrition during gestation and carotid arterial compliance in the adult offspring - the Dutch Famine Birth Cohort

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submitted

Abstract

Objective Experimental evidence indicates that maternal undernutrition during gestation may program hypertension in the offspring. We investigated whether maternal undernutrition leads to increased arterial stiffness.

Methods We measured carotid artery lumen diameter (LD), distensibility (DC), stiffness (β), and compliance (CC) by M-mode ultrasound in 673 people, aged 56-61, who had been born as term singletons around the time of the 1944-'45 Dutch famine.

Results Maternal famine exposure had no effect on any of the measures of carotid size or stiffness in the offspring. Low maternal weight at the end of pregnancy and low birth weight were associated with decreased LD (0.01 mm per kg maternal weight, sex adjusted $p<0.001$; 0.1 mm per kg birth weight, sex adjusted $p=0.08$) and CC (0.002 mm^2/kPa per kg maternal weight, sex adjusted $p=0.001$; 0.03 mm^2/kPa per kg birth weight, sex adjusted $p=0.03$), but neither was associated with increased β, or decreased DC. These effects were not attenuated by adjusting for maternal protein/carbohydrate ratio in the third trimester. The association of low birth weight with increased CC diminished after adjusting for maternal weight. The association of maternal weight with CC was smaller when adjusted for LD.

Conclusions Our findings suggest that small maternal size, not poor maternal diet, in late gestation programs decreased arterial compliance in the adult offspring by affecting vessel size rather than vessel wall stiffness.

Introduction

Arterial stiffness predicts cardiovascular morbidity and mortality independently of conventional risk factors [1-3]. Blood pressure [4], mechanical vessel wall properties including elastin, collagen and extracellular matrix content of the arterial media [5] as well as arterial tone [6] all contribute to arterial stiffness.

People who were small at birth have increased rates of hypertension [7], coronary heart disease [8] and stroke [9]. Increased vessel stiffness may be one of the links between compromised fetal growth and poor health in adult life. Restricted intrauterine growth may lead to increased vessel stiffness by decreasing arterial wall elastin endowment at birth [10] or altering cardiovascular control mechanisms [11-13]. Increased arterial stiffness has been demonstrated among people who were small at birth in a number of studies [14-17]. However, a considerable number of studies reported no association between smallness at birth and increased arterial stiffness, or find an effect in subgroups only [18-26].

Part of the discrepancy in results may be due to the fact that size at birth is a composite measure of maternal, fetal and placental factors. In rats, maternal dietary manipulation can increase arterial stiffness in the offspring, without affecting birth weight [27, 28]. Moreover, growth restriction caused by nutritional factors can produce different effects on vascular physiology in later life compared to growth restriction of placental origin [29]. Maternal dietary manipulation has been shown to program hypertension in the offspring in both animals and humans [30-33]. The effects of maternal nutrition on the offspring's arterial stiffness have not been studied in humans.

The Dutch famine offers a unique opportunity to directly study the effects of maternal undernutrition during gestation on the offspring's adult health. The 5-month famine in the winter of 1944-'45 started after the German occupation placed an embargo on food transports to the west of the Netherlands, and ended abruptly upon liberation, in early May 1945. Ration records allow the study of the effects of maternal dietary caloric restriction as well as of protein, carbohydrate and fat components on health in later life.

Previously we reported that maternal famine exposure during gestation did not affect blood pressure in the offspring [34], but that a low maternal protein/carbohydrate ratio in the third trimester led to increased blood pressure in the offspring [31]. Maternal famine exposure during gestation was associated with increases in various other markers of cardiovascular risk [35-40].

In this paper, we investigate whether measures of maternal undernutrition during gestation increase carotid arterial stiffness in the offspring.

Subjects and Methods

Selection procedure

The Dutch Famine Birth Cohort consists of 2414 live-born, term singletons, born in the Wilhelmina Gasthuis in Amsterdam, the Netherlands. All babies were born between November 1st 1943 and February 28th 1947. The selection procedure has been described in detail elsewhere [35].

For this study, cohort members were eligible for participation if they lived in the Netherlands on September 1st 2002 and their address was known to the investigators. 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.

Maternal nutritional intake and exposure to famine

The official rations were determined every week by the National Bureau for Food Ditribution during War Time. We used weekly protein and carbohydrate intake to calculate the percentage of energy derived from protein and carbohydrate during each week, and also to calculate the protein/carbohydrate ratio [31]. We estimated the intake in the third trimester of gestation as the average intake during week 27 to 39.

We defined famine exposure according to the daily official food-rations for adults. In addition to the official rations, food from other sources, such as church organizations, central kitchens, the 'black market', was also available. People may have had access to up to double the rationed amount at the peak of the famine. The rations adequately reflect the fluctuation of food availability during the famine [41].

A person was considered prenatally exposed to famine if the average daily ration for adults during any 13–week period of gestation was less than 1000 calories. Therefore, people born between January 7th 1945 and December 8th 1945 were exposed prenatally. People born between November 1st 1943 and January 6th 1945 (born before the famine) and between December 9th 1945 and February 28th 1947 (conceived after the famine) were thus unexposed to famine in utero.

We defined periods of 16 weeks each to differentiate between those who were exposed in late gestation (born between January 7th and April 28th 1945), mid gestation (born between April 29th and August 18th 1945) and in early gestation (born between August 19th and December 8th 1945), in correspondence with previous publications on this cohort [34-40, 42-44] (see Figure 1).

Data collection

The medical birth records provided information about the mother, the course of the pregnancy and the size of the baby at birth [35]. The local Medical Ethics Committee approved the study.

The participants attended the clinic for the data collection protocol, which encompassed a full day. The mean age of participation in the study was 58 years (range 56 to 61). All participants gave written informed consent. Trained nurses carried out all measurements and interviews. We measured height with a fixed or a portable stadiometer and weight with Seca scales or Tefal portable scales. 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. Pulse pressure was calculated by subtracting mean diastolic from mean systolic blood pressure. Participants underwent a standard oral glucose tolerance test (OGTT). Known diabetics were excluded from OGTT. Known diabetes was defined as the use of anti-diabetic medications (oral or injected). Blood was drawn for analysis of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol.

Participants were asked about their current smoking status. We defined current socio-economic status (SES) according to the participant's occupation or their partner's; whichever was highest, using the ISEI-92. Values ranged from 16 (low status) to 87 [45].

Standard 12-lead electrocardiograms (ECG) were made of participants. Trained technicians blinded to clinical data scored the ECG-s according to the Minnesota criteria. 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 recorded all current medications. All classes of blood pressure regulating medication were coded as anti-hypertensive medication.

Distal carotid lumen diameter measurements and vessel wall stiffness measurements

Lumen diameter (LD) and stiffness parameters were measured in the distal common carotid arteries (CCA). Two trained investigators (RCP and SdR) carried out the majority (92%) of the ultrasound examinations. Four other experienced sonographers carried out the remainder. The coefficient of variation for paired replicate images performed by the same sonographer within the same subject may not exceed 10% in order for the sonographer to gain certification, and was 5.4% in similar studies (personal communication E. de Groot).

The ultrasounds were performed with subjects in a supine position using an Acuson 128XP/10v (Acuson Corp, Mountain view, Calif) ultrasound instrument equipped with a 5-10 MHz L7 (Acuson L7) and Extended Frequency software, version 7.02 (Acuson

Corp). The left and right far-walls of the CCA were imaged in B-mode standardized magnification (Regional Expansion Selection 2 x 2 cm). The B-mode image was used to direct the M-mode ultrasound beam perpendicular to the walls of the CCA. In the 1/3 B-mode / 2/3 M-mode duplex setting, wall motion was recorded for at least two consecutive heartbeats and then frozen and saved as a JPEG still image (Sony DKR-700P video still image recorder).

After the completion of the data collection, two image analysts batch-read all ultrasound images. They were blinded to the identity of the subject. The images read by the image analysts overlapped. These overlapping scans (n=20) were provided to the analysts in a blinded fashion. The coefficient of variation of the paired differences of the lumen was 1.1%. The arterial lumen was defined as the distance between the contours of the near wall intima-lumen and the far wall lumen-intima interfaces as traced by automated contour detection software (eTrack, version 2.3, W.J. Stok, Dept of Physiology, AMC, University of Amsterdam) [46]. The mean distal common carotid LD was calculated by averaging the distance between the near and far wall interfaces for at least two heartbeats. The mean of left and right LD measurements was calculated. If either of these was missing, the carotid LD measurement of the remaining side was used.

Brachial blood pressure was measured ipsilaterally using an automated device (Omron 705CP/IT; Omron Healthcare UK, West Sussex, UK) while the M-mode image recording took place.

Three parameters for functional arterial wall alterations were calculated: the distensibility coefficient (DC), the vessel stiffness (β) and the compliance coefficient (CC), defined as:

Distensibility coefficient (DC) = (2 (ΔD /D)) / PP	10^{-3}/kPa
Vessel stiffness (β) = [ln (SBP/DBP)]/ (ΔD /D)	no unit
Compliance coefficient (CC) = (π*ΔD*D)/ (2*PP)	mm^2/kPa

SBP=systolic blood pressure (kPa), DBP=diastolic blood pressure (kPa), PP=pulse pressure (kPa), ΔD= diameter change (mm), D= diastolic diameter (mm)

The parameters were calculated as the average of their value on the left and right side. If either the right or left value was missing, the remaining available value was used instead of the mean value.

DC is the fractional change in arterial cross-sectional area per unit in (local) pressure change, i.e. it describes the amount of diameter expansion expressed as a percentage of the initial diameter of the artery in relation to the force that causes the expansion (transmural pressure).

β is a pressure standardized measure of vessel distensibility.

CC describes the absolute diameter expansion in relation to the force that causes the expansion. DC is therefore a measure of the elastic properties of the artery, whereas CC is a measure of the local vessel capacity to store extra volume (buffering capacity).

We calculated that, at a significance level of 0.05, we would have a power of 80% to detect a 6% rise in arterial compliance in the smallest exposure group (exposed in early gestation, n=51) compared to unexposed.

Statistical Methods

We used linear and logistic regression analyses to compare the characteristics of individuals exposed or not exposed to famine in gestation. Linear regression was also applied to explore the association between carotid LD, DC, β and CC and the characteristics of the participants. In all analyses we corrected for gender. We additionally corrected for other potential confounders, including measures of adult body size, blood pressure and life-style.

Variables with skewed distributions, including DC, β and CC were log transformed before the analyses and are reported as geometric means and log-scale regression coefficients.

Table 1 Birth, adult and carotid artery characteristics for participants in the Dutch Famine Birth Cohort Study, according to timing of exposure to famine. Means and standard deviations (SD), except where given as percentages.

	Exposure to famine					
	born before	**late**	**mid**	**early**	**conceived after**	**all (SD)**
Number	212	114	96	51	200	673
Men (%)	50	46	41	41	51	47
Birth characteristics						
Birth weight (g)	3405	3196[1]	3192[1]	3432	3483	3364 (459)
Head circumference (cm)	32.9	32.6[1]	32.2[1]	32.8	33.2	32.8 (1.5)
Maternal weight end of pregnancy (kg)	66.7	62.8[1]	63.0[1]	68.8	69.6	66.5 (8.5)
Maternal weight gain 3rd trimester (kg)	3.2	0.1[1]	4.9v	5.71	4.2	3.3 (3.2)
Protein/carbohydrate ratio 3rd trimester[2]	0.15	0.15[1]	0.19[1]	0.191	0.18	0.16
Adult characteristics						
Body mass index (kg/m^2)[3]	28	28	28	28	29	28 (5)
Socio-economic status[4]	49	53	52	47	51	50 (14)
Current smoker (%)	21	23	25	28	21	22
Glucose 120 mins (mmol/l)[3]	5.7	6.0	6.2	6.2	5.9	5.9 (2.4)
LDL/HDL ratio[3]	2.5	2.7	2.6	2.9[1]	2.5	2.6 (1.0)
Systolic blood pressure (mmHg)	138	137	136	134	135	136 (18)
Diastolic blood pressure (mmHg)	81	81	80	82	82	81 (10)
Pulse pressure (mmHg)	57	55	56	52	53	55 (12)
Anti-hypertensive medication (%)	26	19	22	16	23	23
Coronary heart disease (%)	6.7	6.3	4.2	8.0	6.6	6.2
Carotid artery characteristics						
Lumen diameter (mm)	6.5	6.6	6.4	6.2	6.4	6.5 (0.8)
Distensibility (10^{-3}/kPa)[3]	24.9	24.8	27.0	26.0	26.2	25.5 (8.5)
Stiffness[3]	6.1	6.1	5.9	5.8	5.8	6.0 (1.8)
Compliance (mm^2/kPa)[3]	0.72	0.74	0.74	0.69	0.75	0.73 (0.27)

[1] p value<0.05 (adjusted for gender) compared to unexposed
[2] median
[3] Geometric mean
[4] Socio-economic status according to the ISEI-92 (range 16-87)

Maternal weight and maternal weight gain at the end of pregnancy were missing in a relatively large number of participants (11% and 29% respectively). Therefore, when we corrected for these variables, we imputed the mean value of the variable if information was missing, and included a separate indicator of missing information. When we studied the effect of both maternal weight at the end of pregnancy as well as birth weight on LD or CC in a multivariable model, we used birth weight adjusted for maternal weight because of the multi-collinearity of their association with LD and CC. We used SPSS 12.0 for all data analyses.

Results

We measured carotid LD in 673 people (47% of eligible cohort members). We were unable to calculate DC, β, and CC in 17 people due to failure of the blood pressure measurement.

Famine exposure and arterial stiffness

Table 1 shows the characteristics of the participants according to timing of exposure to famine in utero. People exposed in late or mid gestation were lighter, and had smaller head circumferences at birth and their mothers weighed less in late pregnancy com-

Table 2 Adult and carotid artery characteristics for participants in the Dutch Famine Birth Cohort Study, according to quartiles of birth weight.

	Birth weight (g)				
	≤3050	-3360	-3680	≥3681	p^1
Number	171	171	167	164	
Men (%)	41	41	53	56	0.9
Adult characteristics					
Body mass index (kg/m^2)[2]	28	28	28	29	0.4
Socio-economic status[3]	49	48	49	47	0.5
Current smoker (%)	22	23	25	19	0.9
Glucose 120 mins (mmol/l)[2]	6.4	6.1	5.8	5.4	<0.0001
LDL/HDL ratio[2]	2.5	2.4	2.3	2.4	0.1
Systolic blood pressure (mmHg)	141	135	134	136	<0.001
Diastolic blood pressure (mmHg)	83	80	80	81	0.001
Pulse pressure (mmHg)	57	55	54	54	0.007
Anti-hypertensive medication (%)	21	23	22	24	0.9
Coronary heart disease (%)	7	5	4	9	0.7
Carotid artery characteristics					
Lumen diameter (mm)	6.4	6.4	6.6	6.5	0.08
Distensibility (10^{-3}/kPa)[2]	25	26	26	26	0.4
Stiffness[2]	6.0	5.9	6.1	6.0	0.7
Compliance (mm^2/kPa)[2]	0.68	0.72	0.76	0.76	0.03

Means and standard deviations (SD), except where given as percentages.
[1] p (adjusted for gender) for birth weight as a continuous measure in linear or logistic regression
[2] Geometric mean
[3] Socio-economic status according to the ISEI-92 (range 16-87)

pared to unexposed people. There were no differences between famine exposed and unexposed people in terms of blood pressure or pulse pressure, LD, or any of the indicators of vessel stiffness.

Low birth weight and arterial stiffness

Table 2 shows that low birth weight was associated with increased blood pressure, elevated 120 minute plasma glucose concentrations and decreased LD (0.1 mm per kg birth weight, sex adjusted p=0.08) and decreased CC (0.03 mm^2/kPa per kg birth weight, sex adjusted p=0.03), but not with decreased DC or increased β.

Maternal size and arterial stiffness

Table 3 shows that low maternal weight at the end of pregnancy was associated with low birth weight, low adult body mass index, elevated 120 minute glucose concentrations, decreased LD (0.01 mm per kg maternal weight, sex adjusted p<0.001) and decreased CC (0.002 mm^2/kPa per kg maternal weight, sex adjusted p=0.001) in the offspring, but not with decreased DC or increased β.

Maternal weight gain in the third trimester did not affect LD (-0.01 mm per kg maternal weight gain, gender adjusted p=0.3), or any of the indicators of vessel stiffness

Table 3 Adult and carotid artery characteristics for participants in the Dutch Famine Birth Cohort Study, according to quartiles of maternal weight at the end of pregnancy

	Maternal weight (kg)				
	<61	**61-66**	**66-72**	**>72**	***p*[1]**
Number	150	149	148	149	
Men (%)	49	50	50	42	0.1
Birth weight (g)	3095	3306	3470	3572	<0.0001
Adult characteristics					
Body mass index (kg/m^2)[2]	28	28	28	30	0.006
Socio-economic status[3]	51	49	54	49	0.6
Current smoker (%)	21	25	25	19	0.9
Glucose 120 mins (mmol/l)[2]	6.2	6.3	5.7	5.5	<0.0001
LDL/HDL ratio[2]	2.5	2.4	2.3	2.3	0.2
Systolic blood pressure (mmHg)	139	137	133	137	0.1
Diastolic blood pressure (mmHg)	83	81	80	82	0.2
Pulse pressure (mmHg)	56	55	54	55	0.2
Anti-hypertensive medication (%)	20	22	24	24	0.3
Coronary heart disease (%)	4	8	9	3	0.9
Carotid artery characteristics					
Lumen diameter (mm)	6.4	6.5	6.5	6.6	0.0003
Distensibility (10^{-3}/kPa)[2]	24.1	26.4	26.0	25.4	0.4
Stiffness[2]	6.3	5.8	6.0	5.9	0.2
Compliance (mm^2/kPa)[2]	0.67	0.75	0.75	0.76	0.001

Means and standard deviations (SD), except where given as percentages.
[1] p (adjusted for gender) for maternal weight as a continuous measure in linear or logistic regression
[2] Geometric mean
[3] Socio-economic status according to the ISEI-92 (range 16-87)

Table 4 Adult and carotid artery characteristics for participants in the Dutch Famine Birth Cohort Study, according to quartiles of protein/carbohydrate ratio (P/CBR) in the maternal diet in the third trimester

	P/CBR				
	<0.15	**-0.16**	**-0.18**	**>0.18**	***p*[1]**
Number	169	171	168	165	
Men (%)	52	46	51	41	0.1
Maternal weight at the end of pregnancy (kg)	64	65	67	67	<0.001
Birth weight (g)	3230	3342	3387	3374	0.003
Adult characteristics					
Body mass index (kg/m^2)[2]	29	28	29	28	0.4
Socio-economic status[3]	50	49	51	50	0.8
Current smoker (%)	21	23	23	23	0.9
Glucose 120 mins (mmol/l)[2]	5.9	5.8	5.9	6.0	0.6
LDL/HDL ratio[2]	2.4	2.4	2.4	2.4	0.9
Systolic blood pressure (mmHg)	137	138	135	135	0.5
Diastolic blood pressure (mmHg)	81	81	82	82	0.2
Pulse pressure (mmHg)	56	57	53	54	0.03
Anti-hypertensive medication (%)	29	23	22	16	0.01
Coronary heart disease (%)	4	9	7	6	0.5
Carotid artery characteristics					
Lumen diameter (mm)	6.6	6.5	6.5	6.3	0.001
Distensibility (10^{-3}/kPa)[2]	25.1	25.0	25.7	26.4	0.2
Stiffness[2]	6.1	6.1	5.9	5.8	0.07
Compliance (mm^2/kPa)[2]	0.74	0.71	0.75	0.72	0.6

Means and standard deviations (SD), except where given as percentages.
[1] *p* (adjusted for gender) for P/CBR as a continuous measure in linear or logistic regression
[2] Geometric mean
[3] Socio-economic status according to the ISEI-92 (range 16-87)

(DC <0.001 10^{-3}/kPa gender adjusted *p*=0.8; β <0.001 gender adjusted *p*=0.9 and CC -0.002 mm^2/kPa gender adjusted *p*=0.3; all per kg maternal weight gain).

A smaller external conjugate diameter (ECD) of the mother's pelvis was associated with increased systolic blood pressure in the adult offspring (1.0 mmHg per cm maternal ECD, gender adjusted *p*=0.05) and decreased LD (-0.04 mm per cm maternal ECD, gender adjusted *p*=0.06), CC (-0.01 mm^2/kPa per cm maternal ECD, gender adjusted *p*=0.02) and birth weight (-38 g per cm maternal ECD, gender adjusted *p*=0.003).

Maternal dietary composition and arterial stiffness

Table 4 shows that a low protein/carbohydrate ratio in the maternal diet was associated with low maternal weight at the end of pregnancy, low birth weight, high pulse pressure, more use of antihypertensive medication and increased LD in the offspring, but not with changes in any of the measures of vessel stiffness.

Multivariable model, lumen diameter and arterial compliance

We investigated the associations between carotid LD and CC with cardiovascular risk factors, famine exposure during different stages of gestation, maternal protein/carbo-

hydrate intake in the third trimester, maternal weight and birth weight in univariate and multivariable regression (Table 5). Low maternal protein/carbohydrate intake in the third trimester and low maternal weight at the end of pregnancy were both associated with a decrease in LD, which was independent of adult body size, blood pressure and cardiovascular risk markers. The children of mothers who had been light at the end of pregnancy also had decreased CC. Adjusting for cardiovascular risk markers did not diminish the association of low maternal weight with decreased CC in a multivariable regression model. However, when we additionally adjusted for LD, the effect of maternal weight on CC was greatly attenuated.

Low birth weight was associated with a small decrease in LD, which was independent of adult body size, blood pressure and cardiovascular risk markers (-0.2 mm per kg birth weight decrease, p=0.008, adjusted for covariates in Table 5, except maternal weight and diet). People who had been light at birth also had decreased CC (-0.03 mm^2/kPa per kg birth weight decrease, p adjusted for sex=0.03). Adjusting for cardiovascular risk markers and maternal protein/carbohydrate intake in the third trimester did not diminish the association of low birth weight with decreased CC in a multivariable regression model (-0.03 mm^2/kPa per kg birth weight decrease, p=0.05). However, when we additionally adjusted for maternal weight at the end of pregnancy, the effect of birth weight on LD (-

Table 5 Linear regression coefficients (RC) and p-values for carotid lumen diameter (LD, mm) and compliance coefficient (CC, mm^2/kPa) per Z-score of cardiovascular risk factors, birth weight, maternal weight at the end of gestation, and famine exposure.

	LD (univariate*)		LD (multivariable)¶		CC ∫ (univariate*)		CC ∫ (multivariable)¶	
	RC	*p*	RC	*p*	RC	*p*	RC	*p*
Male sex	0.63	<0.001	0.59	<0.001	0.027	0.02	0.037	0.002
BMI	0.18	<0.001	0.16	<0.001	-0.013	0.03	<0.001	0.97
Socio-economic status (ISEI-92)	-0.06	0.04	-0.02	0.4	-0.008	0.2	-	-
Current smoking	0.18	0.006	0.24	<0.001	0.058	<0.001	0.040	0.004
Glucose 120 minutes	0.03	0.4	-	-	-0.015	0.02	-0.004	0.5
LDL/HDL ratio	0.04	0.1	-	-	-0.006	0.3	-	-
Systolic BP	0.17	<0.001	0.06	0.3	-0.040	<0.001	-0.056	<0.001
Diastolic BP	0.10	0.001	-	-	-0.042	<0.001	-	-
Pulse pressure	0.18	<0.001	0.12	0.02	-0.025	<0.001	0.027	0.02
Anti-hypertensive medication	0.22	<0.001	0.04	0.6	0.002	0.9	-	-
Famine exposure late∞	0.09	0.2	-	-	0.002	0.9	-	-
Famine exposure mid ∞	-0.03	0.7	-	-	0.003	0.9	-	-
Famine exposure early∞	-0.19	0.07	-	-	-0.024	0.3	-	-
Maternal protein/carbohydrate intake 3rd trimester	-0.09	0.001	-0.09	0.001	-0.003	0.6	-	-
Maternal weight end of pregnancy	0.10	<0.001	0.09	<0.001	0.018	0.001	0.016#	0.007#
Birth weight	0.05	0.08	0.04‡	0.2	0.013	0.03	0.006‡	0.3

*Adjusted for sex ,¶ multivariable models contain only the covariates in this column, ∫ log transformed, ∞ RC and p compared to unexposed (born before and conceived after the famine) ‡ Birth weight adjusted for maternal weight (birth weight and maternal weight have a collinear relation to carotid LD and CC) # RC=0.006 p=0.3 after additional correction for LD

0.04 mm per kg birth weight decrease, multivariable adjusted p=0.2) and CC was greatly attenuated (-0.006 mm^2/kPa per kg birth weight decrease, p=0.03) (see Table 5).

Low birth weight and blood pressure

The association between low birth weight and increased blood pressure (+5 mmHg per kg birth weight decrease, p<0.001) was not attenuated by adjusting for low CC (+5 mmHg per kg birth weight decrease, p=0.001), maternal protein/carbohydrate intake in the third trimester (+5 mmHg per kg birth weight decrease, p<0.001) or maternal weight at the end of gestation (+6 mmHg per kg birth weight decrease, p<0.001).

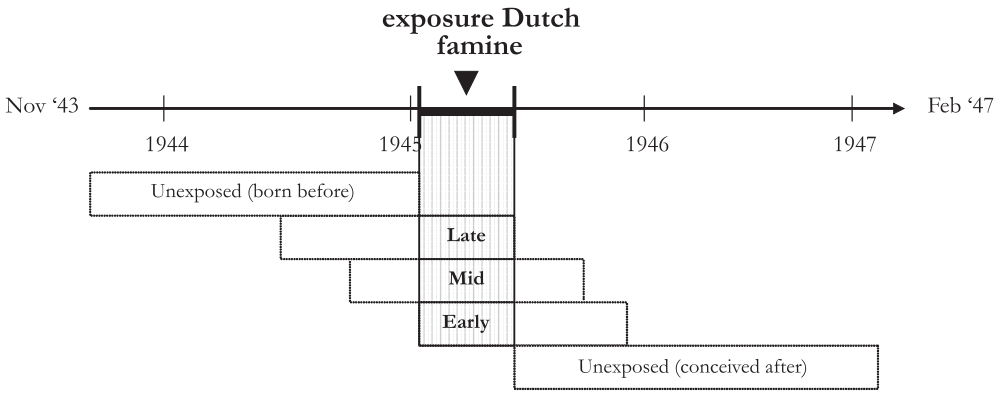

Figure 1 The Dutch famine birth cohort: famine exposure and gestation in relation to the timing of the Dutch famine.

Discussion

In this study, we investigated whether maternal undernutrition contributes to decreased arterial compliance. Maternal famine exposure during gestation did not affect CC, DC or β. We found that low maternal weight and low birth weight were associated with small decreases in LD and CC, but that they did not affect DC or β. When we adjusted for low maternal weight, the association between low birth weight and low LD and CC was greatly attenuated. Our findings thus suggest that mothers who were light, irrespective of their diets during gestation, had lighter offspring with smaller LD and lower CC, in the absence of changes to vessel wall characteristics.

Our findings correspond with those from an Indian study, which found that arterial compliance was reduced among babies of mothers who were lighter or had smaller pelvic measurements [18]. The authors hypothesize that reduced maternal skeletal growth in childhood may underlie this finding [47]. Unfortunately, we are uninformed about maternal pre-pregnancy weight and stature. We are thus unable to investigate how these factors, as well as genetic factors or poor maternal childhood growth may contribute to the association of low maternal weight and reduced CC.

The fact that we did not have access to maternal weight at the end of pregnancy in a considerable number of cases may present a limitation of the study. We imputed the mean of the available weights in cases where this information was missing, as well as adding an indicator of missingness to regression models. This method may potentially have introduced an element of bias. However, regression models which employed values generated by multiple imputation (results not shown) yielded similar results. It therefore seems unlikely that the fact that maternal weights were missing in 11% of our participants has biased our findings.

The link between low birth weight, low maternal weight and decreased CC seems to be the result of small LD. Small vessel diameter independent of adult body dimensions has also been described in other studies among children and adolescents who were growth retarded as babies [19, 48], and indeed drove the association between low birth weight and decreased CC in a study among 36-year olds [25]. This suggests that, although LD may dynamically respond to changes in blood pressure, the reduction in LD among individuals who were small at birth persists from a young age. Moreover, these studies reported smaller LD in the carotid, popliteal, brachial and coronary arteries as well as in the aorta. The fact that small LD is present in many different arterial segments, in particular the coronary circulation, may have repercussions for coronary artery disease susceptibility. To our knowledge, our study is the first to report the fact that maternal weight and maternal diet at the end of pregnancy contribute to small LD.

Although we found that, in the carotid artery, low maternal weight at the end of pregnancy and low birth weight were associated with decreased CC, the effect was not attributable to increased DC and β. This suggests that the elastic properties of the vessel wall of this conduit artery are not affected by poor intrauterine growth or maternal dietary factors. However, we cannot exclude that vessel wall stiffness of the aorta [49], or that of other conduit arteries may be affected by maternal famine exposure or other aspects of maternal diet. During fetal development in a poor intrauterine environment, the carotid artery may be at an advantage in comparison to other vessels because it supplies the brain, which is ultimately the last organ to suffer decreased blood flow [50, 51]. This notion is supported by evidence from a study which found that, among individuals who had been small at birth, femoral arteries were stiffer than among normal birth weight peers, while carotid arterial stiffness was not affected by birth weight [25].

In concordance with a large number of studies, we found that adults who had been small at birth had higher blood pressure [7]. We found this association to be independent of the fact that people with low birth weight had less compliant carotid vessels. This finding does not support the hypothesis that structural arterial wall alterations contribute to the blood pressure increase among low birth weight individuals [10]. Although it is statistically significant, the 4% decrease in compliance per kilogram reduction in birth weight is too small to produce a clinically relevant increase in blood pressure. Other mechanisms, including low nephron endowment [52, 53] and alterations in car-

diovascular control [54, 55], may play a more important role in the pathophysiology of hypertension after prenatal growth restriction.

Our findings suggest that small maternal size, not poor maternal diet, in late gestation programs decreased arterial compliance in the adult offspring by affecting vessel size rather than vessel wall stiffness. In contrast, and in keeping with previous reports, poor fetal growth programmes hypertension.

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Two children eating a soup kitchen meal during the Dutch Famine (Photo NIOD)

chapter

6

Reduced intima media thickness in adults after prenatal exposure to the Dutch Famine

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Abstract

Background Restricted prenatal growth is associated with an increased risk of coronary heart disease morbidity and mortality. We studied the effects of exposure to famine during gestation on intima media thickness (IMT) in later life.

Methods and Results We studied 730 people aged 58 years who were born as term singletons around the time of the 1944-'45 Dutch famine. Persons exposed to famine during gestation (n=293) had reduced carotid artery IMT compared to people who had not been exposed to famine in utero (n=437) (mean 0.71 mm, SD 0.16 mm compared to 0.75 mm, SD 0.15 mm, sex adjusted p=0.001). Femoral artery IMT was also thinner among people exposed to famine during gestation compared to people unexposed in utero (mean 0.64 mm, SD 0.20 mm, compared to 0.68 mm, SD 0.24), although the difference did not achieve statistical significance.

Conclusion Exposure to famine in utero may reduce IMT. However, it does not reduce the risk of coronary heart disease among famine exposed people.

Introduction

Low birth weight is associated with increased cardiovascular disease morbidity and mortality later in life [1,2]. Although biological risk factors for CHD, including raised lipids [3], hypertension [4] and type II diabetes [5,6] are also linked to low birth weight, the mechanisms underlying the association between restricted intrauterine growth and subsequent cardiovascular disease remain poorly understood [7]. A number of studies have suggested that the increase in CHD risk among low birth weight individuals may be mediated by an increased propensity for atheroma development. The aortic wall is thicker at birth among small babies [8]. The prevalence and severity of carotid stenosis is higher among 70-year old men and women who were small at birth [9]. However, the associations between low birth weight and carotid intima media thickness (IMT) - a validated marker of present atherosclerosis and future atheroslerotic disease risk [10] - have been inconsistent [11-15]. Birth weight is a marker of fetal nutrition, but, in humans and animals, restricted maternal nutrition during gestation has been shown to have permanent effects on adult health, without affecting birth size [16-18].

The Dutch famine offers a unique opportunity to directly study the effects of maternal undernutrition during gestation on the offspring's adult health. The 5-month famine in the winter of 1944-'45 started after the German occupation placed an embargo on food transports to the west of the Netherlands, and ended abruptly upon liberation, in early May 1945. The cities, including Amsterdam, were worst affected by the famine. Official rations were cut to 400-800 calories a day: less than a quarter of the pre-war rations. The relatively short duration of the famine enables us to study the effects of famine exposure during specific periods of gestation on adult health.

Previously we have reported impaired glucose tolerance among people exposed to famine in mid and late gestation [19] and more microalbuminuria [20] after exposure in mid gestation. After exposure in early gestation we found an excess in dyslipidemia [18], obesity [21], fibrinogen [22] and a tripling of CHD prevalence at age 50 [23]. The effects were independent of size at birth as well as adult risk factors. Little is known about the mechanisms responsible for the effects of prenatal exposure to famine on subsequent adult disease.

In the current study, we investigate the hypothesis that prenatal exposure to famine leads to an increase in IMT.

Subjects and Methods

Selection procedure

The Dutch Famine Birth Cohort consists of 2414 live-born, term singletons, born in the Wilhelmina Gasthuis in Amsterdam, the Netherlands. All babies were born between November 1st 1943 and February 28th 1947. The selection procedure has been described in detail elsewhere [19]. Subjects were eligible for participation if they were cohort members, lived in the Netherlands on September 1st 2002 and their address was known to the investigators. 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.

Exposure to famine

We defined famine exposure according to the daily official food-rations for adults. In addition to the official rations, food from other sources, such as church organizations, central kitchens, the 'black market', was also available. People may have had access to up to double to rationed amount at the peak of the famine. The rations do however adequately reflect the fluctuation of food availability during the famine [24].

A person was considered prenatally exposed to famine if the average daily ration for adults during any 13–week period of gestation was less than 1000 calories. Therefore, people born between January 7th 1945 and December 8th 1945 were exposed prenatally. People born between November 1st 1943 and January 6th 1945 (born before the famine) and between December 9th 1945 and February 28th 1947 (conceived after the famine) were thus unexposed to famine in utero and acted as the control group.

We defined periods of 16 weeks each to differentiate between those who were exposed in late gestation (born between January 7th and April 28th 1945), mid gestation (born between April 29th and August 18th 1945) and in early gestation (born between August 19th and December 8th 1945), in correspondence with previous publications on this cohort [18-23; 25-28].

Data collection

The medical birth records provided information about the mother, the course of the pregnancy and the size of the baby at birth [19]. All participants gave written informed consent. The local Medical Ethics Committee approved the study.

The participants attended the clinic for the data collection protocol, which encompassed a full day. Trained nurses carried out all measurements and interviews. We measured waist circumference with a flexible tape measure, height with a fixed or a por-

table stadiometer and weight with Seca scales or Tefal portable scales. 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. Pulse pressure was calculated by subtracting mean diastolic from mean systolic blood pressure. Participants underwent a standard oral glucose tolerance test (OGTT). Known diabetics were excluded from OGTT. Known diabetes was defined as the use of anti-diabetic medications (oral or injected). Blood was drawn for analysis of low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL).

Participants were asked about their smoking history. We calculated the number of pack years by dividing the total number of cigarettes smoked per day by twenty (a pack of cigarettes was assumed to contain 20 cigarettes) and multiplying this number by the total number of years that person was a smoker. We defined current socio-economic status (SES) according to the participant's occupation or their partner's; whichever was highest, using the ISEI-92. Values ranged from 16 (low status) to 87 [29]. We asked participants about their current medication, and coded all medications that belong to the class of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors.

Standard 12-lead electrocardiograms (ECG) were made of participants. Trained technicians blinded to clinical data scored the ECG-s according to the Minnesota criteria.

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).

IMT measurements

We performed B-mode ultrasound examinations of the arterial walls of the common, bulb and internal carotid artery segments, and the common and superficial femoral artery segments. Two trained investigators (RCP and SdR) carried out the majority (92%) of the ultrasound examinations. Four other experienced sonographers carried out the remainder. The ultrasounds were performed using an Acuson 128XP/10v (Acuson Corp, Mountain view, Calif) ultrasound instrument equipped with a 5-10 MHz L7 (Acuson L7) and Extended Frequency software, version 7.02 (Acuson Corp). The left and right far-walls of the carotid and femoral artery segments were imaged in standardized magnification (Regional Expansion Selection 2 x 2 cm). Vessel lumen diameter was measured in the distal common carotid artery only. In the 1/3 B-mode / 2/3M-mode duplex setting, wall motion was recorded for at least two consecutive heartbeats and then frozen. The sonographer saved stills of the B-mode and M-mode images as 4:1 compressed JPEG files (Sony DKR-700P video still image recorder). After the completion of the data collection, two image analysts batch-read all ultrasound images. They were blinded to the identity of the subject. In-house designed software for image analy-

sis (eTrack, version 2.3, W.J. Stok, Dept of Physiology, AMC, University of Amsterdam) was used as previously published [30].

Mean carotid IMT was defined as the mean IMT in mm of the right and left common artery, carotid bulb, and the internal carotid far wall segments. If either the right or left value was missing for any given carotid segment, the remaining available segment was used to calculate the mean carotid IMT. If both right- and left-sided values were missing for any given carotid segment, the mean carotid was coded missing. Mean femoral artery IMT was defined as the mean of the right and left common femoral artery and the right and left superficial femoral artery. The same procedure applied for missing measurements of the femoral artery.

The arterial lumen was defined as the distance between the contours of the near wall intima-lumen and the far wall lumen-intima interfaces as traced by automated contour detection software (eTrack, version 2.3, W.J. Stok, Dept of Physiology, AMC, University of Amsterdam). The mean distal common carotid lumen diameter was calculated by averaging the distance between the near and far wall interfaces for at least two heartbeats. The mean of left and right carotid diameter measurements was calculated. If either of these was missing, the carotid diameter measurement of the remaining side was used.

Statistical Methods

We used linear or logistic regression analyses to compare the characteristics of individuals exposed or not exposed to famine in gestation. Linear regression was also applied to explore the association between IMT (carotid and femoral) and common carotid vessel diameter and the characteristics of the participants. In all analyses we corrected for gender. We additionally corrected for other potential confounders. When correcting for waist circumference and common carotid diameter in a multivariable model, we used the common carotid diameter adjusted for waist circumference because of the multi-collinearity of their association with carotid IMT. For 87 participants maternal weight at the end of pregnancy was not available. We imputed the mean weight (66 kg) for missing cases and added an indicator of missingness when we used this variable in regression models. Variables with skewed distributions, such as fasting glucose and SES, were log transformed before the analyses and are reported as geometric means. We used SPSS 12.0 for all data analyses.

Results

Participants

At age 58 (range 56-61 years) all 1423 eligible cohort members were invited to participate. Seven hundred and forty people (52%) participated in the study. The mean birth weights of the 740 participants (3363 g) did not differ significantly from the mean birth

weights of eligible people who did not participate (3343 g, p=0.4). IMT measurements were made in 730 people. In 10 participants IMT measurements failed due to technical or logistical problems. Common carotid diameter measurements were available for 673 people; in 57 people the M-mode still was of insufficient quality.

Famine exposure and IMT

Table 1 shows the characteristics of the participants who were exposed or not exposed to famine. People born before and conceived after the famine had a similar mean IMT for carotid and femoral (0.76 mm compared to 0.74 mm, p=0.3). The mean carotid IMT was reduced in people that had been exposed to famine in utero compared to unexposed people (0.71 mm compared to 0.75 mm, p=0.001). Carotid IMT was reduced to a similar extent regardless of whether exposure to famine had occurred in late (0.71 mm, sex adjusted p=0.03 compared to unexposed), mid (0.70 mm, sex adjusted p=0.01 compared to unexposed) or early gestation (0.71 mm, sex adjusted p=0.07 compared to unexposed). Similarly, mean femoral IMT was lower among those exposed to famine (0.64 mm compared to 0.68 mm), though the difference was not statistically significant.

Table 1 Maternal, birth and adult characteristics for participants in the Dutch Famine Birth Cohort Study

	Exposed to famine in gestation		
	yes[a]	no[a]	p[c]
Number	293	437	
Male (%)	44	49	0.13
Maternal & Birth characteristics			
Maternal weight at the end of pregnancy (kg)	64 (8)	66 (9)	<0.0001
Birth weight (g)	3259 (433)	3436 (469)	<0.0001
Head circumference (cm)	32 (1)	33 (2)	<0.0001
Adult characteristics			
Mean carotid IMT (mm)	0.71 (0.16)	0.75 (0.15)	0.001
Common carotid IMT (mm)	0.69 (0.14)	0.71 (0.16)	0.06
Mean femoral IMT (mm)	0.64 (0.20)	0.68 (0.24)	0.07
Common femoral IMT (mm)	0.76 (0.35)	0.83 (0.45)	0.03
Mean common carotid diameter (mm)	6.44 (0.77)	6.49 (0.78)	0.9
Coronary heart disease prevalence (%)	6	7	0.7
HDL cholesterol (mmol/l)[d]	1.4 (0.4)	1.5 (0.4)	0.02
HDL:LDL cholesterol [d,b]	2.5 (1.0)	2.3 (0.9)	0.05
HMG-CoA reductase inhibitor use (%)	21	18	0.4
Systolic blood pressure (mmHg)	136 (18)	137 (18)	0.8
Diastolic blood pressure (mmHg)	81 (10)	81 (10)	0.3
Pulse pressure (mmHg)	54 (11)	54 (12)	0.7
Fasting glucose (mmol/l)[b]	5.6 (0.8)	5.6 (0.8)	0.9
Waist circumference (cm)	96 (13)	98 (13)	0.2
Pack years	16 (23)	16 (21)	0.9

[a] mean (SD), except where given as percentages
[b] high density lipoprotein: low density lipoprotein ratio
[c] p for difference between exposed and unexposed, sex adjusted (logistic or linear regression)
[d] geometric mean

The IMT of the common carotid and common femoral arterial segments showed a similar pattern in relation to famine exposure in utero.

Common carotid vessel diameter was lowest among people exposed to famine in early gestation (6.25 mm), although the difference in comparison to people who were unexposed to famine (6.49 mm) did not achieve statistical significance (sex adjusted p=0.07). The common carotid vessel diameter among those exposed in mid gestation (6.40 mm) and those exposed in late gestation (6.56 mm) was similar to that of unexposed individuals.

HDL-cholesterol concentrations were lower, and HDL:LDL cholesterol was raised among people who had been exposed to famine in utero, in comparison to unexposed people.

The exposed and unexposed groups were similar in terms of CHD prevalence, blood pressure, fasting plasma glucose concentrations and number of pack years.

Famine exposure, cardiovascular risk factors and IMT

Table 2 describes the association between cardiovascular risk factors and carotid and femoral IMT. Table 2 also shows that correcting for the cardiovascular risk factors in a multivariable model did not attenuate the association between famine exposure and reduced carotid IMT (adjusted reduction –0.04 mm, p=0.002).

Table 2 Linear regression coefficients (β) for carotid and femoral intima media thickness (IMT) and cardiovascular risk factors and famine exposure

	Carotid IMT (mm) (univariate[a])		Carotid IMT (mm) (multivariate)		Femoral IMT (mm) (univariate[a])	
	β	*p*	β	*p*	β	*p*
Male sex	0.06	<0.0001	0.02	0.09	0.12	<0.0001
Waist circumference (cm)	0.001	0.05	0.001	0.21	<0.001	0.59
Mean common carotid diameter (mm)	0.05	<0.0001	0.03[d]	<0.0001[d]	0.04	0.001
HDL cholesterol (mmol/l)[c]	0.006	0.90	-	-	0.04	0.54
HDL:LDL cholesterol [b,c]	0.05	0.15	-	-	0.02	0.66
HMG-CoA reductase inhibitor use	0.01	0.54	-	-	0.02	0.40
Systolic blood pressure (mmHg)	0.002	<0.0001	-	-	0.001	0.008
Diastolic blood pressure (mmHg)	0.002	0.004	-	-	0.001	0.53
Pulse pressure (mmHg)	0.003	<0.0001	0.002	0.003	0.002	<0.0001
Fasting glucose (mmol/l)[c]	0.24	0.02	0.16	0.17	0.22	0.14
Socio-economic status (ISEI-92)[c]	-0.07	0.07	-	-	-0.09	0.15
Pack years	0.001	<0.0001	0.001	0.04	0.002	<0.0001
Famine exposure	-0.04	0.001	-0.04	0.002	-0.03	0.07

[a] adjusted for sex

[b] high density lipoprotein: low density lipoprotein ratio

[c] log transformed

[d] common carotid diameter adjusted for waist circumference (waist circumference and common carotid diameter have a collinear relation to carotid IMT)

Famine exposure, coronary heart disease and IMT

Every 0.1 mm increase in carotid IMT was associated with a 1.3-fold increase of CHD prevalence (95% CI 1.1 to 1.5, sex adjusted p=0.008). We examined the effects of increased IMT on CHD prevalence among people exposed or unexposed to famine in gestation. Among people who were exposed to famine, every 0.1 mm rise in carotid IMT was associated with a larger increase in CHD prevalence (odds ratio 1.5, 95% confidence interval 1.2 to 2.0, p=0.002, adjusted for sex), than among people who were not exposed to famine (odds ratio 1.1, 95% confidence interval 0.9 to 1.4, p=0.50, adjusted for sex). The interaction between the effect of carotid IMT and famine exposure (p=0.07) did however not achieve statistical significance.

Maternal weight, fetal body size and IMT

We examined the effects of maternal and fetal body size on carotid IMT. Maternal weight at the end of pregnancy was positively associated with carotid IMT (p=0.03). Correcting for maternal weight did not attenuate the association between famine exposure and reduced carotid IMT (adjusted reduction –0.03 mm, p=0.004).

The effects of size at birth on adult IMT depended on sex (p for interaction =0.04). Women that were larger at birth had greater IMT-s. There was a 0.05 mm increase in femoral IMT per kg increase in birth weight (p<0.01) and 0.01 mm increase in carotid IMT for every cm increase in head circumference at birth (p=0.04). There were no significant associations between size at birth and IMT in men. Correcting for measures of size at birth did not attenuate the association between famine exposure and reduced carotid IMT (birth weight adjusted reduction –0.04 mm, p=0.001).

Discussion

We found that exposure to famine in utero was associated with thinner IMT of the carotid artery. Based on the previous publications which reported an increased prevalence of CHD [23] and its biological risk factors among people that were exposed to famine in utero [18,19,21], we had postulated that exposure to famine would lead to increased atherosclerosis, as expressed by IMT. Our findings do not support this hypothesis, and in fact indicate that prenatal famine exposure is associated with IMT reduction, although it does paradoxically not protect against CHD.

Two recent studies have investigated the effects of maternal nutrition during gestation and intima media thickness in the offspring [15,31]. Skilton et al found that the offspring of rats that had been fed a low protein diet throughout gestation had reduced aortic media thickness, which is in line with our findings [31]. In contrast, another study reported that low maternal energy intake during gestation was associated with an increased intima media thickness in the 9-year old offspring [15]. The fact that

these findings are at odds with each other may be due to the fact that the latter was an observational study. Although the authors attempted to account for many factors, the apparent effects of maternal diet may in fact be due to confounders, including postnatal food choices. Alternatively, the discrepancy may be due to the fact that the nutritional insult in the experimental study as well as that imposed by the Dutch famine was much more severe than the observed variation in caloric intake in the study by Gale et al.

Previously, we reported a three-fold increase in CHD prevalence [23] and a younger age at onset among people exposed in early gestation [32], which would appear to be at odds with the fact that we found a reduction in IMT in this group of people. The small number of CHD cases (n=5 people exposed in early gestation) in the current study, however, precludes us from exploring the reasons for this apparent contradiction.

Despite the wealth of literature showing associations between carotid IMT and cardiovascular disease [10,33], carotid IMT is not synonymous to atherosclerosis. The IMT increase with increasing age or CHD risk is due to smooth muscle proliferation and cellular infiltration [34]. Maternal diet may affect smooth muscle cell number [35], possibly leading to reduced IMT. Alternatively, carotid IMT may not adequately reflect the degree of atherosclerosis present in the coronary arteries. Adverse conditions in utero may have an adverse effect on coronary artery development rather than on the carotid arteries [36].

While many studies have described an increase in CHD among people that were small at birth [1,2], the inverse association between IMT and birth weight has been inconsistent [11-15]. Smallness at birth is associated with smaller arterial diameter [37; 38]. Smaller vessel diameter is associated with reduced IMT. In our study, however, reduced vessel diameter was not responsible for lower IMT among people exposed to famine in utero. The link between low birth weight and later CHD may be mediated by processes other than atherosclerosis. A number of alternative mechanisms, including hormonal axis programming [39,40], enhanced sympathetic tone [41,42], altered myocardial structure [43,44], altered arterial structure [45; 45] accelerated aging [46,47] and endothelial dysfunction[48,49] have been proposed.

There was significant infant mortality within the cohort [26]. The largest contrasts in terms of infant mortality were between the people born before the famine and those conceived after the famine. These two groups had similar IMT measurements. We therefore conclude that selective infant survival is not likely to have influenced our findings. On the other hand, selective adult survival may be an issue in the interpretation of our findings. We found more type II diabetes [19], CHD [23] and respiratory disease [25] among people exposed to famine in a previous study at age 50. These factors may have led to excess mortality and disability among those exposed to famine in utero, resulting in selective participation of only the healthier individuals among those subjects exposed to prenatal famine. A recent follow-up study of adult survival, however,

found no evidence for excess mortality among subjects exposed to famine in utero [28]. Nonetheless, selective participation may have had an influence on our findings.

Maternal hypercholesterolemia is associated with increased atherosclerosis in both humans and animals [50; 51]. Our findings may reflect the cholesterol content of the maternal diet during the Dutch famine. We were unable to investigate the effects of specific components of maternal diet on the offspring's IMT measurements.

In summary, exposure to famine in utero may reduce carotid IMT. However, it does not reduce the risk of coronary heart disease among famine exposed people.

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Mother with baby and child battling the hardships of the Dutch Famine (Photo NIOD)

chapter

7

Microalbuminuria in adults after prenatal exposure to the Dutch Famine

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Abstract

Maternal undernutrition during gestation is associated with an increase in cardiovascular risk factors in the offspring in adult life. The effect of famine exposure during different stages of gestation on adult microalbuminuria (MA) was studied. MA was measured in 724 people, aged 48 to 53, who were born as term singletons in a university hospital in Amsterdam, the Netherlands, around the time of the Dutch famine 1944 to 1945. Twelve percent of people who were exposed to famine in mid gestation had MA (defined as albumin/creatinine ratio ≥ 2.5) compared with 7% of those who were not prenatally exposed to famine (odds ratio 2.1; 95% confidence interval 1.0 to 4.3). Correcting for BP, diabetes, and other influences that affect MA did not attenuate this association (adjusted odds ratio 3.2; 95% confidence interval 1.4 to 7.7). The effect of famine was independent of size at birth. Mid gestation is a period of rapid increase in nephron number, which is critical in determining nephron endowment at birth. Fetal undernutrition may lead to lower nephron endowment with consequent MA in adult life.

Introduction

Individuals with low birth weight are at increased risk for developing type 2 diabetes [1,2], hypertension [3], and cardiovascular disease [4], as well as microalbuminuria (MA) [5-8] and end-stage renal failure [9] as adults. These findings led to the fetal origins hypothesis [10], which states that an adverse intrauterine environment, in particular undernutrition, increases the risk for chronic disease in later life. Fetal organs are especially vulnerable to the effects of a reduced supply of nutrients when they are differentiating—so-called critical periods. Suboptimal development during these critical periods leads to permanent changes in organ structure or function and may have detrimental effects on health in later life [11,12].

A reduced number of glomeruli is considered a predisposing factor for the development of glomerular damage. Increased filtration through each glomerulus leads to hypertrophy and hyperfiltration injury, which is marked by the onset of MA, and may eventually lead to a reduction in renal function [13,14].

In animal models, mothers that are undernourished during gestation produce offspring that have fewer glomeruli [15] and develop higher BP in later life [16]. In humans, small infants have reduced nephron numbers at birth [17,18]. Brenner and colleagues [19-22] postulated that the reduced number of nephrons of people who had low birth weight is one of the mechanisms underlying the link between low birth weight and later hypertension.

In the human fetus, glomerular number increases slowly between the 10th week and the 18th week of gestation and rapidly between the 18th week and 32nd week, after which time no more glomeruli are formed [23,24]. The period of rapid increase in glomerulus number is crucial in determining the eventual total glomerular endowment. Maternal undernutrition during mid gestation therefore could reduce the number of glomeruli and increase the individual's risk for developing MA and possibly impaired renal function.

During the winter of 1944 to 1945, the western part of the Netherlands was struck by a period of severe food scarcity. The previously and subsequently well-nourished Dutch population's daily rations dropped acutely to as little as 400 to 800 calories during the 5 to 6 mo of famine [25]. The Dutch Famine Birth Cohort study offers a unique opportunity to study the long-term effects of maternal starvation during specific stages of pregnancy on health in later life. This study showed that undernutrition during early gestation was linked to a more atherogenic lipid profile [26] and an increased risk for coronary heart disease [27], whereas undernutrition in late gestation was linked to glucose intolerance in adulthood [25]. This article describes the long-term effects on MA and renal function.

Materials and Methods

All participants were part of a cohort of 2414 liveborn term singletons, who were born at the Wilhelmina Gasthuis in Amsterdam between November 1, 1943, and February 28, 1947. The selection procedure for this clinical study has been described in detail elsewhere [25]. The local Medical Ethics Committee approved the study, and all participants gave informed consent. The study was carried out in accordance with the declaration of Helsinki. A urine sample was collected from 724 of the 741 people who visited the clinic. Their mean age at clinic visit was 50 yr (range 48 to 53 yr). Their mean birth weight (3352 g) did not differ significantly from the 1690 who were not included (mean 3343 g).

We defined the famine period as the time that the official adult food rations were under 1000 calories per day: November 26, 1944, to May 12, 1945. Although supplementation of the official food ration by church organizations, the black market, and central kitchens was possible and indeed total food intake may have been up to twice as high as the official rations indicate, the official rations do adequately reflect the variation over time of total food availability throughout the famine period [28].

We considered fetuses to have been exposed to famine when the average daily rations for adults during any 13-wk period of gestation were <1000 calories. Therefore, infants who were born between January 7, 1945, and December 8, 1945, were exposed to famine in utero. Three 16-wk periods were used to differentiate between people who were exposed in late gestation (born between January 7 and April 28, 1945), in mid gestation (born between April 29 and August 18, 1945), and in early gestation (born between August 19 and December 8, 1945). Children who were younger than 1 yr were relatively protected during the famine, because their official daily rations never fell below 1000 calories, and the specific nutritional components were always above the standards used by the Oxford Nutritional Survey [29]. As in all previous publications from this study [25–27,30–33], the nonexposed group comprises those who were born before the famine and those who were conceived after the famine. The medical birth records provided information about the mother, the course of the pregnancy, and the size of the infant at birth (birth weight, length, ponderal index, and head circumference).

An overnight urine sample was collected from 10 p.m. the evening before the clinic visit. Plasma and urinary creatinine were measured. Urinary MA was assessed using immunonephelometry. An albumin/creatinine ratio (ACR) was calculated by dividing urinary microalbumin (mg/L) by urinary creatinine (mmol/L). MA was defined as an ACR ≥2.5 g/mol, according to the hospital laboratory's guidelines. Creatinine clearance was estimated using the Cockcroft-Gault formula [34]:

$$\text{creatinine clearance (ml/min)} = \frac{(140 - \text{age}) \times (\text{weight, kg})}{\text{plasma creatinine } (\mu\text{mol/L})} \times 1.23 \text{ (men) or } 1.05 \text{ (women)}$$

The validity of the Cockcroft-Gault formula in the general population has been confirmed in a number of studies [34–36].

During the clinic visit, a standard oral glucose tolerance test [25] was performed, an electrocardiogram (ECG) was made, weight and height were measured, BP was measured using an automated auscultatory device (Profilomat; Disentronic Medical Systems AG, Burgdorf, Switzerland) [37], and serum cholesterol was measured. A cardiologist, blinded to clinical data, examined the ECG and marked them either (borderline) normal or abnormal. Diabetes or impaired glucose tolerance (IGT) was defined as either self-reported diabetes (type 1 and type 2) or a 2-h blood glucose >7.8 mmol/L [25].

Health, medication use, lifestyle, and socioeconomic data had been recorded during a home interview. People who were receiving hypertension treatment were not excluded from analyses. Socioeconomic status (SES) was determined from the participant's or the partner's occupation, whichever was highest, according to the socioeconomic index (ISEI-92) scale ranging from 16 for the lowest to 87 for the highest status.

Body mass index (BMI) and 2-h glucose concentrations were log transformed because they had skewed distributions; therefore, results are reported as geometric means and SD. We were not able to transform the skewed ACR distribution, so ACR results are reported using the dichotomized MA definition only.

Statistical Analyses

We calculated odds ratios (OR) with 95% confidence intervals (CI) using logistic regression to compare the prevalence of MA in people who were exposed in early, mid, or late gestation to that in nonexposed people, adjusting for gender and famine exposure at other stages of gestation. We considered differences to be statistically significant at $P<0.05$. SPSS 11.0 was used for all statistical analyses.

Results

A total of 288 (40%) of the 724 participants studied had been exposed to famine in utero (Table 1). Weight at the last antenatal clinic visit was lower in mothers of participants who were exposed to famine during late and mid gestation than in mothers of nonexposed individuals. Infants who were exposed to famine during mid or late gestation were lighter and shorter and had smaller placentas than infants who were not exposed to famine in utero. Adult BMI was higher among those who were exposed early in gestation ($p=0.04$).

In total, 52 (7.2%) people had MA (Table 2). People with MA had a higher BMI, had a lower SES, more often were smokers, were older, had higher diastolic and systolic BP, and were more likely to have IGT or diabetes and ECG abnormalities than those without MA. The prevalence of MA did not differ between men (7.3%) and women (7.1%;

Table 1. Maternal characteristics, birth outcomes, adult characteristics according to timing of prenatal exposure to famine.

	Exposure to famine						
	born before	**late**	**mid**	**early**	**conceived after**	**all (SD)**	**N**
General							
number	207	119	104	65	229	724	
proportion female (%)	50	53	58	54	47	51	724
Birth outcome							
birth weight (gram)	3386	3167*	3226*	3456	3444	3352 (471)	724
birth length (cm)	50.6	49.6*	49.9*	51.1	50.5	50.3 (2.1)	717
placental area (cm^2)	379	345*	336*	353	351	356 (88)	613
Maternal characteristics							
weight at last prenatal visit (kg)	66	63*	63*	68	69	66 (9)	634
Adult characteristics							
age (years)	51.4	50.8	50.5	50.3	49.5	50.5 (0.95)	724
BMI (kg/m^2)[1]	26.6	26.6	26.7	27.9	27.1	26.9 (1.17)	676
current smoker (%)	37	34	31	42	33	35 (5)	724
SES ISEI-92	46	50	48	48	48	48 (13.4)	719
mean systolic BP (mmHg)	126	127	124	124	125	125 (15)	675
mean diastolic BP (mmHg)	86	86	83	85	85	86 (10)	675
mean total cholesterol (mmol/l)	6.0	5.8	5.8	6.0	6.0	6.0 (1.0)	717
IGT or NIDDM (2 hour >7.8 or known diabetic) (%)[2]	18	20	17	17	17	18 (38)	720
abnormal ECG (%)	18	13	7	12	17	15 (36)	717
creatinine clearance female (ml/min)	110	115	110	128	119	115 (30)	341
creatinine clearance male (ml/min)	120	128	121	127	127	124 (25)	326
ACR ≥ 2.5 (%)	8	7	12*	9	4	7	724
OR (95% confidence interval)[3]		1.14(0.50-2.59)	2.07(1.01-4.27)*	1.61(0.64-4.08)			
OR adjusted (95% confidence interval)[4]		1.27 (0.49-3.26)	3.22 (1.34-7.65)*	1.89 (0.59-6.11)			

* Significant ($p<0.05$) difference compared to those not exposed to famine during gestation.

[1] Geometric mean and standard deviation

[2] Impaired glucose tolerance or non insulin dependent diabetes mellitus.

[3] OR compared to non exposed group, adjusted for sex

[4] OR compared to non exposed group, adjusted for sex, age, adult BMI, smoking, SES (ISEI-92), systolic blood pressure, IGT or NIDDM (2-hour glucose>7.8 or known diabetic), cholesterol and ECG abnormalities.

p=0.89). People with MA tended to have been smaller at birth than people without MA (3269 and 3358 g; p=0.2, corrected for gender).

The prevalence of MA was significantly higher in those who were exposed to famine in mid gestation (12%) than in people who were not exposed prenatally (7%; p=0.05). The prevalence of MA in those who were exposed in early (9%; p=0.3) or late gestation (7%; p=0.8) was not significantly increased.

The effect of exposure to famine in mid gestation was independent of maternal weight (adjusted OR 2.1; 95% CI 1.0 to 4.4), birth weight (adjusted OR 1.9; 95% CI 0.9

Table 2. Maternal, birth and adult characteristics for people with and without microalbuminuria.

	microalbuminuria	no microalbuminuria	*p*-value (adjusted for sex)	N
N	52	672		724
female	50%	51%	0.89	724
Birth characteristics				
birth weight (gram)	3269	3358	0.17	724
birth length (cm)	50	50	0.27	717
placental area (cm^2)	332	358	0.07	613
Maternal characteristics				
weight at last prenatal visit (kg)	64	66	0.10	634
Adult characteristics				
age (years)	50.7	50.4	0.03	724
body mass index (kg/m^2)[1]	29	27	0.01	676
smoking	52%	33%	0.01	724
alcohol (units per week)	12	9	0.06	724
SES ISEI-92	44	48	0.04	719
mean systolic BP (mmHg)	135	125	<0.01	675
mean diastolic BP (mmHg)	91	85	<0.01	675
mean total cholesterol (mmol/l)	5.7	6.0	0.10	717
IGT or NIDDM (2 hour glucose >7.8 or known diabetic)[2]	40%	16%	<0.01	720
HbA1c[3]	6.2%	5.4%	<0.01	714
abnormal ECG	25%	14%	0.04	717
creatinine clearance (ml/min)	136	119	0.01	667

Means or percentages, *p*-value of difference adjusted for sex calculated with logistic regression.
[1] Geometric mean
[2] Impaired glucose tolerance or non insulin dependent diabetes mellitus.
[3] Glycosylated hemoglobin fraction of total hemoglobin

to 4.0), and other measures of size at birth. None of the parameters of size at birth were themselves associated with MA.

After adjusting for variables that are known to influence MA, including BMI, gender, age, smoking, IGT or diabetes, systolic BP, the presence of ECG abnormalities and serum cholesterol, and adult SES in a multivariate logistic regression model, the effect of exposure to famine in mid gestation on adult MA remained significant (OR 3.2; p=0.01). The most marked increase in the point estimate for the OR was observed when adjusting for BMI. Again, there was no significant association with exposure in early (p=0.3) or late (p=0.6) gestation after adjusting for these risk factors in a multivariate model (Table 1). The association between mid gestational exposure to famine and MA was independent of the use of antihypertensive medication, diagnosed hypertension, and pulse pressure. In a gender-stratified analysis, we found no significant modification of the effect of famine exposure on MA prevalence.

We were able to estimate creatinine clearance in 667 of the 724 participants: Body weight measurements were missing in 48, and plasma creatinine was missing in 9 participants. The overall mean creatinine clearance was 115 ml/min in women and 124

ml/min in men. Lower SES, higher fasting glucose, higher systolic BP, and higher adult weight were associated with higher creatinine clearance.

The mean creatinine clearance of individuals who were born before the famine was significantly lower than the mean clearance in the group that was conceived and born after the famine (age- and gender-adjusted $p<0.01$). We therefore used the conceived after group as the control group for analyses of creatinine clearance, comparing creatinine clearance per exposure group adjusting for confounders using linear regression.

Those who were conceived after the famine had the highest mean clearance. In comparison, those who were born before the famine had a gender- and age-adjusted decrease of 16.4 ml/min (95% CI 7.4 to 25.4; $p<0.01$), whereas people who were exposed to famine in mid gestation had a decrease of 11.9 ml/min (95% CI 4.0 to 19.8; $p<0.01$). Adjusting for confounders such as adult weight, systolic BP, fasting glucose, adult SES, as well as for measures of size at birth or maternal parameters, did not attenuate this association. Neither measures of size at birth nor maternal parameters were associated with mean creatinine clearance.

Discussion

We found that people who were exposed to famine in mid gestation had higher rates of MA at age 50 compared with people who had not been exposed to famine in utero. This is the first direct evidence suggesting that maternal starvation during gestation is linked to impaired renal function in the offspring. This effect was not mediated by reduced maternal weight or small size of the infant at birth. Adjusting for variables that are known to influence MA did not alter the effect.

We did not find a significant association between small birth size and MA, although people with MA tended to have been smaller at birth than people without MA. Some studies have found an association of either birth weight [6,7] or ponderal index with MA [8], whereas other studies were able to demonstrate only a trend to that effect [38,39]. This inconsistency may be because small size at birth is a crude marker of fetal undernutrition [40]. In female rats that were exposed to a low-protein diet for 1 wk in mid pregnancy, the nephron number of the offspring was profoundly reduced, whereas the effect on birth weight was negligible [16]. This illustrates that organ-specific effects of a short period of undernutrition during gestation may be independent of effects on total body size.

Previously, we found people who were exposed to famine in late gestation to have reduced glucose tolerance [25], whereas exposure to famine in early gestation was linked to higher rates of obesity [30], a more atherogenic lipid profile [26], and cardiovascular disease [27]. We now have evidence that mid gestational exposure to famine is linked to reduced renal function. This distinct relation between prenatal exposure to famine

at different stages of gestation provides additional evidence that long-term effects of maternal undernutrition depend on its timing during gestation.

Our findings are consistent with the hypothesis that mid gestational undernutrition leads to reduced glomerular endowment at birth and a consequent increased risk for MA as a result of single nephron hyperfiltration [41,42]. We are limited, however, by the small number of individuals in each of the exposure groups in our ability to make firm statements about the precise timing of the effects per trimester of exposure. Our conclusions are based on noninvasive outcome measures only. Renal biopsy, allowing glomerular counting and sizing, is not available in this cohort of healthy volunteers because of ethical considerations. We are limited by the small sample size in this study in drawing conclusions for the repercussions of our findings for ESRD rates. However, the literature suggests that adverse intrauterine conditions do contribute to higher ESRD prevalence [5,9].

We conclude that exposure to famine in mid gestation is linked to a 3.2-fold increase in occurrence of MA in adulthood and a 10% decrease in creatinine clearance, neither of which can be explained by cardiovascular confounders. We propose that mid gestational exposure to famine may prevent formation of sufficient glomeruli and thus increase the risk for MA and possibly affect renal function in adulthood. This supports the concept that intrauterine conditions during distinct, organ-specific periods of sensitivity may permanently determine health outcome in later life.

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Photo taken with a concealed camera of the queue outside a grocery store (Photo NIOD)

chapter

8

A possible link between prenatal exposure to famine and breast cancer – a preliminary study

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Abstract

In a study of 475 women born around the 1944-'45 Dutch famine, women exposed to prenatal famine more often reported a history of breast cancer than non-exposed women (hazard ratio 2.6, 95% confidence interval 0.9-7.7). They also had alterations in reproductive risk factors. Prenatal famine may increase breast cancer incidence.

Introduction

Maternal diet during gestation can affect the risk of breast cancer in the offspring in animal models [6,7]. Whether and to what extent maternal caloric restriction during gestation might be involved in the early origins of breast cancer in humans is unclear. The Dutch famine was a five-month period of severe food shortage during the last year of World War II. In this paper, we investigate the role of maternal undernutrition during gestation on cumulative breast-cancer incidence up to age 61.

Subjects and Methods

The Dutch Famine Birth Cohort consists of 2414 live-born term singletons, of which 1174 were females, born in the Wilhelmina Gasthuis in Amsterdam, the Netherlands, between November 1st 1943 and February 28th 1947. The selection and tracing procedures and loss to follow-up have been described in detail elsewhere [12-14].

The local Medical Ethics Committee had approved the study.

A person was considered prenatally exposed to famine if the average daily ration for adults during any 13 week period of gestation was less than 1000 calories. Using this definition, all cohort members born between January 7th 1945 and December 8th 1945 had been exposed prenatally. We defined periods of 16 weeks each to differentiate between those who were exposed in late gestation (born between January 7th and April 28th 1945), mid gestation (born between April 29th and August 18th 1945) and in early gestation (born between August 19th and December 8th 1945).

All eligible women in the cohort were invited to visit the hospital. Three hundred ninety one women visited the hospital, 50 women were visited at home and 34 women were interviewed by telephone.

Women were asked whether they had ever been diagnosed with cancer, and, if so what type of cancer and at what age. Spontaneous menopause was defined as the cessation of menstrual periods, not due to surgery of the ovaries or uterus, for a period of longer than 12 months. We assessed maternal breast cancer history by asking all participants whether their mothers had any health problems, and scoring all mentions of breast malignancies. Information on whether or not women had a history of hormone replacement therapy use was not collected.

We used the Cox proportional hazards model to calculate hazard ratios (HR) to compare the incidence of breast cancer in women who were exposed to famine in early, mid or late gestation with that in unexposed women (born before or conceived after). We added covariates to the Cox model in order to adjust for potential confounders. Due to the small number of cases, we limited multivariable models to one added covariate.

Results

Of the 1174 women in the cohort, 141 women had died before the start of the study, 114 had emigrated and 60 women refused permission for their addresses to be included in the study database. The municipalities were unable to trace a further 96 women to a correct address. Seven hundred and sixty three women were eligible for the current study, of whom 475 women (62%) participated in the study. Participation rates did not differ between women that had (65%) and had not been exposed to famine (60%, p=0.2). Birth weights of women included in the study (mean 3293 g) did not differ significantly from birth weights of eligible women not included (mean 3286 g, p=0.9).

Table 1 describes the maternal, birth and adult characteristics as well as breast cancer prevalence among the women in the study, according to the timing of exposure to famine in utero. The breast cancer incidences within the control group (born before or conceived after the famine) did not differ significantly (p=0.3).

Table 1 Breast cancer cumulative incidence and maternal, birth and adult characteristics for women that participated in the Dutch Famine Birth Cohort[1].

	Exposure to famine						
	born before	**late**	**mid**	**early**	**conceived after**	**all (SD)**	**N**
Number	144	82	77	46	126		475
Breast cancer (%)	**2.8**	**3.7**	**3.9**	**8.7*** **	**0.8**	**3.2**	**475**
Breast cancer (n)	**4**	**3**	**3**	**4**	**1**	**15**	**475**
Maternal characteristics							
Maternal socio-economic status low (manual, %)	86	71	69	54*	68	72	381
Primiparous (%)	31	27	33	50*	37	40	475
Maternal breast cancer (%)	3.5	4.9	3.9	2.2	5.6	4.2	473
Birth characteristics							
Birth weight (g)	3356	3140*	3144*	3448	3354	3293 (450)	475
Adult characteristics							
Age at interview (years)	59.2	58.5	58.3	58.1	57.5	58.4 (0.9)	475
Body mass index (kg/m^2)[2]	27.8	28.1	28.1	26.9*	29.1	28.2 (5.2)	440
Socio-economic status (ISEI-92)[3,2]	44	46	50*	45	49	47 (15)	465
Nulliparous (%)	16	13	5***	4	12	12	472
Number of children	1.7	1.9	2.0**	2.0**	1.7	1.8 (1.0)	472
Age at menarche (years)	13.2	12.7	12.9	12.9	12.7	12.9 (1.7)	470
Age at first child (years)[4]	23.2	23.6	23.5***	22.9***	24.2	23.3 (3.8)	416
Age at spontaneous menopause (years)	50.7	49.3	52.1***	48.7	49.3	50.1 (5.0)	325

[1] Means and standard deviations (SD), except where given as percentages

[2] Geometric mean

[3] Socio economic status scale according to International Socio Economic Index-92 ranges from 16-87

[4] Median age

* p<0.05 compared to unexposed (logistic or linear regression)

** p<0.05 compared to unexposed (Mann-Whitney test)

*** p<0.05 compared to unexposed (Cox regression)

Table 2 shows the unadjusted hazard ratios and hazard ratios adjusted for maternal, birth and adult risk factors for breast cancer among women exposed to famine in utero compared to unexposed women. Women exposed to famine in utero more frequently reported a history of breast cancer than unexposed women (unadjusted HR 2.6, 95% confidence interval 0.9 to 7.7).

The birth weights of women with breast cancer (mean 3191 g) did not differ significantly from those of women without breast cancer (3296 g, p=0.4).

Table 2 Hazard ratios (95% confidence intervals) for breast cancer among women exposed to famine in utero compared to unexposed women, unadjusted and adjusted for risk factors

	Exposure to famine during gestation			
	late	**mid**	**early**	**all exposed**
Unadjusted	2.0 (0.5-8.3)	2.1 (0.5-8.7)	4.8 (1.2-17.8)	2.6 (0.9-7.7)
Adjusted for:				
Maternal socio-economic status low (manual)	2.2 (0.5-9.3)	2.3 (0.5-9.6)	5.8 (1.5-21.7)	3.0 (1.0-8.7)
Maternal parity	1.9 (0.4-8.0)	2.1 (0.5-8.6)	5.1 (1.4-19.4)	2.6 (0.9-7.7)
Maternal breast cancer status	2.0 (0.5-8.2)	2.1 (0.5-8.8)	4.8 (1.3-18.1)	2.6 (0.9-7.7)
Birth weight	1.8 (0.4-7.5)	1.9 (0.4-7.9)	5.0 (1.3-18.9)	2.5 (0.8-7.4)
Body mass index	2.9 (0.6-15.6)	3.3 (0.7-8.4)	7.1 (1.6-32.0)	4.0 (1.1-14.5)
Socio-economic status	2.0 (0.5-8.3)	2.0 (0.5-8.4)	5.0 (1.3-18.7)	2.6 (0.9-7.7)
Nulliparity	2.0 (0.5-8.3)	2.2 (0.5-9.2)	5.0 (1.3-18.8)	2.7 (0.9-7.9)
Age at first child	0.6 (0.1-5.6)	1.9 (0.4-7.8)	4.4 (1.2-16.6)	2.0 (0.6-6.0)

Discussion

Women who had been exposed to famine in utero had a higher incidence of breast cancer compared to unexposed women, although the effect failed to reach statistical significance. The effect was largest, and statistically significant, among women who were conceived during the famine. The effect of maternal starvation during gestation on breast cancer incidence in the offspring was not explained by differences in known risk factors for breast cancer, including parity and body mass index. The number of cases in this study is small, and the reported effect may be spurious or associated with selective participation. However, this study may present the first direct evidence that maternal undernutrition during gestation may be linked to breast cancer risk in the offspring.

Due the small size of the cohort and relatively young age at the time of data collection, this study did not have sufficient power to detect modest effects of undernutrition during gestation on subsequent breast cancer at a statistically significant level.

Sixty-two percent of eligible women participated in this study. Selective participation may therefore present a source of bias. However, birth weights did not differ between participants and non-participants, and participation rates were similar among famine exposed and unexposed women.

Women were less likely to conceive during the famine, and those who did conceive may have been of a different constitution; They may have had enhanced reproductive capacity or a more efficient metabolism. The effect of prenatal exposure to famine on adult breast cancer incidence was however not limited to daughters of mothers who conceived during the famine. We found no indication for more breast cancer among the mothers of the study participants exposed to famine during gestation. This suggests that selective fertility among women more prone to develop breast cancer – making their daughters more susceptible by familial inheritance – is unlikely to be wholly responsible for the effect of prenatal famine exposure on subsequent breast cancer.

A previously published study into adult mortality in the same cohort suggested higher cancer mortality among women exposed in early gestation [12]. The finding may reflect the fact that the higher breast cancer morbidity after famine exposure in utero is followed by higher cancer mortality. Higher cancer mortality may have led to an underestimation of the effect of famine exposure because only women who were alive at age 58 could participate in the study.

In a different study, childhood famine exposure was linked to breast cancer [4]. In accordance with these findings, women born before the famine, who were exposed as infants, had more breast cancer than those conceived after. Although this difference was not statistically significant, and the records show that young infants received adequate rations during the famine [2], the fact that we adhered to the pooled control group model [13] may thus have led to an underestimation of the effect of prenatal famine exposure on breast cancer incidence.

Little is known about the pathophysiology of the association between prenatal factors and subsequent cancer risk. High birth weight has been associated with an excess of cancer, particularly breast cancer [1, 11], although other studies [3, 8], including our own, could not confirm this association. Rapid postnatal growth following fetal growth restriction has been associated with permanently raised levels of growth factors [9], possibly propagating pre malignant cell growth [5]. The restoration of an adequate diet after a period of relative growth restriction due to maternal malnutrition, as was present in women exposed to famine during gestation, could also be associated with increased levels of growth factors, though this is speculative.

Alternatively, hormonal axis programming may be involved in programming subsequent breast cancer excess [15]. In rats, maternal starvation has a programming effect on the fetal hypothalamic pituitary gonadal (HPG) axis [10], possibly with lasting repercussions for breast tissue. There is circumstantial evidence for HPG programming in our data. Women exposed to famine during gestation had more children and were less likely to be childless; women exposed in mid or early gestation had children at a younger age; menopause was delayed in women exposed in mid gestation.

The increase in breast cancer among women who were in utero during famine reported here is based on very small numbers. In the future, we hope to be able to link this

unique birth cohort to the national hospital admissions database, in order to objectify our finding that prenatal famine exposure is associated with increased breast cancer prevalence.

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Nurse from Amsterdam's inner city Binnengasthuis preparing and weighing liquid food (protein hydrosylate), used for the treatment of severe cases of malnutrition (Photo NIOD, May/June 1945)

chapter

9

Adult mortality at age 57 after prenatal exposure to the Dutch famine

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Abstract

Prenatal famine exposure has previously been shown to be associated with cardiovascular disease and type II diabetes in adulthood. In the current study, we could not demonstrate an effect of prenatal exposure to famine in 2254 term singletons born during the 1944–1945 Dutch famine on adult mortality up to the age of 57 years. Follow-up of this cohort will resolve whether famine exposure is linked to increased adult mortality.

Maternal undernutrition during gestation may reduce the offspring's adult survival. The lifespan of rats whose mothers were malnourished during pregnancy was reduced by 25% [1]. Studies in the Gambia have shown that people born during the wet or 'hungry' season have a 10 times higher mortality than those born in the harvest season [2], although this finding could not be replicated in similar studies among young adults [3, 4]. In the Gambian study, the excess mortality among adults was mainly due to infectious causes. Little research has been done on the effect of exposure to famine in utero on all-cause mortality in the middle aged.

The Dutch Hunger winter was a five-month period of severe food shortage during the last year of World War II. The Dutch Famine Birth Cohort study offers a unique opportunity to study the long-term effects of maternal starvation during specific stages of pregnancy on health in later life. We previously found excess infant mortality among those born before and during the famine. People conceived during the famine were more obese as adults [5] and had a more atherogenic lipid profile [6] and three times more coronary heart disease (CHD) [7] than people not exposed to famine in utero. Mid gestational exposure to famine was linked to more obstructive pulmonary disease [8] and microalbuminuria [9]. Exposure to famine in late gestation was linked to 10% higher 2-hour glucose levels [10].

We postulated that the increased prevalence of CHD and its biological risk markers after exposure to famine in utero would be reflected in higher adult all cause mortality rates.

The Dutch famine birth cohort consists of 2,254 term singletons, born alive between November 1st 1943 and February 28th 1947 in Amsterdam. The babies' birth and the mother's obstetrical records have been preserved, providing data on antenatal examination, pregnancy duration and outcome, as well as size at birth. The selection procedure for this study has been described in detail elsewhere [11]. In summary: Of the 2,254 cohort-members, 93 (4%) were not traceable to a place of residence, 163 (7%) did not consent to their address being made available to the Academic Medical Centre, and 200 (9%) people had emigrated. Prior to 1996, 263 (12%) people had died. In addition, the mortality after 1996 was assessed by Statistics Netherlands (SN), which is able to link address and date of birth data to a unique personal identifier number, which in turn can be linked to the deaths registry. One thousand four hundred and sixty-two (95%) of the 1,535 persons available for follow up after 1996 were linked to an A-number. The survival times of 73 subjects SN was unable to link to an A-number were censored at the date at which the municipal registry had provided their address. The 1,462 A-numbers were linked to the national deaths register (complete through 2001). Forty-seven (3%) people had died between January 1st 1996 and January 1st 2001. Their primary causes of death were provided by SN. Mortality up to the age of 18 in this cohort has been described in detail elsewhere [11]. In this paper we therefore report adult mortality only.

The causes of death were coded according to the International Classification of Disease (ICD) coding system used at the time of death [11]. ICD-10 was used from 1996 onwards. For the purpose of the analyses, we subsequently categorized them into infections (ICD-10 codes A00-B99), coronary heart disease (ICD-10 codes I20–I25), cancer (ICD-10 codes C00-D48) and others or unknown cause of death.

We defined the famine period according to the official daily food rations for the general adult population [11,12]. Children under 1 year of age were relatively protected during the famine. Official daily rations for this group were higher than 1,000 calories a day [13]. However, infant mortality among this group was high during the famine [11]. During the famine the protein/carbohydrate ratio remained stable, while caloric intake was reduced to 40% of prefamine levels [14].

A person was considered exposed in utero if the average daily adult ration was less than 1,000 calories during any 13-week-period of gestation. We defined three periods of 16 weeks each, leading to three groups: those that had been either exposed in late gestation (born between January 7th 1945 and April 28th 1945), mid gestation (born between April 29th 1945 and August 18th 1945) or early gestation (born between August 19th 1945 and December 8th 1945).

We constructed Kaplan–Meier cumulative adult mortality curves as a function of age per famine exposure group: subjects born before the famine, those exposed to famine in late, mid or early gestation, and those conceived after the famine. We used the proportional hazards model to explore the influence of exposure to famine, maternal characteristics and birth size on overall and cause specific cumulative adult mortality (>18 years) always adjusting for sex as a covariate. Results are reported as hazard ratios with 95% confidence interval.

The date of emigration was missing for 22 persons who had emigrated before 1996. We imputed the median age of emigration (18 years).

The size of the cohort would allow a power of 80% to detect a 60% increase in all-cause mortality among people exposed to famine during gestation, or a tripling of coronary heart disease mortality among people exposed to famine in early gestation, using a 0.05 significance level. Although the study was underpowered to detect a more modest rise in mortality, we nevertheless carried out the study to assess the potential bias originating from the selective participation of live members of the cohort in a follow up clinical study that commenced in 2002.

Of the 2,254 people included in the cohort, 778 (35%) had been exposed to famine in utero (Table 1). At age 18, 1,991 people were available for follow-up, of whom 135 (6.8%) had died by the end of follow-up in 2001 (Table 2). The mean age of the survivors was 56.5 years (range 54.8–58.2 years). Men had a higher all-cause adult mortality than women (8.3 vs. 5.3%) (hazard ratio 1.6, 95% confidence interval 1.1–2.3). Forty percent of adult mortality was due to cancer (27 men, 28 women), 20% was due to coronary heart disease (21 men, 7 women), the remainder was due to other or unknown causes.

Table 1 Characteristics of the Dutch famine birth cohort according to exposure to famine.

	Exposure to famine						
	Born before	Late exposure	Mid exposure	Early exposure	Conceived after	All (SD)	n
					after		
Number	728	295	278	205	748	2254	
Proportion of men (%)	52	46	46	47	52	50	
Birth outcomes							
Birth weight (g)[1]	3370	3135	3215	3452	3418	3343 (478)	2254
Birth length (cm)[1]	50.5	49.5	49.8	50.9	50.5	50.3 (2.2)	2224
Head circumference (cm)[1]	32.9	32.3	32.1	32.8	32.2	32.8 (1.6)	2238
Ponderal index (kg/m^3)[1]	26.2	25.8	26.0	26.1	26.5	26.2 (2.4)	2224

[1] mean (SD)

People born before the famine had the highest mortality (9.1%), with a hazard ratio of 1.3 (95% CI: 0.9–2.0, p = 0.22) compared to people conceived after the famine (Figure 1).

People exposed to famine in late gestation had the lowest overall adult mortality (4.4%) (Table 2). The sex adjusted hazard ratio for this group was 0.7 (95% CI: 0.3–1.3, p = 0.21) compared to those conceived after the famine. People exposed to famine in mid and early gestation had hazard ratios of 1.0 (95% CI: 0.5–0.7) and 1.0 (95% CI: 0.5–2.0), respectively.

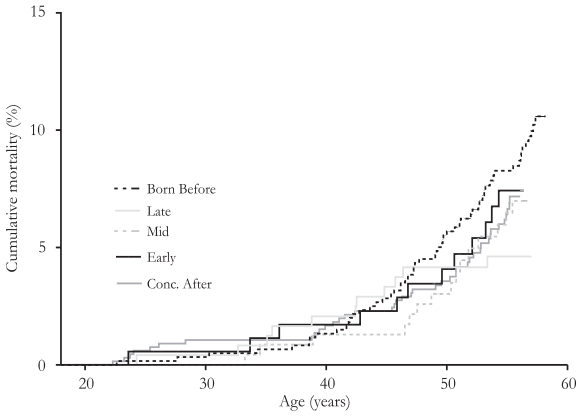

Figure 1. Cumulative mortality (%) per exposure group after age 18.

Table 2 Mortality among men and women between 18 and 57 years of age according to timing of exposure to famine

	Exposure to famine														
	Born before			Late exposure			Mid exposure			Early exposure			Conceived after		
Number at birth	728			295			278			205			748		
Number at risk at age 18	626			251			248			184			682		
All-cause mortality[1]	57	9.1%		11	4.4%		15	6.0%		12	6.5%		40	5.9%	
Cancer[2]	21	3.4%	37%	4	1.6%	36%	6	2.4%	40%	8	4.3%	67%	16	2.3%	40%
Coronary heart disease[2]	12	1.9%	21%	3	1.2%	27%	3	1.2%	20%	2	1.1%	17%	8	1.2%	20%
Other/unknown[2]	24	3.8%	42%	4	1.6%	36%	6	2.4%	40%	2	1.1%	17%	16	2.3%	40%

[1] n, absolute %

[2] n, absolute %, relative %

Twenty-eight deaths were due to coronary heart disease. There was no association between famine exposure in any part of gestation and coronary heart disease related mortality (all $p>0.3$, compared to those conceived after).
No measure of size at birth was related to all-cause mortality. The sex adjusted hazard ratio per kilogram decrease in birth weight was 0.98 ($p = 0.95$).

Fifty-five deaths were due to cancer. Cancer mortality was highest among women (7.1%), but not men (1.2%), exposed in early gestation, although it did not differ significantly from women that were conceived after the famine (3.4%) (p for interaction = 0.092). We observed an association between increasing birth weight and increased cancer mortality. The hazard ratio per kilogram increase in birth weight adjusted for sex was 1.8 (95% CI: 1.0–3.1, $p = 0.04$). This effect was not modified when corrected for famine exposure.

Although earlier findings from the Dutch famine birth cohort have revealed a threefold increase in the prevalence of coronary heart disease among people exposed to famine in utero in early gestation [5–7, 15], and more type II diabetes among those exposed to famine in late gestation [10], we found no excess mortality among any of the groups exposed to famine during gestation.

The highest mortality occurred in people born just before the famine. Although this excess was not statistically significant, and may reflect the fact that this group is also the oldest in the cohort, it is of interest because infant (0–1 year) mortality was increased in this group [11] and poor growth during infancy is known to be associated with increased risk of coronary heart disease independently of birth weight [16]. However, infants had access to adequate rations protecting them from postnatal undernutrition [13]. Therefore it is unlikely for the excess infant mortality in this group to have been due to poor nutrition during infancy alone. Because information on postnatal growth is not available for this cohort, we were not able to directly assess the effect of postnatal growth that may have on adult mortality.

We found an association between increasing birth weight and cancer mortality, which is consistent with reported associations between increasing birth weight and breast cancer [17] and other types of cancer [18, 19]. The link between birth weight and cancer was not mediated by famine exposure.

Animal studies have shown that life span can be programmed in utero [1, 20], especially when animals that were protein-restricted in utero, have an unrestricted postnatal diet. The Dutch famine occurred in a population that had access to plentiful food before the famine started, and returned to more than adequate rations within weeks of the end of the famine. This period of acute famine may make the Dutch famine cohort a more suitable comparison to animal studies [1, 20], which show a significantly shortened life span after prenatal protein restriction, than studies carried out in populations where prenatal famine is superimposed on chronic malnutrition [2–4, 21] and who, for that reason, are unlikely to have experienced compensatory growth in early child-

hood. Moreover adult mortality in these populations is mainly due to infectious causes, whereas adult mortality in the present-day Netherlands is mainly cardiovascular and cancer related.

In view of the findings in animal studies and the increase in morbidity in those members of this cohort exposed to famine in utero, an increase in mortality after exposure to famine during gestation was predicted. The people born around the time of the Dutch famine are still relatively young. Continued follow-up of this cohort will resolve whether famine exposure in utero is associated with reduced adult survival.

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A typical daily ration during the Dutch Famine would consist of 2 slices of bread, 2 potatoes and half a sugar beet

(Photo NIOD)

chapter

10

Early gestation and cardiovascular disease in later life-implications for future research

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Abstract

Dietary restriction during early gestation has detrimental effects on the offspring's cardiovascular disease risk. At present, prenatal care commences after placentation and organogenesis are complete. This precludes advice aimed at improving maternal nutrition before conception and in early gestation, which may benefit the offspring's cardiovascular health. However, further research is needed before evidence based dietary advice can be developed for preconception dietary counseling. The influence the environment exerts on the early embryo, and its implications for cardiovascular disease risk in the offspring, may also have repercussions for the management of severe morning sickness as well as for assisted reproductive techniques including in vitro fertilization. More research is needed to assess whether the offspring of mothers who suffered from hyperemesis gravidarum have an increased cardiovascular disease risk, and whether nutritional management may lower this risk. Finally, follow-up studies of children born after assisted reproduction should assess blood pressure, impaired glucose tolerance and raised lipid concentrations in addition to neurological outcomes, to establish whether in vitro techniques increase the offspring's cardiovascular disease risk.

Introduction

Epidemiological studies have consistently reported an association between small size at birth, which is a summary measure of fetal environment throughout gestation, and subsequent adult chronic disease [1-5], but they have failed to identify the causal determinant leading to such an association. Experimental evidence from animal studies indicates that early nutrition, specifically maternal nutrition around the time of conception and in early gestation, may play a key role in the early origins of adult chronic disease [6-11]. The Dutch famine study has provided the first direct evidence in humans in support of the hypothesis that early gestational undernutrition has detrimental effects on the developing embryo's future cardiovascular health. Exposure to famine in early gestation is associated with a more atherogenic lipid profile [12], altered clotting [13], obesity [14], an enhanced stress response [15] and more coronary heart disease in the offspring [16, 17]. Interestingly, infants born after exposure to famine in early gestation had normal birth weights. This suggests that maternal diet around conception is an important determinant of adult disease and that its effects are not necessarily mediated by changes in birth weight.

The fact that maternal diet in early gestation has a lasting impact on the metabolic and endocrine function of the offspring may be the result of the susceptibility of rapidly differentiating tissues to alterations in growth and development. During embryonic development tissue differentiates more rapidly than at any other time during gestation, or later life [18]. When faced with a lack of resources, the fetus may need to make trade-offs in order to survive. By sacrificing the growth of less essential organs, more energy becomes available for essential processes [19]. Poor maternal nutrition may also induce alterations in the metabolic and endocrine set-points of the developing organism. The capacity for developmental plasticity is likely to be largest in early gestation. It may be a means by which the developing organism is able to adapt its physiology in preparation for the environment into which it will be born [20]. This notion is supported by findings from a prospective study of 381 mothers and their babies in Southampton, which show that maternal thinness before pregnancy may lead to a diversion of fetal blood flow to the liver [21]. By increasing blood flow to the liver, the fetus may be able to more effectively extract nutrients, and can increase the growth of many fetal organs by increasing growth factor expression, as has been shown in sheep [22]. 'Liver sparing' may have permanent repercussions for cardiovascular health, including impaired lipid and clotting factor homeostasis.

The evidence suggesting the important role of maternal nutrition during gestation, particularly around conception, may have public health implications. This paper discusses the implications for maternal pre-conception nutritional advice, for the management of severe morning sickness and for assisted reproductive techniques.

Implications for Maternal Pre-Conception Dietary Advice in a Western setting

Maternal starvation around the time of conception is linked to cardiovascular disease in the offspring. There is preliminary evidence suggesting that an unbalanced maternal diet prior to conception can have equally detrimental effects on the offspring's cardiovascular disease risk [21, 23-25]. In 2001, almost one in four young women in Europe were attempting to lose weight by dieting or other means. Only one third of all young women ate fresh fruit or vegetables every day. Fizzy drinks and sweets were consumed on a daily basis by one in three women [26]. It is highly likely that women will continue to eat an unbalanced diet, or continue weight loss strategies during early embryo development, before they are aware of the fact that they are pregnant [27].

Although the Dutch famine study has provided us with compelling evidence for the significance of maternal diet during early gestation, such an extreme form of maternal nutritional deprivation is of limited practical value in a contemporary western setting. Langley-Evans et al postulated that, because birth weight is not affected by normal variation in the western maternal diet, it is unlikely that alterations in western maternal diet are responsible for any modification of cardiovascular disease risk [28]. Birth weight and maternal nutrition, however, may each independently contribute to adult disease risk [29]. This is illustrated by the fact that the increases in cardiovascular disease among people conceived in famine were not paralleled by any effects on birth weight. Besides the absolute caloric content, the balance of macro- and micro-nutrients in the maternal diet during gestation may have permanent effects on the offspring's health. Protein [30-32], folate [33], glycine [34] and calcium [35] content have each been reported to influence cardiovascular disease risk in the offspring. The dietary habits of young women in today's western world may thus present a potentially modifiable cardiovascular disease risk factor for future generations. The fact that, in the Netherlands, the vast majority of pregnancies are planned may provide opportunities for pre-conception counseling [36], including advice on which maternal diet will benefit the cardiovascular health of the offspring.

The current recommendations for preconception nutrition, such as those of the American College of Obstetricians and Gynecologists, are largely based on expert clinical opinion [37]. While experimental studies have identified the importance of periconceptional nutrition in animals, there is, to date, limited evidence-based information on preconception nutrition and the offspring's health in later life. A number of large prospective cohort studies, aimed at describing the determinants of maternal and offspring health, are currently being carried out [38-41]. Such studies may help resolve whether maternal dieting or maternal thinness during pregnancy have the detrimental repercussions for the offspring's health predicted by the Dutch famine Study. They may also be able to identify specific dietary components, such as micro-nutrients, which are

potentially beneficial for the offspring's cardiovascular health. However, the design of these studies harbors limitations. Firstly, of these studies, only the Southampton Women's Survey has collected data on diet and body composition prior to pregnancy [39]. Other studies are not able to assess the effects of diet in the pre-conception and early gestational period on later health. More importantly, the studies are observational. The interpretation of the findings may be hampered by confounding factors, including the clustering of poor dietary habits, maternal obesity, maternal smoking, low breast feeding rates and poor antenatal care within families of low socio-economic status, poor education and non-western ethnicity. Recommendations for maternal nutritional prior to and during gestation should therefore be based on randomized intervention studies. The long follow-up and ethical considerations contribute to the fact that, to date, very few such studies have been carried out [35, 42, 43]. Interventions based on expert clinical opinion, such as the recommendation of a high protein and low carbohydrate diet made to pregnant mothers in Scotland in the 1950-s [31, 32], have, in the past, demonstrated that good intentions which are not evidence based can indeed be harmful. It is concerning that in the United States, where multivitamin supplementation is now universally recommended, trials investigating the long term effects of such supplements have never been conducted, and are now considered unethical [44].

At present, prenatal care doesn't commence until placentation and organogenesis are complete. The importance of preconception care is increasingly being acknowledged [45]. In order to develop appropriate dietary recommendations for preconception counseling, well conducted randomized controlled trials of adequate sample size which include measures of effectiveness are needed. Such trials should include food based as well as micronutrient supplement based interventions and should measure fetal growth, maternal metabolism and long term outcomes in the offspring. To shorten the time until completion of the follow-up of cardiovascular end-points, including coronary heart morbidity, trials could measure glucose tolerance, blood pressure and lipid profile, which have been shown to track from childhood [46, 47], in the children. A considerable number of trials have investigated the effects of a number of dietary interventions on short term end points, including maternal and neonatal outcomes [48]. The possibility of tracing and following up the offspring born to mothers who were included in these trials could be explored, as this approach may enable the study of outcomes in older aged offspring. Studies of children born to mothers who were involved in studies carried out in Scotland in the nineteen-fifties, have demonstrated that this approach is possible, and can yield valuable information [31, 32, 49].

Implications for Maternal Diet in Developing Countries

The Dutch famine study has found detrimental effects on cardiovascular disease markers among people conceived in famine, who were exposed to adequate nutrition in later gestation and after birth. Postnatal catch-up growth has been shown to compound the effects of low birth weight or maternal undernutrition [1, 50]. In rat pups subjected to prenatal undernutrition, exposure to a postnatal high-fat diet had much greater effects on the development of obesity and hyperleptinemia and resulted in a 25% shorter lifespan compared to pups born to mothers with a normal dietary intake, but then fed a high-fat diet postnatally [50, 51]. A high fat postnatal diet may be less harmful to cardiovascular health, if it was preceded by a high fat diet during gestation [52]. Possibly, undernutrition in early gestation causes a greater degree of metabolic conflict when it contrasts with adequate nutrition in later gestation or in later life, thus resulting in detrimental effects on cardiovascular disease risk. Our findings can, therefore, not be directly translated to the current situation in many developing countries where, unfortunately, nutritional deprivation commonly persists throughout gestation and later life. They do, however, provide insight in health issues pertinent to populations transitioning from poor nutritional circumstances to western dietary habits.

Cardiovascular disease and diabetes are currently taking on epidemic proportions on the Indian subcontinent, where the urban population is currently making the transition from traditional to a westernized life-style. In India, maternal diets are particularly compromised; young women are traditionally the last member of the household to eat and subsequently have low energy reserves during pregnancy [53]. One third of babies born in India are of low birth weight (<2.5 kg) (UNICEF, 1998). In 2001, over 10% of all Indians –over 1 million people– had impaired glucose tolerance [54]. Poor maternal nutrition may be contributing to the current increase in type 2 diabetes mellitus in India [55]. Improved maternal nutrition may be a useful strategy in the prevention of this huge burden of disease. A number of intervention studies focusing on the benefits of dietary supplementation of pregnant women for maternal and neonatal health outcomes have been carried out in nutritionally compromised populations [35, 42, 43, 56, 57]. To date, only two of these trials have also reported the follow-up of cardiovascular risk markers among the offspring. The results showed that a simple nutritional intervention in nutritionally compromised pregnant women in Guatamala - a protein and calorie dense supplement- can benefit glucose tolerance in the offspring [43]. Calcium supplementation in the second half of gestation led to lower offspring blood pressure [35]. Prospective studies such as the Indian 'Mumbai Randomised Controlled Trial' (http://www.mrc.soton.ac.uk/index.asp?page=46) will test the hypothesis that supplementation of specific foods and micronutrients, including green leafy vegetables, folic acid and vitamin B12 to the maternal diet, can reduce the cardiovascular risk of future

generations. Traditional to westernized lifestyle transition is also occurring in China and countries in South East Asia and South America. Studies indicate that, in these countries, improvements in maternal diet may benefit cardiovascular risk in the offspring [35, 43, 58, 59].

The United Nations Standing Committee on Nutrition has recently acknowledged the fact that improved maternal nutrition will contribute to the prevention of non communicable disease in subsequent generations [60]. The Committee also recognized that the double burden of malnutrition-the coexistence of undernutrition and overnutrition within the same population, or even within the same individual -is a problem that needs to be addressed. Randomized controlled trials including culturally appropriate food-based interventions, which study long-term outcomes in the offspring as well as measures of effectiveness need to be carried out, and should serve as a basis for interventions in nutritionally compromised pregnant women [61]. Cultural changes, including the emancipation of women in developing countries, may bring about further improvements in the nutritional status of fertile women in developing countries.

Implications for the Management of Hyperemesis Gravidarum

Morning sickness is a common condition which occurs frequently during the first trimester; >50% of women experience nausea, >10% of women experience daily vomiting [62]. Severe morning sickness, also called hyperemesis gravidarum, is a condition of intractable vomiting during pregnancy leading to fluid, electrolyte and acid-base imbalance, maternal first trimester weight loss [63] and metabolic changes in the mother which are similar to those seen during starvation. Hyperemesis gravidarum is severe enough to require hospital admission in between 0.3% and 1.5% of pregnancies [64].

Despite decades of research, the pathophysiology of hyperemesis gravidarum remains unclear. The symptoms of morning sickness have been associated with the steep rise in human chorio-gonadotrophic (HCG) hormone which occurs in early pregnancy as well as with gestational transient thyrotoxicosis [64]. Flaxman and Shermann postulated that symptoms of nausea, vomiting and food aversion may play a functional role in maternal avoidance of foods and substances which are potentially harmful for the developing embryo [65]. Lack of knowledge about the aetiology of hyperemesis gravidarum has contributed to the fact that the treatment of this condition is empirical and sub-optimal. Therapeutic options for hyperemesis gravidarum are limited to fluid and salt supplementation, in combination with anti emetic drugs and pyridoxine (vitamin B6). In protracted cases refractory to pharmacological therapy, gastric tube feeding or total parenteral feeding is used to overcome the period of nausea [66].

Hyperemesis gravidarum is associated with poor pregnancy outcome including low birth weight [67]. The association between low birth weight and subsequent cardiovascular disease [1, 2] supports the hypothesis that hyperemesis gravidarum may be associated with cardiovascular disease in the offspring. Independently of birth weight, however, the poor maternal nutritional status during the period of embryogenesis may increase the offspring's cardiovascular risk in later life. The effects of severe morning sickness on the offspring's markers of cardiovascular risk have, however, never been studied. Follow-up studies in large population based cohorts may provide the first evidence of an increase of cardiovascular risk markers among the children of mothers who suffered severe hyperemesis gravidarum. A randomized controlled trial investigating the effects of different feeding strategies for hyperemesis gravidarum on short term outcomes, including improvement of nausea and vomiting, duration of hospitalization and birth weight, has never been conducted [66]. The follow-up of the cardiovascular risk profile of the offspring from a trial of this kind may resolve whether nutritional support may lower cardiovascular risk in the offspring.

Implications for In Vitro Fertilization Techniques

The environment around the time of conception affects the offspring's growth and cardiovascular physiology [6-9, 11, 68]. For example, maternal nutrition around the time of conception may exert a permanent effect on the offspring's phenotype. In experimental models, maternal undernutrition around conception leads to blastocyst abnormalities, hypertension [9], altered stress response [6, 7] and impaired glucose tolerance [8]. Although the evidence in humans is limited, people who were conceived in famine have increased cardiovascular risk profile [12-15, 17, 69]. The molecular mechanisms underlying these permanent effects are unclear, but may include altered gene expression [70]. In the agouti mouse mutant, maternal dietary folate supplementation at conception alters the expression of the imprinted agouti gene by altering the capacity for methylation [71]. Dietary protein restriction induces epigenetic modification of hepatic gene expression in the offspring [72]. Epigenetic processes may have an impact on maternal and paternal gametes as well as on pre-implantation embryo development [73]. This implies that the manipulation of gamete and embryo environment inherent to assisted reproductive techniques may lead to permanent alterations in gene expression.

Epigenetic alterations have been demonstrated to underlie the phenomenon termed large offspring syndrome, which occurs after in vitro fertilization (IVF) in animal husbandry [74]. Besides producing abnormally large offspring, the syndrome is linked to alterations in organ growth, skeletal defects and perinatal mortality [75]. The phenomenon seems to be directly linked to the post-fertilization period culture period in vitro [76]. Interestingly, a study comparing artificial insemination-produced to in vitro-produced offspring, found the IVF calves had altered postnatal thyroid function, leptin

concentrations, compromised post-prandial insulin secretion and a higher gain/feed ratio and growth [77]. This illustrates that manipulation of early embryo environment can lead to a constellation of endocrine and metabolic alterations which may predispose to cardiovascular disease in later life.

The IVF regimens used in humans differ from those commonly used in animal husbandry, in that they make use of ovulation induction, mature oocytes and significantly shorter in vitro culture periods [76]. Nonetheless, there are more major congenital malformations among children born after IVF and intra cytoplasmic sperm injection (ICSI), although the neuropsychological development of children born after assisted reproductive techniques is generally reassuring [78, 79]. Several studies have demonstrated that babies born after IVF are smaller, even when maternal age, parity and previous subfertility are taken into account [80, 81]. A large number of epidemiological studies have demonstrated that low birth weight is associated with increased rates of type 2 diabetes, hypertension and cardiovascular disease [1, 3, 4].

Given the evidence, it is surprising that cardiovascular or metabolic outcomes have, to date, never been investigated in studies following up children born after assisted reproduction. IVF and ICSI are firmly established in the treatment of subfertile couples; in Europe, in 2001, 2-3% of all deliveries pregnancy had been achieved using these techniques. To date, a total of three million children have been conceived by assisted reproduction [82]. Follow up studies of children born after assisted reproduction should include cardiovascular outcomes, including blood pressure, glucose tolerance and lipid concentrations. In the future, aspects of assisted reproductive techniques, including the duration of in vitro culture as well as media composition, may need to be adapted in order to limit cardiovascular risk for generations to come.

Conclusion

The findings from the Dutch famine study highlight the fact that the maternal diet during the embryo's early development is crucial in determining later cardiovascular disease risk. Further research is needed before evidence based dietary advice can be developed for preconception counseling. The long term consequences for the cardiovascular risk of the offspring of mothers who suffered severe nausea and vomiting need to be studied. The management of severe morning sickness may need to focus on nutritional support, in order to limit cardiovascular risk in the offspring. Assisted reproduction in animal husbandry has metabolic and endocrine repercussions for the offspring. These risks need to be addressed in the human situation. Women who became pregnant after in vitro fertilization may have offspring with increased cardiovascular risk, which may be prevented by appropriate early embryo handling.

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Weighing in at one of the Red Cross 'Hunger Clinics'. An extra ration was provided to those who were >25% underweight

(Photo NIOD)

Summary

Cardiovascular disease (CVD) presents a major burden to public health in the Western world, and is an increasing health threat in developing countries. Despite the identification of adult risk factors, the primary prevention of known cardiovascular risk factors is proving to be a challenge.

A large number of epidemiological studies have established the link between low birth weight and cardiovascular morbidity and mortality [1]. Birth weight is a composite marker of placental, fetal and maternal factors. Poor maternal nutrition during gestation is likely to be one of the key determinants of the effects of low birth weight on the offspring's poor cardiovascular health [2]. The effect size associated with maternal dietary restriction is striking: the life-span of rats whose mothers had received low protein rations during pregnancy was reduced by 25% [3].

Ethical and practical considerations limit the scope of investigation of dietary restriction during gestation in human populations. The Dutch famine was a 5-month period of extreme food restriction during the last year of World War II. Rations were cut to a quarter of pre-war levels. The circumstances created by the famine closely mimic the experimental conditions in animal studies, thus offering us the unique opportunity of studying the effects of a discrete period of extreme maternal undernutrition during different periods of gestation on the offspring's health in later life.

The Dutch famine study has been the first study to establish, in a human population, that maternal undernutrition during gestation can have detrimental effects on cardiovascular risk in the offspring (reviewed in *Chapter 2*). Impaired glucose tolerance [4], an atherogenic lipid profile [5], altered clotting [6], obesity [7] and coronary artery disease (CHD) are all more common among people who were in utero during the Dutch famine. This unique study has also illustrated the model of 'critical periods' [8] - during which organ systems undergo fast growth and development - is also likely to apply to human prenatal development.

Chapter 3 describes that coronary artery disease rates among people conceived in famine are double those among unexposed people, and that, interestingly, the onset of symptoms occurs at a younger age. This may imply that famine exposure in utero leads to accelerated ageing, as has been indicated by animal studies. Alterations in vascular properties, including alterations in cardiovascular control mechanisms, decreased vascular compliance, and an increased propensity for atherosclerosis may also contribute to the development of coronary artery disease among individuals who were exposed to famine in utero.

In order to assess whether the increase in coronary heart disease after prenatal famine is mediated by enhanced stress response, for which there is evidence from animal studies, the participating cohort members completed a battery of three psychological stress tests *(Chapter 4)*. Although baseline blood pressure values were similar, the blood pressure rise in response to stress was higher among people conceived in famine com-

pared to unexposed people. Alterations in cardiovascular control mechanisms induced by famine exposure in utero may underlie this finding.

We found no evidence in support of the hypothesis that decreased arterial compliance contributes to increased cardiovascular risk after prenatal famine exposure *(Chapter 5)*. Our findings suggest that small maternal size prior to pregnancy, not poor maternal diet during gestation, programs decreased arterial compliance in the adult offspring by affecting vessel size rather than vessel wall stiffness.

We hypothesized that the increased coronary heart morbidity among people exposed to famine during early gestation would be evident in increased intima media thickness *(Chapter 6)*. In a B-mode ultrasound study of the carotid and femoral arteries, however, we found evidence to the contrary: famine exposure during any period of gestation is associated with a thinner intima media complex. The association, which was of a similar magnitude as the difference in intima media thickness between smokers and non-smokers, was statistically significant for the carotid segment. The fact that they had a thinner intima media complex did, however, not protect people exposed to famine in utero from developing coronary artery disease.

Chapter 7 describes that exposure to famine in mid gestation leads to a 3-fold increase in the prevalence of microalbuminuria, independent of cardiovascular risk factors including hypertension and diabetes. This suggests that mid gestation, during which nephron number increases rapidly [9], is the critical period in determining total nephron endowment at birth. Possibly, people exposed to famine during mid gestation may run a higher risk of renal disease in later life. This hypothesis is supported by the fact that creatinine clearance was also lower among people exposed to famine in mid gestation.

Chapter 8 shows that the effects of prenatal famine on health in later life are not limited to cardiovascular and metabolic disease. This chapter provides the first evidence for an increased incidence of breast cancer after prenatal famine exposure, although this is based on small numbers of cases (n=15). Women exposed to famine, on average, had more children at a younger age and were less likely to remain childless than unexposed women. Women exposed in mid gestation were older at menopause. The fact that the increased breast cancer incidence is paralleled by alterations in markers of reproductive function may imply that alterations in hypothalamic-pituitary-gonad function or ovarian programming may be involved in the pathophysiology of breast cancer after prenatal famine.

Although prenatal exposure to famine has marked effects on cardiovascular and metabolic morbidity, there was no increase in adult all-cause or cardiovascular mortality associated with famine exposure in utero *(Chapter 9)*. This discrepancy may be explained by the fact that the cohort is still relatively young; the mean age at follow-up was 57. Future follow-up in the cohort should resolve whether prenatal famine exposure leads to increased mortality. The study also tells us that it is unlikely that selective mortality

has introduced a large degree of bias into the findings from the clinical study of which the results are described in this thesis.

The findings from the Dutch famine study highlight the fact that the composition of the mother's diet during early embryo development is crucial in determining later cardiovascular disease risk. *Chapter 10* discusses the implications of these findings for future research. At present, antenatal care starts after placentation and organogenesis have been completed. This precludes advice aimed at improving lifestyle and maternal nutrition before conception and in early gestation, which may benefit the offspring's cardiovascular health. However, further research is needed before evidence based dietary advice can be developed for preconception dietary counseling. In the first trimester many women suffer from pregnancy associated vomiting, which can be so severe that it leads to maternal undernutrition. The management of hyperemesis gravidarum may thus need to focus on nutritional support, in order to limit cardiovascular risk in the offspring. Many factors affect the composition of the early embryo's environment, and its subsequent cardiovascular risk. In vitro techniques may produce an early environment which differs significantly from the in vivo situation. Women who became pregnant after in vitro fertilization may therefore have offspring with increased cardiovascular risk, which may be prevented by appropriate early embryo handling.

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Maternity corset, used for the illegal distribution of ration coupons (From the collection of the Dutch Resistance Museum, Amsterdam)

Samenvatting

Hart- en vaatziekten veroorzaken een grote ziektelast in de in de westerse wereld, en vormen een snel groeiend probleem in ontwikkelingslanden. Ondanks de identificatie van risicofactoren, blijkt de primaire preventie van hart- en vaatziekten moeilijk.

Een groot aantal epidemiologische studies heeft aangetoond dat mensen met een laag geboortegewicht later vaker hart- en vaatziekten krijgen. Foetale, maternale en placentaire kenmerken dragen elk bij aan het geboortegewicht. Sub-optimale voeding van de moeder tijdens de zwangerschap speelt waarschijnlijk een sleutelrol in het verband tussen laag geboortegewicht en het optreden van hart-en vaatziekten in het nageslacht. De grootte van de effecten van maternale voeding zijn frappant: het nageslacht van ratten, die tijdens de zwangerschap eiwit-arme voeding hadden gekregen, leefde 25% korter dan dat van ratten die normaal voedsel hadden gekregen.

Naar de rol van de moeder's voeding tijdens de zwangerschap is, uit ethische en praktische overwegingen, bij de mens nog weinig onderzoek gedaan. In de laatste vijf maanden van de Tweede Wereldoorlog speelde zich in de grote steden in het westen van Nederland een humanitair drama af: de Hongerwinter. De rantsoenen daalden naar het niveau van slechts een kwart van die van voor het begin van de oorlog. De omstandigheden tijdens de Hongerwinter bieden aanknopingspunten voor het bestuderen van de rol van extreme maternale ondervoeding in de gezondheid van het kind op latere leeftijd.

Het Hongerwinter Onderzoek heeft als eerste kunnen aantonen dat ondervoeding van de moeder tijdens de zwangerschap het cardiovasculaire risicoprofiel van het nageslacht negatief beinvloedt. Mensen die voor de geboorte werden blootgesteld aan de Hongerwinter hebben een verminderde glucose tolerantie, een atherogener lipidenprofiel, een veranderde stolling, meer obesitas, en lijden vaker aan coronaire hartziekte dan mensen die niet aan de Hongerwinter werden blootgesteld. *Hoofdstuk 2* geeft een overzicht van deze bevindingen. Deze unieke studie heeft ook laten zien, dat het model van 'critical periods'- de in dierexperimenten beschreven periodes waarin, door versnelde ontwikkeling van orgaansytemen, meer vatbaarheid bestaat voor de gevolgen van een tekort aan voedingsstoffen - waarschijnlijk ook van toepassing is op de mens.

Hoofdstuk 3 laat zien dat coronaire hartziekten twee keer zo vaak voorkwamen onder mensen die werden verwekt in de Hongerwinter, maar ook dat klachten zich gemiddeld al op jongere leeftijd openbaarden. Versnelde veroudering, zoals ook aangetoond in dierexperimentele modellen, zou hieraan ten grondslag kunnen liggen. Ook zouden veranderingen in cardiovasculaire controle mechanismen, toegenomen vaatstijfheid of een vergrote neiging tot atherosclerose bijgedragen kunnen hebben aan het versneld optreden van hart- en vaatziekten bij mensen die voor de geboorte aan de Hongerwinter waren blootgesteld.

Een verhoogde reactie op stress zou ook een rol kunnen spelen in de verklaring van het feit, dat mensen die werden verwekt tijdens de Hongerwinter meer hart- en vaatziekten krijgen. Om dit te bestuderen voerden alle deelnemers uit het Hongerwinter cohort een serie van drie stresstesten uit, terwijl hun bloeddruk continue gemeten werd.

De uitgangsbloeddrukwaarden waren vergelijkbaar, maar tijdens stress lieten degenen die verwekt waren in de Hongerwinter een sterkere stijging zien (*Hoofdstuk 4*). Mogelijk treden er permanente veranderingen op in de cardiovasculaire controle mechanismen na Hongerwinter blootstelling in utero.

Er werden geen aanwijzingen gevonden die erop wezen dat verhoogde vaatstijfheid een rol speelt bij het ontstaan van hart- en vaatziekten na prenatale blootstelling aan de Hongerwinter (*Hoofdstuk 5*). De bevindingen wijzen erop, dat een moeder die licht en klein is (alvorens zij zwanger wordt), ongeacht haar voeding tijdens de zwangerschap, nageslacht krijgt met dunnere vaten en een verminderde compliance-overigens zonder dat de vaatwand stijver is.

Aangezien mensen die werden verwekt tijdens de Hongerwinter vaker hart- en vaatziekten krijgen, verwachtten wij dat deze mensen een dikker intima-media complex (IMT) zouden hebben. Wij konden echter vasttstellen de echografisch gemeten IMT van de arteria carotis en arteria femoralis juist was afgenomen onder mensen die voor de geboorte blootgesteld waren aan de Hongerwinter (*Hoofdstuk 6*). Dit verband, dat overigens qua grootte vergelijkbaar was met het IMT verschil tussen rokers en niet rokers, was statistisch significant voor de arteria carotis. Ondanks een dunnere IMT, hadden diegenen die waren blootgesteld aan prenatale ondervoeding geen verminderde kans op coronaire hartziekte.

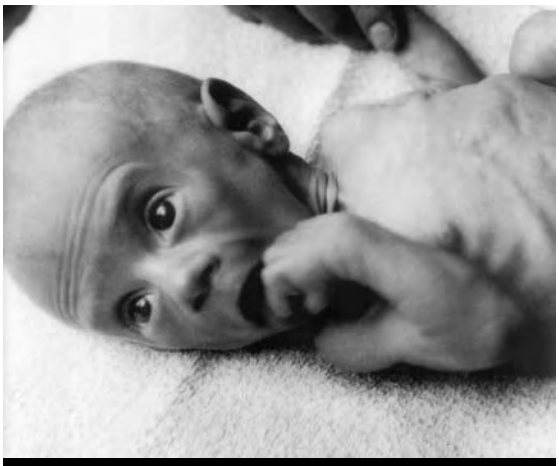
In *Hoofdstuk 7* wordt beschreven dat blootstelling aan de Hongerwinter in het midden van de zwangerschap leidt tot een drie keer grotere kans op het optreden van microalbuminurie. Dit werd niet verklaard door het vaker voorkomen van hypertensie, diabetes of andere cardiovasculaire risicofactoren. Deze bevinding suggereert dat het midden van de zwangerschap - tevens de periode waarin het aantal nefronen zeer snel toeneemt - de zogenaamde 'critical period' zou kunnen zijn waarin het totale aantal nefronen waarmee een individu is uitgerust, wordt bepaald. Dit zou kunnen betekenen dat mensen die in het midden van de zwangerschap aan de Hongerwinter werden blootgesteld een grotere kans hebben op het ontwikkelen van nierziekten op latere leeftijd. Deze hypohese wordt gesteund door het feit dat de creatinine klaring ook lager was onder mensen die in het midden van de zwangerschap aan de Hongerwinter werden blootgesteld.

Hoofdstuk 8 laat zien dat de late effecten van maternale ondervoeding tijdens de zwangerschap zich niet beperken tot cardiovasculaire en metabole ziekten. Ook lijkt er meer borstkanker voor te komen onder vrouwen die tijdens de Hongerwinter in utero waren. Er moet wel worden aangetekend dat deze bevinding gebaseerd is op een klein aantal borstkankerpatiënten (n=15). Opvallend was ook dat vrouwen die voor de geboorte aan maternale ondervoeding waren blootgesteld, zelf later al op jongere leeftijd meer kinderen kregen. Minder van hen bleven kinderloos. Vrouwen die in het midden van de zwangerschap aan de Hongerwinter werden blootgesteld, kwamen zelf pas op latere leeftijd in de menopauze. Het feit dat er naast een verhoogde borstkanker incidentie ook veranderingen optreden in reproductieve parameters onder vrouwen die

voor de geboorte aan maternale ondervoeding werden blootgesteld, zou erop kunnen duiden dat veranderingen in de hypothalamus-schildklier-gonade as functie of ovariële programmering een rol zou kunnen spelen in de oorsprong van borstkanker na prenatale bloostelling aan de Hongerwinter.

Ondanks het feit dat blootstelling aan de Hongerwinter voor de geboorte leidde tot een toename in cardiovasculaire en metabole risicofactoren, was er geen toename in algehele of in de ziektespecifieke sterfte op volwassen leeftijd waarneembaar (*Hoofdstuk 9*). Mogelijk wordt deze discrepantie verklaard uit het feit dat het Hongerwinter cohort nog relatief jong was ten tijde van de studie: 57 jaar. Vervolgstudies van dit cohort zullen in de toekomst uitwijzen of Hongerwinter blootstelling voor de geboorte daadwerkelijk leidt to een toename in cardiovasculaire sterfte. We kunnen overigens ook concluderen dat het onwaarschijnlijk is dat bias, door selectieve sterfte van Hongerwinter blootgestelde mensen, heeft geleid tot grote vertekening van de in dit proefschrift gepresenteerde onderzoeksresultaten.

De bevindingen van het Hongerwinter Onderzoek benadrukken het feit dat de samenstelling van de voeding van de moeder tijdens de vroege embyonale ontwikkeling cruciaal is in het bepalen van het latere cardiovasculaire risico. *Hoofdstuk 10* bespreekt de implicaties van de bevindingen voor toekomstig onderzoek. Op het moment begint de standaard prenatale zorg pas na voltooïng van de placentatie en organogenese. Voedingsadvies aan zwangeren, dat erop gericht is juist in de periode rond de conceptie en tijdens het eerste trimester de voedingstoestand te optimaliseren, met het oogmerk op het verminderen van het cardiovasculaire risico in het nageslacht, kan niet worden gegeven door de late aanvang van de prenatale zorg. Op het moment is er echter nog onvoldoende evidence voorhanden om een goed onderbouwd preconceptioneel voedingsadvies te formuleren. In het eerste trimester heeft een aanzienlijk deel van de zwangeren te maken met zwangerschaps gerelateerde misselijkheid en braken, die in sommige gevallen dusdanig ernstige vormen kan aannemen dat men kan spreken van maternale ondervoeding. De behandeling van hyperemesis gravidarum zou zich in de toekomst mogelijk meer moeten richten op het supplementeren van voeding (bijvoorbeeld in de vorm van sondevoeding), met als doel het verkleinen van het cardiovasculaire risico van het nageslacht. De samenstelling van de omgeving van het embryo, en dientengevolge ook diens toekomstige cardiovasculaire risico, is onderhevig aan allerlei invloeden. De samenstelling van de omgeving tijdens in vitro fertilizatie (IVF) procedures verschillen significant van de in vivo situatie. Vrouwen die zwanger worden via IVF procedures krijgen dus mogelijk nageslacht met een verhoogd cardiovasculair risico, welk te moduleren zou zijn met aangepaste IVF methoden.



Baby boy born during the Dutch Famine (Photo May 1945, NIOD)

List of publication

Publication list

S.R. de Rooij, R.C Painter, T.J. Roseboom
"The effects of prenatal exposure to undernutrition on glucose and insulin metabolism in later life"
in press, *Current Opinion in Endocrinology and Diabetes*, 2006

R.C. Painter, S.R. de Rooij, P.M.M. Bossuyt, C. Osmond, D.J.P Barker, O.P. Bleker,T.J. Roseboom
"A possible link between prenatal exposure to the Dutch famine and breast cancer-a preliminary study"
in press, *Am J Human Biology*, 2006

S.R. de Rooij, R.C. Painter, P.M.M. Bossuyt, E. de Groot, C. Osmond, D.I. Phillips, O.P. Bleker, T.J. Roseboom
"Cortisol response during psychological stress in adults after prenatal exposure to famine"
in press, *Psychoneuroendocrinology*, 2006

R.C. Painter, S.R. de Rooij, P.M.M. Bossuyt, D.I. Phillips, C. Osmond, D.J.P Barker, O.P. Bleker, T.J. Roseboom
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submitted

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"The metabolic syndrome in adults after prenatal exposure to famine"
submitted

Women peeling flower bulbs. There were many recipes available during the Dutch Famine, which used tulip bulbs as a cooking ingredient (Photo NIOD)

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When I joined the Dutch Famine Study in 2002, four years seemed like an awfully long time for a clinician to be carrying out scientific research - an activity that, as I glanced around the Department of Clinical Epidemiology and Biostatistics, seemed to involve a lot of gazing at computer screens and muttering strange statistical phrases. There are many reasons why, four years later, I have proven myself wrong. There is so much more to find out about this fascinating topic and so many other roads to explore.

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Rebecca Painter was born at Sydney's Crown's Street Women's Hospital on the 21st of May 1974 with the modest birth weight of 6 lb 10¼ oz (3012 g). After a few short years in Sydney's Erskineville, the family Painter (now having been completed with the arrival of Tim) moved to Edinburgh, Scotland. During one of the most severe winters in the history of post war Europe (the coldest January on record; snow continued until May), the family moved to Heemstede (the Netherlands) in January of 1979.

After graduating from high school (the Stedelijk Gymnasium, Haarlem), having been discouraged from pursuing a career in medicine by her father, she explored the options in science. After one year of Biomedical Science at Leiden University, however, she decided the laboratory was not for her. A gap year's travel and many odd jobs later, she commenced Medicine at the University of Amsterdam in 1994. During her studies, she held teaching jobs at the Physiology department, collected data for the 'Dyspepsia in general practice study', was involved in the National Growth Study (TNO), and was a genetic field worker at the Foundation for the Identification of Familial Hypercholesterolemia (StOEH). She and her (Austrian) partner Herwig also ran an alpine cheese and dairy farm on the Austrian/Italian border during the summer of 1995. In 1998, Rebecca completed her master's thesis titled '*The significance of the Brenner-Barker hypothesis in the development of renal disease in the Australian Aboriginal population*' at Professor Wendy Hoy's Renal Unit in Darwin, Australia. After completing her clinical internships, including 6 months at Turiani Mission Hospital, Tanzania, Rebecca gained her MD in 2001.

In 2002, after having held a residency in Obstetrics and Gynecology at Almere's Flevoziekenhuis, Rebecca joined the Dutch Famine Study. In 2007 she will start training in Obstetrics and Gynecology.

Despite the adverse climate, Rebecca has persisted in playing field hockey (HC Bloemendaal). Rebecca is married to Herwig Bachmann. They have two sons: Jacob (3 years) and Samuel (10 months).