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Famine in childhood and post-menopausal coronary artery calcification

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ABSTRACT

Objective: To assess if childhood famine exposure does have an effect on coronary calcium deposition and, secondarily, to look at its effect on cardiac valve and aortic calcifications.

Design: a retrospective cohort.

Setting: community.

Patients: 286 postmenopausal women with individual measurements of World War II famine exposure during childhood in the Netherlands.

Intervention/exposure: famine exposure during childhood.

Main outcome measures: coronary artery calcifications measured by CT scan and scored using the Agatston method; calcifications of the aorta and valves (mitral and/or aortic) measured semi-quantitatively. Logistic regression was used with coronary Agatston score of >100 or ≤100, valve or aortic calcifications as dependent variable and an indicator for famine exposure as independent variable. These models were also used for confounder adjustment and stratification based on age groups of 0-9 and 10-17 years old.

Results: In overall analysis, no statistically significant association was found between severe famine exposure in childhood and a high coronary calcium score (OR 1.80, 95%CI 0.87-3.78). However, when looking at specific risk periods, severe famine exposure during adolescence was related to a 3.5 fold higher risk (OR 3.47, 95%CI 1.00-12.07) for a high coronary calcium score than unexposed women, also after confounder adjustment. No statistically significant association was found between childhood famine and valve or aortic calcification (OR 1.66, 95%CI 0.69-4.10).

Conclusion: Famine exposure in childhood, especially during adolescence, seems to be associated with higher risk of coronary artery calcification in late adulthood, whereas its association with cardiac valve or aortic calcification is less clear.

ARTICLE SUMMARY

Article focus

- The association between famine exposure during adolescence and clinically manifest cardiovascular disease has been shown previously.
- We assessed whether childhood famine is associated with coronary calcium deposition as one of the possible underlying mechanisms.

Key messages

- An association between famine in adolescence and increased risk of heavier coronary calcification in post-menopausal women is shown in this study.
- Our findings suggest that the critical period determining future development of cardiovascular pathology and diseases may extend beyond fetal and infancy, emphasizing the importance of maintaining balanced nutrition throughout the developmental years.

Strengths and limitations of this study

- Despite limited statistical power, our study is unique for the combined availability of individual famine exposure data and thoracic CT images, and provides insight into the mechanism of the reported association between famine in adolescence and cardiovascular events.

INTRODUCTION

Cardiovascular diseases remain a leading cause of morbidity and mortality. Globally, ischemic heart and cerebrovascular diseases are top causes of death and it is projected that this will not change in the next 20 years, resulting in 20 million deaths per year in 2030. (1) Despite treatment advances such diseases still have a significant impact on quality of life (2) and cause great economic burden due to their chronic nature, so that effective prevention is essential.

Accumulating evidence suggests that many chronic diseases originate from particular events in early life. Since Barker first proposed the developmental origins of health and diseases (DOHAD) hypothesis,(3, 4) many studies have indicated that adverse influences, such as undernutrition, during human growth and development might result in permanent physiological and metabolic alterations. (5-7) Such alterations are suggested to benefit short-term survival but at the expense of increased risk of later chronic diseases. (8) However, many questions remain on what the exact critical periods are, (9) and specifically whether they extend beyond fetal life and infancy into childhood and adolescence.

Although studies on fetal and infancy periods are numerous, there is little data about the effect of nutrition disturbances in childhood and adolescence on cardiovascular risk. Moreover, most studies evaluated postnatal disturbances as a consequence of disturbances during prenatal development. As suggested by several large cohorts, (10-13) growth patterns after birth seem to be associated with a substantial increase in risk for cardiovascular and metabolic disorders.

Undernutrition in childhood and adolescence may have chronic disease consequences. Studies on the Dutch 1944-45 famine in World War II showed that severe famine exposure in adolescence is associated with a 30% increased risk of coronary heart diseases(14) and a 4-5 fold increased risk of diabetes mellitus and/or peripheral arterial disease in older females. (15) Men exposed to severe starvation around puberty were reported to have a 40-60% increased risk of acute myocardial infarction and stroke. (16) However, despite circumstantial evidence showing that childhood famine may lead to a similar ‘catch up fat phenotype’ as underweight newborns, (17) the mechanism by which food deprivation in these periods increases cardiovascular disease risk remains obscure. Since coronary artery calcium deposition has emerged as a strong predictor for cardiovascular diseases (18, 19) and very likely represents pathologic alterations of the vessel wall underlying clinically manifest coronary disease, (14) we assessed whether childhood famine is associated with coronary calcium deposition. As findings implicating the association between aortic/mitral

valve and aortic calcification with future cardiovascular events are also emerging, (20-22) we secondarily looked at whether undernutrition in childhood induces extra-coronary (valve and aortic) calcification.

SUBJECTS AND METHODS

Study population

Our study was a subset of a larger Dutch cohort called the Prospect-EPIC, part of the European Prospective Investigation into Cancer and Nutrition (EPIC) as described elsewhere. (24) Prospect-EPIC enrolled 17,357 healthy breast-cancer screenees, aged 49 to 70 years, living in Utrecht and surrounding areas between 1993 and 1997.

Our subset comprised 573 post-menopausal women, in whom associations between metabolic and reproductive factors and coronary artery calcium were previously evaluated.(23) For this study, pre-menopausal women were excluded (n=1309). After further selection (Figure 1), we obtained coronary CT scans in 573 women. We further excluded women born after or aged 18 years or over during the famine (n = 64), residing outside occupied Netherlands during the famine (n = 48), unavailable hunger score (n = 170), and non-consenters with follow-up (n = 5), leaving 286 women for our analyses. We collected data from October 2002 until December 2004.

Informed consent was obtained from all women before study inclusion. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center Utrecht.

Famine exposure

We employed a general questionnaire to evaluate to what degree women had been exposed to famine. As written in detail elsewhere, (25) the Dutch famine occurred in winter 1944-1945 in World War II when banned food transport dramatically reduced food supplies in the west of the Netherlands, with official daily rations dropping to 400 to 800 kcal. After 6 months of starvation, the Netherlands was liberated, ending the famine abruptly.

The questionnaire inquired about place of residence and experiences of hunger and weight loss during the Dutch famine. The latter two questions had the answer categories: 'hardly', 'little', or 'very much'. Women who had answered 'not applicable' and 'I don't know' to any of the two questions were excluded. Famine exposure was then categorized into a three-point score: 'severely exposed' if a woman reported having been 'very much' exposed to both hunger and weight loss, 'unexposed' if 'hardly' exposed to either hunger or weight loss, and 'moderately exposed' for all other responses.

Outcome assessment

CT imaging

An unenhanced CT of the heart was performed using a 16-slice CT scanner (Mx8000 IDT 16; Philips Medical Systems, Best, The Netherlands). During a single breath hold, a prospectively ECG-triggered ('step-and-shoot') CT scan was performed. The scan ranged from the tracheal bifurcation to below the apex of the heart. Scan parameters were 16x1.5-mm collimation, 205-mm field of view, 0.42-s rotation time, 0.28-s scan time per table position, 120 kVp, and 40 to 70 mAs (patient weight -70 kg: 40 mAs; 70 to 90 kg: 55 mAs; -90 kg: 70 mAs). Scan duration was approximately 10 s, depending on heart rate and patient size.

Measurement of calcifications

After the CT scan acquisition, CAC was quantified using Agatston's method (26) on the 1.5 mm slices in a separate workstation, with software for calcium scoring (Heartbest-CS, EBW; Philips Medical Systems) by a trained scan reader (AR). The continuous score was dichotomized at a cut off of 100 due to its highly skewed distribution and which is used clinically and scientifically to indicate cardiovascular risk. As published

elsewhere, (27) a cut off above 100 is often referred as moderate calcification while a cut off of 300 reflects heavy calcification. We also performed sensitivity analyses using different cut offs (0 and 300).

Reproducibility was assessed by having 199 scans read by two independent observers and by having 58 women undergo a second scan within 3 months. The inter-reader and inter-scan reproducibility was excellent with within-class correlation coefficients greater than 0.95. (28)

Valve and aortic calcification was quantified on the same CT dataset as used for CAC evaluation. We anatomically divided calcification of the heart valves into what was found in aortic valve leaflets (AVL) and mitral valve leaflets (MVL). The AVL and MVL calcification was graded as 0 (absent), 1 (mild, meaning only one leaflet affected), and 2 (severe, 2 or 3 leaflets affected) as previously described. (20) Due to small numbers, the grade was further dichotomized into absent, and present for mild to severe calcification. We also computed composite valve calcification, which was classified as 'present', if calcification was detected in any of the two valves, or 'absent' if there was no calcification found in either mitral or aortic valve.

For the thoracic aorta, we initially categorized the calcification into absent (none detected), mild (≤4 calcified foci or 1 calcification extending over ≥3 slices), moderate (>4 calcified foci or 2 calcifications extending over ≥3 slices), or severe (calcified aorta covering multiple segments). We later simplified this classification into none-to-mild ('low') and moderate-to-severe ('high') calcifications because of the small number of subjects, especially those with absent calcification. One single reader (PdJ) performed all CT evaluations while blinded for famine exposure status.

Measurement of potential confounders

We collected data on date of birth, cardiovascular disease history, and established risk factors for cardiovascular diseases using questionnaires and standard methods. Smoking was defined as current, past, and never.

We sampled fasting venous blood to measure plasma total cholesterol, triglycerides, and glucose using standard enzymatic procedures, high-density lipoprotein (HDL) cholesterol by direct method (inhibition, enzymatic). For measuring systolic and diastolic blood pressures, we used an automatic device (DINAMAP XL, Critikon; Johnson & Johnson, Tampa, FL) and took the higher value of either measurement on the right or left arm. Height and weight were measured, and the body mass index was calculated as weight divided by height squared (kg/m2), whereas body fat distribution was assessed by measuring waist-to-hip ratio. We defined diabetes mellitus as previously described for the previous study.(29)

Data analysis

Baseline characteristics were tabulated by famine exposure categories for descriptive purposes and confounding assessment, and differences tested using Anova, Chi-square or Kruskal Wallis tests.

We used (adjusted) logistic regression with coronary calcium (Agatston >100 yes/no) as dependent and an indicator for famine exposure levels as independent variable. Because age at famine exposure could be an effect modifier (14), we stratified the analysis on <10 years (pre-adolescence) and 10-18 years (adolescence). We a priori considered smoking a potential effect-modifier as it may increase sensitivity for environmental stressors, and stratified the analysis by smoking status. Adjustments were made for lipid profile, glucose level, waist-to-hip ratio for their known strong association with cardiovascular diseases and – possibly with malnutrition. Similar modeling was used to evaluate calcium deposition in the aorta and mitral/aortic valves. Results are expressed as odds ratios and 95% confidence intervals. All analyses were conducted using SPSS version 19.0 for Mac.

RESULTS

Table 1 shows baseline characteristics of study subjects. Although not statistically significant, subjects exposed to famine were slightly older, and had higher BMI, waist-to-hip ratio, as well as triglyceride levels than the unexposed.

The association between famine exposure and coronary calcium score is shown in Table 2. The overall analysis, both crude and adjusted, showed that severe famine almost doubled the risk for coronary calcium scores > 100 (OR 1.80, 95%CI 0.87-3.78), but not statistically significantly. After stratification for age at famine exposure, those severely exposed to famine as adolescents had higher risks (OR 3.47, 95%CI 1.00-12.07) for coronary calcium scores >100 than unexposed women, which remained significant after adjustment for several variables. Using different cut-off points of the Agatston score (0 and 300) led to similar results (data not shown).

Neither overall nor age-specific analyses indicated associations between famine exposure and calcification of mitral and/or aortic valves (Table 3). Table 4 shows no statistically significant association between famine exposure and aortic calcification, although famine in young children (between 0-9 years of age) may later increase aortic calcium deposition (OR 2.64; 95%CI 0.69-4.10, P = 0.10).

Stratification based on smoking status did not modify the overall results at any calcification site statistically significantly (data not shown).

DISCUSSION

Our study shows that severe famine exposure during childhood, especially in adolescence, is associated with a 3-5 fold increase in the risk of having at least moderate coronary artery calcification in later life compared to those unexposed. Although not statistically significant, famine exposure tended to also increase the risk of moderate-to-severe aortic calcification by approximately 60%, which was more pronounced in subjects severely exposed to famine before the age of 9 years. Famine exposure was not related to mitral or aortic valve calcification.

To appreciate these findings some aspects of the study need to be addressed. Although unique for the combined availability of individual famine exposure data and thoracic CT images, the sample size was small, limiting the statistical power. Also the population is restricted to post-menopausal women and generalizability to men or younger women remains speculative. However, our findings are in line with a previous report on the association between famine exposure in adolescence and late adulthood cardiovascular events, (14) providing further insight into the possible underlying mechanism.

Given previous findings of the possible association between pre-menopausal metabolic factors and both coronary and aortic calcification, (30, 31) there would probably some concerns that they were not accounted in our analysis. However, prior findings also indicated that pre-menopausal and postmenopausal metabolic factors are strongly correlated, given the explainable nature of the effect of post-menopausal factors on calcification by their pre-menopausal counterparts. (30) Therefore, we believe that adjusting for both factors is not necessary and even would be misleading. Moreover, by adjusting for post-menopausal factors, we indirectly take the pre-menopausal and the actual metabolic states at the time of calcium quantification into account. As previously shown, some traditional risk factors, such as lipid profiles,(32) are exaggerated by menopause that accounting for these factors at the postmenopausal stage is important. However, age at menopause itself seems not to be associated with coronary calcification.(23)

The choice of Agatston score of 100 as a somehow arbitrary cut off to rank the severity of coronary calcification might be a limitation of our study. However, despite ongoing controversies on the reliability of a particular cut-off to truly differentiate the risks of having cardiovascular diseases, (33, 34) we used the most commonly applied cut off. A score above 100 has repeatedly been reported to be associated with an approximately 3-7 fold increased risk of coronary events or cardiovascular death, (35, 36) therefore our

choice of cut-off seems justifiable. Moreover, we found that using different cut offs did not led to different results.

A major strength of our study is that we were able to use the unique circumstances of the Dutch famine. As previously described elsewhere, (25) the Dutch famine occurred within an approximately 6-month period between the end of 1944 and mid 1945, when food supplies in the Netherlands were acutely and dramatically dropped to 400-800 kcal/day due to food transport bans and severe winter. After that period, the situation quickly improved, ending the famine abruptly. This allowed for the investigation of the effects of post-natal undernutrition on health as a 'natural experiment', rather than purely observational. Moreover, this allowed for studying the very well documented acute nature of the undernutrition.

Another strength of our study is that we used individual data on famine exposure rather than grouping populations according to their place of residence or time, such that we used more precise exposure measurement. As described previously, our exposure classification agrees with rationing practices at that time, in which individual amount of calories was based on age. Young children (1-3 years) were relatively protected from the famine and received about 50%, whereas adults received about 25% of the distributed amount of calories at the start of the famine. (25) These historical facts are reflected by our data, such that the older the women were at the start of the famine, the higher the proportion of women who reported of having been exposed to famine. This finding may be considered in support of the quality of our exposure data. Although individual data may have some drawbacks related to its subjective nature and can result in misclassification, we believe that this, if anything, will have underestimated the observed effects. We had performed blinded and objective measurements of the calcium content using the CT scan, so that differential scan measurement by exposure knowledge is excluded as an explanation of our findings.

Coronary artery calcifications are thought to be a reflection of the burden of intima lesions; hence coronary atherosclerosis. (37) For aortic calcifications it is less clear that these always reflect atherosclerosis. (38) Most pathological studies found a substantial amount of these calcifications to be located in the tunica media and not the adventitia. Data even suggest that media calcifications develop earlier and more extensively in the aorta compared to intima calcifications. Also for valve calcifications, the biology is now thought to be different from atherosclerosis. Although valve calcification also may involve inflammatory factors, it is also a result of long-standing action of mechanical stress. Changes in valve tissue have been observed in persons who do not exhibit features of atherosclerosis, indicating that early stages of calcification of valve cusps involve different mechanisms from coronary artery calcification.

To our knowledge, there has been no previous study addressing the association between childhood famine exposure and vascular/valve calcifications in later life. Most studies linking malnutrition and coronary

or valve calcification were conducted on patients with end-stage renal disease. (39) Such patients are reported to experience a malnutrition-inflammation-atherosclerosis/calcification (MIAC) syndrome, which is a strong predictor for cardiovascular death. Although a full assessment of the mechanisms underlying the association between undernutrition and coronary artery or valve calcification is beyond the scope of our study, the findings in patients with renal diseases may suggest that a similar interaction between malnutrition, increased levels of pro-inflammatory cytokines, and calcification or atherosclerosis may play a role. (40, 41) Although it has been suggested that caloric restriction in adulthood and animals has a protective effect on atherosclerosis and metabolic syndromes, (43, 44) these findings are not readily translated to undernutrition in early life. This indicates that caloric restriction in different periods of life, may have different effects on atherosclerosis development as suggested by findings from two different studies on the Dutch famine that famine exposure during adolescence increased the risk for cardiovascular events, (14) whereas those occurred prenatally did not.(45)

Our findings do suggest that the critical period determining future development of cardiovascular pathology and diseases may extend beyond fetal and infancy. It also emphasizes that maintaining a balanced life, including a balanced nutrition, is important throughout growth and development. The importance of maintaining a balanced nutrition in early life as from the preconception phase has been quite established (46). For example, the Biafran famine study showed that fetal-infant undernutrition is associated with significantly increased risk of hypertension and impaired glucose tolerance in adulthood, (47) similar to what was found in the Chinese great famine study. (48) Although there has been a limited number of studies evaluating the effect of post-natal events per se on later life health, it seems that maintaining balanced nutrition in later childhood is also crucial, as suggested by the detrimental effects of weight fluctuation or 'yoyo' dieting in young adulthood on coronary heart disease and mortality. (17) Our study only addresses one of the possible mechanisms of how childhood famine exposure may lead to the development of atherosclerosis and cardiovascular diseases, while the biology and mechanics of atherosclerotic plaque formation are complex.

In conclusion, famine exposure in childhood, especially during adolescence, may be associated with higher risk of coronary artery calcification in late adulthood. However, there seems no clear association between childhood famine and calcifications of cardiac valves or the aorta.

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Table 1. Baseline characteristics of the study subjects based on famine exposure level

Variables	Famine exposure			P
	Unexposed n = 139	Moderate n = 103	Severe n = 44	
Age at famine, mean (SD)	7.6 (4.7)	8.9 (5.6)	8.7 (4.3)	0.10
Age category, n(%) through 9 years	98 (70.5)	64 (62.1)	29 (65.9)	0.39
10-18 years	41 (29.5)	39 (37.9)	15 (34.1)	
Age at CT scan	67.73 (4.97)	68.99 (5.41)	68.96 (4.71)	0.11
BMI (kg/m^2), mean (SD)	26.4 (4.4)	27.3 (4.6)	27.8 (4.6)	0.12
Waist-to-hip ratio, mean (SD)	0.84 (0.07)	0.86 (0.07)	0.85 (0.06)	0.06
Highest systolic BP (mmHg), mean (SD)	141.5 (20.4)	143.1 (23.4)	145.0 (21.8)	0.63
Highest diastolic BP (mmHg), mean (SD)	75.4 (9.6)	74.8 (9.6)	74.6 (8.2)	0.87
Cholesterol to HDL ratio, mean (SD)	4.5 (1.2)	4.7 (1.4)	4.8 (1.2)	0.43
Glucose level (mmol/L), mean (SD)	5.6 (1.0)	5.8 (1.2)	5.5 (0.5)	0.35
Triglyceride (mmol/L), median (range)	1.0 (0.5-3.8)	1.1 (0.3-3.7)	1.3 (0.5-3.2)	0.15*
Smoking, n(%) Currently	9 (6.5)	11 (10.7)	3 (6.8)	0.71
Former	57 (41.0)	45 (43.7)	18 (40.9)	
Never	75 (52.5)	47 (45.6)	23 (52.3)	
Pack-years smoked in ever/current smokers, median (range)	6.0 (0.05-94)	7.4 (0.05-73.5)	11.5 (0.03-53.8)	0.66
Metabolic syndrome, n(%)	15 (10.8)	18 (17.5)	3 (6.5)	0.14
Pack-years smoked, median (range)	0.0 (0.0-94)	0.3 (0.0-73.5)	0.0 (0.0-53.8)	0.57*

*Kruskal-Wallis test

Table 2. The effect of famine exposure on coronary artery calcification as reflected by Agatston score

Famine Exposure		Agatston score (n, %)		Crude			Model 1			Model 2		
		<100 n = 204	>100 n = 78	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	104 (51.0)	33 (42.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	72 (35.3)	29 (37.2)	1.27	0.71-2.27	0.42	1.08	0.58-1.99	0.81	1.00	0.53-1.92	0.99
	Severe	28 (13.7)	16 (20.5)	1.80	0.87-3.78	0.11	1.63	0.76-3.46	0.21	1.74	0.79-3.84	0.17
	P for trend				0.11			0.26			0.24	
Age 0-9 years	Unexposed	78 (51.3)	18 (48.6)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	51 (33.6)	13 (35.1)	1.11	0.50-2.45	0.81	1.08	0.48-2.40	0.86	0.92	0.39-2.18	0.84
	Severe	23 (15.1)	6 (16.2)	1.13	0.40-3.18	0.82	1.06	0.37-3.02	0.92	1.22	0.40-3.70	0.17
	P for trend				0.78			0.88			0.24	
Age 10-17 years	Unexposed	26 (50.0)	15 (36.6)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	21 (40.4)	16 (39.0)	1.32	0.53-3.28	0.55	1.07	0.41-2.80	0.89	1.00	0.53-1.92	0.99
	Severe	5 (9.6)	10 (24.4)	3.47	1.00-12.07	0.05	3.65	1.01-13.31	0.04	4.62	1.16-18.43	0.03
	P for trend				0.07			0.09			0.06	

Model 1: Adjusted for age and pack-years smoked

Model 2: Adjusted for age, pack-years smoked, BMI, glucose, triglyceride, waist-to-hip ratio, systolic BP, cholesterol-to-HDL ratio

OR = odds ratio, 95%CI = 95% confidence interval, ref = reference category

Table 3. The effect of famine exposure on aortic and/or mitral valve calcification

Famine Exposure		Valve calcification (n, %)		Crude			Model 1			Model 2		
		No n = 206	Yes n = 80	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	102 (49.5)	37 (46.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	71 (34.5)	32 (40.0)	1.24	0.71-2.18	0.45	1.04	0.57-1.88	0.90	0.98	0.53-1.81	0.94
	Severe	33 (16.0)	11 (13.8)	0.92	0.42-2.00	0.83	0.78	0.35-1.76	0.35	0.68	0.29-1.58	0.37
	P for trend				0.92			0.65			0.44	
Age 0-9 years	Unexposed	74 (50.0)	24 (55.8)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	51 (34.5)	13 (30.2)	0.79	0.37-1.69	0.54	0.72	0.32-1.58	0.41	0.67	0.30-1.54	0.35
	Severe	23 (15.5)	6 (14.0)	0.80	0.29-2.21	0.67	0.63	0.22-1.78	0.38	0.56	0.18-1.71	0.56
	P for trend				0.56			0.30			0.23	
Age 10-17 years	Unexposed	28 (48.3)	13 (35.1)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	20 (34.5)	19 (51.4)	2.05	0.82-5.08	0.12	1.66	0.64-4.30	0.99	1.65	0.52-4.65	0.34
	Severe	10 (17.2)	5 (13.5)	1.08	0.31-3.79	0.91	1.06	0.29-3.92	0.93	0.79	0.20-3.16	0.74
	P for trend				0.53			0.66			0.37	

Model 1: Adjusted for age and pack-years smoked

Model 2: Adjusted for age, pack-years smoked, BMI, glucose, triglyceride, waist-to-hip ratio, systolic BP, cholesterol-to-HDL ratio

OR = odds ratio, 95%CI = 95% confidence interval, ref = reference category

Table 4. The effect of famine exposure on aortic calcification

Famine Exposure		Aortic calcification* (n, %)		Crude			Model 1			Model 2		
		Low n = 209	High n = 77	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	108 (51.7)	31 (40.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	71 (34.0)	32 (41.6)	1.57	0.88-2.80	0.11	1.23	0.65-2.34	0.53	1.17	0.58-2.37	0.65
	Severe	30 (14.4)	14 (18.2)	1.63	0.77-3.44	0.20	1.39	0.61-3.15	0.43	1.66	0.69-4.10	0.26
	P for trend				0.12			0.39			0.28	
Age 0-9 years	Unexposed	85 (53.1)	13 (41.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	53 (33.1)	11 (35.5)	1.36	0.57-3.25	0.49	1.23	0.50-3.07	0.65	1.05	0.39-2.86	0.92
	Severe	22 (13.8)	7 (22.6)	2.08	0.74-5.84	0.16	1.68	0.57-4.91	0.35	2.64	0.82-8.52	0.10
	P for trend				0.17			0.35			0.16	
Age 10-17 years	Unexposed	23 (46.9)	18 (39.1)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	18 (36.7)	21 (45.7)	1.49	0.62-3.60	0.38	1.28	0.51-3.24	0.61	1.34	0.45-4.30	0.60
	Severe	8 (16.3)	7 (15.2)	1.12	0.34-3.67	0.85	1.07	0.31-3.73	0.92	0.91	0.21-3.96	0.90
	P for trend				0.65			0.79			0.92	

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Contributorship statement

The authors' responsibilities were as follows— NSI, SGE, and CSPMU: contributed to the study conceptualisation and design; YTS, AFMA, TJR, PDJ, AR, and SGE: were responsible for data collection; NSI: analysed the data and wrote the manuscript; and YTS, AFMA, TJR, PDJ, AR, DEG, CSPMU, and SGE: provided constructive feedback on each draft of the manuscript. All authors read and approved the final manuscript. None of the authors had any conflicts of interest to disclose.

Data sharing statement

Extra data is available by emailing the corresponding author (NSI).

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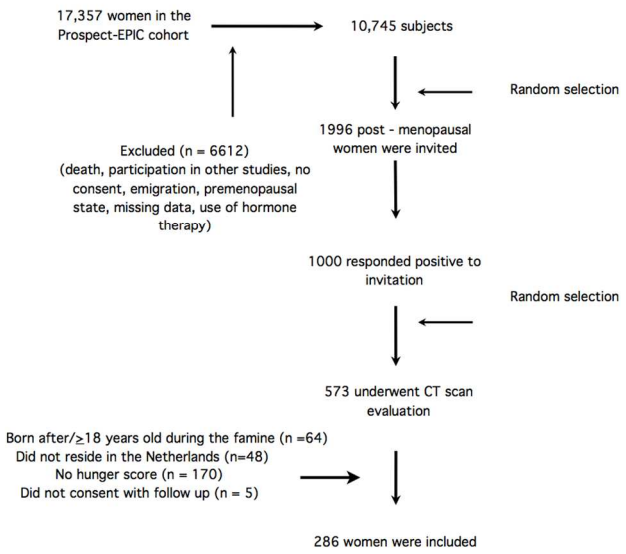
Figure 1. Subjects' recruitment flow



209x297mm (150 x 150 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

		Item No	Recommendation
Title and abstract	Y	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	Y	2	Explain the scientific background and rationale for the investigation being reported
Objectives	Y	3	State specific objectives, including any prespecified hypotheses
Methods			
Study design	Y	4	Present key elements of study design early in the paper
Setting	Y	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	Y	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
	N/A		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	Y	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	Y	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	Y	9	Describe any efforts to address potential sources of bias
Study size	Y	10	Explain how the study size was arrived at
Quantitative variables	Y	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	Y	12	(*a*) Describe all statistical methods, including those used to control for confounding
	Y		(*b*) Describe any methods used to examine subgroups and interactions
	Y		(*c*) Explain how missing data were addressed
	N/A		(*d*) If applicable, explain how loss to follow-up was addressed
	N/A		(*e*) Describe any sensitivity analyses
Results			
Participants	Y	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
	Y		(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	Y	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
	Y		(b) Indicate number of participants with missing data for each variable of

			interest
	N/A**		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	Y	15*	Report numbers of outcome events or summary measures over time
Main results	Y	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
	Y		(*b*) Report category boundaries when continuous variables were categorized
	N/A		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	Y	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	Y	18	Summarise key results with reference to study objectives
Limitations	Y	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	Y	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	Y	21	Discuss the generalisability (external validity) of the study results
Other information			
Funding	Y	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.
** Follow up time is reflected by the age of the patients.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Famine in childhood and post-menopausal coronary artery calcification

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Famine in childhood and post-menopausal coronary artery calcification

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ABSTRACT

Objective: To assess the effects of famine exposure during childhood on coronary calcium deposition and, secondarily, on cardiac valve and aortic calcifications.

Design: Retrospective cohort.

Setting: Community.

Patients: 286 postmenopausal women with individual measurements of famine exposure during childhood in the Netherlands during World War II.

Intervention/exposure: Famine exposure during childhood.

Main outcome measures: Coronary artery calcifications measured by CT scan and scored using the Agatston method; calcifications of the aorta and cardiac valves (mitral and/or aortic) measured semi-quantitatively. Logistic regression was used for coronary Agatston score of >100 or ≤100, valve or aortic calcifications as the dependent variable and an indicator for famine exposure as the independent variable. These models were also used for confounder adjustment and stratification based on age groups of 0-9 and 10-17 years.

Results: In the overall analysis, no statistically significant association was found between severe famine exposure in childhood and a high coronary calcium score (OR 1.80, 95%CI 0.87-3.78). However, when looking at specific risk periods, severe famine exposure during adolescence was related to a higher risk for a high coronary calcium score than non-exposure to famine, both in crude (OR 3.47, 95%CI 1.00-12.07) and adjusted analyses (OR 4.62, 95%CI 1.16-18.43). No statistically significant association was found between childhood famine exposure and valve or aortic calcification (OR 1.66, 95%CI 0.69-4.10).

Conclusion: Famine exposure in childhood, especially during adolescence, seems to be associated with a higher risk of coronary artery calcification in late adulthood. However, the association between childhood famine exposure and cardiac valve/aortic calcification is less clear.

ARTICLE SUMMARY

Article focus

- The association between famine exposure during adolescence and clinically manifested cardiovascular disease has been shown previously.
- We assessed for an association between famine in childhood and coronary calcium deposition, as a possible underlying mechanism of the previously shown association.

Key messages

- An association between famine in adolescence and increased risk of heavier coronary calcification in post-menopausal women is shown in this study.
- Our findings suggest that the critical period determining future development of cardiovascular pathology and disease may extend beyond the fetal and infant periods.

Strengths and limitations of this study

- Despite limited statistical power, our study is unique for the combined availability of individual famine exposure data and thoracic CT images. In addition, this study provides insight into the mechanism of the reported association between famine in adolescence and cardiovascular events.

INTRODUCTION

Cardiovascular diseases remain a leading cause of morbidity and mortality. Globally, ischemic heart and cerebrovascular diseases are major causes of death and it has been projected that this condition will not change in the next 20 years, resulting in 20 million deaths per year in 2030. [1] Despite advances in treatment, such diseases still have a significant impact on quality of life [2] and cause great economic burden. As such, effective prevention is essential.

Evidence suggests that many chronic diseases originate from particular events in early life. Since Barker first proposed the developmental origins of health and diseases (DOHAD) hypothesis, [3,4] many studies have indicated that adverse influences, such as undernutrition during growth and development, might result in permanent physiological and metabolic alterations. [5-7] Such alterations may benefit short-term survival, but at the expense of increased risk of chronic diseases later in life. [8] However, the exact critical periods have yet to be defined [9] as to whether they extend beyond fetal life and infancy into childhood and adolescence.

Although studies on the fetal and infant periods are numerous, there is little data on the effect of childhood and adolescent nutritional disturbances on cardiovascular risk. Moreover, most studies evaluated postnatal disturbances as a consequence of prenatal events. As suggested by several large cohort studies, [10-13] growth patterns after birth seem to be associated with a substantial increase in risk for cardiovascular and metabolic disorders.

Undernutrition during childhood and adolescence may have chronic disease consequences. Studies on the Dutch 1944-45 famine in World War II showed that severe famine exposure in adolescence was associated with a 30% increased risk of coronary heart diseases [14] and a 4-5-fold increased risk of diabetes mellitus and/or peripheral arterial disease in older females. [15] Men who experienced severe starvation around the time of puberty were reported to have a 40-60% increased risk of acute myocardial infarction and stroke.[16] However, despite circumstantial evidence showing that childhood famine may lead to a similar 'catch up fat phenotype' as underweight newborns, [17] the mechanism by which food deprivation in these periods increases cardiovascular disease risk remains obscure. Since coronary artery calcium deposition has emerged as a strong predictor for cardiovascular diseases [18,19] and likely represents pathologic alterations of the vessel wall underlying clinically manifested disease, [14] we investigated an association between childhood exposure to famine and coronary calcium deposition. As findings implicating the association between cardiac valve or aortic calcification and coronary cardiovascular events are also

emerging, [20-22] we also assessed for an association between childhood undernutrition and extra-coronary (valve and aortic) calcification.

SUBJECTS AND METHODS

Study population

Our study was a subset of a larger Dutch cohort, Prospect-EPIC, part of the European Prospective Investigation into Cancer and Nutrition (EPIC), as described elsewhere. [23] Prospect-EPIC enrolled 17,357 healthy 49-70 year old women who underwent breast-cancer screening and lived in Utrecht or the surrounding area between 1993 and 1997.

Our subset was comprised of 573 post-menopausal women, who had been evaluated for associations between metabolic and reproductive factors and coronary artery calcium. [24] For our study, pre-menopausal women were excluded (n=1309). After further selection (Figure 1), we obtained coronary CT scans of 573 women. We further excluded women who were born after or aged 18 years or over during the famine (n = 64), resided outside occupied Netherlands during the famine (n = 48), had unavailable hunger scores (n = 170), or did not consent to follow-up (n = 5), leaving 286 women for our analyses. Data was collected from October 2002 until December 2004.

Informed consent was obtained from all women before their inclusion to the study. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center of Utrecht.

Famine exposure

We employed a general questionnaire to evaluate the subjects' degree of exposure to famine. As detailed elsewhere,[25] the Dutch famine occurred in the winters of 1944-1945 during World War II when banned food transport dramatically reduced food supplies in the western Netherlands, with official daily rations dropping to 400 to 800 kcal. After 6 months of starvation conditions, the Netherlands was liberated, abruptly ending the famine.

The questionnaire was used to collect information on place of residence as well as experiences of hunger and weight loss during the Dutch famine. The latter two questions had answer categories of 'hardly,' 'little,' or 'very much.' Women who answered 'not applicable' or 'I don't know' to either of the two questions were excluded. Famine exposure was then categorized into a three-point score as follows: 'severely exposed' for women who reported having been 'very much' exposed to both hunger and weight loss, 'unexposed' for those who were 'hardly' exposed to either hunger or weight loss, or 'moderately exposed' for all other responses.

Outcome assessment

CT imaging

Unenhanced CT imaging of the heart was performed using a 16-slice CT scanner (Mx8000 IDT 16; Philips Medical Systems, Best, The Netherlands). During a single breath hold, a prospectively ECG-triggered ('step-and-shoot') CT scan was performed. The scan ranged from the tracheal bifurcation to below the apex of the heart. Scan parameters were 16x1.5-mm collimation, 205-mm field of view, 0.42-s rotation time, 0.28-s scan time per table position, 120 kVp, and 40 to 70 mAs (patient weight 70 kg: 40 mAs; 70 to 90 kg: 55 mAs; 90 kg: 70 mAs). Scan duration was approximately 10 s, depending on heart rate and patient size.

Measurement of calcifications

After CT scan acquisition, coronary artery calcification (CAC) was quantified using Agatston's method [26] on the 1.5 mm slices using software for calcium scoring (Heartbest-CS, EBW; Philips Medical Systems) by a trained scan reader (AR). The continuous score was dichotomized at a cut-off of 100 due to its highly skewed distribution. This cut-off value has been used clinically and scientifically to indicate cardiovascular

risk. As published elsewhere, [27] a cut-off above 100 is often referred to as moderate calcification, while a cut-off of 300 reflects heavy calcification. We also performed sensitivity analyses using different cut-off values (0 and 300).

We attempted to ensure reproducibility by having 199 scans read by two independent observers and by having 58 women undergo a second scan within 3 months. The inter-reader and inter-scan agreement was excellent with within-class correlation coefficients greater than 0.95. [28]

Valve and aortic calcification was quantified using the same CT dataset as the CAC evaluation. We divided calcification of the heart valves anatomically into aortic valve leaflet (AVL) and mitral valve leaflet (MVL) findings. The AVL and MVL calcification was graded as 0 (absent), 1 (mild, meaning only one leaflet affected), or 2 (severe, 2 or 3 leaflets affected), as previously described.[20] Due to small numbers of subjects, the grades were further dichotomized into absent, or and present for mild to severe calcification. We also computed assessed composite valve calcification, which was classified as 'present' if calcification was detected in either of the two valves, or 'absent' if no calcification was found in either the mitral or aortic valve.

For the thoracic aorta, we initially categorized the calcification into absent (none detected), mild (≤4 calcified foci or 1 calcification extending over ≥3 slices), moderate (>4 calcified foci or 2 calcifications extending over ≥3 slices), or severe (calcified aorta covering multiple segments). We later simplified this classification into none-to-mild ('low') and moderate-to-severe ('high') calcifications because of the small number of subjects, especially those without calcification. A single reader (PdJ) performed all CT evaluations while blinded for famine exposure status.

Measurement of potential confounders

We collected data on date of birth, cardiovascular disease history, and established risk factors for cardiovascular diseases using questionnaires. Smoking was defined as current, past, or never.

From fasting venous blood specimens, we measured plasma total cholesterol, triglycerides, and glucose using standard enzymatic procedures, as well as high-density lipoprotein (HDL) cholesterol by direct method (inhibition, enzymatic). Systolic and diastolic blood pressures were measured by an automatic device (DINAMAP XL, Critikon; Johnson & Johnson, Tampa, FL). We took the higher value of either the right or left arm measurement. Height and weight were also measured, and the body mass index was calculated as weight divided by height squared (kg/m2). Body fat distribution was assessed by measuring the waist-to-hip ratio. We defined diabetes mellitus as previously described. [29]

Data analysis

Baseline characteristics were tabulated into famine exposure categories for descriptive purposes and confounding assessment, and differences were tested using Anova, Chi-square or Kruskal-Wallis tests. We used (adjusted) logistic regression with coronary calcium (Agatston >100 yes/no) as the dependent variable, and an indicator for famine exposure levels as the independent variable. Because age at famine exposure could be an effect modifier[14] we stratified the analysis by age groups, 0-9 years (pre-adolescence) and 10-18 years (adolescence). Since smoking may be a potential effect-modifier as it may increase sensitivity for environmental stressors, we stratified the analysis by smoking status. Adjustments were made for lipid profile, glucose level, and waist-to-hip ratio for their known strong associations with cardiovascular diseases and possibly with malnutrition. Similar modeling was used to evaluate calcium deposition in the aorta and mitral/aortic valves. Results are expressed as odds ratios and 95% confidence intervals. All analyses were conducted using SPSS version 19.0 for Mac.

RESULTS

Table 1 shows the baseline characteristics of study subjects. Although not statistically significant, subjects exposed to famine were slightly older, and had higher BMI, waist-to-hip ratio, as well as triglyceride levels than the unexposed.

The association between famine exposure and coronary calcium score is shown in Table 2. The overall analysis, both crude and adjusted, showed that severe famine almost doubled the risk for coronary calcium scores > 100 (OR 1.80, 95%CI 0.87-3.78), but not in a statistically significant manner. After stratification for age at famine exposure, those exposed to severe famine as adolescents had higher risks (OR 3.47, 95%CI 1.00-12.07) for coronary calcium scores >100 than unexposed women, which remained significant after adjustment for several variables. Using different cut-off points of the Agatston score (0 and 300) led to similar results (data not shown).

Neither overall nor age-specific analyses indicated associations between famine exposure and calcification of mitral and/or aortic valves (Table 3). Table 4 shows no statistically significant association between famine exposure and aortic calcification, although famine in young children (between 0-9 years of age) may later increase aortic calcium deposition (OR 2.64; 95%CI 0.69-4.10, P = 0.10).

Stratification based on smoking status did not modify the overall results at any calcification site (data not shown).

DISCUSSION

Our study shows that severe famine exposure during childhood, especially in adolescence, is associated with a 3-5-fold risk increase to have at least moderate coronary artery calcification in later life compared to non-exposure to famine. Although not statistically significant, famine exposure also tended to increase the risk of moderate-to-severe aortic calcification by approximately 60%, which was more pronounced in severe exposure to famine before the age of 9 years. Famine exposure was not related to mitral or aortic valve calcification.

To appreciate these findings some study aspects need to be addressed. Although unique for the combined availability of individual famine exposure data and thoracic CT images, the sample size was relatively small, limiting the statistical power. It is possible that effects that would have been seen in a larger study were missed, such as the effect of famine on extra-coronary calcification. Nevertheless, we found an association between famine in adolescence and coronary artery calcification. Also, our study population was restricted to post-menopausal women, so that generalizing to men or to younger women remains speculative. However, our findings are in line with a previous report on the association between famine exposure in adolescence and late adulthood cardiovascular events, [14] providing further insight into the possible underlying mechanism.

Despite a possible association between pre-menopausal metabolic factors and both coronary and aortic calcifications, [30,31] we chose not to adjust our analysis for these factors. As pre-menopausal and postmenopausal metabolic factors are strongly correlated, [30] we believe that adjusting for both factors is not necessary and would even be misleading. By adjusting for post-menopausal factors, we indirectly took the pre-menopausal and the actual metabolic states at the time of calcium quantification into account. As previously shown, some traditional risk factors, such as lipid profiles, [32] are exaggerated by menopause, so that accounting for these factors at the postmenopausal stage is important. However, the age at menopause itself seems not to be associated with coronary calcification. [24]

The choice of Agatston score of 100 as an arbitrary cut-off to rank the severity of coronary calcification might be a limitation of our study. However, despite ongoing controversies on the reliability of a particular cut-off to truly differentiate the risks of having cardiovascular diseases, [33,34] we used the most commonly applied cut-off. A score above 100 has repeatedly been reported to be associated with an

approximately 3-7-fold increased risk of coronary events or cardiovascular death, [35,36] therefore, our choice of cut-off seems justifiable. Moreover, we found that using different cut-offs did not lead to different results.

A major strength of our study was the use of the unique circumstances of the Dutch famine. As previously described, [25] the Dutch famine occurred within an approximately 6-month period between the end of 1944 and mid-1945, when food supplies in the Netherlands acutely and dramatically dropped to 400-800 kcal/day due to food transport bans and severe winter weather. After that period, the situation quickly improved, abruptly ending the famine. This situation allowed us to investigate the effects of post-natal undernutrition on health as a 'natural experiment,' rather than purely observational and to study the well-documented, acute nature of the undernutrition.

Although the measurement of famine exposure by recall may have some drawbacks related to its subjective nature and propensity of misclassification, we believe that if anything had occurred, it would have happened at random and only underestimate the observed effects. On the other hand, the use of individual data on famine exposure rather than grouping populations according to place of residence or time is a strength of our study since we used a more precise exposure measurement. As described previously, our exposure classification agrees with rationing practices at that time, in which individual calorie amounts were based on age. Young children (1-3 years) were relatively protected from the famine and received about 50%, whereas adults received about 25% of the distributed calorie amounts at the start of the famine. [25] These historical facts are reflected by our data, such that the older the women were at the start of the famine, the higher the proportion of women who reported having been exposed to famine. This may be considered in support of the quality of our exposure data. We performed blinded and objective measurements of the calcium content using the CT scan, so that differential scan measurement by exposure knowledge is excluded as an explanation of our findings.

Coronary artery calcifications are thought to be a reflection of the burden of intima lesions; hence, coronary atherosclerosis. [37] For aortic calcifications, it is not clear that these always reflect atherosclerosis. [38] Most pathological studies found a substantial amount of aortic calcifications located in the tunica media and not the intima. Data even suggest that media calcifications develop earlier and more extensively in the aorta compared to intima calcifications. [39] Also for valve calcifications, the biology is now thought to be more on longstanding mechanical stress than inflammation. [40] Changes in valve tissue have been observed in persons who do not exhibit features of atherosclerosis, indicating that early stages of cardiac valve calcification involve mechanisms different from coronary artery calcification. [40]

To our knowledge, there has been no previous study addressing an association between childhood famine exposure and vascular or valve calcifications in later life. Most studies linking malnutrition and

coronary or valve calcification were conducted on patients with end-stage renal disease.[41] Such patients are reported to experience a malnutrition-inflammation-atherosclerosis/calcification (MIAC) syndrome, which is a strong predictor for cardiovascular death. Although a full assessment of the mechanisms underlying the association between undernutrition and coronary artery or valve calcification is beyond the scope of our study, the findings in patients with renal diseases suggest that a similar interaction between malnutrition, increased levels of pro-inflammatory cytokines, and calcification or atherosclerosis may play a role.[42,43] This suggests that the protective effect of caloric restriction in adulthood and animals on atherosclerosis and metabolic syndromes[44,45] may not be readily translated to undernutrition in early life. Caloric restriction in different periods of life probably has different effects on atherosclerosis development, as suggested by findings from two studies on the Dutch famine which showed that famine exposure during adolescence increased the risk for cardiovascular events,[14] whereas prenatal famine exposure did not.[46]

Our findings suggest that the critical period determining future development of cardiovascular diseases may extend beyond fetal life and infancy. Previous studies have demonstrated the role of early life nutrition in the development of chronic diseases in adulthood. For example, the Biafran famine study showed that fetal-infant undernutrition is associated with an increased risk of hypertension and impaired glucose tolerance in adulthood,[47] similar to what was found in the Chinese great famine study.[48] Although few studies have evaluated the effect of post-natal events per se on health in later life, it seems that maintaining balanced nutrition in later childhood is also crucial, as suggested by the detrimental effects of weight fluctuation or 'yoyo' dieting in young adulthood on coronary heart disease.[17] Our study only addresses one of the possible mechanisms of the development of atherosclerosis and cardiovascular diseases by childhood famine exposure. The findings are consistent with previous finding of the association between famine exposure in adolescence and clinical cardiovascular disease. Hence, our findings warrant further studies into the role of calcium deposition in the relationship between acute famine and later life cardiovascular disease.

In conclusion, famine exposure in childhood, especially during adolescence, may be associated with a higher risk of coronary artery calcification in late adulthood. There seems to be no clear association between childhood famine exposure and cardiac valve or aortic calcification.

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Table 1. Baseline characteristics of the study subjects based on famine exposure level

Variables		Famine exposure			P
		Unexposed n = 139	Moderate n = 103	Severe n = 44	
Age at famine, mean (SD)		7.6 (4.7)	8.9 (5.6)	8.7 (4.3)	0.10
Age category, n(%) through 9 years		98 (70.5)	64 (62.1)	29 (65.9)	0.39
	10-18 years	41 (29.5)	39 (37.9)	15 (34.1)	
Age at CT scan		67.7 (5.0)	69.0 (5.4)	69.0 (4.7)	0.11
BMI (kg/m^2), mean (SD)		26.4 (4.4)	27.3 (4.6)	27.8 (4.6)	0.12
Waist-to-hip ratio, mean (SD)		0.84 (0.07)	0.86 (0.07)	0.85 (0.06)	0.06
Highest systolic BP (mmHg), mean (SD)		141.5 (20.4)	143.1 (23.4)	145.0 (21.8)	0.63
Highest diastolic BP (mmHg), mean (SD)		75.4 (9.6)	74.8 (9.6)	74.6 (8.2)	0.87
Cholesterol to HDL ratio, mean (SD)		4.5 (1.2)	4.7 (1.4)	4.8 (1.2)	0.43
Glucose level (mmol/L), mean (SD)		5.6 (1.0)	5.8 (1.2)	5.5 (0.5)	0.35
Triglyceride (mmol/L), median (range)		1.0 (0.5-3.8)	1.1 (0.3-3.7)	1.3 (0.5-3.2)	0.15*
Smoking, n (%)	Currently	9 (6.5)	11 (10.7)	3 (6.8)	0.71
	Former	57 (41.0)	45 (43.7)	18 (40.9)	
	Never	75 (52.5)	47 (45.6)	23 (52.3)	
Pack-years smoked in ever/current smokers, median (range)		6.0 (0.05-94)	7.4 (0.05-73.5)	11.5 (0.03-53.8)	0.66
Pack-years smoked, median (range)		0.0 (0.0-94)	0.3 (0.0-73.5)	0.0 (0.0-53.8)	0.57*
Metabolic syndrome, n(%)		15 (10.8)	18 (17.5)	3 (6.5)	0.14

*Kruskal-Wallis test

Table 2. The effect of famine exposure on coronary artery calcification as reflected by Agatston score

Famine Exposure		Agatston score (n, %)		Crude			Model 1			Model 2		
		<100 n = 204	>100 n = 78	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	104 (51.0)	33 (42.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	72 (35.3)	29 (37.2)	1.27	0.71-2.27	0.42	1.08	0.58-1.99	0.81	1.00	0.53-1.92	0.99
	Severe	28 (13.7)	16 (20.5)	1.80	0.87-3.78	0.11	1.63	0.76-3.46	0.21	1.74	0.79-3.84	0.17
	P for trend				0.11			0.26			0.24	
Age 0-9 years	Unexposed	78 (51.3)	18 (48.6)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	51 (33.6)	13 (35.1)	1.11	0.50-2.45	0.81	1.08	0.48-2.40	0.86	0.92	0.39-2.18	0.84
	Severe	23 (15.1)	6 (16.2)	1.13	0.40-3.18	0.82	1.06	0.37-3.02	0.92	1.22	0.40-3.70	0.17
	P for trend				0.78			0.88			0.24	
Age 10-17 years	Unexposed	26 (50.0)	15 (36.6)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	21 (40.4)	16 (39.0)	1.32	0.53-3.28	0.55	1.07	0.41-2.80	0.89	1.00	0.53-1.92	0.99
	Severe	5 (9.6)	10 (24.4)	3.47	1.00-12.07	0.05	3.65	1.01-13.31	0.04	4.62	1.16-18.43	0.03
	P for trend				0.07			0.09			0.06	

Model 1: Adjusted for age and pack-years smoked

Model 2: Adjusted for age, pack-years smoked, BMI, glucose, triglyceride, waist-to-hip ratio, systolic BP, cholesterol-to-HDL ratio

OR = odds ratio, 95%CI = 95% confidence interval, ref = reference category

Table 3. The effect of famine exposure on aortic and/or mitral valve calcification

Famine Exposure		Valve calcification (n, %)		Crude			Model 1			Model 2		
		No n = 206	Yes n = 80	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	102 (49.5)	37 (46.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	71 (34.5)	32 (40.0)	1.24	0.71-2.18	0.45	1.04	0.57-1.88	0.90	0.98	0.53-1.81	0.94
	Severe	33 (16.0)	11 (13.8)	0.92	0.42-2.00	0.83	0.78	0.35-1.76	0.35	0.68	0.29-1.58	0.37
	P for trend				0.92			0.65			0.44	
Age 0-9 years	Unexposed	74 (50.0)	24 (55.8)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	51 (34.5)	13 (30.2)	0.79	0.37-1.69	0.54	0.72	0.32-1.58	0.41	0.67	0.30-1.54	0.35
	Severe	23 (15.5)	6 (14.0)	0.80	0.29-2.21	0.67	0.63	0.22-1.78	0.38	0.56	0.18-1.71	0.56
	P for trend				0.56			0.30			0.23	
Age 10-17 years	Unexposed	28 (48.3)	13 (35.1)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	20 (34.5)	19 (51.4)	2.05	0.82-5.08	0.12	1.66	0.64-4.30	0.99	1.65	0.52-4.65	0.34
	Severe	10 (17.2)	5 (13.5)	1.08	0.31-3.79	0.91	1.06	0.29-3.92	0.93	0.79	0.20-3.16	0.74
	P for trend				0.53			0.66			0.37	

Model 1: Adjusted for age and pack-years smoked

Model 2: Adjusted for age, pack-years smoked, BMI, glucose, triglyceride, waist-to-hip ratio, systolic BP, cholesterol-to-HDL ratio

OR = odds ratio, 95%CI = 95% confidence interval, ref = reference category

Table 4. The effect of famine exposure on aortic calcification

Famine Exposure		Aortic calcification* (n, %)		Crude			Model 1			Model 2		
		Low n = 209	High n = 77	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	108 (51.7)	31 (40.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	71 (34.0)	32 (41.6)	1.57	0.88-2.80	0.11	1.23	0.65-2.34	0.53	1.17	0.58-2.37	0.65
	Severe	30 (14.4)	14 (18.2)	1.63	0.77-3.44	0.20	1.39	0.61-3.15	0.43	1.66	0.69-4.10	0.26
	P for trend				0.12			0.39			0.28	
Age 0-9 years	Unexposed	85 (53.1)	13 (41.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	53 (33.1)	11 (35.5)	1.36	0.57-3.25	0.49	1.23	0.50-3.07	0.65	1.05	0.39-2.86	0.92
	Severe	22 (13.8)	7 (22.6)	2.08	0.74-5.84	0.16	1.68	0.57-4.91	0.35	2.64	0.82-8.52	0.10
	P for trend				0.17			0.35			0.16	
Age 10-17 years	Unexposed	23 (46.9)	18 (39.1)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	18 (36.7)	21 (45.7)	1.49	0.62-3.60	0.38	1.28	0.51-3.24	0.61	1.34	0.45-4.30	0.60
	Severe	8 (16.3)	7 (15.2)	1.12	0.34-3.67	0.85	1.07	0.31-3.73	0.92	0.91	0.21-3.96	0.90
	P for trend				0.65			0.79			0.92	

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Competing interests: none declared

Contributorship statement

The authors' responsibilities were as follows— NSI, SGE, and CSPMU: contributed to the study conceptualisation and design; YTS, AFMA, TJR, PDJ, AR, and SGE: were responsible for data collection; NSI: analysed the data and wrote the manuscript; and YTS, AFMA, TJR, PDJ, AR, DEG, CSPMU, and SGE: provided constructive feedback on each draft of the manuscript. All authors read and approved the final manuscript. None of the authors had any conflicts of interest to disclose.

Data sharing statement

Extra data is available by emailing the corresponding author (NSI).

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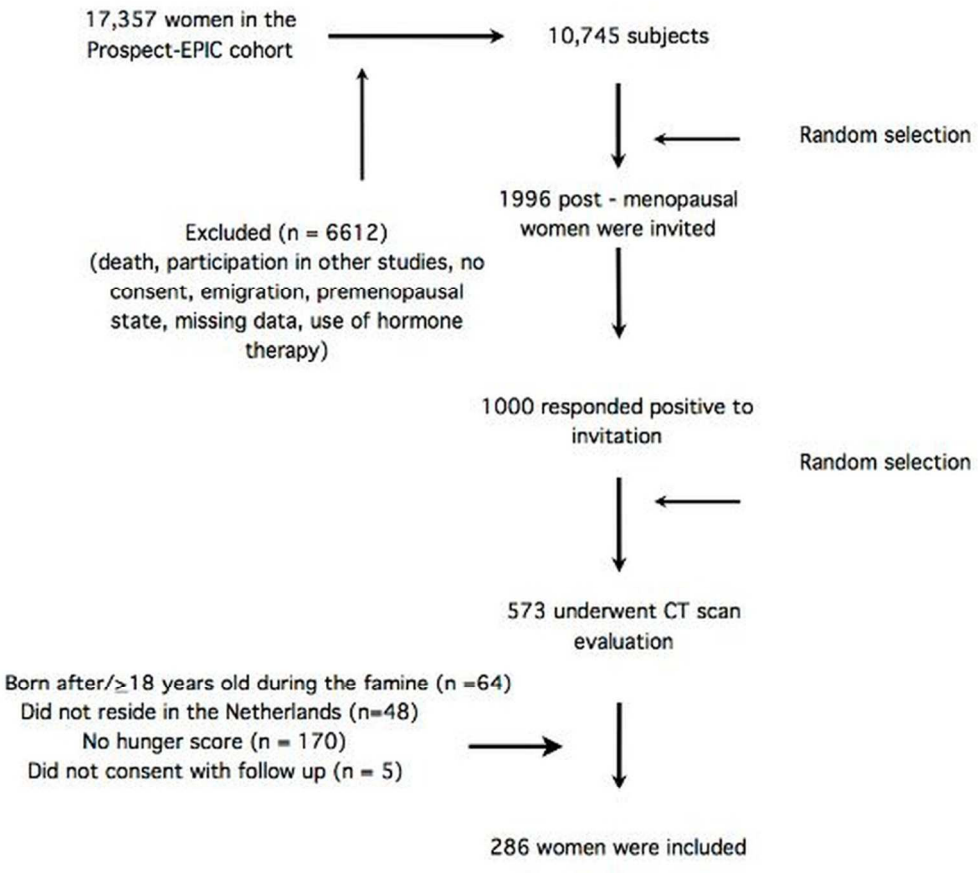
Figure 1. Subjects' recruitment flow



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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

		Item No	Recommendation
Title and abstract	Y	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	Y	2	Explain the scientific background and rationale for the investigation being reported
Objectives	Y	3	State specific objectives, including any prespecified hypotheses
Methods			
Study design	Y	4	Present key elements of study design early in the paper
Setting	Y	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	Y	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
	N/A		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	Y	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	Y	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	Y	9	Describe any efforts to address potential sources of bias
Study size	Y	10	Explain how the study size was arrived at
Quantitative variables	Y	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	Y	12	(*a*) Describe all statistical methods, including those used to control for confounding
	Y		(*b*) Describe any methods used to examine subgroups and interactions
	Y		(*c*) Explain how missing data were addressed
	N/A		(*d*) If applicable, explain how loss to follow-up was addressed
	N/A		(*e*) Describe any sensitivity analyses
Results			
Participants	Y	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
	Y		(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	Y	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
	Y		(b) Indicate number of participants with missing data for each variable of

			interest
	N/A**		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	Y	15*	Report numbers of outcome events or summary measures over time
Main results	Y	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
	Y		(*b*) Report category boundaries when continuous variables were categorized
	N/A		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	Y	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	Y	18	Summarise key results with reference to study objectives
Limitations	Y	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	Y	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	Y	21	Discuss the generalisability (external validity) of the study results
Other information			
Funding	Y	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.
** Follow up time is reflected by the age of the patients.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.