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Caloric restriction reduces age at menopause: the effect of the 1944-1945 Dutch famine

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Abstract

Objective: To assess the effect of caloric restriction, as endured during the 1944-1945 Dutch famine, on the age at which natural menopause occurs and to identify specific vulnerable age periods in which caloric restriction has the largest effect.

Design: This was a population-based cohort study conducted in Utrecht, the Netherlands. Between 1983 and 1986, 9,471 women aged 40 to 73 years at the time of interview were classified regarding their exposure to the famine. Age at natural menopause was obtained from all available data, retrospectively as well as prospectively. We estimated differences in mean age at natural menopause between famine exposure categories (not, moderately, and severely exposed), with adjustment for smoking, parity, socioeconomic status, body mass index, age at menarche, and year of birth.

Results: Women experienced natural menopause on average 0.36 years earlier (95% CI: −0.60, −0.11) when severely exposed to the famine and 0.06 years earlier (95% CI: −0.22, 0.09) when moderately exposed compared with the unexposed women. This effect was particularly pronounced in those severely exposed from 2 to 6 years of age: −1.83 years (95% CI: −3.03, −0.63).

Conclusions: Our findings suggest that caloric restriction decreases age at natural menopause. Early childhood seems to be a particularly sensitive age period for this effect.

Key Words: Cohort studies – Women – Human – Caloric restriction – Menopause – DOM project.

Age at menopause is considered to be an important risk indicator for subsequent morbidity. An early age at menopause may increase the risk of cardiovascular disease,[1] osteoporosis,[2] colorectal cancer,[3] and cognitive decline.[4] In contrast, risks of breast cancer,[5] ovarian cancer, and endometrial cancer may be reduced.[3]

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Ample research has been performed to identify determinants of age at natural menopause. Smoking has consistently been associated with an earlier natural menopause.[2] Other possible determinants of an early menopause have been reported with varying consistency: lower socioeconomic status, nulliparity or a low number of children, early age at menarche, and a low body mass.[2,6,7] These predominantly midlife factors explain only a small part of the total variation of the age at natural menopause, leaving substantial room for early-in-life determinants and heritable effects.[8,9] Evidence is accumulating that early life factors may contribute considerably to later health, fueled by the "fetal origins" hypothesis of diseases by David Barker and colleagues.[10]

Another candidate to affect age at natural menopause could be early nutrition. We used individual data on women regarding fierce caloric restriction in the last

year of World War II in the Netherlands to determine its effect on subsequent age at natural menopause.

This famine evolved from an accumulation of circumstances. In an attempt to hasten the end of World War II in Europe, Allied forces launched an attack on Sept. 17, 1944, to capture the Rhine bridge at Arnhem, the Netherlands. This operation—"Market Garden"—would have facilitated an advance to the Ruhr area, but the bridge at Arnhem could not be reached.[11] To support this offensive, a railroad-strike was ordered by the Dutch government in exile to thwart German transport of troops and ammunition. The German occupier responded with a food embargo, which particularly struck the densely populated western parts of the Netherlands and resulted in a fierce famine in this affluent country.[12] The food situation deteriorated rapidly from October 1944 onwards: the official daily rations per capita for adults dropped from about 1,500 kilocalories in September 1944 to below 700 kilocalories in January 1945. The ratio of proteins, fats, and carbohydrates remained essentially unchanged during this period. After 6 months of starvation, the Netherlands were liberated on May 5, 1945, abruptly ending the famine.[13]

METHODS

Population

Between June 1983 and March 1986, a questionnaire regarding exposure to the 1944-1945 Dutch famine was sent to 19,732 women aged 40 to 73 years who, at that time, were participating in the DOM (Diagnostisch Onderzoek Mammacarcinoom) breast cancer screening project in the Netherlands.[14] These women belong to three distinctive population-based cohorts in the province of Utrecht, in which different breast cancer screening intervals were evaluated. The DOM 1 cohort (born 1911-1925 and recruited 1974-1979) was offered five screening rounds between the ages of 48 and 73; the DOM 2 cohort (born 1926-1931 and recruited 1981-1982) was offered three screening rounds between the ages of 49 and 69; the DOM 3 cohort (born 1932-1941 and recruited 1982-1985) was offered one screening between the ages of 40 and 53. The women reflect a homogenous Caucasian population. The overall participation rate was 70% (range, 44%-72%).

We obtained information on age at menopause, cause of menopause, use of hormone replacement therapy, smoking, reproductive history, age at menarche, and health insurance from questionnaires from 1974 onward. Trained assistants measured height and weight at the time of the first screening and checked the questionnaires for missing information.

Women who lived in the Netherlands, Indonesia, or Germany (the latter two being mainly concentration camp residents) but were not evacuated at the time of the 1944-1945 Dutch famine and could be classified as to their exposure to caloric restriction were eligible for analysis ($N = 16,864$).

We excluded from the analyses women who reported to be premenopausal at the time of the last questionnaire or who reported an unlikely age at menopause (before 24 or after 62; $n = 1,649$), who reported to have ever used hormone replacement therapy, which may obscure the age at menopause ($n = 2,578$), who reported that the cessation of menses was caused by medical treatment ($n = 3,123$), who reported to be already postmenopausal during the 1944-1945 Dutch famine ($n = 2$), or whose body mass index (BMI), socioeconomic status, age at menarche, or smoking habits were not known ($n = 41$).

The final study population consisted of 9,471 women with a natural menopause.

Menopausal age

We defined the age at menopause as the age of last menses, retrospectively evaluated after 12 months of amenorrhoea not the result of pregnancy or lactation. Because comparable data were collected in different screening rounds, we made use of the information obtained nearest to the actual menopause to minimize problems with recall.

Within the DOM 1 study population, 3,352 women reported at the first screening to be postmenopausal from natural causes; 754 reported this at the fifth screening (1984). In the DOM 2 study population 1,993 reported being postmenopausal naturally at the first screening, 582 at the second screening (1982-1986), and 29 at the third screening (1986). In the DOM 3 study population, 610 reported being postmenopausal naturally at the first screening. The age at menopause was extracted from questionnaires in which the participants were asked whether their menses had ceased for more than 12 months and, if so, when the last menses occurred. However, at the second screening of DOM 2, women were asked only if they had reached menopause, but not when. Here, we estimated age at menopause using the interval between the screening rounds (maximum of 4 years), assuming that menses had ceased halfway through the interval minus 12 months.

Since DOM 3 participants were offered only one screening at a relatively young age, not all had reached menopause at that time. Those still premenopausal between 1982 and 1986 received additional questionnaires regarding their menopausal status until 1995.

Response was about 80% at each mailing. In 1992, special efforts by additional questionnaires and by telephone were made to complete follow-up for a random subsample of 4,207 of these women. For the total DOM 3 cohort, follow-up is 82% complete.[15] Consequently, 1,937 women with a known age at natural menopause could be added to the study at hand.

Furthermore, approximately 50% of all the women also participated in the Dutch component of the European Prospective Investigation into Cancer and Nutrition (EPIC).[16] Data from this study, obtained by questionnaires similar to those used in the DOM project, were therefore used to complete information concerning age at menopause for this subset (n = 214).

Famine exposure

Women were asked about their place of residence during the 1944-1945 Dutch famine and about their experiences of hunger, cold, and weight loss during this period. We ranked these last questions into three levels: "hardly," "little," or "very much," excluding answers "not applicable" and "I don't know." We combined the answers into a three-point subjective hunger score: women reporting severe exposure to at least two of these famine characteristics were categorized as "severely exposed." Women reporting no exposure to at least two of these famine characteristics were categorized as "unexposed," and all others as "moderately exposed."

Other variables

We categorized cigarette smoking as ever or never. Parity was defined as giving birth ever or never. We based socioeconomic status on type of health insurance: public health insurance (lower status), civil servant's insurance (intermediate status), or private insurance (higher status). BMI at study recruitment was used to represent premenopausal BMI (mean age, 53 years; standard deviation (SD), 5.5 years). We calculated BMI as weight (kg)/height $(m)^2$ and categorized this into less than 18.5 (low weight), 18.5 to 25 (normal weight), 25 to 30 (overweight), and more than 30 (obese).

Data analysis

We analyzed the data in a number of ways. First, we assessed the crude effect of famine exposure on age at natural menopause with univariate linear regression in the total study population. Multiple linear regression adjusted the effect of famine exposure on age at natural menopause for differences in smoking, parity, socioeconomic status, BMI, age at menarche, and year of birth.

To examine whether there are particular vulnerable periods during female development in which caloric restriction has the largest effect on age at natural menopause, we introduced interaction terms in the model for exposure status and subgroups according to age at the start of the famine (Oct. 1, 1944). These subgroups are based on distinct stages in female development: 2 to 6 years (early childhood), 7 to 9 years (middle childhood), 10 to 12 years (later childhood), 13 to 18 years (adolescence), older than 18 years (adulthood).[17]

Linear regression model assumptions were checked with normal probability plots of the standardized residuals (normality assumption) and scatterplots with standardized residuals versus standardized predicted values (constant variance assumption and linearity assumption). These assumptions were found to be justified.

We performed all statistical analysis with SPSS 10.1, and based P values on two-sided tests with a cutoff level for statistical significance of 0.05.

RESULTS

Population characteristics

Within the study population, 1,074 (11%) women reported having been severely exposed to the 1944-1945 Dutch famine, 3,793 (40%) having been moderately exposed, and 4,604 (49%) having been unexposed. The severely exposed tended to be older during the famine, had a lower socioeconomic status, and were more often obese as adults. There were no differences in mean age at menarche between the famine exposure categories (Table 1).

Famine exposure and age at natural menopause

Women who reported having been severely exposed to the famine experienced natural menopause on average 0.37 years earlier (95% CI: −0.62, −0.13) than the unexposed group. In the moderately exposed group, mean age at natural menopause was 0.08 years earlier (95% CI: −0.24, 0.08). These effects persisted when adjustment was made for smoking, socioeconomic status, parity, BMI, age at menarche, and year of birth: −0.36 years (95% CI: −0.60, −0.11) and −0.06 years (95% CI: −0 .22, 0.09), respectively (Table 2).

The interaction terms for early and middle childhood with severe famine exposure significantly contributed to the model (P = 0.01 and P = 0.03, respectively).

TABLE 1. *Population characteristics according to famine exposure*

		Unexposed to famine (n = 4,604)	Moderately exposed to famine (n = 3,793)	Severely exposed to famine (n = 1,074)
Age at famine exposure (y)[a]		17 (2-33)	19 (2-33)	21 (2-33)
Age at menarche (y)[b]		13.7 ± 1.5	13.6 ± 1.6	13.7 ± 1.8
Age at famine questionnaire (y)		56 (41-73)	59 (42-73)	61 (41-73)
Socioeconomic status (%)				
Low		56.4	59.8	64.2
Intermediate		13.6	13.1	13.5
High		30.0	27.1	22.3
Body mass index (kg/m^2) (%)[c]				
Underweight	(< 18.5)	0.6	0.8	0.9
Normal weight	(18.5-25)	50.7	49.6	49.3
Overweight	(25-30)	38.8	38.4	36.1
Obese	(> 30)	10.0	11.1	13.7
Smoking habit (%)				
Never smoked		68.2	68.2	65.7
Ever smoked		31.8	31.8	34.3
Parity (%)				
0		14.2	17.6	17.4
≥1		85.8	82.4	82.6

[a]Median (range).
[b]Mean ± SD.
[c]Because of rounding, not all percentages total 100.

TABLE 2. *Linear regression: difference in mean age at natural menopause in years*

	Unadjusted model		Adjusted model	
	Change in age at menopause (y)	(95% CI)	Change in age at menopause (y)	(95% CI)
Famine exposure				
Unexposed	reference		reference	
Moderately exposed	−0.08	(−0.24, 0.08)	−0.06	(−0.22, 0.09)
Severely exposed	−0.37	(−0.62, −0.13)	−0.36	(−0.60, −0.11)
Socio-economic status				
Low			reference	
Intermediate			0.69	(0.47, 0.91)
High			0.52	(0.35, 0.70)
Body mass index (kg/m^2)				
Underweight (<18.5)			−1.39	(−2.27, −0.51)
Normal weight (18.5-25)			reference	
Overweight (25-30)			0.16	(0.00, 0.32)
Obese (>30)			0.49	(0.24, 0.74)
Smoking habit				
Never smoked			reference	
Ever smoked			−0.68	(−0.84, −0.52)
Parity				
0			reference	
≥1			0.60	(0.40, 0.81)
Age at menarche (per y)			0.09	(0.05, 0.14)
Year of birth (per y)			−0.014	(−0.02, −0.01)

Therefore we divided the total study group into age-at-exposure subgroups and performed further analyses within each of these subgroups. The adjusted effect was most pronounced in women who were severely exposed to the famine at 2 to 6 years of age, amounting to a decrease in the mean age at natural menopause by 1.83 years (95% CI: −3.03, −0.63) (Table 3).

Other determinants of age at natural menopause

High or intermediate socioeconomic status, higher BMI, later age at menarche, and multiparity were associated with a later mean age at natural menopause. Having smoked resulted on average in an earlier natural menopause (Table 2).

TABLE 3. *Linear regression: difference in mean age at natural menopause in years from famine exposure according to age at exposure*[a]

	Age at exposure				
Famine exposure	2-6 (n = 851)	7-9 (n = 856)	10-12 (n = 1,037)	13-18 (n = 1,992)	>18 (n = 4,735)
Unexposed	reference	reference	reference	reference	reference
Moderately exposed					
Change in age at menopause (y)	−0.16	−0.22	−0.03	−0.04	−0.01
(95% CI)	(−0.69, 0.36)	(−0.70, 0.26)	(−0.47, 0.41)	(−0.37, 0.29)	(−0.25, 0.23)
Severely exposed					
Change in age at menopause (y)	−1.83	−1.30	−0.47	−0.32	−0.14
(95% CI)	(−3.03, −0.63)	(−2.18, −0.42)	(−1.24, 0.31)	(−0.84, 0.21)	(−0.48, 0.19)

[a]Adjusted for smoking, socioeconomic status, body mass index, parity, age at menarche, and year of birth.

DISCUSSION

Our findings in a cohort of close to 10,000 women show that severe caloric restriction, as experienced during the 1944-1945 Dutch famine, is independently associated with an earlier age at natural menopause, particularly if this restriction occurs before the age of 10.

To appreciate these findings, some strengths and limitations of our study need to be addressed. Because of sad circumstances in the Netherlands at the end of World War II, we were able to study the effects of a short but severe famine in otherwise well-nourished girls and women. For a large population, we were able to use individual data on famine exposure instead of classifying populations using, for instance, urbanization grade. This individual famine exposure score is based on recollection and, therefore, prone to misclassification. We excluded from our analyses women who answered "I don't know" and 'not applicable' on the famine questions to limit this misclassification. If the subjective nature of famine exposure classification caused misclassification, this most likely was unrelated to menopausal age. It then has caused a dilution of the observed effect.

We found a good correlation between place of residence during the war and the recalled individual famine exposure,[18] even in the youngest two age groups at the time of the famine (2-6 years and 7-9 years). This reflects differences in exposure severity between people residing in rural versus urbanized areas.[12] Also, the finding that the young were less exposed than the old is historically accurate. Throughout World War II, the allocated individual amount of calories was based on age: special effort was made to maintain adequate rations for the young. At the nadir of the famine, those aged 1 to 3 years received about 50%, whereas those aged over 18 years received about 25% of the distributed amount of calories at the start of the famine (Oct. 1, 1944).[13]

In addition, data on age at natural menopause may have introduced some misclassification. For 79.5% of our eventual study population, menopausal age was obtained retrospectively. In a subset of these women, who reported their age at natural menopause twice, 7 to 9 years apart, author Isolde den Tonkelaar found that 71% reported the same menopausal age, to within 1 year.[19] Misclassification, therefore, seems limited.

Women were classified according to age at famine exposure. The period of famine is clearly demarcated in the Netherlands (October 1944 to May 1945). Some women (4.3% of the study population) resided in Germany or Indonesia at that time, mostly in concentration camps, where they also suffered from food deprivation but not only during this particular period. Analysis of the data solely with women residing in the Netherlands did not change the results.

Women who were still premenopausal at the end of the follow-up period were excluded from the analyses. Because women from the younger birth cohorts were generally younger at the end of follow-up, older ages at menopause were underrepresented in these cohorts, which probably explains the decrease in mean age at natural menopause with time (Table 2). We can only speculate about the effect of this exclusion on our estimates. Those still premenopausal are more often less exposed (57% unexposed compared with 49% in the postmenopausal group) and will experience natural menopause at later ages. Therefore, the measured effect of famine exposure is, if anything, likely to be underestimated.

The associations of smoking, socioeconomic status, parity, age at menarche, and BMI with age at natural menopause are consistent with the literature.[2,6,7] Adjustment for these covariables did not change the famine estimates (Table 2), although residual confounding may be a possibility because many of these variables are rather crudely defined. To address this issue for

smoking habits, which was the strongest determinant of age at natural menopause in our cohort, we reanalyzed the data in women who reported to have never smoked (n = 6,432). This increased the famine estimates about 5%. Residual confounding thus seems unlikely to explain the results.

Until now, few studies have reported an association of caloric intake with the age at natural menopause. One study investigating the effect of midlife diet on age at natural menopause found no association with total energy intake.[20] Midlife dietary restriction, defined as being on a weight-reduction diet, has been associated with early natural menopause.[21] Three studies reported findings concerning birth weight, as a measure of nutritional status at birth, in relation to age at natural menopause. One of these studies in twins did not find any association.[22] The second and third study also could not disclose an association between birth weight and age at natural menopause, but both reported that women with a low weight at 1 year[23] or at 2 years[24] tended to experience natural menopause at a younger age. In New Guinea, a mean age at menopause of 46.1 years was found in a group of women who had sustained severe and prolonged malnutrition compared with a mean age of 47.6 years in a better nourished group from the same region.[25] These results in a small population are in accordance with our findings.

The menopausal transition is marked by a rapid loss of ovarian follicles as well as hypothalamic-pituitary changes. Which one is the driving force remains an issue of debate.[26] The number of follicles in full-term infants is on average 700,000 at birth,[27] which declines during life until menopause.[28] Caloric restriction in early life may act through an acceleration in the loss of follicles to advance menopause. On the other hand, caloric restriction may cause changes in neuroendocrine pathways,[29] which in turn may be another possible mechanism for the association found.

CONCLUSION

Our data provide evidence that caloric restriction decreases age at natural menopause, especially when exposure takes place in early life.

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REFERENCES

1. van der Schouw YT, van der Graaf Y, Steyerberg EW, Eijkemans JC, Banga JD. Age at menopause as a risk factor for cardiovascular mortality. *Lancet* 1996;347:714-718.
2. Sowers MR, LA Pietra MT. Menopause: its epidemiology and potential association with chronic diseases. *Epidemiol Rev* 1995;17: 287-302.
3. La Vecchia C, Brinton LA, McTiernan A. Menopause, hormone replacement therapy and cancer. *Maturitas* 2001;39:97-115.
4. Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M. Minireview: neuroprotective effects of estrogen-new insights into mechanisms of action. *Endocrinology* 2001;142:969-973.
5. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet* 1997;350:1047-1059.
6. Gold EB, Bromberger J, Crawford S, et al Factors associated with age at natural menopause in a multiethnic sample of midlife women. *Am J Epidemiol* 2001;153:865-874.
7. Sherman B, Wallace R, Bean J, Schlabaugh L. Relationship of body weight to menarcheal and menopausal age: implications for breast cancer risk. *J Clin Endocrinol Metab* 1981;52:488-493.
8. van Noord PAH, Peeters PH, Grobbee DE, Dubas JS, te Velde E. Onset of natural menopause. *J Clin Epidemiol* 1999;52:1290-1292.
9. de Bruin JP, Bovenhuis H, van Noord PAH, et al The role of genetic factors in age at natural menopause. *Hum Reprod* 2001;16:2014-2018.
10. Barker DJ. The fetal and infant origins of adult disease. *BMJ* 1990; 301:1111.
11. Montgomery BL. *The Memoirs of Field-Marshal the Viscount Montgomery of Alamein.* London: Collins Clear-Type Press, 1958.
12. de Jong L. *The Kingdom of the Netherlands in the Second World War* [in Dutch]. The Hague: General State Printing Office, 1981.
13. Burger GCE, Sandstead HR, Drummond JC. *Malnutrition and Starvation in Western Netherlands, September 1944 to July 1945*. Part I and II. The Hague: General State Printing Office, 1948.
14. de Waard F, Collette HJ, Rombach JJ, Baanders-van Halewijn EA, Honing C. The DOM project for the early detection of breast cancer, Utrecht, The Netherlands. *J Chronic Dis* 1984;37:1-44.
15. de Vries E, den Tonkelaar I, van Noord PAH, van der Schouw YT, te Velde E, Peeters PH. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod* 2001;16:1657-1662.
16. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997;26(Suppl 1):S6-S14.
17. Bogin B. *Patterns of Human Growth*, 2nd ed. Cambridge, England: Cambridge University Press, 1999.
18. Elias SG, van Noord PAH, Peeters PHM, den Tonkelaar I, Grobbee DE. The 1944-1945 Dutch famine and age at natural menopause—the value and validity of individual exposure assessment. *IARC Sci Publ* 2002;156:311-313.
19. den Tonkelaar I. Validity and reproducibility of self-reported age at menopause in women participating in the DOM-project. *Maturitas* 1997;27:117-123.
20. Nagata C, Takatsuka N, Kawakami N, Shimizu H. Association of diet with the onset of menopause in Japanese women. *Am J Epidemiol* 2000;152:863-867.
21. Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prospective study of the determinants of age at menopause. *Am J Epidemiol* 1997;145:124-133.
22. Treloar SA, Sadrzadeh S, Do KA, Martin NG, Lambalk CB. Birth weight and age at menopause in Australian female twin pairs: exploration of the fetal origin hypothesis. *Hum Reprod* 2000;15:55-59.
23. Cresswell JL, Egger P, Fall CH, Osmond C, Fraser RB, Barker DJ. Is the age of menopause determined in-utero? *Early Hum Dev* 1997; 49:143-148.

24. Hardy R, Kuh D. Does early growth influence timing of the menopause? Evidence from a British birth cohort. *Hum Reprod* 2002;17: 2474-2479.
25. Scragg RFR. Menopause and reproductive span in rural Niugini. *Annual Symposium of the Papua New Guinea Medical Society* 1973;126-131.
26. Wise PM, Krajnak KM, Kashon ML. Menopause: the aging of multiple pacemakers. *Science* 1996;273:67-70.
27. Block E. A quantitative morphological investigation of the follicular system in newborn female infants. *Acta Anatomica* 1953;17: 201-206.
28. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab* 1987;65:1231-1237.
29. Han ES, Lu DH, Nelson JF. Food restriction differentially affects mRNAs encoding the major anterior pituitary tropic hormones. *J Gerontol A Biol Sci Med Sci* 1998;53:B322-B329.