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SHORT REPORT

# LONG TERM CONSEQUENCES OF THE 1944–1945 DUTCH FAMINE ON THE INSULIN-LIKE GROWTH FACTOR AXIS

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**The insulin-like growth factor axis is highly responsive to nutritional status and may be involved as one of the underlying mechanisms through which caloric restriction could affect cancer risk. High levels of circulating insulin-like growth factor (IGF)-I, or IGF-I relative to IGF binding protein (IGFBP)-3 have been related to various human cancer types. In a group of 87 postmenopausal women, we found that childhood exposure to the 1944–1945 Dutch famine was associated with increased plasma levels of IGF-I and IGFBP-3, whereas IGFBP-1 and -2 levels were weakly decreased. These results are opposite to immediate responses seen under starvation and we hypothesize that this could indicate a permanent overshoot upon improvement of nutritional status after the famine.**


**Key words:** *caloric restriction; IGF-I; IGFBP*

Caloric restriction extends the lifespan and reduces the risk of age-related pathologies, such as cancer, in various species.[1] One of the possible mechanisms leading to these long term beneficial effects may involve changes in insulin[2] and the insulin-like growth factor axis,[3] both highly responsive to energy availability and nutritional status. Plasma insulin-like growth factor (IGF)-I levels are decreased in calorie-restricted rodents, which has led to the suggestion that caloric restriction causes a shift in IGF-I homeostasis towards an increased paracrine activity and decreased plasma concentrations.[4]

IGF-I has strong mitogenic and antiapoptotic potential,[5] and high plasma levels have been linked to risk of various human cancer types, including premenopausal breast cancer, colorectal, prostate and lung cancers.[6] In addition, the IGF binding proteins (IGFBP) may modulate this risk by regulating the bioavailability of IGF-I.[7] For example, IGFBP-3, together with a so-called acid labile subunit, binds over 90% of circulating IGF-I and inhibits its tissue availability since this ternary complex is too large to pass the capillary barrier.[7] Moreover, IGFBP-3 is reported to have direct apoptotic effects in breast cancer cells, independent of IGF-I.[8]

In humans, underfeeding also changes circulating levels of IGF-I and its binding proteins. Key factors that regulate this response are insulin and growth hormone (GH). With fasting, insulin levels decrease, causing resistance of IGF-I synthesis to GH stimuli, which is compensated by increased GH production from the pituitary.[7]

We hypothesize that, besides these immediate responses, caloric restriction may permanently affect the insulin-like growth factor axis. At the end of World War II, people in the Western Netherlands were seriously deprived of food for almost 6 months. The official daily rations dropped from about 1,500 kilocalories in September 1944 to below 700 kilocalories in January 1945, but remained nutritionally balanced. With liberation on May 5, 1945, the famine came to an abrupt end.[9] To examine the hypothesis of permanent effects, we investigated within a population-based female cohort whether exposure to the 1944–1945 Dutch famine during childhood has affected postmenopausal IGF-I, IGFBP-1, -2, -3 and C-peptide (a proxy for insulin) concentrations.

## SUBJECTS AND METHODS

### *The Prospect-EPIC cohort*

The Prospect-EPIC cohort, one of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts, consists of 17,357 women, aged 50–69, recruited from participants of the national breast cancer screening program living in Utrecht, the Netherlands, and its vicinity (response rate 35%).[10] At recruitment between 1993 and 1997, a nonfasting blood sample was taken, which was stored under liquid nitrogen until analysis. Furthermore, participants underwent physical examination and filled out extensive questionnaires. Two questions were asked about individual experiences of weight loss and hunger during the 1944–1945 Dutch famine (ranked absent, moderate or severe), which were combined into an individual famine exposure score. Women who responded to both questions with absent or, respectively, severe exposure, were classified as unexposed or severely exposed. All other women were considered moderately exposed after excluding women with missing data.

The study population consisted of 163 women selected from Prospect-EPIC to serve as a control group in a previous study on postmenopausal breast cancer risk and the insulin-like growth factor axis.[11] At the moment of blood donation, these women were postmenopausal, did not use hormone replacement therapy or insulin and were free from cancer. We excluded for the present study, women who were younger than 2 years of age during the famine ($n = 33$), who could not be classified to famine exposure status (n=35), or who did not reside in the occupied Netherlands during the famine ($n = 8$), leaving 87 women for analyses.

### *Laboratory analyses*

Plasma concentrations were either measured by radioimmunoassay (IGF-I, IGFBP-1 and -3) or by competitive radioimmunoassay (IGFBP-2 and C-peptide) in the Hormones and Cancer Group, at the International Agency for Research on Cancer in Lyon, France. Before measurement, an acid-precipitation procedure extracted the IGF-I from its binding proteins. Intra- and interassay coefficients of variation were 2.6% and 6.4%, respectively, for IGF-I (at 150 ng/mL); 3.3% and 9.9% for IGFBP-1 (at 10 ng/mL); 8.5% and 7.7% for IGFBP-2 (at 400 ng/mL); 1.1% and 4.7% for IGFBP-3 (at 4,000 ng/mL); 8.4% and 10.9% for C-peptide (at 1.8 ng/mL).[11]

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*Data analyses*

All concentrations (expressed in ng/mL) as well as the molar ratio of IGF-I to IGFBP-3 were logarithmically transformed to achieve normal distributions. Geometric means of IGF-I, IGFBP-1, -2, -3, C-peptide and the IGF-I to IGFBP-3 molar ratio were determined according to famine exposure status by analysis of covariance. Trends were tested in linear regression models, where famine exposure was quantitatively scored as 1, 2 or 3 with increasing exposure status.

We considered body mass index, waist/hip ratio, cigarette smoking habits (ever/never), age at recruitment and the time that had passed between last meal and blood donation and last drink and blood donation, to be potential confounders. To adjust for the strongest determinants of each outcome, a backward selection strategy was adopted. Covariables were excluded from the linear regression models – which always included the famine exposure score – in a stepwise manner based on the strength of the associations with the outcome. This procedure was repeated until all remaining covariables in the model showed at least associations at the $p = 0.15$ level.

Statistical analyses were performed with SPSS 11 and all tests were 2-sided.

## RESULTS

In total, 14 women reported to be severely, 28 to be moderately and 45 to be unexposed to the 1944–1945 Dutch famine. The overall median age during the famine was 12 years (range: 2–20 years). As shown in Table I, the famine exposure groups were comparable with regard to most covariables, although severely exposed women reported more often to have ever smoked cigarettes.

We found a tendency of increased plasma concentrations of IGF-I and IGFBP-3 with severity of famine exposure, whereas IGFBP-1 and -2 concentrations showed a weak decline. No differences were found for C-peptide levels. Adjustment for potential confounders did not materially change these results, although trends of IGF-I and IGFBP-3 with famine exposure reached statistical significance (Table II). All associations showed a dose-response relation to famine exposure, except for IGFBP-2 and C-peptide.

Molar ratios of IGF-I to IGFBP-3 were similar in women who were not exposed to the famine (geometric mean = 0.16; 0.15–0.17) compared to moderately (geometric mean = 0.16; 0.15–0.18) or severely exposed women (geometric mean = 0.17; 0.15–0.19). Adjustment for body mass index, cigarette smoking habits, age at recruitment and time between blood donation and last drink yielded similar results (data not shown).

## DISCUSSION

The results of our study suggest that a relatively short period of marked caloric restriction at young ages (2–20 years of age), as was experienced during the 1944–1945 Dutch famine, may be associated with long term levels of circulating IGF-I, IGFBP-1, -2 and -3, but not of C-peptide. IGF-I and IGFBP-3 concentrations were found to be increased with degree of famine exposure in a dose-response manner, whereas IGFBP-1 and -2 levels were somewhat lower in severely famine-exposed women. We found no effects on the molar ratio of IGF-I to IGFBP-3. Despite the small number of observations in our study, the effects of famine exposure on IGF-I and IGFBP-3 were strong enough to reach statistical significance. Inspection of the data did not reveal any outliers that could explain these results.

The 1944–1945 Dutch famine, albeit a black page in history, makes it possible to study the long-term effects of severe short-term caloric restriction in humans. We were able to classify women according to their individual famine exposure status, which provides us – in our belief – with a more precise tool compared to, for instance, geographic classifications. Nevertheless, this exposure score was based on recollection and may therefore be affected by misclassification. Since it is very unlikely that the degree of misclassification is related to the serum concentrations under investigation, this should have led to an underestimation of the results, if anything. In a similar group of women who answered comparable questions regarding their famine exposure, we found a strong correlation between the degree of recalled exposure and urbanization grade,[12] reflecting the historical situation.[9]

Given the relation between high levels of IGF-I and cancer risk,[6] and the protective potential of caloric restriction, the increase of circulating IGF-I with famine exposure was unanticipated. However, the molar ratio of IGF-I to IGFBP-3 did not differ between the famine exposure groups, suggesting that famine exposure does not change the amount of bioavailable IGF-I, which may be stronger related to cancer risk than total IGF-I.[6] The increased IGF-I levels with famine exposure could also be relevant to other health related parameters. It has been described that increased serum levels of IGF-I relate to decreased risk of cardiovascular disease[13] and osteoporosis.[14]

As already stated in the introduction, the human insulin-like growth factor axis is highly responsive to nutritional status. During starvation, circulating levels of IGF-I and IGFBP-3 are decreased and levels of IGFBP-1 and -2 are increased, potentially favoring tissue availability of IGF-I, since IGF-I bound to IGFBP-3 cannot pass the capillary barrier whereas IGF-I bound to IGFBP-1 or -2 can.[7] Our results on long term consequences are exactly opposite to these immediate responses, which may indicate a permanent overshoot after the famine, when food was abundant again. We hypothesize that the observed overshoot in IGF-I in the severely famine exposed women could be mediated by changes in "set-points" within the hypothalamus or pituitary. More elevated levels of IGF-I could explain the decreases in IGFBP-1 and -2, and the increases in IGFBP-3. Indeed, there is evidence from crosssectional data that IGF-I is inversely related to both IGFBP-1 and -2, and positively related to IGFBP-3.[15,16] Furthermore, experimental

**TABLE I** – BASELINE CHARACTERISTICS OF THE STUDY POPULATION ACCORDING TO FAMINE EXPOSURE STATUS

| Characteristics | Unexposed $n = 45$ | | Moderately exposed $n = 28$ | | Severely exposed $n = 14$ | |
|---|---|---|---|---|---|---|
| Age during the famine (years)[1] | 12 | (3–19) | 12 | (2–18) | 12 | (5–20) |
| Age at study recruitment (years)[1] | 63 | (52–69) | 62 | (53–69) | 63 | (56–69) |
| Body mass index[2] | 26.4 | ±4.2 | 26.0 | ±4.3 | 27.7 | ±4.0 |
| Waist/hip ratio[2] | 0.81 | ±0.04 | 0.78 | ±0.05 | 0.82 | ±0.04 |
| Time between blood donation | | | | | | |
| And last meal (hours)[1] | 2.6 | (0.6–17.2) | 2.1 | (0.4–15.2) | 2.3 | (1.0–11.9) |
| And last drink (hours)[1] | 1.7 | (0.6–5.3) | 1.9 | (0.7–8.7) | 2.2 | (0.8–3.8) |
| Cigarette smoking habits | | | | | | |
| Ever | 49% | | 54% | | 57% | |
| Never | 51% | | 46% | | 43% | |

[1]Median (range).–[2]Mean ± SD.

**TABLE II** – GEOMETRIC MEANS[1] AND 95% CONFIDENCE INTERVALS OF CIRCULATING LEVELS OF IGF-I, IGFBP-1, -2, -3 AND C-PEPTIDE ACCORDING TO FAMINE EXPOSURE STATUS

| | Unexposed | | Moderately exposed | | Severely exposed | | $p$ trend |
|---|---|---|---|---|---|---|---|
| | Geometric mean | 95% CI | Geometric mean | 95% CI | Geometric mean | 95% CI | |
| Unadjusted | | | | | | | |
| IGF-I | 133.8 | (121.8–146.9) | 142.0 | (126.1–159.9) | 159.0 | (134.5–188.1) | 0.078 |
| IGFBP-1 | 12.3 | (9.5–16.1) | 11.6 | (8.3–16.2) | 7.9 | (4.9–12.8) | 0.149 |
| IGFBP-2 | 388.6 | (336.6–448.5) | 343.4 | (286.3–411.9) | 326.6 | (250.0–426.5) | 0.183 |
| IGFBP-3 | 3,175.4 | (3031.1–3326.6) | 3,222.8 | (3040.3–3416.3) | 3,526.1 | (3247.0–3829.2) | 0.050 |
| C-peptide | 3.3 | (2.7–3.9) | 3.1 | (2.5–3.9) | 3.6 | (2.6–5.0) | 0.704 |
| Adjusted[2] | | | | | | | |
| IGF-I | 132.9 | (121.4–145.6) | 142.0 | (126.6–159.3) | 162.3 | (138.0–191.0) | 0.038 |
| IGFBP-1 | 12.6 | (10.0–16.0) | 10.2 | (7.5–13.8) | 9.6 | (6.2–14.7) | 0.179 |
| IGFBP-2 | 388.2 | (341.2–441.7) | 335.4 | (284.7–395.2) | 344.6 | (270.8–438.7) | 0.219 |
| IGFBP-3 | 3,170.7 | (3029.0–3319.1) | 3,236.9 | (3053.1–3431.7) | 3,511.8 | (3235.6–3811.7) | 0.045 |
| C-peptide | 3.3 | (2.8–3.8) | 3.3 | (2.7–4.1) | 3.2 | (2.4–4.3) | 0.946 |

[1]Expressed in ng/ml.–[2]IGF-I levels were adjusted for time between blood donation and last drink, cigarette smoking habits and age at recruitment; IGFBP-1 levels were adjusted for body mass index and waist/hip ratio; IGFBP-2 levels were adjusted for body mass index; IGFBP-3 levels were adjusted for waist/hip ratio and age at recruitment; C-peptide levels were adjusted for time between blood donation and last meal, body mass index and waist/hip ratio.

data show that elevated IGF-I levels may lead to decreased IGFBP-1 and -2, and increased IGFBP-3 synthesis in the liver.[17–19]

Similar to our observations, Reed *et al.*[20] reported that calorie-restricted mice showed higher plasma levels of IGF-I and IGFBP-3 upon refeeding compared to mice fed ad libitum throughout life. However, in contrast to our findings, IGFBP-2 levels were also increased in this experiment.

In conclusion, our results suggest that childhood famine exposure may have long-term consequences for the insulin-like growth factor axis. As the sample size was very small, we were not able to explore whether the reported effects of famine exposure depended on the age at which exposure took place. Further research into this topic, in a larger study population, would be informative.


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