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Cancer Epidemiology

The energy restriction (ER)-colorectal cancer (CRC) association is inconsistent in literature. To strengthen the biological plausibility of the ER-CRC association, we investigated whether genetic variation in the insulin-like growth factor (IGF) pathway, a putative underlying mechanism, modulated this association in the Netherlands Cohort Study. Participants completed a questionnaire ($n = 120,852$) and provided toenail clippings for DNA (~75%) at baseline. Individuals living in a Western city during the Hunger Winter (1944–45) or Western rural versus non-Western area were exposed to (severe) ER at young age. Genotyping was performed for 3,768 subcohort members and 2,580 CRC cases (case-cohort with 16.3 years follow-up). Cox hazard ratios for CRC were estimated across combined categories of ER and a genetic sum score of unfavorable alleles based on 18 single nucleotide polymorphisms in IGF-related genes and ER and an *IGF1* 19-CA repeat polymorphism. The reference included ER exposed individuals, so that increased hazard ratios were expected in higher combined categories for calculating relative excess risks due to interaction (additive interactions). Wald tests for multiplicative interactions were also performed. Multiplicative and additive interactions were nonsignificant. Combined ER-genetic sum score categories showed increasing CRC risks in men, but confidence intervals were wide. Women carrying two variant *IGF1* 19-CA repeat alleles versus those carrying two wild type *IGF1* 19-CA repeat alleles were at an ~50% decreased CRC risk, irrespective of ER exposure. In conclusion, data indicate that the IGF pathway might be involved in the ER-CRC association in men, but not women, although interactions were nonsignificant, hampering definite conclusions.

Animal research on energy restriction (ER) and cancer has shown that ER up to 65%, as compared to *ad libitum* levels, is one of the most effective measures to increase lifespan and reduce cancer risk.[1,2] These data are intriguing in this era of obesity, with obesity being the result of a positive energy balance and having many adverse health effects, including an increase in colorectal cancer (CRC),[3] which is a frequent health problem.[4] Experimental studies on ER in humans are sparse for ethical and methodological reasons and do not include the investigation of long-term effects, such as changes in CRC risk.[5–7] Ecological data showed a drop in CRC incidence for birth cohorts born around World War 2 in Norway[8] and Estonia[9], against a background of rising CRC incidences over time. Observational data on the association between ER during World War 2 and CRC in cohorts from Russia,[10] the Netherlands,[11] and Israel[12] have shown a decreased CRC risk,[11] a decreased CRC death risk[10] and an increased CRC risk, respectively.[12] The latter study included Israeli Jews who were potentially exposed to the Holocaust

Key words: colon neoplasms, energy restriction, insulin-like growth factors, polymorphisms, rectal neoplasms

Abbreviations used: ADIPOQ: adiponectin; BMI: body mass index; CI: confidence interval; CRC: colorectal cancer; ER: energy restriction; FFQ: food-frequency questionnaire; GH1: growth hormone 1; HR: hazard ratio; HWE: Hardy-Weinberg Equilibrium; IGF1: insulin-like growth factor 1; IGF1R: insulin-like growth factor receptor 1; IGFBP: insulin-like growth factor binding protein; IRS: insulin-receptor substrate; PPARG: peroxisome proliferator-activated receptor gamma; RERI: relative excess risk due to interaction; SNP: single nucleotide polymorphism.

Additional Supporting Information may be found in the online version of this article.

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What's new?

Whether energy restriction (ER) impacts colorectal cancer (CRC) risk in humans is unclear, though the potential for such an association is favored by the insulin-like growth factor (IGF) pathway, which can be influenced by ER and putatively stimulates malignant growth. Here, ER at young age and varians in IGF-related genes were simultaneously investigated in relation to CRC in participants of the Netherlands Cohort Study on diet and cancer. The results show that the IGF pathway may be involved in associations between ER at young age and CRC in men, though interactions were not statistically significant. No clear risk pattern was detected in women.

versus those who were not. A possible explanation for the inconsistent finding in this study as opposed to the other studies is that both inverse and positive ER-CRC associations can exist depending on the duration of ER, the severity of ER, the quality of the diet, and the timing of exposure to ER in life. In a systematic literature review, we recently observed that a longer duration of ER might be associated with an increased cancer risk.[13] Alternatively, there may have been other residual confounding factors present in potential Holocaust survivors, *e.g.* stress, sleep deprivation, and hygienic circumstances.

Considering the uniqueness of ER exposure, one way to strengthen the evidence for an ER-CRC association is to investigate the mechanisms through which ER influences CRC risk. This will support the biological plausibility of the ER-CRC association, which is an important criterion for establishing causality.[14] A plausible biological mechanism is the insulin-like growth factor (IGF) pathway, considering findings that link together ER and IGF-related factors, and IGF-related factors and CRC risk. Firstly, a decrease in IGF-1 levels and in the IGF-1:IGF binding protein 3 (IGFBP-3) ratio has been found in caloric restricted animals, although a similar decrease in humans may require a restriction in protein intake specifically.[6,15] Secondly, adiponectin levels increased with increasing age in caloric restricted animals,[16] and nuclear protein, mRNA, and DNA binding activity levels of peroxisome proliferator-activator receptors (PPARs) remained constant with increasing age in caloric restricted rats, whereas (activity) levels decreased with age in *ad libitum* fed rats.[17] These effects of caloric restriction on adiponectin and PPARs contribute to better insulin sensitivity, possibly lowering insulin and IGF-1 levels. Lastly, increased IGF-1 levels have been found significantly associated with a 25% increased CRC risk (95% CI = 1.08–1.45), particularly colon cancer risk, in a meta-analysis of human epidemiological studies.[18] IGFBP-1 and −3 levels were non-significantly associated with a reduced CRC risk in this meta-analysis.[18]

Here, we investigated interactions between exposure to ER and genetic variants in the IGF pathway, adiponectin (receptors), and PPARG, as markers for IGF pathway involvement, in relation to CRC risk by sex and subsite and we characterized joint effects.

Subjects and Methods

Population and design

The Netherlands Cohort Study on diet and cancer (NLCS) includes 120,852 men and women who were between 55 and 69 years old at baseline in 1986, when completing a self-administered questionnaire.[19] ~75% of participants also returned toenail clippings. The NLCS was approved by the review boards of the TNO Nutrition and Food Research Institute (Zeist, the Netherlands) and Maastricht University (Maastricht, the Netherlands). The NLCS is characterized by a case-cohort approach for reasons of efficiency, in which a subcohort ($n = 5000$), randomly selected immediately after baseline and independent of any exposure, is followed up through linkage to the Central Bureau of Genealogy and municipal registries to estimate the accumulated person-time at risk (>99.9% completeness). The whole cohort is followed up for incident cancer cases through linkage to the population-based cancer registry and PALGA (the Netherlands pathology database) (>96% completeness).[20,21] Participants who reported a history of cancer (other than skin cancer) on the baseline questionnaire were excluded from follow-up. This left 4,774 subcohort members and 3,440 incident CRC cases (ICD-O 153–154) after a follow-up of 16.3 years. Toenail DNA was isolated according to the DNA extraction protocol of Cline *et al.*[22] with some adjustments.[23] Toenails are a valid long-term DNA source for SNP genotyping in large-scale epidemiological studies.[24] Toenail DNA was available for 3,768 subcohort members (78.9%) and 2,580 CRC cases (75.0%), of which 114 CRC cases were also subcohort members. Consequently, we had available a total of 6,234 unique toenail DNA samples for genotyping.

ER exposure and covariates

ER exposure was based on area-exposure data derived from the baseline questionnaire. Subcohort members and CRC cases were between 12 and 28 years old at the time of the Dutch Hunger Winter in 1944–45. According to Stein *et al.*[25] 11 Western cities are considered famine cities: Amsterdam, Rotterdam, The Hague, Utrecht, Zaandam, Hilversum, Amersfoort, Dordrecht, Vlaardingen/Schiedam, Delft and Leiden. At the height of the famine, between December 1944 and April 1945, official daily rations per capita were 400–800 kilocalories, although the diet remained nutritionally balanced.[26,27] We distinguish between participants who lived in a non-Western area during the Hunger Winter, Western rural area, and Western city, as reported by participants on the baseline questionnaire. Other available proxy variables for ER were the place of residence during the War Years in 1940–44 (urban versus rural) and whether or not an individual's father was employed during the Economic Depression in 1932–40. Nutritional differences existed

during these years, although the contrast was smaller than during the Hunger Winter, *i.e.* sufficient calories were available, but the variation in the food pattern was more limited. For this reason, we view the Hunger Winter as our main exposure (further information is available in the Online Supplemental Material).

Covariates were also derived from the baseline questionnaire, which included a semi-quantitative 150-item food frequency questionnaire (FFQ). Comparison with a 9-day dietary record showed that it ranked subjects adequately according to intake of the food groups and nutrients investigated.[28] The FFQ was found to be a good indicator of nutrient intake over a period of $\geq$5 years.[29]

Single nucleotide polymorphisms in IGF-related genes

Our selection of single nucleotide polymorphisms (SNPs) in IGF-related genes was based on literature and has been described before.[30] The quality control of genotyping resulted in some exclusions, leaving 5,697 samples with at least 95% call rate (*i.e.* one SNP was missing at most).[30] We generated an unweighted genetic sum score of unfavorable alleles using 18 SNPs in 8 genes, as shown in Supporting Information Table 1, for which the literature was unequivocal about which allele was the unfavorable allele. The genetic sum score was divided into tertiles as based on the distribution in the subcohort to maintain optimal power for estimating the CRC risk associated with combined categories of ER and the genetic sum score. One hundred and thirty-four subcohort members and 120 CRC cases could not be categorized because of missing SNP data. Additional details are available in the Online Supplemental Material.

IGF1 19-CA repeat polymorphism

In addition to SNPs, an *IGF1* 19-CA repeat polymorphism was selected for genotyping, because it has been associated with CRC risk.[31–34] Genotyping was performed as described before[30] and in the Online Supplemental Material. The *IGF1* 19-CA repeat polymorphism was categorized according to Rosen *et al.*[35] distinguishing between individuals homozygous for the wild type allele (19/19 CA repeats), heterozygous individuals (19/non-19 CA repeats), and individuals carrying two variant alleles (non-19/non-19 CA repeats). Previous studies showed increased[31–33] and decreased CRC risks[34] for variant repeat alleles.

Statistics

Baseline differences according to ER exposure were studied using χ^2 and ANOVA tests, as appropriate. We estimated sex- and subsite-specific hazard ratios (HRs) for CRC and 95% confidence intervals (95% CIs) for linear combinations of ER and genetic variables using Cox regression, while adjusting for potential confounders. Potential confounders were predefined on the basis of literature[3,11,30]: age, first-degree family history of CRC, smoking status, alcohol intake, meat intake, processed meat intake, total energy intake, body mass index (BMI), and occupational physical activity in men[36] and non-occupational physical activity in women.[36] ER exposed individuals (*i.e.* Western city residents during the Hunger Winter) in the lowest genetic sum score tertile were used as the reference group. This is contrary to what we did in previous studies, in which those who lived in a non-Western area during the Hunger Winter were used as the reference group, yielding a decreased CRC risk to be associated with ER exposure in men.[11] However, it was necessary to switch reference groups for ER variables because the relative excess risk due to interaction (RERI), which is a measure for additive interaction, can only be calculated when coding variables such that the highest combined category is expected to increase risk. Even though we previously observed decreased CRC risks across subsites in women carrying two variant *IGF1* 19-CA repeat alleles versus those carrying two wild type *IGF1* 19-CA repeat alleles, we refrained from recoding the *IGF1* 19-CA repeat polymorphism. Reasons for this were inconsistent literature findings[31–34] and that we previously also observed an increased overall CRC risk in men carrying two variant *IGF1* 19-CA repeat alleles versus those carrying two wild type *IGF1* 19-CA repeat alleles, when analyzing this repeat polymorphism separately[30] and in combination with body size.[37] For comparability, we kept the coding of the *IGF1* 19-CA repeat polymorphism the same in men and women, but we did not show RERIs in women, because these are only interpretable when higher combined categories are expected to increase risk. The RERI was derived from the formula $RERI = RR_{11} - RR_{10} - RR_{01} + 1$,[38] in which *RR* denotes relative risk and *1* and *0* denote comparison versus reference categories, with the position of this number denoting the first and second exposure of two combined exposures. Corresponding 95% bias-corrected confidence intervals were estimated by bootstrapping (n bootstrap samples = 1,000).[39] In addition, we tested multiplicative interactions using the Wald test. To account for the additional variance introduced by sampling the subcohort from the entire cohort, standard errors were estimated using the robust Huber-White sandwich estimator.[40] The proportional hazards assumption was tested using the scaled Schoenfeld residuals and by visually inspecting the -log-log-transformed hazard curves (there were no apparent violations). To check for the influence of preclinical disease, a sensitivity analysis was conducted in which the first 2 years of follow-up were excluded (with no essential changes in results). Analyses were conducted using Stata version 12 (Stata Corp., College Station, TX). Statistical significance was indicated by a p value <0.05 for two-sided testing.

Results

Baseline characteristics

Figure 1 shows a flow diagram of the available number of subcohort members and CRC cases for each combination of ER variables and genetic variables (irrespective of missing values on potential confounders). Participants with an inconsistent or incomplete FFQ were excluded. The genetic sum score as based on the distribution in the subcohort was generated after this exclusion step and the range of unfavorable alleles was 6–14, 15–18, and 19–29 in the lowest, middle, and highest tertile, respectively.

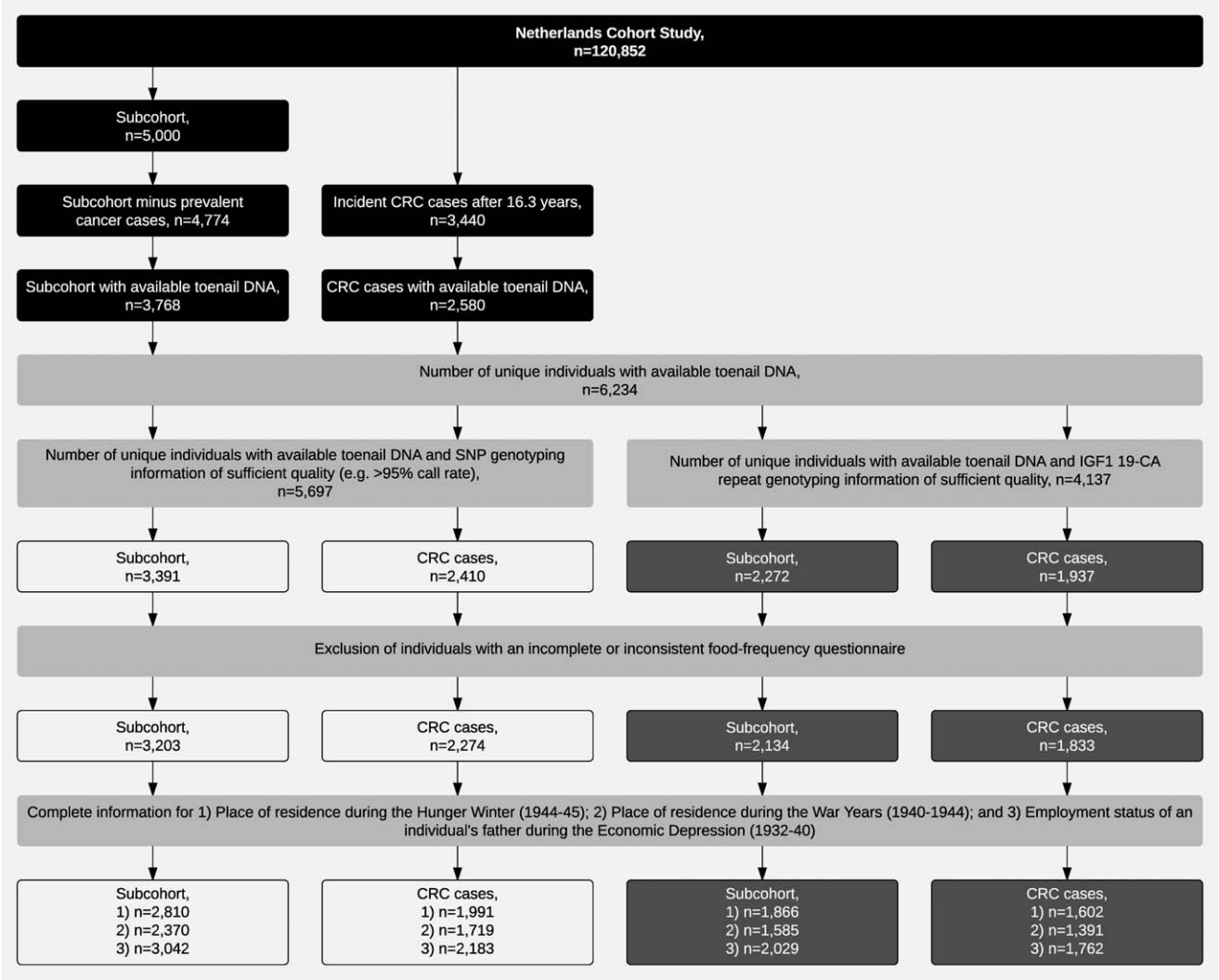

Figure 1. Flow diagram of available subcohort members and colorectal cancer cases in this study within the Netherlands Cohort Study.

Table 1 shows the number of male and female subcohort members across categories of genetic variables and potential confounders according to the place of residence during the Hunger Winter. In general, men living in a non-Western area during the Hunger Winter were younger (p value = 0.02), had a higher intake of meat (p value = 0.02) and processed meat (p value < 0.001), had a higher BMI (p value < 0.001), and were relatively less often engaged in high levels of occupational physical activity (p value = 0.003) than men living in a Western area during the Hunger Winter. Women living in a non-Western area during the Hunger Winter were less often (ex-)smokers (p value = 0.02) and had a higher intake of processed meat (p value < 0.001) and total energy at baseline (p value = 0.03) than women living in a Western area during the Hunger Winter. There were no significant differences in genetic variables, first-degree family history of colorectal cancer and alcohol intake in men and women, smoking and total energy intake in men, and meat intake, BMI and non-occupational physical activity in women according to the place of residence during the Hunger Winter.

Joint effects of ER exposures and genetic variation in the IGF pathway

There were no multiplicative or additive interactions between the place of residence during the Hunger Winter and the genetic sum score of unfavorable alleles in the IGF pathway in relation to the risk of CRC overall and by subsite in both men (Table 2) and women (Table 3). Increasing CRC risks were observed across combined categories of exposure to the Hunger Winter and the genetic sum score in men, but such a trend was not evident in women. Hazard ratios (95% CIs) for CRC comparing ER exposed men in the highest genetic sum score tertile (genetic effect), ER unexposed men in the lowest genetic sum score tertile (effect of absence of ER), and

Table 1. Baseline characteristics of participants in the Netherlands Cohort Study according to place of residence during the Dutch Hunger Winter in 1944–45

	Place of residence during the Dutch Hunger Winter of male subcohort members						Place of residence during the Dutch Hunger Winter of female subcohort members					
	Western city		Western rural area		Non-western area		Western city		Western rural area		Non-western area	
Baseline characteristic in 1986	***N*** **(%)**	**Mean (SD)**	***N*** **(%)**	**Mean (SD)**	***N*** **(%)**	**Mean (SD)**	***N*** **(%)**	**Mean (SD)**	***N*** **(%)**	**Mean (SD)**	***N*** **(%)**	**Mean (SD)**
Age (years)*		61.2 (4.5)		60.1 (4.1)		60.9 (4.2)		61.5 (4.3)		60.9 (4.1)		61.0 (4.2)
Genetic sum score[1]												
T1	121 (42.5)		62 (36.5)		230 (36.1)		113 (35.7)		47 (34.1)		199 (36.5)	
T2	99 (34.7)		67 (39.4)		249 (39.0)		115 (36.3)		51 (37.0)		207 (37.9)	
T3	65 (22.8)		41 (24.1)		159 (24.9)		89 (28.1)		40 (29.0)		140 (25.6)	
IGF1 **19-CA repeat polymorphism‡**												
19/19 CA repeats	73 (35.3)		51 (41.1)		157 (34.5)		64 (27.0)		34 (35.8)		108 (30.2)	
19/non-19	86 (41.6)		45 (36.3)		187 (41.1)		84 (35.4)		30 (31.6)		132 (36.9)	
Non-19/non-19	48 (23.2)		28 (22.6)		111 (24.4)		89 (37.6)		31 (32.6)		118 (33.0)	
Family history of colorectal cancer (% yes)§	18 (6.1)		8 (4.5)		32 (4.8)		15 (4.5)		7 (4.8)		41 (7.2)	
Smoking status¶												
Never	37 (12.5)		25 (14.0)		91 (13.8)		166 (49.7)		88 (60.3)		330 (58.1)	
Ex	172 (58.1)		97 (54.5)		349 (52.7)		79 (23.7)		36 (24.7)		117 (20.6)	
Current	87 (29.4)		56 (31.5)		222 (33.5)		89 (26.7)		22 (15.1)		121 (21.3)	
Alcohol intake°												
0 g/d	46 (15.5)		23 (12.9)		83 (12.5)		95 (28.4)		37 (25.3)		190 (33.5)	
0.1–29.0	209 (70.6)		127 (71.4)		470 (71.0)		226 (67.7)		104 (71.2)		360 (63.4)	
≥30	41 (13.9)		28 (15.7)		109 (16.5)		13 (3.9)		5 (3.4)		18 (3.2)	
Meat intake, g/d**		99.7 (42.6)		101.2 (42.3)		107.5 (41.6)		89.6 (38.4)		88.7 (38.9)		95.0 (40.2)
Processed meat intake, g/d††		13.6 (13.2)		16.2 (17.0)		18.7 (18.9)		9.1 (9.8)		9.8 (9.4)		12.2 (13.6)
Total energy intake, kcal/d‡‡		2,107 (467)		2,172 (475)		2,188 (496)		1,657 (369)		1,741 (391)		1,720 (405)
Body mass index, kg/m^2§§		24.7 (2.5)		24.2 (2.6)		25.1 (2.5)		25.2 (3.7)		25.2 (3.5)		24.9 (3.6)
Occupational physical activity¶¶												
<8 kJ/day	30 (10.1)		29 (16.3)		99 (15.0)							
8–12	65 (22.0)		36 (20.2)		192 (29.0)							
>12	201 (67.9)		113 (63.5)		371 (56.0)							

Table 1. Baseline characteristics of participants in the Netherlands Cohort Study according to place of residence during the Dutch Hunger Winter in 1944–45 (Continued)

	Place of residence during the Dutch Hunger Winter of male subcohort members						Place of residence during the Dutch Hunger Winter of female subcohort members					
	Western city		Western rural area		Non-western area		Western city		Western rural area		Non-western area	
Baseline characteristic in 1986	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)	Mean (SD)
Non-occupational physical activity[∞]												
≤30 min/d							84 (25.3)		34 (23.8)		118 (20.9)	
>30–60							84 (25.3)		34 (23.8)		129 (22.9)	
>60–90							103 (31.0)		52 (36.4)		194 (34.4)	
>90							61 (18.4)		23 (16.1)		123 (21.8)	

Abbreviations: SD, standard deviation.
Notes: *p*-Values for χ^2 or ANOVA tests indicating potential differences between categories of ER exposure and baseline characteristics were *0.02, †0.45, ‡0.73, §0.66, ¶0.64, °0.68, **0.02, ††<0.001, ‡‡0.06, §§<0.001 and ¶¶0.003 in men, respectively, and *0.27, †0.90, ‡0.50, §0.20, ¶0.02, °0.29, **0.07, ††<0.001, ‡‡0.03, §§0.45 and °°0.43 in women, respectively.
[1]The range in tertiles of the genetic sum score was 6–14 (T1), 15–18 (T2), and 19–29 (T3) unfavorable alleles.

ER unexposed men in the highest genetic sum score tertile (joint effect) relative to ER exposed men in the lowest genetic sum score tertile, which were hypothesized to be at lowest risk and thus used as reference, were: 1.23 (0.74 to 2.04), 1.51 (1.03 to 2.20), and 2.04 (1.39 to 3.00), respectively. However, even though several hazard ratios across combined categories reached statistical significance, confidence intervals were wide and overlapping. Results by subsite did not clarify associations further.

There were also no interactions between the place of residence during the Hunger Winter and the *IGF1* 19-CA repeat polymorphism in relation to the risk of CRC overall and by subsite in both men (Table 4) and women (Table 5). Women carrying two variant repeat alleles (non-19 CA repeats) versus women carrying two wild type alleles (19 CA repeats) were found to have strongly decreased CRC risks, irrespective of ER exposure. This result was consistent across colon subsites, but not observed in relation to rectal cancer.

When considering the place of residence during the War Years and the employment status of an individual's father during the Economic Depression in interaction with the genetic sum score, no interactions were observed in relation to the risk of CRC overall and by subsite in men and women (Supporting Information Tables 2–5). When considering the place of residence during the War Years and the employment status of an individual's father during the Economic Depression in interaction with the *IGF1* 19-CA repeat polymorphism, multiplicative interactions in relation to CRC, in particular proximal colon cancer, were observed in women but not men (Supporting Information Tables 6–9). Similar to results for combined categories of ER exposure during the Hunger Winter and *IGF1* 19-CA repeat status, women carrying two variant repeat alleles as compared to women carrying two wild type repeat alleles were at a decreased risk of CRC, irrespective of ER exposure during the War Years or Economic Depression (Supporting Information Tables 7 and 9).

Discussion

We investigated whether genetic variation in IGF-related genes modified the ER-CRC association. Multiplicative and additive interactions between ER variables and genetic variation in the IGF pathway in relation to CRC were not statistically significant in men and women. There was indication of a pattern of increasing CRC risks across combined categories of ER-genetic sum score combinations in men, but not women. However, confidence intervals around hazard ratios were wide and overlapping, making it difficult to conclude whether hazard ratios in higher combined categories differed significantly, and chance findings cannot be excluded. It should be noted that for the purpose of assessing additive interactions, we changed the reference group of the ER variables to ER exposed individuals instead of unexposed individuals, which were used as reference in our previous studies. Previously, in the NLCS, ER exposed versus unexposed men, but not women, were found to be at a decreased CRC risk.[11] The genetic

Table 2. Hazard ratios and 95% confidence intervals for colorectal cancer by subsite for combined categories of energy restriction during the Dutch Hunger Winter and tertiles of the genetic sum score of unfavorable alleles in IGF-related genes in men in the Netherlands Cohort Study (1986–2002)

		Place of residence during the Dutch Hunger Winter (1944–45), indicating exposure to energy restriction											
		Western city (exposed)			Western rural area			Non-Western area (non-exposed)			*p* for multiplicative interaction	RERI[2]	(95% CI)[2]
		N cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)			
Colorectum													
Genetic sum	T1 (lowest)	69/1,690	1	reference	44/850	1.30	(0.78, 2.17)	190/3,157	1.51	(1.03, 2.20)			
score[3]	T2	90/1,371	1.65	(1.06, 2.55)	50/934	1.54	(0.93, 2.53)	242/3,423	1.73	(1.19, 2.50)			
	T3 (highest)	47/888	1.23	(0.74, 2.04)	42/586	1.86	(1.07, 3.22)	193/2,243	2.04	(1.39, 3.00)	0.52	0.31	(−0.71, 1.04)
Colon													
Genetic sum	T1 (lowest)	48/1,690	1	reference	27/850	1.22	(0.67, 2.20)	115/3,157	1.34	(0.87, 2.06)			
score[3]	T2	62/1,371	1.65	(1.01, 2.69)	29/934	1.30	(0.73, 2.32)	148/3,423	1.57	(1.03, 2.39)			
	T3 (highest)	34/888	1.27	(0.72, 2.23)	33/586	2.08	(1.14, 3.80)	130/2,243	2.02	(1.31, 3.10)	0.45	0.41	(−0.53, 1.30)
Proximal colon													
Genetic sum	T1 (lowest)	18/1,690	1	reference	16/850	1.98	(0.92, 4.24)	59/3,157	1.76	(0.97, 3.20)			
score[3]	T2	25/1,371	1.65	(0.83, 3.29)	13/934	1.56	(0.71, 3.45)	63/3,423	1.73	(0.95, 3.13)			
	T3 (highest)	14/888	1.32	(0.60, 2.91)	14/586	2.22	(0.98, 5.02)	61/2,243	2.45	(1.35, 4.45)	0.56	0.37	(−1.20, 1.65)
Distal colon													
Genetic sum	T1 (lowest)	27/1,690	1	reference	11/850	0.87	(0.39, 1.93)	53/3,157	1.13	(0.66, 1.94)			
score[3]	T2	32/1,371	1.60	(0.88, 2.93)	15/934	1.18	(0.57, 2.46)	81/3,423	1.59	(0.95, 2.65)			
	T3 (highest)	19/888	1.31	(0.66, 2.61)	18/586	2.12	(1.02, 4.39)	67/2,243	1.91	(1.13, 3.23)	0.70	0.46	(−0.87, 1.39)
Rectum													
Genetic sum	T1 (lowest)	16/1,690	1	reference	11/850	1.25	(0.53, 2.97)	62/3,157	2.02	(1.10, 3.74)			
score[3]	T2	22/1,371	1.74	(0.85, 3.56)	13/934	1.66	(0.73, 3.75)	70/3,423	2.01	(1.09, 3.70)			
	T3 (highest)	9/888	1.02	(0.42, 2.49)	4/586	0.78	(0.24, 2.51)	41/2,243	1.81	(0.96, 3.41)	0.62	−0.23	(−2.38, 0.88)

Abbreviations: CI, confidence interval; HR, hazard ratio; IGF, insulin-like growth factor; PY, person-years at risk, RERI, relative excess risk due to interaction; T1–3, tertile 1–3.
[1]Adjusted for age, first-degree family history of colorectal cancer, smoking status, alcohol intake, meat intake, processed meat intake, total energy intake, occupational physical activity, and body mass index.
[2]Relative excess risk due to interaction (RERI) and bias-corrected confidence interval. The RERI requires variables to be coded such that the highest combined category is expected to increase risk.
[3]The range in tertiles of the genetic sum score was 6–14 (T1), 15–18 (T2), and 19–29 (T3) unfavorable alleles.

Table 3. Hazard ratios and 95% confidence intervals for colorectal cancer by subsite for combined categories of energy restriction during the Dutch Hunger Winter and tertiles of the genetic sum score of unfavorable alleles in IGF-related genes in women in the Netherlands Cohort Study (1986–2002)

		Place of residence during the Dutch Hunger Winter (1944–45), indicating exposure to energy restriction											
		Western city (exposed)			Western rural area			Non-Western area (non-exposed)			*p* for multiplicative interaction	RERI[2]	(95% CI)[2]
		N cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)			
Colorectum													
Genetic sum	T1 (lowest)	58/2,073	1	reference	48/971	1.80	(1.10, 2.95)	140/4,120	1.18	(0.81, 1.72)			
score[3]	T2	94/2,095	1.59	(1.05, 2.40)	51/1,019	1.76	(1.08, 2.87)	154/3,976	1.26	(0.86, 1.84)			
	T3 (highest)	56/1,514	1.29	(0.81, 2.05)	28/888	1.16	(0.66, 2.04)	113/2,792	1.33	(0.89, 1.99)	0.23	−0.14	(−1.03, 0.48)
Colon													
Genetic sum	T1 (lowest)	47/2,073	1	reference	38/971	1.78	(1.04, 3.02)	102/4,120	1.06	(0.70, 1.60)			
score[3]	T2	76/2,095	1.59	(1.02, 2.47)	36/1,019	1.51	(0.88, 2.59)	116/3,976	1.18	(0.78, 1.78)			
	T3 (highest)	39/1,514	1.12	(0.67, 1.88)	23/888	1.17	(0.64, 2.13)	80/2,792	1.16	(0.75, 1.80)	0.35	−0.01	(−0.91, 0.56)
Proximal colon													
Genetic sum	T1 (lowest)	35/2,073	1	reference	25/971	1.59	(0.87, 2.92)	48/4,120	0.67	(0.40, 1.10)			
score[3]	T2	48/2,095	1.35	(0.81, 2.24)	18/1,019	1.00	(0.52, 1.94)	68/3,976	0.94	(0.58, 1.52)			
	T3 (highest)	19/1,514	0.72	(0.38, 1.38)	14/888	0.95	(0.46, 1.93)	43/2,792	0.83	(0.49, 1.41)	0.19	0.44	(−0.30, 1.01)
Distal colon													
Genetic sum	T1 (lowest)	12/2,073	1	reference	12/971	2.16	(0.91, 5.12)	49/4,120	2.01	(1.02, 3.98)			
score[3]	T2	26/2,095	2.16	(1.04, 4.48)	17/1,019	2.82	(1.26, 6.31)	46/3,976	1.84	(0.92, 3.65)			
	T3 (highest)	18/1,514	2.07	(0.93, 4.57)	8/888	1.59	(0.61, 4.14)	34/2,792	1.97	(0.97, 4.00)	0.25	−1.11	(−4.41, 0.43)
Rectum													
Genetic sum	T1 (lowest)	8/2,073	1	reference	7/971	1.83	(0.63, 5.30)	28/4,120	1.67	(0.72, 3.84)			
score[3]	T2	12/2,095	1.45	(0.57, 3.70)	14/1,019	3.65	(1.44, 9.27)	23/3,976	1.28	(0.54, 2.99)			
	T3 (highest)	12/1,514	2.06	(0.80, 5.30)	3/888	0.96	(0.24, 3.85)	26/2,792	2.24	(0.96, 5.19)	0.09	−0.49	(−4.29, 1.23)

Abbreviations: CI, confidence interval; HR, hazard ratio; IGF, insulin-like growth factor; PY, person-years at risk, RERI, relative excess risk due to interaction; T1–3, tertile 1–3.
[1]Adjusted for age, first-degree family history of colorectal cancer, smoking status, alcohol intake, meat intake, processed meat intake, total energy intake, non-occupational physical activity, and body mass index.
[2]Relative excess risk due to interaction (RERI) and bias-corrected confidence interval. The RERI requires variables to be coded such that the highest combined category is expected to increase risk.
[3]The range in tertiles of the genetic sum score was 6–14 (T1), 15–18 (T2), and 19–29 (T3) unfavorable alleles.

Table 4. Hazard ratios and 95% confidence intervals for colorectal cancer by subsite for combined categories of energy restriction during the Dutch Hunger Winter and *IGF1* 19-CA repeat status in men in the Netherlands Cohort Study (1986–2002)

		Place of residence during the Dutch Hunger Winter (1944-45), indicating exposure to energy restriction											
		Western city (exposed)			Western rural area			Non-Western area (non-exposed)			*p* for multiplicative interaction	RERI[2]	(95% CI)[2]
		N cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)			
Colorectum													
IGF1 repeat	19/19 repeats	71/1,096	1	reference	43/726	0.95	(0.54, 1.67)	184/2,242	1.20	(0.78, 1.83)			
polymorpism	19/non-19	67/1,289	0.70	(0.43, 1.14)	44/700	0.95	(0.55, 1.64)	209/2,770	1.01	(0.66, 1.53)			
	Non-19/non-19	50/723	1.01	(0.58, 1.75)	24/401	0.93	(0.46, 1.85)	135/1,559	1.19	(0.76, 1.86)	0.89	−0.02	(−0.98, 0.60)
Colon													
IGF1 repeat	19/19 repeats	53/1,096	1	reference	29/726	0.87	(0.47, 1.62)	119/2,242	1.06	(0.67, 1.69)			
polymorpism	19/non-19	50/1,289	0.71	(0.42, 1.21)	29/700	0.85	(0.47, 1.56)	137/2,770	0.89	(0.57, 1.40)			
	Non-19/non-19	34/723	0.94	(0.52, 1.73)	14/401	0.76	(0.35, 1.66)	77/1,559	0.92	(0.56, 1.52)	0.92	−0.08	(−1.10, 0.51)
Proximal colon													
IGF1 repeat	19/19 repeats	18/1,096	1	reference	14/726	1.34	(0.59, 3.06)	62/2,242	1.70	(0.90, 3.22)			
polymorpism	19/non-19	17/1,289	0.75	(0.35, 1.63)	14/700	1.28	(0.57, 2.85)	61/2,770	1.22	(0.65, 2.29)			
	Non-19/non-19	19/723	1.58	(0.72, 3.49)	6/401	0.99	(0.34, 2.94)	34/1,559	1.18	(0.59, 2.35)	0.37	−1.11	(−3.58, 0.14)
Distal colon													
IGF1 repeat	19/19 repeats	29/1,096	1	reference	15/726	0.77	(0.35, 1.66)	57/2,242	0.91	(0.52, 1.59)			
polymorpism	19/non-19	30/1,289	0.76	(0.41, 1.42)	14/700	0.73	(0.34, 1.54)	70/2,770	0.83	(0.48, 1.42)			
	Non-19/non-19	14/723	0.71	(0.33, 1.54)	8/401	0.77	(0.30, 1.97)	42/1,559	0.96	(0.53, 1.75)	0.94	0.34	(−0.75, 0.96)
Rectum													
IGF1 repeat	19/19 repeats	15/1,096	1	reference	8/726	0.80	(0.30, 2.09)	54/2,242	1.54	(0.79, 3.00)			
polymorpism	19/non-19	15/1,289	0.69	(0.30, 1.55)	7/700	0.67	(0.25, 1.79)	55/2,770	1.21	(0.62, 2.37)			
	Non-19/non-19	9/723	0.79	(0.31, 2.01)	6/401	0.97	(0.32, 2.93)	40/1,559	1.55	(0.78, 3.08)	0.99	0.22	(−1.29, 1.14)

Abbreviations: CI, confidence interval; HR, hazard ratio; IGF, insulin-like growth factor; PY, person-years at risk, RERI, relative excess risk due to interaction.
[1]Adjusted for age, first-degree family history of colorectal cancer, smoking status, alcohol intake, meat intake, processed meat intake, total energy intake, occupational physical activity, and body mass index.
[2]Relative excess risk due to interaction (RERI) and bias-corrected confidence interval. The RERI requires variables to be coded such that the highest combined category is expected to increase risk.

Table 5. Hazard ratios and 95% confidence intervals for colorectal cancer by subsite for combined categories of energy restriction during the Dutch Hunger Winter and *IGF1* 19-CA repeat status in women in the Netherlands Cohort Study (1986–2002)

		Place of residence during the Dutch Hunger Winter (1944–45), indicating exposure to energy restriction									*p* for multiplicative interaction	RERI[2]	(95% CI)[2]
		Western city (exposed)			Western rural area			Non-Western area (non-exposed)					
		N cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)	*N* cases/PY	HR[1]	(95% CI)			
Colorectum													
IGF1 repeat	19/19 repeats	75/1,237	1	reference	38/657	1.02	(0.58, 1.78)	120/2,322	0.82	(0.54, 1.24)			
polymorpism	19/non-19	86/1,499	0.93	(0.60, 1.45)	46/787	1.00	(0.59, 1.69)	124/2,806	0.66	(0.44, 0.99)			
	Non-19/non-19	32/1,549	0.34	(0.21, 0.58)	31/737	0.73	(0.41, 1.29)	83/2,589	0.52	(0.34, 0.79)	0.14	-	-
Colon													
IGF1 repeat	19/19 repeats	65/1,237	1	reference	29/657	0.89	(0.49, 1.62)	93/2,322	0.73	(0.47, 1.13)			
polymorpism	19/non-19	65/1,499	0.81	(0.50, 1.29)	39/787	0.98	(0.56, 1.71)	89/2,806	0.54	(0.35, 0.83)			
	Non-19/non-19	24/1,549	0.30	(0.17, 0.53)	22/737	0.60	(0.32, 1.11)	59/2,589	0.42	(0.27, 0.66)	0.19	-	-
Proximal colon													
IGF1 repeat	19/19 repeats	39/1,237	1	reference	13/657	0.68	(0.32, 1.45)	54/2,322	0.71	(0.42, 1.19)			
polymorpism	19/non-19	41/1,499	0.82	(0.47, 1.43)	27/787	1.13	(0.59, 2.13)	47/2,806	0.47	(0.28, 0.79)			
	Non-19/non-19	15/1,549	0.31	(0.16, 0.62)	13/737	0.58	(0.27, 1.24)	28/2,589	0.34	(0.19, 0.59)	0.22	-	-
Distal colon													
IGF1 repeat	19/19 repeats	24/1,237	1	reference	16/657	1.25	(0.58, 2.69)	38/2,322	0.80	(0.43, 1.46)			
polymorpism	19/non-19	22/1,499	0.76	(0.39, 1.46)	10/787	0.67	(0.29, 1.54)	39/2,806	0.65	(0.36, 1.16)			
	Non-19/non-19	9/1,549	0.30	(0.13, 0.69)	9/737	0.65	(0.27, 1.55)	30/2,589	0.57	(0.31, 1.05)	0.42	-	-
Rectum													
IGF1 repeat	19/19 repeats	6/1,237	1	reference	6/657	2.13	(0.61, 7.50)	20/2,322	1.74	(0.66, 4.62)			
polymorpism	19/non-19	16/1,499	2.34	(0.86, 6.36)	6/787	1.65	(0.48, 5.66)	24/2,806	1.65	(0.63, 4.28)			
	Non-19/non-19	7/1,549	0.93	(0.30, 2.93)	7/737	2.17	(0.65, 7.26)	17/2,589	1.35	(0.50, 3.61)	0.41	-	-

Abbreviations: CI, confidence interval; HR, hazard ratio; IGF, insulin-like growth factor; PY, person-years at risk, RERI, relative excess risk due to interaction.
[1]Adjusted for age, first-degree family history of colorectal cancer, smoking status, alcohol intake, meat intake, processed meat intake, total energy intake, non-occupational physical activity, and body mass index.
[2]Relative excess risk due to interaction (RERI) and bias-corrected confidence interval. The RERI requires variables to be coded such that the highest combined category is expected to increase risk. Since decreased risks were observed for variant repeat allele carriers, irrespective of energy restriction during the Hunger Winter, as compared to the reference in this table, we did not show RERIs, because these are not interpretable.

sum score of unfavorable alleles in IGF pathway genes was previously associated with an increased CRC risk in men, but not women.[30] When we previously considered interactions between indicators of body size and this genetic sum score, we found that a larger body size was a CRC risk factor in men, but not women, in the presence of an accumulation of unfavorable alleles in the IGF pathway, although also no significant interactions were observed.[37] Variant *IGF1* 19-CA repeat alleles have been consistently associated with decreased CRC risks in women in the NLCS, irrespective of ER in the present study and body size in a previous study.[37]

The absence of significant interactions in the presence of a CRC risk pattern in men across categories of ER exposure and the genetic sum score prevents definite conclusions. Nonsignificant interactions could be because of insufficient power, even though large case numbers were available. It is known that a four times larger sample size is needed to detect an interaction effect as compared to a marginal effect of similar magnitude,[41] making power a common issue in gene-environment interaction (GxE) research. Still, a higher number of significant interactions has been reported in the GxE field as would be expected on the basis of chance alone, but few interactions have been consistently replicated, which suggests publication bias or false positives.[42,43] In this regard, there may often be a a trade-off between high-quality exposure data and sample size.[42] The present study included detailed baseline exposure information and had a nearly complete follow-up. In addition, next to testing both multiplicative and additive interactions, because interactions may be scale-dependent, we felt it important to characterize joint effects by looking at potential risk patterns.

The observed CRC risk pattern in men across categories of ER exposure and the genetic sum score is biologically plausible, as described in the introduction. The biological plausibility of involvement of the IGF pathway in the ER-CRC association is further indicated by mice studies that showed that caloric restriction, inducing a lean phenotype, decreased IGF-1 blood levels[44–46] and increased IGFBP-3[46] and adiponectin blood levels[45], next to increasing the time to palpable tumor[44] and decreasing the volume[45] or number of (induced) tumors.[46] These animal models cannot explain the heterogeneity found in results between men and women. However, it has long been recognized that insulin-like growth factor, insulin, and hormonal axes may interact, explaining differences in obesity-CRC associations between men and women. For example, an association has been observed between obesity and CRC in men and premenopausal women, but less clearly in postmenopausal women.[47] A putative explanation for this relates to that obesity is correlated with estrogen levels in postmenopausal women, in which androgen to estrogen conversion in adipose tissue is the main source of estrogen, whereas ovarian production of estrogens mainly determines estrogen levels in premenopausal women.[47] Higher postmenopausal endogenous hormone levels[48] and postmenopausal hormone use[49] have been associated with a decreased CRC risk. Therefore, it is thought that estrogens may counter the risk-increasing influence of obesity in postmenopausal women.[47] In parallel, in response to moderate aerobic exercise combined with caloric restriction, estrogen levels have been reported to decrease in premenopausal women,[50] and the protective effect of ER in women at young age, which favorably influences insulin-like growth factor and insulin levels, might be weakened by a simultaneous drop in estrogen levels. In the present study, ER exposure occurred at young age before, during or after menarche, but the vast majority of women in the NLCS was exposed after menarche[11] and thus exposure was premenopausal. NLCS participants came under observation since 1986 when at least 55 years old, rendering the vast majority of incident female CRC cases in this study postmenopausal. It would be interesting for future research to explore a three-way interaction between ER at young age, BMI at adult age, and genetic variation in the IGF pathway, for which we had too limited power at present.

Strengths of the present study include the prospective character and long follow-up. The long follow-up for cancer incidence (16.3 years) resulted in large case numbers and substantial power, but, as a limitation, may have attenuated associations with increasing follow-up time. Unfortunately, the relatively small proportion of individuals exposed to ER did not allow for the exploration of combined categories of ER and genetic variables in relation to CRC risk according to different follow-up periods. The use of area-exposure data as proxies for ER was validated within our cohort, as 80% of women who reported severe hunger also reported living in a Western city during the Hunger Winter.[51] Strengths of this study also include the availability of information on many potential confounders in diet and lifestyle, minimizing residual confounding. This is important, because in any study investigating ER exposure at young age and later life CRC risk, other risk and protective factors will have accumulated in individuals between the age of ER exposure and baseline measurements. We did not adjust for multiple testing, because our study was hypothesis-based and the genetic sum score greatly reduced the number of tests that had to be performed. Although this may be considered a limitation by some, we were most interested in characterizing joint effects between ER and genetic variation in the IGF pathway, as opposed to discovering genetic variants affecting the risk of CRC, for which guarding for type I errors is most important (though increasing the chances of type II errors).[42,52] In retrospect, adjustment would not have changed our results (*i.e.* most results were nonsignificant).

To conclude, these unique data on ER and CRC indicate that the IGF pathway might be involved in the ER-CRC association in men, but not women, although multiplicative and additive interactions were nonsignificant, hampering definite conclusions.

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