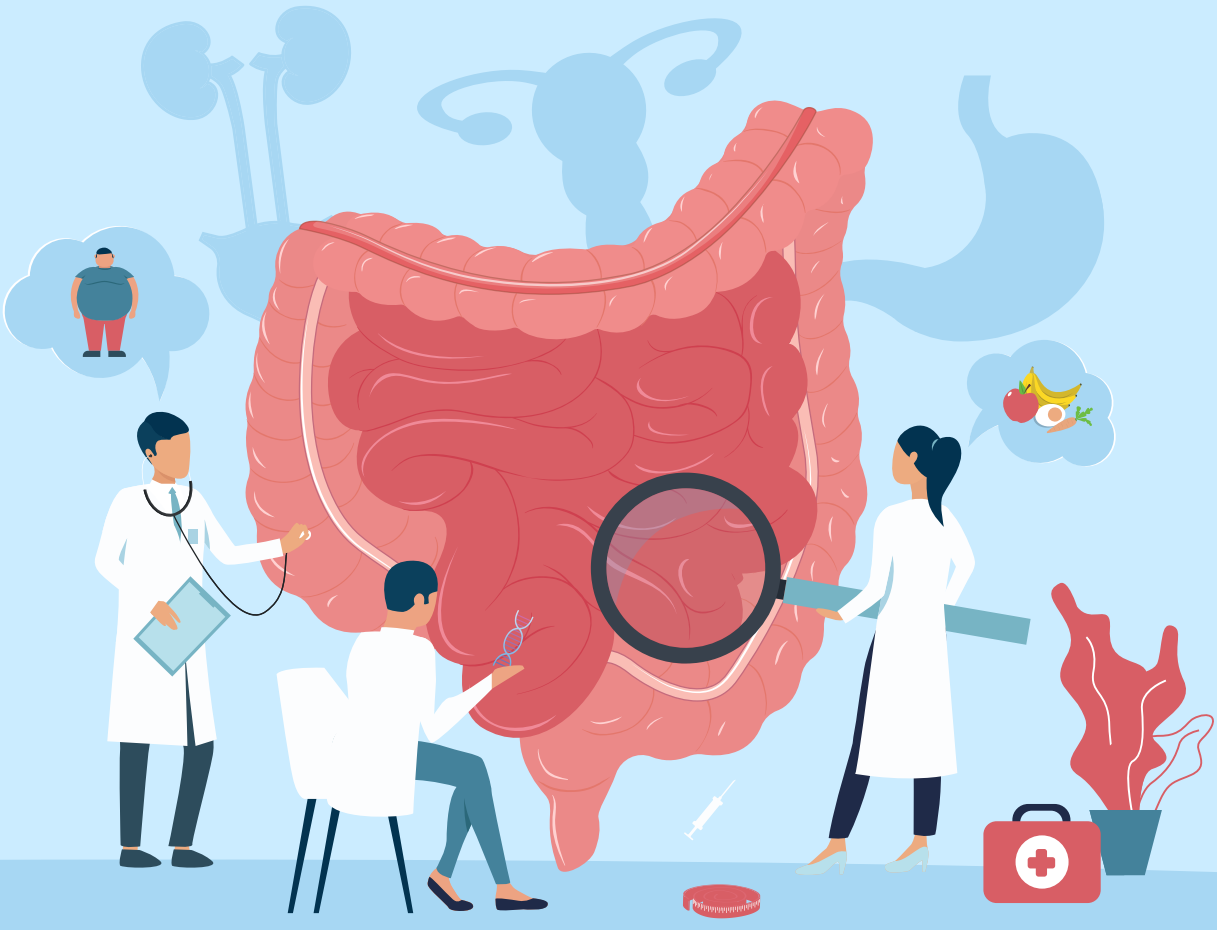
TUMOUR DEVELOPMENT IN LYNCH SYNDROME

GENES LOAD THE GUN, LIFESTYLE PULLS THE TRIGGER?

Jesca G.M. Brouwer



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This thesis was conducted under auspices of the Graduate School VLAG (Advanced Studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

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Thesis

Submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.K. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 16 September 2020
at 1:30 p.m. in the Aula.

Jesca G.M. Brouwer
Tumour development in Lynch syndrome. *Genes load the gun, lifestyle pulls the trigger?*
170 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2020)
With references, with summary in English

ISBN: 978-94-6395-296-5
DOI: https://doi.org/10.18174/512910

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CHAPTER 1

GENERAL INTRODUCTION

Lynch syndrome

In 1913, Aldred Scott Warthin was the first to describe a family with a 'family susceptibility' to cancer[1] (Figure 1). The disease running in family "G" was later identified as Lynch syndrome (LS)[2]. LS is caused by a dominantly inherited pathogenic variant in one of the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2*[3], or by a deletion of the *EPCAM* gene leading to epigenetic silencing of *MSH2*[4]. An estimated 1 in 279 persons has LS[5]. Depending on the study, the lifetime risk of colorectal and endometrial cancer are estimated to be 12% to 97%[6-10] and 12% to 54%[6-8, 10, 11], respectively. This is substantially higher compared with a lifetime risk of 4% to 7% for colorectal and 2% to 3% for endometrial cancer in the general population of Western countries[12, 13]. LS is responsible for 1% to 3% of the colorectal cancer burden[14] and 1% to 4%[15, 16] of the endometrial cancer burden. Apart from an increased risk of colorectal and endometrial cancer, persons with LS are also more often diagnosed with colorectal adenomas[17, 18], a precursor lesion of colorectal cancer[19], and with cancer at other sites including the stomach, pancreas, small bowel, biliary tract, urinary tract and possibly breast[8, 10, 20-22]. Moreover, the median age of LS-associated cancers is estimated to be 4 to 11 years lower compared to the median age of cancer diagnosis for the general population[8, 20, 23].

Cancer risk estimates are highly variable within and between families with the same mutated gene[6-8, 10]. For example, for family "G", in which a *MSH2* gene mutation was responsible for LS[2], initially a family susceptibility to gastric and endometrial cancer was described which shifted over generations to colorectal and endometrial cancer (Figure 1). The high variability in cancer risk estimates and the shift of the predominant cancers over time, suggests that, apart from having a LS-causing germline gene mutation, other factors may also be involved in cancer development for these persons.

Lifestyle-related factors and tumour risk for persons with LS

In the general population, it is suggested that about 40% of all cancer diagnoses can be prevented by a healthy lifestyle and avoidance of certain infectious agents, environmental pollution, occupational carcinogens and ionizing radiation[24]. There is convincing evidence that smoking, several dietary factors, physical activity, body fatness and height influence cancer risk[25]. Generalizing these results to persons with LS may be hampered due to the suggested distinctive molecular pathway of LS-associated tumour development compared to the majority of tumours diagnosed in the general population demonstrated by, for example, the presence of microsatellite instability (MSI) in almost all LS-associated tumours while MSI is present only

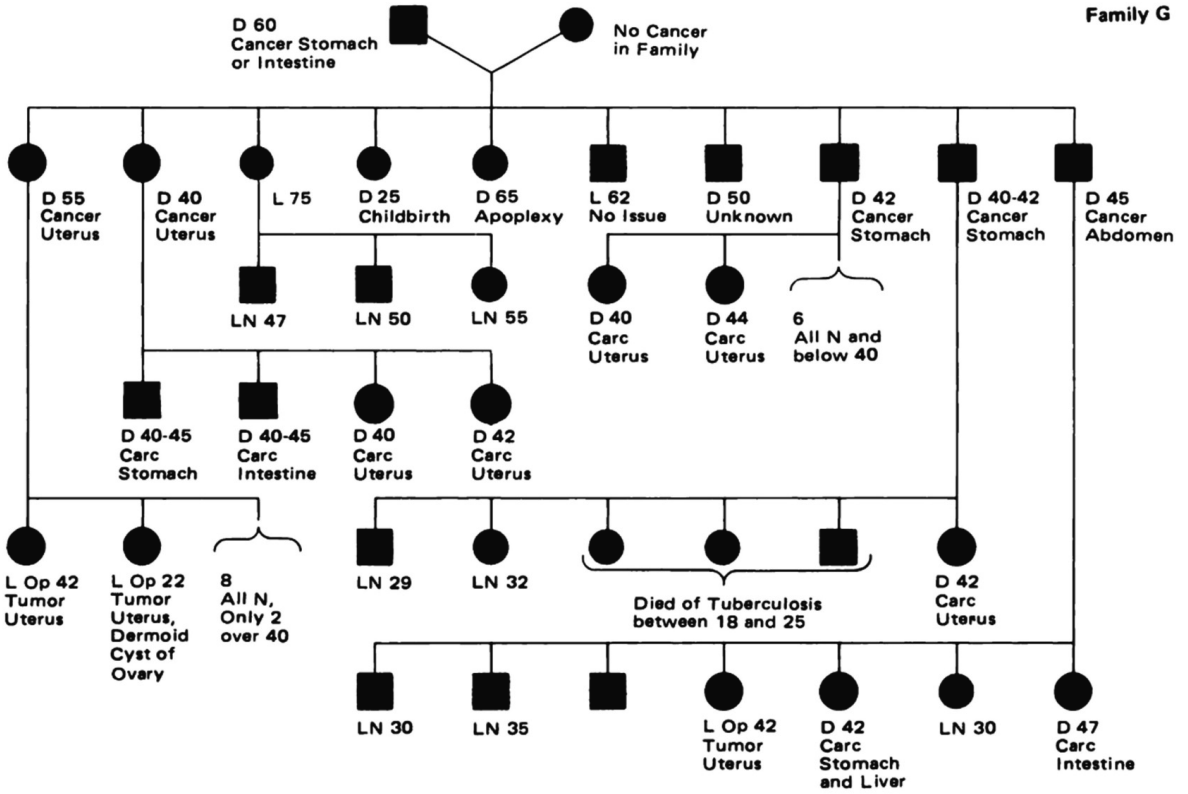


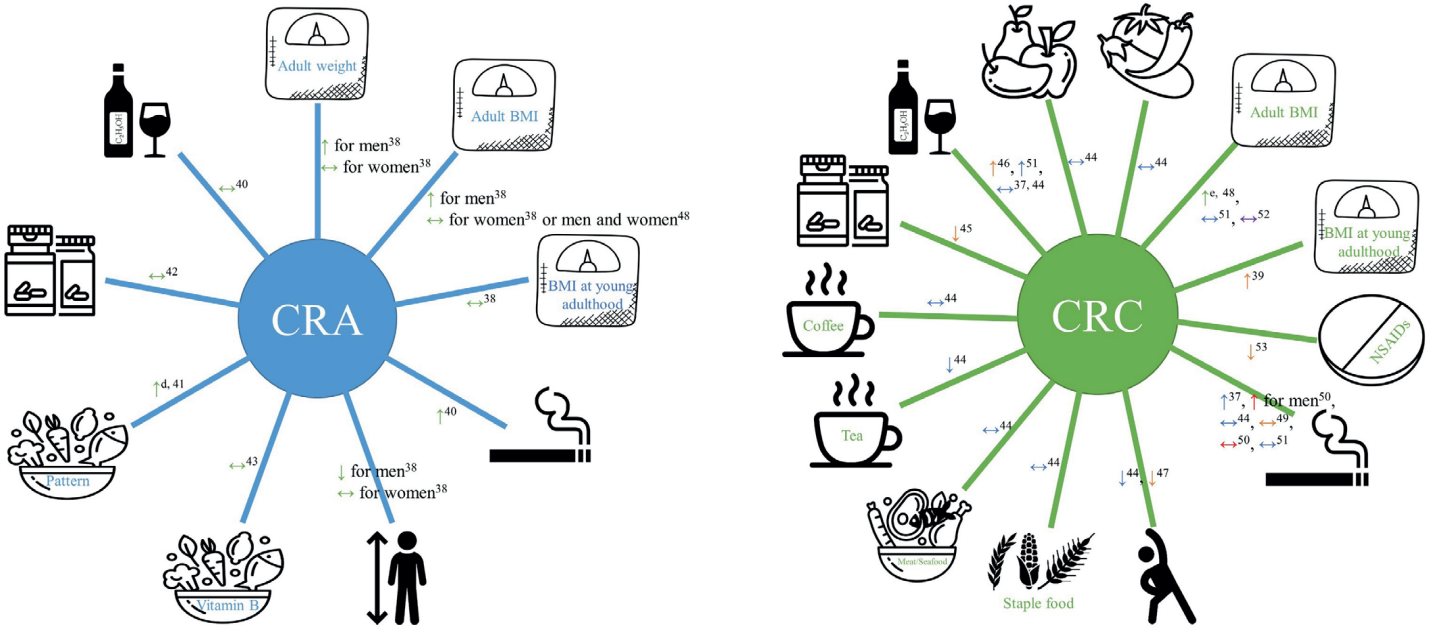
Figure 1. Family tree of the disease running in family "G" as described by Warthin[1]. A germline mutation in the *MSH2* gene leading to Lynch syndrome was later identified to cause this disease[2]. L, living; N, normal; Op, operated; Carc, carcinoma.

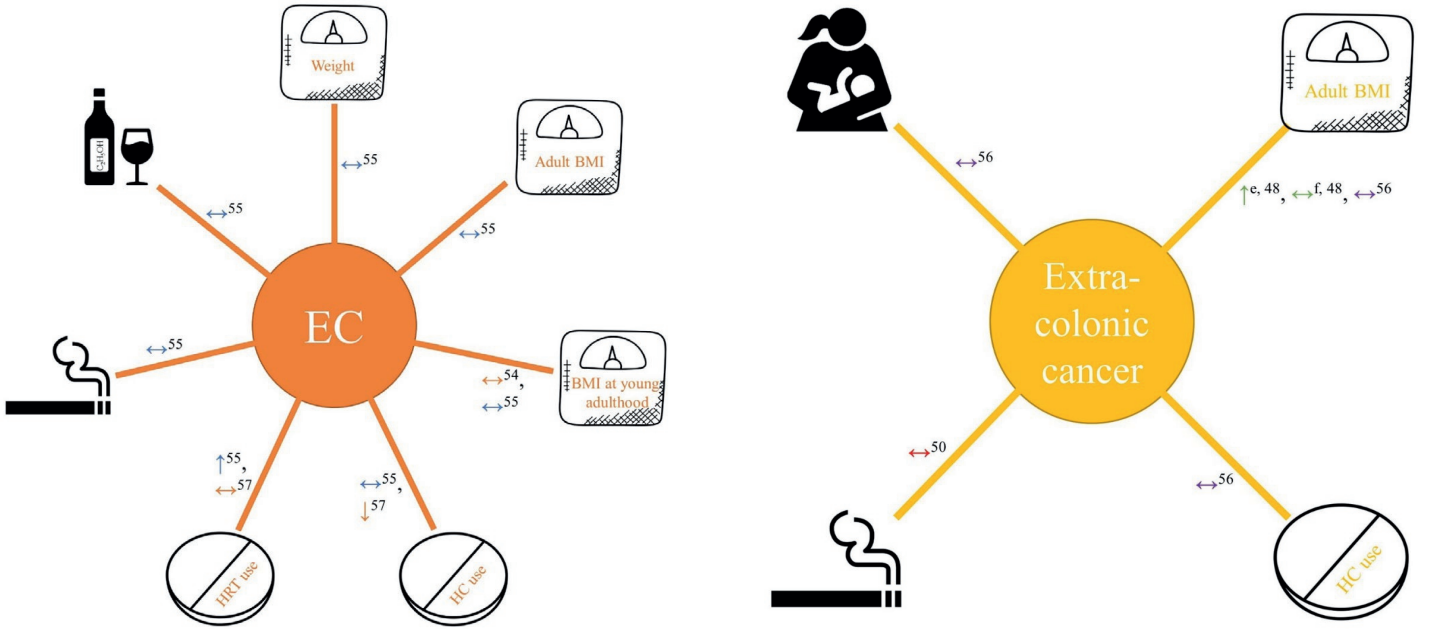
in the minority of tumours diagnosed in the general population[26-36]. Studies on the influence of lifestyle-related factors on cancer risks for persons with a LS-causing germline mutation have been published previously, but numbers are limited and the studies mainly focused on the risk of colorectal adenomas and colorectal carcinomas (i.e. colorectal tumours, Figure 2)[37-52]. The majority of these studies were performed with data of persons with LS participating in a prospective cohort study established by the Wageningen University to investigate the influence of Genetic, Environmental and Other factors on tumour risk for persons with LS (GEOLynch), and in the Colon Cancer Family Registry (CCFR).

The seventeen performed studies on lifestyle-related factors and colorectal tumour risk suggest that smoking[37, 40] and being overweight in adulthood (especially for men)[38, 48, 52] increase the risk for colorectal adenomas and/or colorectal carcinomas. Some studies showed an increased risk of colorectal adenomas and/or colorectal carcinomas with a higher body mass index (BMI) at young adulthood[39], a higher alcohol intake[46, 51], having a manual occupation[44], when adhering more to a dietary "Snack" pattern[41], and for each 5 kg weight gain in men[38]. In contrast, in other studies no associations were observed between adult BMI[51, 52], smoking[44, 49-51], alcohol use[37, 40, 44] and the risk of colorectal adenomas or colorectal carcinomas. Performing regular physical activity[44, 47], long-term use of multivitamins and calcium supplements[45], using non-steroidal anti-inflammatory drugs (NSAIDs)[53], ever consumption of tea[44] and being taller for men[38] were observed to decrease colorectal adenoma or colorectal carcinoma risk for persons with LS. In contrast, no association was observed between dietary supplement use during the month before study inclusion[42] and the risk of colorectal adenomas. In several studies, no associations were observed between various lifestyle factors including coffee consumption[44], dietary vitamin B intake[43], fruit[44], vegetable[44], seafood[44], staple food[44] or meat intake[44], and the risk of colorectal adenomas or colorectal carcinomas.

For persons with LS, six studies have been published on the association between lifestyle-related factors and the risk of cancer outside the colorectum (extra-colonic) (Figure 2)[48, 50, 54-57]. For endometrial cancer, in one study was observed that using postmenopausal hormone replacement therapy increased the risk of endometrial cancer[55]. No associations were observed between BMI at young adulthood[54, 55], adult BMI[55], weight gain[55], smoking[55], alcohol intake[55], hormonal contraceptive use[55], and postmenopausal hormone replacement therapy use[57], and endometrial cancer. In contrast, using hormonal contraceptives was observed to be associated with a decreased endometrial cancer risk in one study[57]. The few studies that investigated lifestyle-related factors and other (LS-associated) extra-colonic cancers, such as gastric, ovarian and breast cancer among LS populations, did not report evidence for

Figure 2. Observed associations between lifestyle-related factors and several tumour types for persons with Lynch syndrome.[a,b,c]





[a]Associations are only presented for studies that included persons with genetically confirmed Lynch syndrome (LS) and/or persons who were obligate/inferred for LS. Studies in persons suspected for LS based on a strong family history, i.e. based on agreement with the Amsterdam Criteria (II) or Revised Bethesda Guidelines, are not considered in this figure. [b]Observed associations were categorized as positive (i.e. increased risk [↑]), inverse (i.e. decreased risk [↓]), no association (i.e. no increased or decreased risk [↔]). Categorization of the association was based on statistically significant adjusted hazard ratios or odds ratios, or statistically significant differences in percentage (two-sided p-value<0.05). If no adjusted risk estimates were reported in the publication, the crude risk estimate was used to categorize the association. [c]The study design is reflected in the colour of the arrow. Green arrows reflects a prospective cohort study, orange arrows reflect a weighted cohort study, blue arrows reflect a retrospective cohort study, purple arrows reflect a case-control study and red arrows reflect a cross-sectional study. [d]Refers to adhering more to a dietary "Snack" pattern. [e]For *MLH1* gene mutation carriers. [f]For *MSH2* mutation carriers. BMI, body mass index; C_2H_5OH, molecular formula of alcohol; CRA, colorectal adenoma; CRC, colorectal cancer; EC, endometrial cancer; extra-colonic cancer, cancer located outside the colorectum; HC, hormonal contraceptive; HRT, hormonal replacement therapy; NSAID, non-steroidal anti-inflammatory drug. All lifestyle-related icons were obtained from the Noun project (www.thenounproject.com).

associations with adult BMI[48, 56], smoking[50], breastfeeding duration[56], and hormonal contraceptive use[56].

In summary, studies on lifestyle-related factors and tumour risk for persons with LS are limited, often inconsistent or show null results. The inconsistency in results may be due to differences in study design (e.g. prospective cohort vs. case-control study), exposure (e.g. categorical vs. continuous), outcome (colorectal adenoma vs. carcinoma) (Figure 2), while the null results may be due to a limited power to detect weak associations or limited power as a consequence of subgroup analyses. These inconsistent and limited results for the influence of lifestyle-related factors on colorectal tumours and the lack of knowledge of those factors on extra-colonic cancers, warrants studies to identify such factors. Therefore, this thesis aims to evaluate associations between lifestyle-related factors and colorectal tumours, endometrial cancer and cancer at other sites for persons with LS.

Knowing which lifestyle factors are associated with the risk of cancer for persons with LS, may provide support to change lifestyle habits. Until now, no quantitative research has been published in which prospectively measured changes in lifestyle habits after a tumour diagnosis were evaluated for persons with LS. Hence, this thesis also aimed at exploring if a colorectal tumour diagnosis is associated with changes in lifestyle habits for persons with LS.

Aim of this thesis

The overall aim of this thesis was to evaluate associations between lifestyle-related factors, i.e. the inflammatory potential of the diet, height and BMI at young adulthood (for rationale see below), and colorectal tumours and/or cancer at several sites for persons with LS. It was also explored whether a colorectal tumour diagnosis is associated with a change in lifestyle habits among persons with LS. The overall aim will assist in providing an answer to the question mentioned in the subtitle of this thesis: *Genes load the gun, lifestyle pulls the trigger?*.

Rationale research questions, methods and thesis outline

As previously described, the use of NSAIDs has been associated with a decreased risk of colorectal cancer for persons with LS in a prospective study[53]. A similar result was observed in a randomized controlled trial including persons with LS based on germline mutation testing or on a clinical suspicion for LS due to their personal and family history[58]. Both NSAIDs and diet are suggested to modulate (low-grade chronic) inflammation[59-61]. A previously developed dietary index that reflects the inflammatory potential of an individual's habitual dietary intake, i.e. the (adapted) dietary inflammatory index, was shown to be associated with systemic low-

grade chronic inflammation[62-64]. Therefore, the association between diet, as reflected by the inflammatory potential of the diet, and colorectal tumours has been evaluated for persons with LS in **chapter 2** of this thesis.

For persons with LS or suspected to have LS, conflicting results are reported for the association between height and colorectal tumours[38, 65] while studies on height and extra-colonic cancers are lacking. An increment in BMI, as a reflection of body fatness, at young adulthood was associated with a higher risk of colorectal cancer[39], but not with colorectal adenomatous polyps[38] or endometrial cancer[54, 55]. Hence, evaluating the association between both height and BMI at young adulthood and the risk of cancer for persons with LS is desirable. However, obtaining sufficient power for research on LS-associated cancers, especially LS-associated extra-colonic cancers, is difficult because, despite the high relative increased risks of extra-colonic cancers for persons with LS, the absolute number of persons diagnosed with these cancers is still small. Therefore, to investigate height and BMI at young adulthood in this thesis, the number of participants of the GEOLynch study which started in 2006 in the Netherlands was extended and an international collaboration was established to obtain a substantial number of persons with LS who have been diagnosed with several types of cancer. Within this international collaboration data of persons with LS included in the GEOLynch study[38] and data of persons with LS from the CCFR[66, 67] – which were recruited between 1997 and 2007 in Australia, New Zealand, Canada and the USA - has been harmonized. With this harmonized data, the association between height and the risk of both colorectal and endometrial cancer has been investigated in **chapter 3**. The harmonized data was also used to investigate the association between BMI at young adulthood and the risk of cancer at all sites and at extra-colonic sites as presented in **chapter 4**.

In **chapter 5** it is explored if a colorectal tumour diagnosis is associated with a change in lifestyle habits for persons with LS with the use of GEOLynch data. Finally, in **chapter 6**, the results of this thesis are discussed, clinical implications are mentioned and recommendations for future research directions are provided.

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CHAPTER 2

THE INFLAMMATORY POTENTIAL OF THE DIET AND COLORECTAL TUMOR RISK IN PERSONS WITH LYNCH SYNDROME

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American Journal of Clinical Nutrition 2017; 106: 1287-1294

Abstract

Background Persons with Lynch syndrome (LS) have a high lifetime risk of developing colorectal tumors (CRTs) because of a germline mutation in one of their mismatch repair (MMR) genes. An important process in the development of CRTs is inflammation, which has been shown to be modulated by diet.

Objective We aimed to investigate the association between the inflammatory potential of the diet and the risk of CRTs in persons with LS.

Design We used dietary intake of 457 persons with LS from a prospective cohort study to calculate the adapted dietary inflammatory index (ADII). The ADII was split into tertiles in which the highest tertile reflects the most pro-inflammatory potential of the diet. Cox proportional hazard models, with robust sandwich variance estimates to adjust for dependency within families, were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) of CRTs by ADII tertile. HRs were adjusted for age, smoking status, education level and number of colonoscopies as time-dependent variable. Potential effect measure modification was explored by stratifying the results by mutated MMR gene, sex and a history of CRTs. We performed sensitivity analyses by repeating the analyses in non-nonsteroidal anti-inflammatory drug (NSAID) users (N=315).

Results During a median follow-up time of 59 months, 200 (43.8%) participants developed CRTs. No statistically significant association was found in the highest ADII tertile compared with the lowest ($HR_{highest\ vs.\ lowest\ tertile}$: 1.37 [95% CI: 0.80, 2.34]). Stratification by mutated MMR gene, sex and CRT history did not show significantly differential associations (P-interactions≥0.64). In non-NSAID users, an $HR_{highest\ vs.\ lowest\ tertile}$ of 1.60 (95% CI: 0.88, 2.93) was shown. No significant effect modification was shown in this group either (P-interactions≥0.24).

Conclusion A pro-inflammatory potential of the diet does not seem to be statistically significantly associated with CRT risk in persons with LS.

Introduction

Lynch syndrome (LS) is the most commonly occurring type of hereditary colorectal cancer responsible for 1% to 3% of the total colorectal cancer burden[1]. This autosomal dominant condition is caused by germline mutations in DNA mismatch repair (MMR) genes, i.e. *MLH1, MSH2, MSH6* or *PMS2*[2], or by a mutation in the *EPCAM* gene which causes epigenetic silencing of *MSH2*[3]. Carriers of those gene mutations have an increased risk of developing colorectal adenomas and the subsequent progression to carcinomas is accelerated compared with non-carriers[4-6]. Depending on the mutated gene, persons with LS have a lifetime risk of 22-79% to develop colorectal cancer before the age of 70 years compared with about 5% in the general population[3, 7-9].

The high variability in lifetime risk of developing colorectal adenomas and carcinomas (i.e. colorectal tumors (CRTs)) in persons with LS, even if they carry the same mutation, supports the need to investigate potential modifiable risk factors. Diet has consistently been shown to modulate inflammation[10]. Chronic (low grade) inflammation has been directly linked to a higher risk of developing cancer in general[11, 12]. The role of low grade inflammation in the development of CRTs is well established by the results of several observational and intervention studies[13, 14]. In addition, non-steroidal anti-inflammatory drugs (NSAIDs) decreased the risk of sporadic as well as hereditary CRTs in many observational studies and randomized controlled trials[15-17]. However, because the use of aspirin and other NSAIDs is associated with adverse side effects, such as gastrointestinal bleeding, alternatives to the use of NSAIDs should be explored[18-21].

Dietary patterns are related to levels of inflammatory cytokines[10]. Therefore, Cavicchia *et al.* developed and validated the dietary inflammatory index (DII) which assesses the inflammatory potential of the diet based on literature-derived dietary inflammatory weights of energy and several nutrients[22, 23]. Subsequently, van Woudenbergh *et al.* developed and validated the adapted dietary inflammatory index (ADII). This adjusted DII reduces the between-person variation in dietary intake, avoids that the variation in the DII was driven by a few dietary components with a large range in intake, and avoids an overestimation of the inflammatory effect of energy, fat and ethanol[24].

By using these indexes, it was observed that a diet with a high inflammatory potential was associated with a 20% to 22% increased incidence of sporadic colorectal cancer in two prospective cohort studies in postmenopausal women[25, 26]. Similar associations were reported in three case-control studies and two prospective cohort study which included men and women

of all ages[27-31]. However, in the prospective studies, increased associations in both men and women were found but it was not always significant in women[30, 31] .

Hence, diet may be a promising modifiable alternative to the use of NSAIDs to decrease chronic low grade inflammation and consequently the development of CRTs in persons with LS. Therefore, we aimed to prospectively investigate the association between the inflammatory potential of the diet and the risk of CRTs among MMR gene mutation carriers.

Methods

Study population

For this study, we used data from participants of the GEOLynch study[32]. Briefly, this prospective cohort study started in 2006 after approval of the Medical Ethical Review Committee Region Arnhem-Nijmegen. Persons with LS, i.e. with a confirmed mutation in one of the DNA MMR genes *MLH1*, *MSH2*, *MSH6* or *PMS2*, were included. Between July 2006 and July 2008, eligible participants were identified through the Netherlands Foundation for the Detection of Hereditary Tumors in Leiden, the Radboud University Medical Center in Nijmegen and the University Medical Center in Groningen, the Netherlands. Participants, aged 18 to 80 years, had to be Dutch-speaking, mentally competent to participate and undergo regular colonoscopy surveillance. Exclusion criteria included terminally ill participants, those living outside the Netherlands and those with familial adenomatous polyposis, inflammatory bowel diseases, proctocolectomy or colostomy, resulting in 686 presumed eligible participants. Seventy-three percent (n=501) agreed to participate and gave written informed consent (Figure 1). Nine participants appeared ineligible after signing the informed consent and were excluded. Additionally for this study, participants with incomplete questionnaires (n=11), incomplete medical data (n=23) or who were pregnant (n=1) were excluded, resulting in 457 participants for the analyses.

Exposure assessment

Dietary intake was assessed using a self-administered food frequency questionnaire (FFQ) developed and validated by the Division of Human Nutrition, Wageningen University & Research[33, 34]. The FFQ contained 183 items and was designed to assess habitual food intake during the previous month by asking the frequency and amounts of eaten food items. All food items were converted to intake of energy and nutrients by using the Dutch Food Composition Database 2006 (NEVO). Caffeine intake was not included in the NEVO and therefore estimated

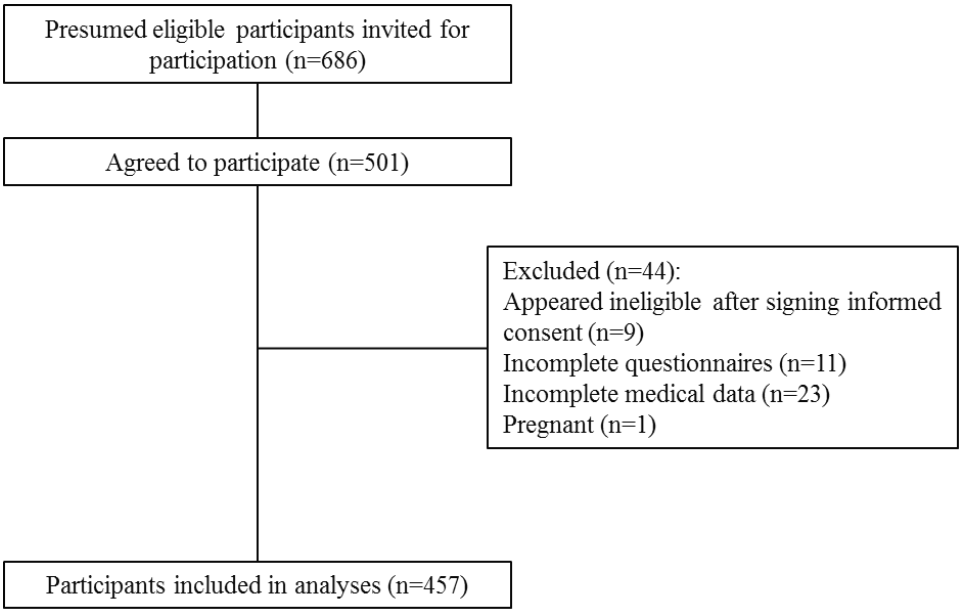

Figure 1. Flowchart of included participants between July 2006 and July 2008 in the GEOLynch cohort study.

based on mean caffeine concentrations of 68.0 mg/100 g coffee[35] and 20 mg/100 g black or green tea[36].

We assessed the inflammatory potential of the diet by calculating the ADII as described by van Woudenbergh *et al.*[24]. Briefly, we used the residual method[37] to retrieve energy-adjusted intakes of saturated fatty acids, trans fatty acids, carbohydrates, cholesterol, vitamin B12, iron, protein, monounsaturated fatty acids (MUFA), riboflavin, thiamine, caffeine, omega-6 polyunsaturated fatty acids (PUFA), omega-3 PUFA, folate, selenium, niacin, ethanol, zinc, vitamin B6, vitamin A, vitamin E, vitamin C, vitamin D, quercetin, magnesium, tea, beta-carotene and fiber. Intakes of eugenol, flavan-3-ol, flavones, flavonones, isoflavones, anthocyanidins, garlic, ginger, saffron, pepper, thyme/oregano, rosemary, onions and turmeric could not be calculated with the FFQ and were therefore not taken into account to investigate the inflammatory potential of the diet. Subsequently, the energy-adjusted intakes were standardized by subtracting the participants' mean intake from the individual intake and then dividing the difference by the standard deviation (SD) of the participants' intake which resulted in an individual *Z*-score for each food component. Next, *Z*-scores were multiplied by their corresponding inflammatory weight (Table 1)[23]. An inflammatory weight of zero was allocated

Table 1. Dietary components included in the adapted dietary inflammatory index and their inflammatory weights.

Components	Units	Inflammatory weight[1]
Saturated fatty acids	g/d	0.373
Trans fatty acids	g/d	0.229
Cholesterol	mg/d	0.110
Vitamin B12	µg/d	0.106
Carbohydrate	g/d	0.097
Iron	mg/d	0.032
Protein	g/d	0.021
MUFA	g/d	-0.009
Riboflavin	mg/d	-0.068
Thiamine	mg/d	-0.098
Caffeine	g/d	-0.110
*n*6 PUFA	g/d	-0.159
Folate	µg/d	-0.190
Selenium	mg/d	-0.191
Niacin	mg/d	-0.246
Ethanol[2]	g/d	-0.278
Zinc	mg/d	-0.313
Vitamin B6	mg/d	-0.365
Vitamin A	µg/d	-0.401
Vitamin E	mg/d	-0.419
Vitamin C	mg/d	-0.424
*n*3 PUFA	g/d	-0.436
Vitamin D	µg/d	-0.446
Quercetin	mg/d	-0.467
Magnesium	mg/d	-0.484
Tea[3]	g/d	-0.536
Beta-carotene	µg/d	-0.584
Fiber	g/d	-0.663

[1]Dietary components with a positive inflammatory weight were considered pro-inflammatory, while those with a negative inflammatory weight were considered anti-inflammatory[23]. [2]Ethanol is not likely to be anti-inflammatory when intakes exceeds 40 grams/day[38]. Hence, the dietary inflammatory weight was assumed to be zero for alcohol intake over 40 grams/day. [3]Tea intake was included because epicatechin intake could not be calculated. MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

to ethanol if ethanol intake exceeded 40 grams per day because the anti-inflammatory effects of ethanol seem to diminish with an intake that exceeds 40 grams a day[38]. The multiplied *Z*-scores were subsequently summed to create one ADII score in which a negative score indicates an anti-inflammatory potential of the diet while a positive score indicates a pro-inflammatory potential of the diet.

Identification of colorectal tumor cases

Participants were followed prospectively by regularly reviewing medical records and pathology reports to obtain medical information about performed colonoscopies, surgical interventions and diagnoses of colorectal adenomas and carcinomas. Also, information on all previously performed colonoscopies, surgical interventions and diagnoses of colorectal adenomas and carcinomas was collected.

Covariate assessment

Demographic and lifestyle information was collected through a self-administered questionnaire about current height and weight, sex, date of birth, education level (low, i.e. finished primary school or lower vocational or lower general secondary education; middle, i.e. finished general secondary school, pre-university education or vocational education; high, i.e. finished higher professional education or university), smoking habits (current, former, never), NSAID use (never, i.e. less than once a month, vs. ever, i.e. equal to or more than once a month) and physical activity. The body mass index (BMI) was calculated by dividing the weight (kg) by the squared height (m) and subsequently categorized as being overweight (BMI $\geq$25 kg/m^2) or not (BMI <25 kg/m^2)[39]. Physical activity was measured with a modified Baecke questionnaire[40, 41] and categorized in tertiles representing an inactive, moderately active and highly active life style.

Data analyses

Summary statistics were used to describe the baseline characteristics of the total cohort and stratified by ADII tertiles with the lowest tertile reflecting the most anti-inflammatory diet. Differences in baseline characteristics between ADII tertiles were tested with a Chi-squared test or Fisher's exact test for categorical variables and an ANOVA or Kruskall-Wallis test for continuous variables. The contribution of the individual dietary components to the variation in the ADII between participants was assessed by using forward linear regression. The partial R^2

of the components in the final model was used to estimate those component's contribution to the ADII adjusted for the influence of other included dietary components.

Cox proportional hazard models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) of the association between the inflammatory potential of the diet, reflected by the ADII score, and risk of developing CRTs. A robust sandwich covariance estimate was used to account for dependence of observations within families[42, 43]. Person time started on the colonoscopy date closest to questionnaire completion and ended on the date halfway between the colonoscopy in which the first pathology-confirmed CRT was diagnosed and the previous clean colonoscopy. Participants without a CRT diagnosis during follow-up were censored at the date of their last known colonoscopy. Participants who died during follow-up (n=31) were censored at their last clean colonoscopy if no CRT was diagnosed before death. Nine participants were included in a trial during follow-up and hence censored to prevent an interference with our results. Their person time ended at the date of their last known colonoscopy before trial-inclusion if no CRT was diagnosed before that date.

The selection of potential confounders was based on literature and a significant association with the exposure and outcome in univariate analyses. HR were adjusted for age (years), smoking status, education level and number of colonoscopies as a time-dependent covariate. In addition, in the main analysis in all participants, a model was run that also included BMI and physical activity because published studies have shown that BMI and physical activity are associated with CRT risk[32, 44]. Tests for linear trend across tertiles were conducted by modelling the median value of each tertile as a continuous variable in the model. The proportionality assumption was tested for statistical significance with Schoenfeld residuals. All covariates met the proportional hazard assumption.

Stratification by the two predominant mutated genes (*MLH1* and *MSH2*), by sex and by a history of CRTs were performed to explore potential effect measure modification. Interaction terms between the covariate and the ADII tertiles were added to the model to determine a significant ($p<0.05$) heterogeneity across the strata.

To investigate whether NSAID use affects the association between ADII and CRTs, NSAID users (i.e. those who used NSAIDs equal to or more than once a month) were excluded in the sensitivity analyses, leaving 69% of the cohort (n=315).

All statistical tests were two-sided and a p-value<0.05 was considered significant. Data were analyzed with the use of SAS software version 9.3 of the SAS System for Windows (SAS Institute Inc.).

Results

During a median follow-up time of 58.8 [quartile 1-quartile 3: 33.7-74.8] months, 200 (43.8%) individuals developed a CRT (182 colorectal adenomas and 18 colorectal carcinomas). ADII scores ranged from -11.7 to 8.4 with a mean of -0.9 (SD 2.6) (Table 2). The variance in ADII scores was mainly explained by the intake of quercetin (48%) followed by folic acid (15%) and trans fatty acids (14%) (Table 3). Individuals in the first ADII tertile (i.e. with the most anti-inflammatory potential of the diet) were significantly older, less often current smokers and had a higher energy intake than those of individuals in the second ADII tertile (i.e. hardly any pro- or anti-inflammatory potential of the diet) and third ADII tertile (i.e. with the most pro-inflammatory potential of the diet). Moreover, individuals in the first ADII tertile were more often men, were less often overweight, were higher educated and were more often highly active, but these differences did not reach statistical significance (Table 2).

Adapted dietary inflammatory index and colorectal tumor risk in all participants

A HR of 1.37 (95% CI: 0.80, 2.34) for CRT risk was shown in participants in the third ADII tertile compared with participants in the first ADII tertile after correction for the effect of age, smoking status, education level and number of colonoscopies during follow-up (Table 4). No significant linear trend across the ADII tertiles was observed (P-trend=0.33). The risk estimate did not change after further adjusting for BMI and physical activity ($HR_{ADII\ tertile\ 3\ vs.\ 1}$: 1.44, 95% CI: 0.88, 2.34; P-trend=0.25) (data not shown).

Stratified colorectal tumor risk estimates in all participants

Stratification by mutated gene, resulted in HRs of 1.67 (95% CI: 0.90, 3.12) and 1.29 (95% CI: 0.52, 3.18) in those with a mutated *MLH1* and *MSH2* gene, respectively, in the third ADII tertile compared with in the first ADII tertile, which were not significantly different (P-interaction=0.64) (Supplemental table S1).

In addition, significantly different HRs were not shown after stratification by sex or CRT history when participants in the third ADII tertile were compared with participants in the first ADII tertile in the adjusted model (P-interactions of 0.66 and 0.82 respectively) (Supplemental table S1).

Table 2. Baseline characteristics of the participants by tertiles of the adapted dietary inflammatory index (N=457).[1]

	All participants (-11.7, 8.4) (N=457)	**ADII tertile 1** (-11.7, <-1.8) (n=152)	**ADII tertile 2** (-1.8, <0.3) (n=152)	**ADII tertile 3** (0.3, 8.4) (n=153)
Age (years)[2]	49.5 ± 11.5	52.0 ± 10.8	50.1 ± 11.6	46.4 ± 11.6
Follow-up time (months)[3]	58.8 [33.3-73.9]	52.5 [33.3-73.9]	59.3 [35.8-76.0]	59.6 [30.3-75.7]
Men	187 (40.9)	67 (44.1)	60 (39.5)	60 (39.2)
BMI				
≥25 (kg/m^2)	191 (41.8)	55 (36.2)	61 (40.1)	75 (49.0)
Education level[4]				
Low	143 (31.3)	45 (29.6)	42 (27.6)	56 (36.6)
Middle	151 (33.0)	42 (27.6)	59 (38.8)	50 (32.7)
High	158 (34.6)	64 (42.1)	49 (32.2)	45 (29.4)
Physical activity tertiles[4]				
Low	146 (32.0)	44 (28.9)	42 (27.6)	60 (39.2)
Moderate	155 (33.9)	50 (32.9)	57 (37.5)	48 (31.4)
High	148 (32.4)	56 (36.8)	49 (32.2)	43 (28.1)
Smoking status[2,4]				
Current	81 (17.7)	15 (9.9)	23 (15.1)	43 (28.1)
Former	201 (44.0)	78 (51.3)	74 (48.7)	49 (32.0)
Never	174 (38.1)	59 (38.8)	54 (35.5)	61 (39.9)
Energy intake (kcal/day)[2,3]	2067.6 [1690.5-2557.0]	2337.5 [1836.6-2719.8]	1952.2 [1614.1-2453.5]	2004.1 [1664.7-2508.7]
No. of colonoscopies[5]				
≤2	208 (45.5)	65 (42.8)	72 (47.4)	71 (46.4)
3	129 (28.2)	43 (28.3)	39 (25.7)	47 (30.7)
≥4	120 (26.3)	44 (28.9)	41 (27.0)	35 (22.9)
NSAID use (yes)	132 (28.9)	48 (31.6)	36 (23.7)	48 (31.4)
Colorectal tumor history (yes)	228 (49.9)	78 (51.3)	73 (48.0)	77 (50.3)

Table 2 continued.

	All participants	ADII tertile 1	ADII tertile 2	ADII tertile 3
MMR genes				
MLH1	176 (38.5)	61 (40.1)	62 (40.8)	53 (34.6)
MSH2	184 (40.3)	59 (38.8)	57 (37.5)	68 (44.4)
MSH6	94 (20.6)	30 (19.7)	32 (21.1)	32 (20.9)
PMS2	3 (0.7)	2 (1.3)	1 (0.7)	-

[1]Characteristics expressed as mean ± SD or n (%). [2]P-value < 0.05. Differences between the ADII tertiles were tested with a Chi-squared test or Fisher's exact test for categorical variables and an ANOVA or Kruskall-Wallis test for continuous variables. [3]Energy intake and follow-up time are expressed as median [quartile 1-quartile 3]. [4]The sum of the percentages doesn't reach 100% due to eight missing values for physical activity, one missing value for smoking and five missing values for education level. [5]Total number of colonoscopies during follow-up time. ADII: adapted dietary inflammatory index, BMI: body mass index, MMR: mismatch repair, NSAID: non-steroidal anti-inflammatory drug.

Table 3. Explained inter-individual variance in the adapted dietary inflammatory index by dietary components included in the calculation of the adapted dietary inflammatory index (N=457).[1]

Components	Partial R^2
Quercetin	0.48
Folic acid	0.15
Trans fatty acids	0.14
Vitamin E	0.07
Carbohydrate	0.03
Fiber	0.03
Tea	0.02
MUFA	0.01
Niacin	0.01
Vitamin D	0.01
Other components	0.04

[1]Forward linear regression was used to calculate the partial R^2. Components that explained more than 1% of the inter-individual variation in the final model are shown. MUFA: monounsaturated fatty acids.

Adapted dietary inflammatory index and colorectal tumor risk in non-NSAID users

In the sensitivity analyses, NSAID-users were excluded leaving 315 individuals with a median follow-up time of 59.2 months (quartile 1-quartile 3: 31.2-74.8) in which 145 (46%) individuals were diagnosed with a CRT (133 colorectal adenomas and 12 colorectal carcinomas). Compared with individuals in the first ADII tertile, individuals in the third ADII tertile had a

HR of 1.60 (95% CI: 0.88, 2.93) to develop CRTs (Table 4). No linear trend across the ADII tertiles was observed (P-trend=0.15).

Table 4. Hazard ratios (HRs) and 95% confidence intervals (CI) for colorectal tumor risk across tertiles of the adapted dietary inflammatory index for all participants (N=457) and in non-NSAID users only (N=315).

ADII	Cases (N)	Total follow-up time (Months)	Crude model		Adjusted model[1]	
			HR	95% CI	HR	95% CI
All participants						
Tertile 1	67	7983.5	1.00	Reference	1.00	Reference
Tertile 2	57	8393.0	0.80	0.58, 1.11	0.70	0.43, 1.15
Tertile 3	76	8077.6	1.11	0.79, 1.57	1.37	0.80, 2.34
P-trend[2]	-	-	0.61		0.33	
Non-NSAID users[3]						
Tertile 1	49	5593.4	1.00	Reference	1.00	Reference
Tertile 2	39	5817.2	0.76	0.51, 1.14	1.01	0.63, 1.62
Tertile 3	57	5344.2	1.21	0.83, 1.78	1.60	0.88, 2.93
P-trend[2]	-	-	0.45		0.15	

[1]Hazard ratio adjusted for age, smoking status, education level and number of colonoscopies as time-dependent variable. [2]Two-sided p-value for test of linear trend calculated using median values for each tertile of the adapted dietary inflammation index. [3]ADII tertile range among non-NSAID users: tertile 1: -9.1, <-1.6, tertile 2: -1.6, <0.3 and tertile 3: 0.3, 8.4. ADII: adapted dietary inflammatory index, NSAID: non-steroidal anti-inflammatory drug.

Stratified colorectal tumor risk estimates in non-NSAID users

Stratification of the non-NSAID users by mutated gene resulted in an increased HR among *MLH1* mutation carriers (Supplemental table S2). For these carriers, a HR of 2.36 (95% CI: 1.05, 5.30) was found for individuals in the third ADII tertile compared with those in the first ADII tertile. In non-NSAID using *MSH2* mutation carriers, a HR of 1.17 (95% CI: 0.45, 3.06) was shown (Supplemental table S2). Again, the associations were not significantly different between *MLH1* and *MSH2* mutation carriers (P-interaction=0.52).

Stratifying the results of participants who did not use NSAIDs by sex or history of CRT did not show significant interactions when participants in the third ADII tertile were compared with participants in the first ADII tertile (P-interactions of 0.24 and 0.55 respectively) (Supplemental table S2).

Discussion

We did not observe a significant association between a pro-inflammatory potential of the diet and the risk of LS associated CRTs. Repeating the analyses among non-NSAID users only, revealed a slightly higher, but still not significant HR.

To the best of our knowledge, this is the first study that investigated the association between the inflammatory potential of the diet and CRT risk in persons with a genetic predisposition to cancer. Earlier studies have been performed on the inflammatory potential of the diet and colorectal cancer risk in the general population. In those studies, increased risks of 20% to 116% were found when individuals with the most pro-inflammatory potential of their diet were compared with those with the most anti-inflammatory potential of their diet[25-31]. In our study, we found statistically non-significant results in the same direction with a HR of 1.37 (95% CI: 0.80, 2.34) when all participants were included and a HR of 1.60 (95% CI: 0.88, 2.34) in non-NSAID users only. Associations in the same direction in non-NSAID users for colorectal cancer risk were found among postmenopausal women by Shivappa *et al.* with a $HR_{\text{quintile 5 vs. quintile 1}}$ of 2.02 (95% CI: 1.21, 3.39) and by Tabung *et al.* with a $HR_{\text{quintile 5 vs. quintile 1}}$ of 1.31 (95% CI: 1.05, 1.65)[25, 26]. These results could be in line with evidence suggesting a protective role of anti-inflammatory drugs on CRT risk[15-17] because a pro-inflammatory potential of the diet tends to increase CRT risk, especially in non-NSAID users.

A pro-inflammatory potential of the diet may influence colorectal cancer risk systemically by increasing insulin resistance[45-47]. The metabolic consequences of insulin resistance, e.g. hyperinsulinemia, promote colorectal cell proliferation and reduce apoptosis[46]. Moreover, diet may also influence focal loss of the epithelial cell barrier function which may lead to an inflammatory response and ultimately colorectal cancer[45]. However, the importance of these mechanisms in LS associated CRT development may be relatively small compared with the influence of the MMR gene mutation that causes microsatellite instability (MSI) high colorectal cancers[48-51]. The MSI pathway to colorectal cancer is often seen in colorectal cancers of persons with LS but is less common among colorectal cancer in the general population[48, 49]. However, the presence of tumor infiltrating lymphocytes (TILs) and Crohn's like lymphocytes in many LS related CRT tissues indicate an important role of inflammation in LS too[52-54]. Nevertheless, this local inflammatory response is expected to suppress instead of promote tumorigenesis since the presence of TILs in colorectal cancers improve survival[55] and diminishing the immune response found in mucosa of persons with LS seems to trigger the development of colorectal cancer[56]. This inflammatory response is suggested to be a consequence of the loss of (functional) MMR proteins[52, 56] and is therefore probably not the

results of systemic chronic inflammation, which is assessed by the ADII. The statistically non-significant findings of our study could hence reflect reality and might support a hypothesis that a less pro-inflammatory diet may be more beneficial to decrease sporadic colorectal cancer in the general population than it is for CRTs in persons with LS.

In this study, most (91.0%) of the diagnosed CRTs during follow-up were colorectal adenomas (i.e. the precursor lesion of colorectal cancer[57]). Not all colorectal adenomas will progress to cancer. Hence, the association between the inflammatory potential of the diet and colorectal adenoma or colorectal cancer risk may differ. However, to the best of our knowledge, no studies investigating the association between the inflammatory potential of the diet and colorectal adenoma risk have been published. Nevertheless, some studies have been performed to investigate the influence of single food items or nutrients, or food patterns on CRT risk in (suspected) MMR gene mutation carriers. In the same direction as our results, fruit and fiber, which contain mainly food components with an anti-inflammatory diet potential, seemed to be inversely associated with CRT risk in persons with confirmed or suspected LS[58]. Moreover, a HR of 2.16 (95% CI: 1.03, 4.49) for colorectal adenomas risk was found among MMR gene mutation carriers in the highest tertile of the "Snack" pattern, which is mainly loaded on food items that consist of components with a pro-inflammatory diet potential, compared with the lowest tertile[59]. In contrast to what would be expected based on their inflammatory weight, no statistically significant associations for alcohol and vitamin B intake and CRT risk were found [58, 60-62]. No association between meat intake and CRT risk was found either[58, 63] which cannot be easily compared with our results because meat contains proinflammatory (e.g. saturated fat) as well as anti-inflammatory (e.g. vitamin B6) food components. Therefore, based on the results of earlier published studies and our result, we cannot yet conclude if the influence of the inflammatory potential of the diet may be different for colorectal adenoma risk compared with colorectal cancer risk in persons with LS.

Strengths of the current study include the inclusion of confirmed MMR gene mutation carriers only, the high participation rate and the prospective design with a relatively long follow-up. In addition, we were able to measure a large number of potential confounders and a validated FFQ[33, 34] was used to measure each individual's dietary intake.

In contrast to most published studies, we used the ADII while the DII was used in the majority of studies in which the inflammatory potential of the diet was investigated. In our study, the DII was mostly explained (72%) by the intake of fiber and repeating the analyses with DII tertiles resulted in similar and weaker associations compared with using ADII tertiles (data not shown). The ADII better reflected the inflammatory potential of the complete diet in

this study. In addition, the ADII has been validated in adults against a summary score of low-grade inflammation including C-reactive protein (CRP), interleukin (IL) 6, IL-8, tumor necrosis factor alpha (TNF-α), serum amyloid A and soluble intercellular adhesion molecule 1[24] while the DII has been validated against CRP, IL-6, TNFα-receptor 2 and homocysteine[64-66]. Hence, the ADII is suitable to estimate the inflammatory potential of the diet and was preferred in this study.

For our ADII calculations, we used 28 out of 45 food components with an inflammatory weight[23]. Three (total fat, total energy intake and PUFA) of the 45 components were excluded to avoid overestimation of the inflammatory effect[24] and 14 of the 45 components were excluded because the dietary intake could not be measured with the used FFQ. All the unmeasured components had an anti-inflammatory diet potential according to their inflammatory weight with the lowest inflammatory weight of turmeric (-0.785) and the highest of rosemary (-0.013). This may have resulted in non-differential misclassification and hence an underestimation of the results. However, the ADII measured with 28 included food components still reflects the inflammatory potential of the diet as it has been validated against a summary score of low-grade inflammation[24].

Finally, our study is one of the largest studies with confirmed MMR gene mutation carriers to date. With our number of participants, a power of at least 80% was reached for an effect size equal to or larger than 1.63 at a 5% significance level. Although similar effect estimates have been observed in other publications[27, 29], our effect sizes were mostly below 1.63, and thus, our study with 457 participants may thus still have resulted in limited power.

In conclusion, our results do not show a significant association between a pro-inflammatory potential of the diet and CRT risk in persons with LS. The results might support previous evidence that CRTs in persons with LS arise from a different pathway than sporadic CRTs. Verification of these results in another and larger prospective cohort study among persons with LS would be desirable before investigating if and how modifying the diet of persons of LS in clinical practice could be useful to decrease CRT risk.

Acknowledgements

We are indebted to all study participants for their cooperation. We thank professor Hendriek Boshuizen (Wageningen University & Research) for her clarification of statistical procedures. We would also like to thank Merel Snellen and Mariska Vos (Wageningen University & Research), Corinne van der Kaa and Corien Meijvogel (Netherlands Foundation for the Detection of Hereditary Tumors), and Maria van Vugt (Radboud University Medical Center Nijmegen) for their assistance with participant recruitment and data collection. The medical specialist of the participants are gratefully acknowledged for their collaboration.

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Supplemental tables

Supplemental table S1. Hazard ratios (HRs) and 95% confidence intervals (CI) for colorectal tumor risk across tertiles of the adapted dietary inflammatory index stratified by the two predominant mutated genes (*MLH1* and *MSH2*), sex and colorectal tumor history.

ADII	**Cases**	**Total follow-up time**	**Crude model**		**Adjusted model[a]**	
	(N)	**(Months)**	**HR**	**95% CI**	**HR**	**95% CI**
Mutated gene						
MLH1 (N=176)						
Tertile 1	25	3235.8	1.00	Reference	1.00	Reference
Tertile 2	22	3477.6	0.83	0.53, 1.29	0.44	0.22, 0.90
Tertile 3	29	2635.9	1.42	0.83, 2.43	1.67	0.90, 3.12
P-trend[b]	-	-		0.26		0.30
MSH2 (N=184)						
Tertile 1	30	3095.0	1.00	Reference	1.00	Reference
Tertile 2	18	3260.0	0.56	0.32, 0.98	0.69	0.33, 1.47
Tertile 3	36	3561.4	1.02	0.60, 1.75	1.29	0.52, 3.18
P-trend[b]	-	-		0.98		0.58
P-interaction[c]	-	-		0.55		0.64
Sex						
Women (N=270)						
Tertile 1	35	4578.4	1.00	Reference	1.00	Reference
Tertile 2	33	5280.0	0.80	0.51, 1.28	0.84	0.43, 1.64
Tertile 3	43	4859.0	1.15	0.72, 1.84	1.52	0.81, 2.86
P-trend[b]	-	-		0.60		0.22
Men (N=187)						
Tertile 1	32	3405.1	1.00	Reference	1.00	Reference
Tertile 2	24	3113.0	0.82	0.51, 1.31	0.56	0.29, 1.08
Tertile 3	33	3218.6	1.10	0.68, 1.76	1.17	0.52, 2.62
P-trend[b]	-	-		0.77		0.91
P-interaction[c]	-	-		0.98		0.66
Colorectal tumor history						
No (N=229)						
Tertile 1	30	4060.6	1.00	Reference	1.00	Reference
Tertile 2	26	4552.4	0.77	0.46, 1.28	0.62	0.27, 1.43

Supplemental table S1 continued.

ADII	Cases	Total follow-up time	Crude model		Adjusted model[a]	
	(N)	(Months)	HR	95% CI	HR	95% CI
Tertile 3	31	4204.5	1.00	0.58, 1.72	1.42	0.68, 2.99
P-trend[b]	-	-	0.94		0.92	
Yes (N=228)						
Tertile 1	37	3922.9	1.00	Reference	1.00	Reference
Tertile 2	31	3840.6	0.84	0.54, 1.32	0.64	0.36, 1.13
Tertile 3	45	3873.1	1.21	0.77, 1.88	1.08	0.57, 2.07
P-trend[b]	-	-	0.45		0.87	
P-interaction[c]	-	-	0.85		0.82	

[a]Hazard ratio adjusted for age, smoking status, education level number of colonoscopies as time-dependent variable. [b]Two-sided p-value for test of linear trend calculated using median values for each tertile of adapted dietary inflammation index. [c]The p-value for interaction was calculated by adding an interaction term with the covariate and ADII tertile in the model. ADII: adapted dietary inflammatory index.

Supplemental table S2. Hazard ratios (HRs) and 95% confidence intervals (CI) for colorectal tumor risk in non-NSAID users (N=315) across tertiles of the adapted dietary inflammatory index stratified by the two predominant mutated genes (*MLH1* and *MSH2*), sex and colorectal tumor history.

ADII[a]	Cases (N)	Total follow-up time (Months)	Crude model HR	Crude model 95% CI	Adjusted model[b] HR	Adjusted model[b] 95% CI
Mutated gene						
MLH1 (N=121)						
Tertile 1	12	2235.5	1.00	Reference	1.00	Reference
Tertile 2	14	2480.3	1.05	0.49, 2.27	0.98	0.43, 2.26
Tertile 3	22	1933.6	2.11	1.03, 4.32	2.36	1.05, 5.30
P-trend[c]	-	-		0.06		0.07
MSH2 (N=120)						
Tertile 1	25	2175.7	1.00	Reference	1.00	Reference
Tertile 2	11	2060.6	0.45	0.24, 0.85	0.58	0.28, 1.20
Tertile 3	25	2037.0	1.06	0.59, 1.88	1.17	0.45, 3.06
P-trend[c]	-	-		0.92		0.83
P-interaction[d]	-	-		0.23		0.52
Sex						
Women (N=183)						
Tertile 1	26	3268.2	1.00	Reference	1.00	Reference
Tertile 2	23	3432.1	0.83	0.49, 1.40	1.38	0.75, 2.55
Tertile 3	33	3120.9	1.32	0.80, 2.19	1.78	0.86, 3.70
P-trend[c]	-	-		0.36		0.12
Men (N=132)						
Tertile 1	23	2325.1	1.00	Reference	1.00	Reference
Tertile 2	16	2385.0	0.68	0.38, 1.24	0.67	0.29, 1.60
Tertile 3	24	2223.3	1.10	0.66, 1.83	1.42	0.59, 3.40
P-trend[c]	-	-		0.87		0.51
P-interaction[d]	-	-		0.84		0.24
Colorectal tumor history						
No (N=157)						
Tertile 1	24	2562.4	1.00	Reference	1.00	Reference
Tertile 2	20	3181.7	0.66	0.38, 1.17	1.26	0.50, 3.20
Tertile 3	26	2852.4	0.98	0.55, 1.75	1.20	0.38, 3.75

Supplemental table S2 continued.

ADII[a]	Cases (N)	Total follow-up time (Months)	Crude model HR	Crude model 95% CI	Adjusted model[b] HR	Adjusted model[b] 95% CI
P-trend[c]	-	-	0.85		0.74	
Yes (N=158)						
Tertile 1	25	3031.0	1.00	Reference	1.00	Reference
Tertile 2	19	2635.5	0.84	0.46, 1.54	1.00	0.49, 2.04
Tertile 3	31	2491.8	1.44	0.84, 2.48	1.53	0.66, 3.53
P-trend[c]	-	-	0.25		0.34	
P-interaction[d]	-	-	0.59		0.55	

[a]ADII tertile range among non-NSAID users: tertile 1: -9.1, <-1.6, tertile 2: -1.6, <0.3 and tertile 3: 0.3, 8.4. [b]Hazard ratio adjusted for age, smoking status, education level and number of colonoscopies as time-dependent variable. [c]Two-sided p-value for test of linear trend calculated using median values for each tertile of adapted dietary inflammation index. [d]The p-value for interaction was calculated by adding an interaction term with the covariate and ADII tertile in the model. ADII: adapted dietary inflammatory index, NSAID: non-steroidal anti-inflammatory drug.

CHAPTER 3

HEIGHT AND COLORECTAL AND ENDOMETRIAL CANCER RISK FOR PERSONS WITH LYNCH SYNDROME

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Accepted for publication in the American Journal of Epidemiology

Abstract

Background Persons with Lynch syndrome (LS – carrying a pathogenic mutation in a DNA mismatch repair gene) have an increased colorectal cancer (CRC) and endometrial cancer (EC) risk. However, a high reported variability in cancer risk suggests the existence of risk factors that modify cancer risk for LS. Height is positively associated with both CRC and EC risk for the general population but studies for persons with LS are limited. Therefore, we aimed to investigate the association between height and CRC and EC for persons with LS using two large studies.

Methods Information of 1155 men and 1553 women with LS from the Colon Cancer Family Registry and the GEOLynch cohort study was harmonized. We used weighted Cox proportional hazard regression with age on the time-axis to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for each 5 cm increment in self-reported height. HRs were adjusted for education level, ethnicity, smoking habits, country of residence, year of birth and age at menarche (for EC only).

Results CRC was diagnosed in 947 persons during 65 369 person years of observation and 171 women were diagnosed with EC during 39 227 person years of observation. Height was not associated with CRC for men (HR 1.00 per 5 cm, 95% CI 0.91-1.11) or women (HR 1.01 per 5 cm, 95% CI 0.92-1.11). Nor was height associated with EC (HR 1.08 per 5 cm, 95% CI 0.94-1.24).

Conclusions We observed no evidence for an association of height with either CRC or EC for persons with LS.

Introduction

Lynch syndrome (LS) is defined by a germline mutation in one of the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* or *PMS2*[1], or the *EPCAM* gene[2]. In persons with such MMR gene mutations, a disrupted DNA MMR system causes an increased risk of several cancer types. Even though not all persons with LS develop cancer, LS is the most common cause of hereditary colorectal cancer (CRC) and endometrial cancer (EC)[3]. LS also increases the risk of colorectal adenomas (a precursor lesion of CRC[4]) as well as ovarian, stomach, small bowel, pancreas and several other cancers[2, 5-12].

Cancer risk estimates for persons with LS are highly variable between and within families, even for those with the same mutated gene[2, 8, 13]. This suggests that factors other than the germline mutation may also influence cancer risk for persons with LS[14].

Height is a factor of interest since a person's tallness may be a surrogate for factors that may influence cancer development, i.e. the number of a person's body cells, a person's genetic make-up, exposure to environmental factors and exposure to several hormones and growth factors during maturation[15]. For the general population, there is strong evidence that height is associated with the risk of sporadic colorectal, kidney, pancreatic, prostate, ovarian, endometrial, pre- and postmenopausal breast cancer and malignant melanomas[16]. LS-related tumors develop via a distinctive molecular pathway compared with non-LS related tumors[17-26], and therefore study findings from the general population might not be directly translatable to persons with LS.

Only two studies have been published on the association between height and colorectal neoplasia risk for persons with LS, with conflicting results. For persons suspected to have LS based on their family history, women taller than 1.55 meters were found to have a 47% to 127% increased CRC risk compared with those shorter than 1.55 meters in a Canadian study while no evidence for an association was found for men[27]. In contrast, for persons confirmed to have LS we reported a 57% decreased risk of colorectal adenomas for each 5 cm increment in height in men while no association was found for women in our previous analyses within a Dutch study (GEOLynch)[28]. The conflicting results might be due to different study samples (suspected for LS vs. confirmed to have LS), exposure (categorical vs. continuous), outcome (CRC vs. colorectal adenoma) and study design (case-control vs. prospective cohort). In these analyses, we aimed to investigate the association between adult attained height and CRC and EC risk for men and women with LS separately using data from a large sample of persons confirmed to have LS.

Methods

Study population

For this study, we harmonized data of 2849 persons confirmed to have LS from two separate studies: the GEOLynch study[28] (ClinicalTrials.gov identifier NCT03303833) and the Colon Cancer Family Registry (CCFR)[29].

Briefly, within the GEOLynch study, persons with LS, i.e. a pathogenic variant in one of the MMR or the *EPCAM* genes, were recruited actively since 2006 through the Netherlands Foundation for the Detection of Hereditary Tumors and two university medical centers (Radboudumc and University Medical Center Groningen, all in the Netherlands). Since 2012 participants were also passively recruited through information published in a magazine of and on a website of the Lynch Polyposis society, a Dutch patient association. Adults with LS both with and without a cancer diagnosis before study enrolment were eligible for study inclusion[28].

The CCFR is an international consortium of six centers in North America and Australia. Its design and recruitment are described in detail by Newcomb *et al.*[29] and Jenkins *et al.*[30]. Briefly, in all six centers population-based probands were recently diagnosed CRC cases identified via cancer registries. Additionally, four centers also used identified clinic-based probands, i.e. cancer-affected and cancer-unaffected persons from families with multiple CRC cases presenting at familial cancer clinics. Population-based probands with MMR-deficient CRC and all clinic-based probands were tested for germline mutations in a DNA MMR gene. A pathogenic variant was identified as LS. Subsequently, where possible, first- and/or second-degree relatives of identified probands with LS were recruited for study participation and germline mutation testing of the variant found in their proband. In this study, we included population-based and clinic-based probands and their relatives with a confirmed germline MMR gene mutation.

Both studies were approved by local medical ethical review committees. Additionally, all participants provided informed consent.

Data collection

For both studies, self-reported height and other self-reported personal information (smoking habits, weight and for women: menstrual and reproductive history and menopausal status) and demographic characteristics (age, sex, ethnicity, education level) were collected at recruitment via study- and/or center- specific standardized questionnaires. Clinical information regarding bowel diseases, colorectal surgeries and hysterectomy were obtained from medical records, pathology reports and/or were self-reported (CCFR).

Cancer diagnoses

Cancer diagnoses were identified by several mechanisms. For GEOLynch, the majority of the participants (80.1%) provided consent for a linkage with the Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA foundation). PALGA has a full coverage of pathology tests since 1991. Reported cancer diagnoses within PALGA after 1991 were therefore used to identify any cancer diagnosis among GEOLynch participant with a linkage to PALGA. Cancer diagnoses obtained from medical records were used for those who did not give consent for a linkage with PALGA and for cancer diagnoses before 1991 which were not reported in PALGA.

In CCFR data, cancer diagnoses were obtained from cancer registries for population-based probands. Self- and/or second-hand reports by relatives of cancer diagnoses at study enrolment and/or 5-year follow up were confirmed, where possible, using pathology reports, medical records, and/or death certificates for all enrolled participants[29, 30].

Study sample

For this study, we excluded participants with missing information on mutated gene (*n*=3), who also carried a germline *BRCA1* mutation (*n*=1), with missing clinical data (*n*=26), aged <18 years at questionnaire completion (*n*=1), with familial adenomatous polyposis (*n*=35), with missing data on height (*n*=44), missing age at cancer diagnosis (*n*=14) and participants with a cancer diagnosed before 18 years of age (*n*=5) (Figure 1). Additionally, for CRC analyses, persons were excluded if they had a total proctocolectomy but missing age at total proctocolectomy (*n*=3) or if no person time could be calculated (*n*=9). For EC analyses, women with a hysterectomy but missing age at hysterectomy (*n*=16) and women without person time (*n*=1) were excluded (Figure 1). Characteristics of the participants included for the analyses were similar to those of the total cohort (data not shown).

Statistical analyses

We used summary statistics to describe the study population across sex-specific medians of height.

Cox proportional hazard (PH) regression with age as the time scale was used to calculate hazard ratios (HRs) including 95% confidence intervals (CI) for height and CRC and EC. Height (cm) was modeled per 5 cm increase for CRC and EC since no evidence for any

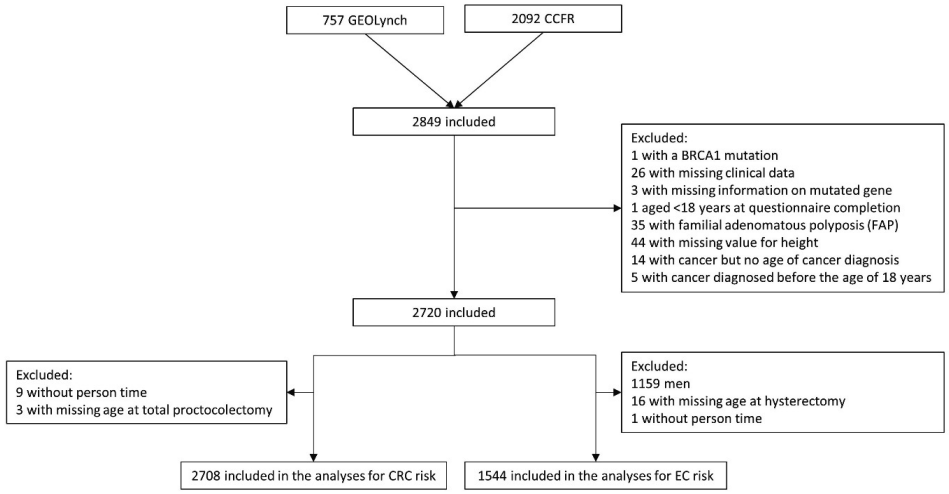

Figure 1. Flowchart of included participants. CCFR, Colon Cancer Family Registry; CRC, colorectal cancer; EC, endometrial cancer.

departure from a linear association was observed by using restricted cubic splines in Cox regression.

A weighted model was chosen in the HR calculations to adjust for ascertainment bias, which may occur due to oversampling of cancer cases in our population (Supplemental tables S1-S3)[31]. By using this method, ascertainment bias will be removed in case of accurate specification of the expected incidence rates of the external referent population and it will be reduced if specification is not completely accurate[31]. Additionally, a robust sandwich-covariance estimate by clustering on family membership was applied to account for any dependence of observations within families[32, 33].

We used a retrospective approach to calculate CRC and EC risk estimates. For CRC, person time started at the age of 18 years since height plateaus around the age of 18 years for men and women[34]. Person time ended at the age of the first occurrence of any of the following events: first diagnosed cancer excluding non-melanoma skin cancer, baseline interview (CCFR), first colonoscopy of the first series of regular colonoscopies (GEOLynch; defined as at least two colonoscopies performed with an interval of maximal 2.5 years between the colonoscopies), last update of the medical records (GEOLynch), last linkage to PALGA (GEOLynch), or age at total proctocolectomy that diminishes the risk to develop CRC.

To calculate EC risk estimates, person time also started at the age of 18 years and ended at the age of the first occurrence of one of the following events: first diagnosed cancer excluding

non-melanoma skin cancer, death, last contact (CCFR), clinical trial enrolment (GEOLynch), lost to follow-up (GEOLynch), last update of the medical records (GEOLynch), last linkage to PALGA (GEOLynch), or age at hysterectomy since a hysterectomy eliminates the risk to develop EC.

Risk estimate were adjusted for a priori identified confounding covariates[35]: education level (low, middle, high), ethnicity (Caucasian vs. non-Caucasian), smoking status at the age of 18 years (ever vs. never), year of birth and country of residence (Australasia, Canada, the Netherlands, USA). Risk estimates for EC were additionally adjusted for age at menarche.

Schoenfeld residuals were used to judge if the PH assumption was met. Violation of the assumption was observed for height in the association between height and CRC for men. Therefore, CRC risk estimates for men were additionally partitioned at the age of 55 years. Moreover, year of birth was added as time-varying variable in regressions for CRC and EC risk estimates were calculated with a stratified Cox procedure over the strata of country of residence to correct for violation of the PH assumption seen for those variables.

Heterogeneity of the effect of height on the three CRC risk estimate, i.e. for men aged <55 years, men aged ≥55 years and women, was explored by adding an interaction term of height and those three groups into the model. Moreover, to explore a potential differential effect by cohort (CCFR vs. GEOLynch), an interaction term of height and cohort was added to the models for CRC and EC to determine heterogeneity by cohort.

Two sensitivity analyses were performed. At first, to assess if self-reported cancer cases or reported cancer cases by relatives and/or spouses influenced the results, we excluded those cancer diagnosis (n=399). Secondly, since Møller *et al*.[36] showed that the incidence of a second primary cancer diagnosis in persons with LS was similar to the incidence of a first primary cancer diagnosis, a sensitivity analyses was performed in which person time ended at the first diagnosed CRC or EC only instead of the first diagnosed cancer.

All p-values were two-sided. Data analyses were performed in SAS software version 9.4 of the SAS System for Windows (SAS Institute Inc.).

Results

Participants' characteristics

A total of 1155 men and 1553 women contributed to 28 279 and 37 090 person years respectively. Median height (range) for men was 180.0 (150.0-213.0) cm and 165.0 (134.0-190.0) cm for women. Taller participants were heavier at young adulthood, more often highly educated and were more often enrolled in the GEOLynch study compared with shorter

participants. Ever smoking at the age of 18 years was less often reported by taller men compared with shorter men. Person time ended less often at CRC diagnosis for taller compared with shorter participants. For taller women, person time ended less often at the age of EC diagnosis compared with shorter women (Table 1). Person time ended more often at CRC (40.9% vs. 18.7%), but not EC (10.9% vs. 11.6%), diagnosis for CCFR participants compared with GEOLynch participants (data not shown).

Table 1. Characteristics of study participants by sex-specific median of height.[a]

	Men		**Women**	
	<180.0 cm	*≥180.0 cm*	*<165.0 cm*	*≥165.0 cm*
	N=577	N=578	N=698	N=855
Age (yr) at study enrolment, mean ± SD	50.2 ± 13.4	46.3 ± 13.7	50.8 ± 14.1	46.5 ± 14.0
Smoking at age 18 years, n (%)				
Ever	238 (41.3)	201 (34.8)	200 (28.7)	246 (28.8)
Weight (kg) at young adulthood[b], median [Q1, Q3]	70.0 [64.0, 77.0]	79.0 [72.0, 85.0]	54.0 [50.0, 59.0]	60.0 [55.0, 67.0]
Age (yr) at menarche, mean ± SD	-	-	12.8 ± 1.5	13.2 ± 1.6
Education level[c], n (%)				
Low	144 (25.0)	100 (17.3)	223 (32.0)	164 (19.2)
Medium	273 (47.3)	258 (44.6)	338 (48.4)	390 (45.6)
High	157 (27.2)	216 (37.4)	133 (19.1)	296 (34.6)
Mutated MMR gene, n (%)				
MLH1	201 (34.8)	211 (36.3)	263 (37.7)	299 (35.0)
MSH2	271 (47.0)	243 (42.0)	306 (43.8)	362 (42.3)
MSH6	69 (12.0)	84 (14.5)	90 (12.9)	122 (14.3)
PMS2	31 (5.4)	33 (5.7)	29 (4.2)	61 (7.1)
EPCAM	5 (0.9)	7 (1.2)	10 (1.4)	11 (1.3)
Ethnicity, n (%)				
Caucasian	535 (92.7)	562 (97.2)	656 (94.0)	823 (96.3)
Country of residence, n (%)				
Australasia	257 (44.5)	202 (35.0)	345 (49.4)	274 (32.1)
Canada	66 (11.4)	45 (7.8)	86 (12.3)	90 (10.5)
The Netherlands	93 (16.1)	202 (35.0)	115 (16.5)	316 (37.0)

Table 1 continued.

	Men		Women	
	<180.0 cm	*≥180.0 cm*	*<165.0 cm*	*≥165.0 cm*
USA	161 (27.9)	129 (22.3)	152 (21.8)	175 (20.5)
Cohort, n (%)				
CCFR	484 (83.9)	376 (65.1)	583 (83.5)	539 (63.0)
GEOLynch	93 (16.1)	202 (35.0)	115 (16.5)	316 (37.0)
End of person time due to CRC diagnosis, n (%)	278 (48.2)	233 (40.3)	210 (30.1)	226 (26.4)
End of person time due to EC diagnosis[d,e], n (%)	-	-	90 (13.0)	81 (9.5)
Age (yr) at the end of person time for CRC[f], mean ± SD	44.4 ± 11.9	40.6 ± 11.9	43.8 ± 12.1	40.3 ± 11.8
Age (yr) at the end of person time for EC [d,e,g], mean ± SD	-	-	44.5 ± 11.0	42.5 ± 10.2

[a]Characteristics based on number of participants included in CRC (N=2708) analyses unless specified differently. [b]Weight at young adulthood reflects weight at the age of 18 years for GEOLynch participants and weight at the age of 20 years for CCFR participants. [c]Values do not add up to 100% due to 7 and 9 missing values for education level in men and women respectively. [d]Women with missing age of hysterectomy were excluded for the EC analyses, i.e. 7 of the 701 women <165.0 cm and 9 of the 860 women ≥165.0 cm. One woman ≥165.0 cm without person time was also excluded. [e]Based on number of women for EC analyses (N=1544). [f]Age of the first occurrence of one of the following events: first diagnosed cancer excluding non-melanoma skin cancers, baseline interview (CCFR), first colonoscopy of the first series of regular colonoscopies (GEOLynch), last update of the medical records (GEOLynch), last linkage to PALGA (GEOLynch) or age at total proctocolectomy. [g]Age of the first occurrence of one of the following events: first diagnosed cancer excluding non-melanoma skin cancers, death, last contact (CCFR), last update of the medical records (GEOLynch), last linkage to PALGA (GEOLynch), trial inclusion (GEOLynch), age at study exclusion (GEOLynch) or age at hysterectomy**.** BMI: body mass index, CCFR: Colon Cancer Family Registry, CRC: colorectal cancer, EC: endometrial cancer, PALGA: the Nationwide Network and Registry of Histo- and Cytopathology in the Netherland, Q: quartile, SD: standard deviation, USA: United States of America.

Colorectal cancer

A 5 cm increment in height was not associated with the risk of CRC in men (HR 1.00, 95% CI: 0.91-1.11) (Table 2). When we partitioned CRC risk estimates for men because the PH assumption was violated for height, we observed a HR of 1.03 (95% CI 0.93-1.14) per 5 cm increment in height for CRC for men aged <55 years, and a HR of 0.72 (95% CI 0.51-1.02) per 5 cm increment in height for men aged ≥55 years (Table 2). No evidence for an association between height and CRC was observed for women (HR 1.01, 95% CI 0.92-1.11).

Heterogeneity of the effect of height on CRC between men aged <55 years, men aged ≥55 years and women was not observed (p-value=0.09). No evidence for heterogeneity by cohort was found either (p-value=0.58).

Endometrial cancer

A 5 cm increment in height was not associated with EC (HR 1.08, 95% CI 0.94-1.24) (Table 3). No evidence for a differential effect of height on EC by cohort was observed (p-value=0.40).

Sensitivity analyses

Excluding self-reported cancer diagnoses and cancer diagnoses reported by relatives or spouses, or ending person time at the first diagnosed CRC or EC only instead of the first diagnosed any cancer did not result in different CRC or EC risk estimate for both men and women (data not shown).

Table 2. Hazard ratio and 95% confidence intervals of colorectal cancer for each 5 cm increment in height.

	Total number	Number of CRC cases	Total person years	Crude analysis			Multivariable analysis[a]		
				HR	95% CI	P-value	HR	95% CI	P-value
Men[b]									
All men	1155	511	28 279	0.95	0.87-1.04	0.25	1.00	0.91-1.11	1.00
<55 yr	1155	449	27 016	0.98	0.89-1.07	0.58	1.03	0.93-1.14	0.60
≥ 55 yr	171	62	1263	0.68	0.50-0.92	0.01	0.72	0.51-1.02	0.06
Women	1553	436	37 090	0.97	0.89-1.05	0.41	1.01	0.92-1.11	0.84

[a]Adjusted for education level, ethnicity, smoking at the age of 18 years, year of birth and country of residence. Year of birth was added as time-varying covariate since year of birth violated the proportional hazard assumption. [b]Violation of the proportional hazard assumption was observed for height in men. Therefore, CRC risk estimates for men were also partitioned at the age of 55 years. CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; yr, year.

Table 3. Hazard ratio and 95% confidence intervals of endometrial cancer for each 5 cm increment in height.

	Total number	Number of EC cases	Total person years	Crude analysis			Multivariable analysis[a]		
				HR	95% CI	P-value	HR	95% CI	P-value
Women	1544	171	39 227	1.01	0.90-1.14	0.81	1.08	0.94-1.24	0.29

[a]Adjusted for education level, ethnicity, smoking at the age of 18 years, year of birth and age at menarche and stratified for country of residence due to a violation of the proportional hazard assumption. CI, confidence interval; EC, endometrial cancer; HR, hazard ratio.

Discussion

In this study with a large number of persons with LS, we did not observe evidence for an association between height and CRC for men and women. Height was not associated with EC for women with LS either.

To the best of our knowledge, this is the first study that investigated the association between height and both CRC and EC in persons confirmed to have LS. While we did not observe evidence for an association between height and CRC, a 4% (95% CI 1.02-1.05) increased CRC risk per 5 cm increment in height was suggested for men and women in the general population [37]. Moreover, being taller increased CRC risk for women but not for men in a Canadian study with persons suspected for LS based on their family cancer history[27]. Our current analyses in persons with a germline MMR gene mutation leading to LS only may show different results compared to analyses performed among persons suspected to have LS, since persons expected to have LS will consist of persons with LS but also of persons with sporadic cancers or other familial cancer syndromes. Additionally, our observation of no association between height and CRC for men is in contrast to the results of our previous analyses in the GEOLynch study in which a 5 cm increment in height was associated with a 57% decreased risk of colorectal adenomas for men with LS. However, for women, results of the current study are consistent with our previous analyses in the GEOLynch study since no evidence for an association between height and colorectal adenoma risk was found for women with LS in the previous analysis[28].

For EC, we did not find evidence for an association between height and EC risk for persons with LS (HR per 5 cm increment in height 1.08, 95% CI 0.94-1.24). In the general population, evidence has been presented in a meta-analysis for a 15% (95% CI 1.09-1.22) increased EC risk for each 10 cm increment in height[38] which is similar to the risk estimate observed in our current analyses if an increment in height of 10 instead of 5 cm is used (HR per 10 cm increment in height 1.16, 95% CI 0.88-1.53).

Strengths of this study include the large number of persons confirmed to have LS from three continents. Additionally, we were able to adjust for confounding covariates, we used a weighted cohort approach to reduce potential ascertainment bias and a robust co-variance estimate was used to adjust for any dependence of observations within families.

It should be noted that the retrospective approach of our data analyses may have introduced survival bias since the mean age at study enrolment was 48.4 years while person time started at the age of 18 years. This may have influenced our results if many CRC- or EC-related deaths occurred between the age of 18 years and the moment of participant recruitment. Survival after

a CRC or EC diagnosis in persons with LS, however, is high with an estimated 5- and 10-year survival of 96% and 88% for colon cancer and 93% and 93% for EC respectively[39]. Hence, we do not expect a large impact of this potential bias on our risk estimates. Additionally, height was self-reported instead of measured which may have led to an inflated reported height[40, 41]. Though, the correlation between self-reported height and measured height is reported to be high (r>0.9)[41]. Nevertheless, even though an inflated report of height may have occurred, this is expected to be non-differential with respect to CRC/EC diagnosis and therefore any estimates of associations would be expected to be biased towards the null. Moreover, participants were asked to report their current height instead of their height at the age of 18 years which may not reflect their tallest adult-attained height since aging comes with a decrease in height[42]. As a consequence, height reported at study enrolment of older participants versus younger participants is more likely to be an underestimation of the tallest adult-attained height. However, self-reported current height is not expected to be differentially reported for those with a taller vs. shorter adult-attained height. Using self-reported current height instead of height at the age of 18 years may hence have introduced a bias towards the null for our risk estimates. Finally, the majority of our participants were of Caucasian origin. Therefore, generalizability of our results to non-Caucasian LS populations may be hampered.

In conclusion, no evidence was observed for an association between height and both CRC and EC for men and women with LS.

Acknowledgements

We thank all GEOLynch and CCFR study participants and staff for their cooperation and contribution to the study. The funding sources were not involved in the study design; in collection, analysis, and interpretation of data; in writing of the manuscript; or in the decision to submit the manuscript for publication.

Funding

This work was supported by the Wereld Kanker Onderzoek Fonds (WCRF) Netherlands as part of the WCRF International grant program [2014/1184] and by the National Cancer Institute (NCI) of the U.S. National Institutes of Health (NIH) under Award Number U01CA167551 and through NCI/NIH cooperative agreements with the following CCFR centers: Australasian Colorectal Cancer Family Registry [U01 CA074778 and U01/U24 CA097735], Mayo Clinic Cooperative Family Registry for Colon Cancer Studies [U01/U24 CA074800], Ontario Familial Colorectal Cancer Registry [U01/U24 CA074783], Seattle Colorectal Cancer Family Registry [U01/U24 CA074794], University of Hawaii Colorectal Cancer Family Registry [U01/U24 CA074806 and R01 CA104132 to L LeMarchand], USC Consortium Colorectal Cancer Family Registry [U01/U24 CA074799]. Additional support for case ascertainment was provided from the Surveillance, Epidemiology and End Results (SEER) Program of the NCI to Fred Hutchinson Cancer Research Center (Control Nos. N01-CN-67009 and N01-PC-35142, and Contract No. HHSN2612013000121], the Hawaii Department of Health [Control Nos. N01-PC-67001 and N01-PC-35137, and Contract No. HHSN26120100037C], and the California Department of Public Health [contracts HHSN261201000035C awarded to the University of Southern California and HHSN261201000140C awarded to the Cancer Prevention Institute of California], the following U.S. state cancer registries: AZ, CO, MN, NC, NH, and by the Victorian Cancer Registry, Australia and the Ontario Cancer Registry, Canada.

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Supplemental tables

Supplemental table S1. Calculation of the weights applied to men with colorectal cancer (affected) and without colorectal cancer (unaffected) for the analyses regarding height and colorectal cancer risk.[a]

				Person years in age group					
Mutated gene	**Age group (years)**	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**Population incidence rate[b]**	**External rate[c]**	**Affected weight**	**Unaffected weight**
MLH1	<35	58	83	632	710	0.000028	0.00378	0.3644	1.4441
	35-40	42	30	87	48	0.000072	0.00972	0.2581	2.0386
	40-45	44	30	75	52	0.000178	0.00926	0.1584	2.2343
	45-50	35	16	51	29	0.000328	0.01706	0.2240	2.6975
	50-55	19	16	42	32	0.000618	0.02163	0.3031	1.8276
	55-60	11	13	20	27	0.000824	0.02884	0.3249	1.5712
	60-65	1	6	1	10	0.001188	0.01069	0.5485	1.0752
	≥65	3	5	7	21	0.001982	0.00864	0.0952	1.5429
MSH2/EPCAM[d]	<35	50	104	567	938	0.000028	0.00378	0.5599	1.2116
	35-40	30	45	69	93	0.000072	0.00972	0.5326	1.3116
	40-45	35	37	82	70	0.000178	0.00926	0.3349	1.6291
	45-50	45	38	94	63	0.000328	0.01706	0.3236	1.8010
	50-55	33	35	69	69	0.000618	0.02163	0.3312	1.6305
	55-60	13	23	15	35	0.000824	0.02884	0.5373	1.2615
	60-65	9	10	16	13	0.001188	0.01069	0.1429	1.7714
	≥65	7	12	33	64	0.002190	0.01068	0.1536	1.4937

Supplemental table S1 continued.

Mutated gene	Age group (years)	Affected (N)	Unaffected (N)	Person years in age group: Affected	Person years in age group: Unaffected	Population incidence rate[b]	External rate[c]	Affected weight	Unaffected weight
MSH6	<35	5	21	41	208	0.000028	0.00034	0.1538	1.2015
	35-40	3	9	8	13	0.000072	0.00086	0.1708	1.2764
	40-45	4	13	16	29	0.000178	0.00214	0.2830	1.2206
	45-50	16	8	30	14	0.000328	0.00394	0.1014	2.7972
	50-55	5	15	5	32	0.000618	0.00402	0.2501	1.2500
	55-60	5	18	10	39	0.000824	0.00536	0.2192	1.2169
	60-65	6	11	16	19	0.001188	0.00772	0.1288	1.4752
	≥65	3	11	12	36	0.001982	0.01288	0.1986	1.2186
PMS2	<35	2	9	18	63	0.000028	0.00018	0.0843	1.2035
	35-40	3	3	7	2	0.000072	0.00047	0.0374	1.9626
	40-45	3	2	8	2	0.000178	0.00102	0.0730	2.3906
	45-50	8	5	16	8	0.000328	0.00187	0.0388	2.5379
	50-55	2	2	1	5	0.000618	0.00254	0.1704	1.8296
	55-60	3	2	9	5	0.000824	0.00338	0.1271	2.3093
	60-65	3	5	4	5	0.001188	0.00297	0.0675	1.5595
	≥65	5	7	12	49	0.002352	0.00589	0.0963	1.6455

Supplemental table S1 continued.

[a]Weights for the model were calculated by comparing the incidence of cancer affected and cancer unaffected participants in our study sample with those of an external referent population. [b]Age-specific population incidences for men were obtained from the *Cancer Incidence in Five Continents* report[1] and included the incidence in Australia, the Netherlands and the USA. [c]External rate is calculated by multiplying the population incidence rate with age- and gene-specific hazard ratios for colorectal cancer in men with Lynch syndrome published in literature[2-4].[d]Numbers of *EPCAM* mutation carriers were small. Therefore, *EPCAM* mutation carriers were added to *MSH2* mutation carriers because Kempers *et al.* did not observe evidence for a different cumulative colorectal cancer risk before the age of 70 years for *EPCAM* vs. *MSH2* mutation carriers[5].

Supplemental table S2. Calculation of the weights applied to women with colorectal cancer (affected) and without colorectal cancer (unaffected) for the analyses regarding height and colorectal cancer risk.[a]

				Person years in age group					
Mutated gene	**Age group (years)**	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**Population incidence rate[b]**	**External rate[c]**	**Affected weight**	**Unaffected weight**
MLH1	<35	54	128	548	964	0.000028	0.0028	0.3853	1.2593
	35-40	32	51	69	123	0.000092	0.0091	0.4785	1.3272
	40-45	43	48	88	94	0.000163	0.0085	0.2383	1.6823
	45-50	32	53	55	107	0.000275	0.0143	0.3456	1.3951
	50-55	14	41	22	82	0.000482	0.0149	0.4666	1.1821
	55-60	15	21	27	34	0.000579	0.0179	0.2500	1.5357
	≥60	6	24	16	206	0.001444	0.0080	0.3271	1.1682
MSH2/EPCAM[d]	<35	44	160	414	1373	0.000028	0.0028	0.6005	1.1099
	35-40	25	72	49	154	0.000092	0.0091	0.7811	1.0760
	40-45	48	90	94	195	0.000163	0.0085	0.2730	1.3877
	45-50	35	75	82	162	0.000275	0.0143	0.3841	1.2874
	50-55	12	61	27	89	0.000482	0.0149	0.5563	1.0873
	55-60	8	23	11	40	0.000579	0.0179	0.5214	1.1665
	≥60	4	32	8	207	0.000797	0.0050	0.2842	1.0895
MSH6	<35	4	39	29	388	0.000028	0.0003	0.2628	1.0756
	35-40	4	17	6	23	0.000092	0.0011	0.2121	1.1854
	40-45	13	22	35	41	0.000163	0.0020	0.0950	1.5348

Supplemental table S2 continued.

Mutated gene	Age group (years)	Affected (N)	Unaffected (N)	Person years in age group: Affected	Person years in age group: Unaffected	Population incidence rate[b]	External rate[c]	Affected weight	Unaffected weight
MSH6	45-50	7	20	4	38	0.000275	0.0033	0.2259	1.2709
	50-55	6	26	9	45	0.000482	0.0031	0.1698	1.1916
	55-60	4	22	8	43	0.000579	0.0038	0.1796	1.1492
	≥60	2	26	15	175	0.001444	0.0094	0.8909	1.0084
PMS2	<35	2	12	26	122	0.000028	0.0002	0.1233	1.1461
	35-40	5	9	8	17	0.000092	0.0006	0.0401	1.5333
	40-45	5	7	7	18	0.000163	0.0009	0.0528	1.6766
	45-50	5	6	13	11	0.000275	0.0016	0.0684	1.7764
	50-55	2	7	1	14	0.000482	0.0021	0.1757	1.2355
	55-60	2	9	8	24	0.000579	0.0025	0.1572	1.1873
	≥60	3	16	21	138	0.001444	0.0035	0.1923	1.1515

[a]Weights for the model were calculated by comparing the incidence of cancer affected and cancer unaffected participants in our study sample with those of an external referent population. [b]Age-specific population incidence for women were obtained from the *Cancer Incidence in Five Continents* report[1] and included the incidence in Australia, the Netherlands and the USA. [c]External rate is calculated by multiplying the population incidence rate with age- and gene-specific hazard ratios for colorectal cancer in women with Lynch syndrome published in literature published in literature[2-4]. [d]Numbers of *EPCAM* mutation carriers were small. Therefore, *EPCAM* mutation carriers were added to *MSH2* mutation carriers because Kempers *et al.* did not observe evidence for a different cumulative colorectal cancer risk before the age of 70 years for *EPCAM* vs. *MSH2* mutation carriers[5].

Supplemental table S3. Calculation of the weights applied to women with endometrial cancer (affected) and without endometrial cancer (unaffected) for the analyses regarding height and endometrial cancer risk.[a]

				Person years in age group					
Mutated gene	**Age group (years)**	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**Population incidence rate[b]**	**External rate[c]**	**Affected weight**	**Unaffected weight**
***MLH1/EPCAM*[d]**	<40	7	213	125	2836	0.000036	0.0027	4.1514	0.8964
	40-45	8	114	20	226	0.000136	0.0103	1.8599	0.9397
	45-50	14	91	23	158	0.000229	0.0174	1.0516	0.9921
	50-55	7	51	9	92	0.000432	0.0097	0.6597	1.0467
	55-60	7	29	7	61	0.000719	0.0161	0.6120	1.0937
	60-65	2	16	1	17	0.000915	0.0205	1.2575	0.9678
	≥65	1	20	2	154	0.000831	0.0186	2.7212	0.9139
MSH2	<40	17	231	262	3184	0.000036	0.0027	1.9615	0.9292
	40-45	19	137	47	257	0.000136	0.0103	0.8690	1.0182
	45-50	17	112	37	208	0.000229	0.0174	0.9158	1.0128
	50-55	22	54	26	101	0.000432	0.0097	0.1801	1.3340
	55-60	4	20	10	35	0.000719	0.0161	0.7825	1.0435
	60-65	1	14	0	23	0.000915	0.0205	1.9758	0.9303
	≥65	2	13	1	59	0.000920	0.0206	0.6476	1.0542
MSH6	<40	3	53	53	799	0.000036	0.0027	3.7652	0.8435
	40-45	5	35	9	73	0.000136	0.0103	1.3571	0.9490
	45-50	3	36	6	53	0.000229	0.0174	2.5611	0.8699
	50-55	11	22	16	33	0.000432	0.0097	0.2326	1.3837

Supplemental table S3 continued.

Mutated gene	Age group (years)	Affected (N)	Unaffected (N)	Person years in age group: Affected	Person years in age group: Unaffected	Population incidence rate[b]	External rate[c]	Affected weight	Unaffected weight
MSH6	55-60	7	11	14	24	0.000719	0.0161	0.3769	1.3965
	60-65	3	5	8	4	0.000915	0.0205	0.6493	1.2104
	≥65	5	12	7	104	0.000831	0.0186	0.4832	1.2154
PMS2	<40	1	20	6	185	0.000047	0.0003	0.3493	1.0325
	40-45	1	18	0	39	0.000136	0.0008	0.2265	1.0430
	45-50	1	14	0	37	0.000229	0.0013	0.2807	1.0514
	50-55	1	10	2	7	0.000432	0.0025	0.3171	1.0683
	≥55	2	22	7	235	0.000822	0.0047	0.5840	1.0378

[a]Weights for the model were calculated by comparing the incidence of cancer affected and cancer unaffected participants in our study sample with those of an external referent population. [b]Age-specific population incidence obtained from the *Cancer Incidence in Five Continents* report[1] and included the incidence in Australia, the Netherlands and the USA. [c]External rate is calculated by multiplying the population incidence rate with age- and gene-specific hazard ratios for endometrial cancer in women with Lynch syndrome[4, 6].[d]The number of *EPCAM* mutation carriers was small. Therefore, *EPCAM* mutation carriers were added to *MLH1* mutation carriers because Kempers *et al.* did not observe evidence for a different cumulative endometrial cancer risk before the age of 70 years for *EPCAM* compared with *MLH1* mutation carriers[5].

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CHAPTER 4

A HIGHER BODY MASS INDEX AT YOUNG ADULTHOOD IS ASSOCIATED WITH AN INCREASED RISK OF CANCER AT ALL SITES FOR WOMEN WITH LYNCH SYNDROME

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Abstract

Background Persons with Lynch syndrome (LS), an inherited predisposition to cancer, have a high life-time risk of several cancer types and develop the disease at an earlier age compared with the general population. Previously, for persons with LS, body mass index (BMI) at young adulthood has been associated with colorectal cancer (CRC), but not endometrial cancer. Little is known about its association with other cancer types. Therefore, we aimed to investigate the association between BMI at young adulthood and the risk of cancer at all sites, cancer outside the colorectum (extra-CRC), and for women, cancer outside both the colorectum and endometrium (extra-CRCEC).

Methods We used harmonized data of 1044 men and 1446 women with LS from the Colon Cancer Family Registry and the GEOLynch study. BMI at young adulthood was based on self-reported height and recalled weight in young adulthood. Weighted Cox regression models were used with age on the time axis to calculate adjusted hazard ratios (HRs) and 95% confidence intervals (CI) for each 5 kg/m^2 increment in BMI at young adulthood. HRs were adjusted for education level, smoking habits, physical activity level, ethnicity, country of residence, year of birth and for women, age at menarche and hormonal contraceptive use.

Results A HR of 1.27 (95% CI 1.10-1.47) and 1.00 (95% CI 0.85-1.17) for cancer at all sites was observed with each 5 kg/m^2 increment in BMI at young adulthood for women and for men, respectively. No association was observed between BMI at young adulthood and extra-CRC or extra-CRCEC.

Conclusion A higher BMI at young adulthood is associated with an increased risk of cancer at all sites for women, but not for men, with LS.

Introduction

An inherited pathogenic variant in one of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* or *PMS2*, or the *EPCAM* gene results in Lynch syndrome (LS)[1, 2]. LS is characterized by an increased cancer risk of mainly the colorectum and endometrium but also of the ovaries, stomach, urinary tract, brain, biliary tract, small bowel, pancreas and possibly female breast[3-7]. Median age at LS-associated cancer diagnosis is, depending on the cancer type, estimated to be 4 to 11 years lower compared with the median age at cancer diagnosis in the general population[3, 4, 7]. The phenotypic expression of LS is variable which is attributed to differences in mutated gene and sex[2, 3, 8]. For example, overall cumulative risk estimates at the age of 75 years may reach 45.8% in *MLH1* mutation carriers for colorectal cancer (CRC) and 24.9% in *MSH2* mutation carriers for cancers in the urinary tract[9]. Additionally, lifestyle factors are suggested to influence the phenotypic expression[10].

Considering lifestyle factors, more body fatness, as represented by a higher body mass index (BMI), is a known risk factor that increases the risk of twelve cancer types in the general population[11]. Both a higher body fatness at adulthood and a higher body fatness at a young age are suggested to increase the risk of several cancer types including many LS-associated cancers[11-16].

Associations between body fatness and CRC or endometrial cancer risk in persons with LS have been studied before. No association between adult BMI and CRC risk was observed in a small subsample of persons with LS in a case-control study[17]. However, our previous prospective analyses within the GEOLynch study showed that for men with LS, a 5 kg/m^2 increment in adult BMI was associated with a 84% (95% confidence interval (CI) 1.13-3.02) increased risk of colorectal adenomatous polyps[18], a precursor lesion of CRC[19]. Regarding BMI at young adulthood, a 5 kg/m^2 increment in BMI seemed to increase LS-associated CRC risk by 30% (95% CI 1.08-1.58) for both men and women in data of the Colon Cancer Family Registry (CCFR)[20], while a higher BMI at young adulthood was not found to be associated with the risk of colorectal adenomatous polyps in the GEOLynch study[18]. For endometrial cancer, data of the CCFR did not show an association between BMI at young adulthood and endometrial cancer risk for women with LS[14].

Even though several studies have been conducted on the association between BMI and CRC or endometrial cancer for persons with LS, little attention has been paid to its association with cancer at other sites. To the best of our knowledge, only two small studies have been published in which conflicting results regarding an association between adult BMI and extra-colonic cancer risk for persons with LS were observed[21, 22]. No studies could be found that

investigated the association between BMI at young adulthood and all, extra-colonic and/or extra-endometrial cancer risk in persons with LS, while this knowledge is important due to the high lifetime risk and early age of development of all cancers in this population.

Therefore, we aimed to prospectively investigate the association between BMI at young adulthood and risk of cancer at all and at extra-colonic sites for men and women with LS, and also at both extra-colonic and extra-endometrial sites for women with LS.

Methods

Study population

Data of participants of the GEOLynch study[18] (ClinicalTrials.gov identifier NCT03303833), *n*=757, and of participants with LS from the CCFR[23], *n*=2092, has been harmonized and used for this study.

Briefly, for the GEOLynch study, cancer-free and cancer-affected persons with LS – i.e. persons with a pathogenic variant in the *MLH1*, *MSH2*, *MSH6*, *PMS2* or *EPCAM* gene – were actively recruited for participation since 2006. Persons with LS were identified from the Netherlands Foundation for the Detection of Hereditary Tumours, Radboud University Medical Center and the University Medical Center Groningen (all in the Netherlands). Since 2012, persons with LS were also recruited passively from volunteers self-identified through information published in a magazine and on the website of the Lynch Polyposis society, a Dutch patient association. Actively or passively recruited persons with LS were eligible for participation if they were Dutch speaking, mentally competent to participate, aged 18-80 years and lived in the Netherlands. Those diagnosed with familial adenomatous polyposis (FAP) were excluded.

The CCFR is an international consortium of six centres in North-America and Australia that has been described in detail elsewhere[23, 24]. In short, participants were recruited population-based in all six centres and also clinic-based in four centres. Persons with CRC identified from cancer registries reflect population-based recruitment. All population-based CRC-affected recruited persons with a LS-causing germline mutation were included in this study. Clinic-based recruited probands were cancer-affected and cancer-unaffected persons presenting at familial cancer clinics with early onset CRC, with a strong family history of CRC, or from a family with a known or probable pathogenic germline mutation in a MMR gene. All population- and clinic-based probands and their family members with a LS-causing germline mutation were included in this study.

The GEOLynch and CCFR were approved by local ethical review committees. All participants provided written informed consent.

Data collection

All participants completed study and/or centre specific standardized questionnaires by personal interviews, telephone interviews or mail. The questionnaires included questions about current height and weight at the age of 18 years (GEOLynch) or weight at the age of 20 years (CCFR). Additionally, demographic characteristics (age, sex, ethnicity, education level), smoking habits including e.g. age at smoking commencement and age at smoking cessation, current physical activity and physical activity in the age period 20-29 years (CCFR), and for women: menstrual and reproductive history, were included. Clinical information regarding bowel diseases, colorectal surgeries, hysterectomy and bilateral oophorectomy were obtained from medical records, pathology reports and/or were self-reported.

Cancer diagnoses were obtained from pathology reports and/or medical records for GEOLynch participants. For CCFR participants, cancer diagnoses were identified from cancer registries for cancer-affected population-based participants and from clinical records for cancer-affected clinic-based participants. Additionally, self- and/or second-hand reports of cancer diagnoses at study enrolment and/or 5-year follow-up surveys were confirmed, where possible, using pathology reports, medical records, cancer registry reports and/or death certificates[23, 24]. Cancer types were categorized into cancer at all sites, at extra-colonic (extra-CRC) sites, and for women at both extra-CRC and extra-endometrial (extra-CRCEC) sites (Supplemental table S1).

Population for analyses

For this study, we excluded participants with FAP (*n*=35), a germline *BRCA1* mutation (*n*=1), missing clinical data (*n*=26), missing data on mutated gene (*n*=2), aged <18 years at study enrolment (*n*=1), missing values for height (*n*=44), missing values for weight at young adulthood (*n*=231), with a cancer diagnosis but unknown age of cancer diagnosis (*n*=14) or with a cancer diagnosis before the age of 18 years (*n*=5) (Figure 1). For the extra-CRC and extra-CRCEC analyses, one additional participant was excluded because no observation time could be calculated. Finally, 2490 participants were included for analyses of cancer at all sites, 2489 participants for analyses of extra-CRC and 1446 women for analyses of extra-CRCEC.

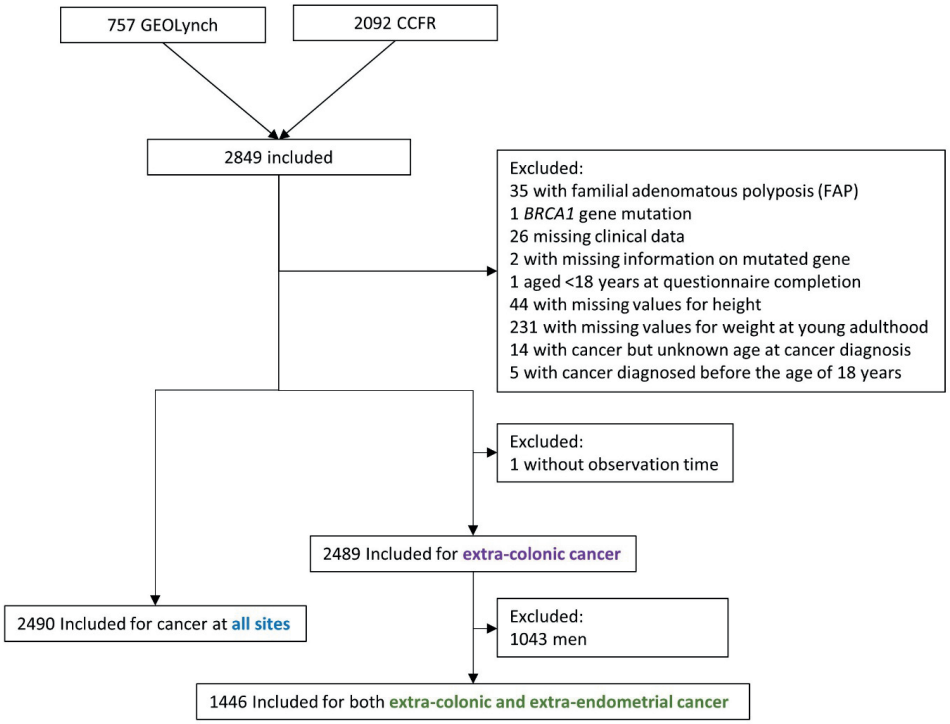

Figure 1. Flowchart of study participants. Extra-colonic cancer: cancers located outside the colorectum. Extra-endometrial cancer: cancers located outside the endometrium.

Statistical analyses

BMI at young adulthood was calculated by dividing self-reported weight (kg) at the age of 18 years (GEOLynch) or 20 years (CCFR) by the squared self-reported height (m). Summary statistics were used to describe the population for analyses for each sex by BMI at young adulthood (<25.0 kg/m^2 vs. ≥25.0 kg/m^2).

Weighted Cox proportional hazard regression models[25] were used to calculate hazard ratios (HRs) and 95% CI of cancer at all sites, extra-CRC and extra-CRCEC for each 5 kg/m^2 increment in BMI at young adulthood and in categories based on BMI classification of the World Health Organization[26] (Supplemental tables S2-S5). A robust sandwich-covariance estimate was used to account for any dependency of observations within families[27, 28].

For the analyses of cancer at all sites, person time started at the age of 18 years and ended at the age of the first occurrence of the following events: first primary cancer diagnosis excluding non-melanoma skin cancers, baseline interview (CCFR), first surveillance colonoscopy (GEOLynch), last update of the medical records (GEOLynch) or last linkage to

the Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA, GEOLynch).

For the analyses of extra-CRC and extra-CRCEC, person time started at the age of 18 years and ended at the age of the first occurrence of the following events: first primary cancer diagnosis excluding non-melanoma skin cancers, death, last contact (CCFR), clinical trial enrolment (GEOLynch), lost to follow-up (GEOLynch), last update of the medical records (GEOLynch) or last linkage to PALGA (GEOLynch).

All models were adjusted for birth year and country of residence to take a potential birth cohort or country effect into account. Additionally, HRs were adjusted for literature-based a priori defined covariates including education level (low, medium, high), smoking habits in young adulthood (ever vs. never), physical activity level at young adulthood (low, medium, high), ethnicity (non-Caucasian vs. Caucasian) and for women, age at menarche and hormonal contraceptive use in young adulthood (ever vs. never).

Tests for linear trend across categories of BMI at young adulthood were conducted by modelling the median value of each category as a continuous variable in the model. Schoenfeld residuals were used to identify any variable in the Cox model that violates the proportional hazard (PH) assumption.

Heterogeneity by sex and study cohort (CCFR vs. GEOLynch) was explored by adding an interaction term of BMI at young adulthood with either sex or cohort to the models.

A sensitivity analysis was performed in which self-reported cancer diagnoses and cancer diagnoses reported by relatives or spouses were excluded (n=399). A second sensitivity analysis was performed to investigate whether censoring at the age of events that diminish or eliminate the risk to develop a specific cancer type, such as age at hysterectomy, will modify the risk estimates. Finally, since Møller *et al.*[29] did not observe a significantly higher incidence of subsequent cancer for LS persons with a previous cancer vs. those without a previous cancer, observation time was discontinued at the age of the first diagnosed extra-CRC or extra-CRCEC instead of the age of first diagnosed any cancer for the extra-CRC and extra-CRCEC analyses, respectively.

A two-sided p-value<0.05 was considered statistically significant. All analyses were generated by using SAS software, Version 9.4 of the SAS system for Windows (SAS Institute Inc.).

Results

A total of 1044 men and 1446 women with LS contributed to a total 25 905 and 34 738 person years for men and women respectively. A BMI ≥25.0 kg/m^2, i.e. being overweight or obese, at young adulthood was reported by 291 (27.9%) men and 175 (13.8%) women (Table 1). Participants with a BMI ≥25.0 kg/m^2 at young adulthood (i.e. being overweight or obese) were younger at study enrolment, less often highly educated and more often enrolled in the CCFR compared with participants with a BMI <25.0 kg/m^2 at young adulthood (i.e. having a normal weight or being underweight). Overweight or obese women less often had a high physical activity level compared with women with a normal weight while overweight or obese men vs. normal weight men were more often highly physically active at young adulthood. Ever use of hormonal contraceptives at the age of 18 years was more often reported in women with a BMI at young adulthood ≥25.0 vs. <25.0 kg/m^2. Person time ended more often at the age of cancer diagnosis at all sites and less often at the age of extra-CRC for participants with a BMI ≥25.0 vs. <25.0 kg/m^2 at young adulthood.

For women, a 5 kg/m^2 increment in BMI at young adulthood resulted in a HR of 1.27 (95% CI 1.10-1.47) for cancer at all sites, whereas a HR of 1.00 (95% CI 0.85-1.17) was observed for men (Figure 2). Similarly, while for women being obese (HR 2.54, 95% CI 1.51-4.27), but not overweight (HR 1.38, 95% CI 0.97-1.99), compared with normal weight was associated with increased risk of cancer at all sites, no association was observed for obese (HR 1.07, 95% CI 0.59-1.97) or overweight (HR 1.01, 95% CI 0.76-1.34) men compared with normal weight men (Table 2). No differential effect of BMI at young adulthood on cancer at all sites by sex or cohort was observed (p-value for interaction of 0.33 and 0.40 respectively).

A HR of 0.89 (95% CI 0.67-1.19) and 1.12 (95% CI 0.93-1.35) was observed for extra-CRC with each 5 kg/m^2 increment in BMI at young adulthood for men and women, respectively (Figure 2). Being overweight or obese vs. normal weight resulted in a HR of 0.69 (95% CI 0.38-1.23) and 1.09 (95% CI 0.42-2.86) for men, and 1.49 (95% CI 0.92-2.42) and 1.09 (95% CI 0.42-2.79) for women respectively (Table 2). No differential effect of BMI at young adulthood on extra-CRC risk by sex or cohort was observed (p-value for interaction of 0.21 and 0.67 respectively).

Table 1. Characteristics of study participants with a BMI <25.0 kg/m^2 and a BMI ≥25.0 kg/m^2 by sex.[a]

	Men		**Women**	
	<25.0 kg/m^2	*≥25.0 kg/m^2*	*<25.0 kg/m^2*	*≥25.0 kg/m^2*
	N=753	N=291	N=1271	N=175
Weight (kg) at young adulthood[b], median [Q1, Q3]	70.0 [65.0, 76.0]	86.0 [81.0, 93.0]	56.0 [51.0, 61.0]	76.0 [70.0, 83.0]
Height (cm), mean ± SD	178.7 ± 7.7	178.1 ± 7.3	165.0 ± 7.4	164.3 ± 7.6
Age (yr) at study enrolment, mean ± SD	48.9 ± 13.2	46.2 ± 13.7	49.2 ± 13.9	43.3 ± 13.9
Education level[c], n (%)				
Low	150 (19.9)	56 (19.2)	313 (24.6)	40 (22.9)
Medium	345 (45.8)	148 (50.9)	597 (47.0)	88 (50.3)
High	257 (34.1)	84 (28.9)	356 (28.0)	45 (25.7)
Smoking habits at young adulthood, *ever*, n(%)	288 (38.3)	117 (40.2)	367 (28.9)	52 (29.7)
Physical activity level at young adulthood[c,d]				
Low	210 (27.9)	72 (24.7)	442 (34.8)	73 (41.7)
Medium	249 (33.1)	88 (30.2)	409 (32.2)	56 (32.0)
High	275 (36.6)	126 (43.3)	386 (30.4)	42 (24.0)
Mutated MMR gene, n (%)				
MLH1	258 (34.3)	116 (39.9)	450 (35.4)	66 (37.7)
MSH2	338 (44.9)	124 (42.6)	566 (44.5)	73 (41.7)
MSH6	103 (13.7)	33 (11.3)	173 (13.6)	19 (10.9)
PMS2	45 (6.0)	15 (5.2)	66 (5.2)	14 (8.0)
EPCAM	9 (1.2)	3 (1.0)	16 (1.3)	3 (1.7)
Ethnicity, *Caucasian*, n (%)	710 (94.3)	280 (96.2)	1209 (95.1)	167 (95.4)
Age (yr) at menarche, mean ± SD	-	-	13.0 ± 1.6	12.5 ± 1.4
Hormonal contraceptive use at age 18 years, *ever*, n (%)	-	-	406 (32.2)	77 (44.0)
Country of residence, n (%)				
Australasia	304 (40.4)	136 (46.7)	515 (40.5)	86 (49.1)
Canada	72 (9.6)	33 (11.3)	154 (12.1)	17 (9.7)

Table 1 continued.

	Men		Women	
The Netherlands	195 (25.9)	20 (6.9)	328 (25.8)	22 (12.6)
USA	182 (24.2)	102 (35.1)	274 (21.6)	50 (28.6)
Cohort, n(%)				
CCFR	558 (74.2)	271 (93.1)	943 (74.2)	153 (87.4)
GEOLynch	195 (25.9)	20 (6.9)	328 (25.8)	22 (12.6)
Number of persons who end person time at the age of a cancer diagnosis at all sites, n (%)	390 (51.8)	157 (54.0)	623 (49.0)	93 (53.1)
Number of persons who end person time at the age of an extra-CRC diagnosis, n (%)[e]	84 (11.2)	25 (8.6)	343 (27.0)	41 (23.4)
Number of persons who end person time at the age of an extra-CRCEC diagnosis[f], n (%)	-	-	168 (13.2)	22 (12.6)
Age (yr) at the end of person time for cancer at all sites[g], mean ± SD	43.3 ± 12.0	41.7 ± 11.8	42.5 ± 11.9	38.3 ± 11.5
Age (yr) at the end of person time for extra-CRC[e, h], mean ± SD	47.8 ± 12.2	45.7 ± 12.2	47.3 ± 12.2	42.1 ± 11.8
Age (yr) at the end of person time for extra-CRCEC[f, h], mean ± SD	-	-	47.3 ± 12.2	42.1 ± 11.8

[a]Characteristics based on the population for the analyses of cancer at all sites unless stated otherwise. [b]Weight at young adulthood reflects weight at the age of 18 years for GEOLynch participants and weight at the age of 20 years for CCFR participants. [c]Values do not add up to 100% due to 4 and 7 missing values for education level and 24 and 38 missing values for physical activity in men and women respectively. [d]Cohort specific tertiles of physcial activity. For GEOLynch participants, current physical activity levels were used to reflect physical activity at young adulthood while for CCFR participants, physical activity in the age group 20-29 years was used. [e]N (%) based on the population for extra-CRC analyses (n=2488). [f]N(%) based on the population for extra-CRCEC analyses (n=1446). [g]Age of the first occurrence of one of the following events: first diagnosed cancer excluding non-melanoma skin cancers, baseline interview (CCFR), first surveillance colonoscopy (GEOLynch), last update of the medical records (GEOLynch) or last linkage to PALGA (GEOLynch). [h]Age of the first occurence of one of the following events: first primary cancer diagnosis excluding non-melanoma skin cancers, death, last contact (CCFR), clinical trial enrolment (GEOLynch), lost to follow-up (GEOLynch), last update of the medical records (GEOLynch) or last linkage to PALGA (GEOLynch). BMI: body mass index, CCFR: Colon Cancer Family Registry, CRC: colorectal cancer, EC: endometrial cancer, extra-CRC: outside the colorectum, extra-CRCEC: outside the colorectum and endometrium, PALGA: the Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands, Q: quartile, SD: standard deviation, USA: United States of America.

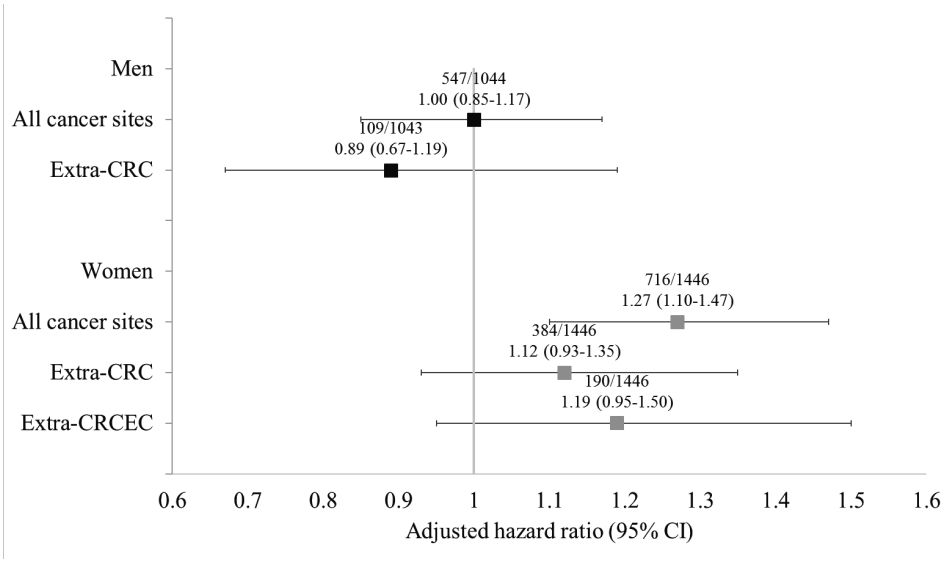

Figure 2. Cancer cases/total number of persons and adjusted hazard ratios (95% confidence interval) for a 5 kg/m^2 increment in body mass index at young adulthood by sex and cancer site. Hazard ratios are adjusted for year of birth, country of residence, education level, smoking habits at young adulthood, physical activity level at young adulthood and ethnicity. For women an additional adjustment for age at menarche and hormonal contraceptive use in young adulthood has been applied. CI, confidence interval; extra-CRC, cancers located outside the colorectum; extra-CRCEC, cancer located outside the colorectum and endometrium.

For extra-CRCEC, a HR of 1.19 (95% CI 0.95-1.50) was observed for women with a 5 kg/m^2 increment in BMI at young adulthood (Figure 2) and a HR of 1.44 (95% CI 0.80-2.61) for overweight and 1.59 (95% CI 0.60-4.23) for obese women compared with normal weight women (Table 2). The effect of BMI at young adulthood on extra-CRCEC risk did not differ by cohort (p-value for interaction=0.29).

The sensitivity analyses, i.e. excluding self-reported cancer diagnoses and cancer diagnoses reported by relatives or spouses, censoring at the age of events that diminish or eliminate the risk to develop a specific type of cancer or ending person time at the first diagnosed extra-CRC or extra-CRCEC, did not result in a different conclusion (data not shown).

Table 2. Hazard ratio and 95% confidence intervals of cancer for categorized body mass index[a] at young adulthood.

	Total number of persons	Number of cancer cases	Total person years	Univariable analysis		Multivariable analysis[b]	
				HR	95% CI	HR	95% CI
Men							
All cancer sites							
Underweight	33	16	852	0.75	0.37-1.51	0.64	0.37-1.44
Normal	720	374	18 183	1.00	Reference	1.00	Reference
Overweight	241	131	5831	1.19	0.88-1.60	1.01	0.76-1.34
Obese	50	26	1056	1.62	0.88-2.98	1.07	0.59-1.97
P-value for trend[c]	-	-	-	0.05		0.61	
Extra-CRC							
Underweight	33	2	1039	0.49	0.10-2.34	0.45	0.10-2.05
Normal	719	82	21 401	1.00	Reference	1.00	Reference
Overweight	241	20	6805	0.82	0.48-1.40	0.69	0.38-1.23
Obese	50	5	1242	1.28	0.53-3.06	1.09	0.42-2.86
P-value for trend[c]	-	-	-	0.76		0.83	
Women							
All cancer sites							
Underweight	193	91	4660	1.07	0.77-1.47	0.95	0.67-1.33
Normal	1078	532	26 520	1.00	Reference	1.00	Reference
Overweight	129	67	2670	1.47	1.03-2.09	1.38	0.97-1.99
Obese	46	26	888	2.44	1.42-4.21	2.54	1.51-4.27
P-value for trend[c]	-	-	-	0.00		0.00	

Table 2 continued.

	Total number of persons	Number of cancer cases	Total person years	Univariable analysis		Multivariable analysis[b]	
				HR	95% CI	HR	95% CI
Women							
Extra-CRC							
Underweight	193	46	5614	1.06	0.71-1.60	1.03	0.68-1.58
Normal	1078	297	31 685	1.00	Reference	1.00	Reference
Overweight	129	33	3203	1.41	0.90-2.22	1.49	0.92-2.42
Obese	46	8	1008	1.19	0.51-2.77	1.09	0.42-2.79
P-value for trend[c]	-	-	-	0.31		0.34	
Extra-CRCEC							
Underweight	193	24	5614	1.10	0.67-1.82	1.00	0.59-1.71
Normal	1078	144	31 685	1.00	Reference	1.00	Reference
Overweight	129	16	3203	1.40	0.79-2.50	1.44	0.80-2.61
Obese	46	6	1008	1.99	0.85-4.68	1.59	0.60-4.23
P-value for trend[c]	-	-	-	0.17		0.18	

[a]A body mass index at young adulthood of 18.5-25.0 kg/m^2 reflects a normal weight, of <18.5 kg/m^2 reflects underweight, of 25.0-30.0 kg/m^2 reflects overweight and of ≥30.0 kg/m^2 reflects obesity. [b]Adjusted for education level (low, medium, high), smoking habits in young adulthood (ever vs. never), physical activity level at young adulthood (low, medium, high), ethnicity (non-Caucasian vs. Caucasian), country of residence, year of birth and for women, age at menarche and hormonal contraceptive use in young adulthood (ever vs. never). For men, year of birth was added as time-varying variable in the analyses for cancer at all sites since the proportional hazard (PH) assumption was violated for year of birth. For women, the PH assumption of education level was violated for the extra-CRCEC analyses and therefore a stratified weighted Cox regression model over strata of education level was used. [c]Two sided p-values for tests for linear trends were calculated by adding the median value of each category as continuous variable into the model. BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; extra-CRC: outside the colorectum, extra-CRCEC: outside the colorectum and endometrium; HR, hazard ratio; PH, proportional hazard; yr, year.

Discussion

For each 5 kg/m^2 increment in BMI at young adulthood, we observed a 27% increased risk of cancer at all sites for women with LS, while no association was observed for men with LS. No association was found between BMI at young adulthood and the risk of extra-CRC for men and women, and extra-CRCEC for women.

Although the association between BMI at young adulthood and the risk of cancer at all sites did not differ statistically significantly by sex, we did observe a positive association for women but no association for men. The lack of association in men was unexpected since a 5 kg/m^2 increment in BMI at young adulthood was previously reported to increase the risk of CRC[20], which was the majority (86.8%) of the diagnosed cancers at all sites in men in our study. Interestingly, our participating men with a BMI≥25.0 kg/m^2 more often reported a high level of physical activity at young adulthood compared with men with a BMI<25.0 kg/m^2. Since adjusting our analyses for physical activity did not change the results, this may not explain the results observed for men. However, it may indicate that the BMI≥25.0 kg/m^2 is reflecting muscle mass instead of body fatness for men.

A recent meta-analysis in the general population reported increased risks of cancer at several sites with increasing BMI at young adulthood for men and women combined and/or separately[16]. However, one study that was not included in the meta-analysis also reported an increased sporadic CRC risk with increasing BMI at young adulthood for women, but not for men[30]. Generally, it is suggested that biological mechanisms are responsible for any observed sex difference in risk estimates. However, these mechanisms are considered to be similar for men and women[31, 32]. It is hence not clear why we observed an association for women but not for men. Future studies that also include measures besides BMI to reflect body fatness, such as waist-to-hip ratio, may help to delineate any difference in the association between body fatness at young adulthood and cancer risk for men and women with LS.

No association was observed for extra-CRC and extra-CRCEC, which is unexpected because the underlying mechanisms that may cause an association between BMI at young adulthood and extra-CRC or extra-CRCEC are assumed to be similar to those previously mentioned for cancers at all sites. Though, for women in the general population, there is probable evidence that more body fatness at young adulthood protects against both pre- and postmenopausal breast cancer[16, 32]. Since breast cancer cases contributed to 14.1% of the extra-CRC cases and 28.4% of the extra-CRCEC cases, this may have masked or diluted a potential positive association between BMI at young adulthood and the risk of cancers outside the breast. It would be interesting to obtain risk estimates by obesity dependent cancers (e.g. breast cancer,

and cancers of the stomach, gallbladder, ovary, pancreas, kidney, and thyroid[16, 32]) separately according to findings in the general population, but the numbers for obesity dependent cancers besides CRC and/or EC in our population were too small to allow any analyses with sufficient power.

One of the limitations of our study is that we used self-reported height and recalled weight in young adulthood to calculate BMI at young adulthood. Self-reported height tends to be overestimated while recalled weight tends to be underreported, especially in those being overweight or obese[33, 34]. The self-reported BMI at young adulthood may hence be an underestimation. Nonetheless, a strong correlation is reported between measured and self-reported height (r>0.9)[35] and between measured and recalled weight (r>0.73)[36, 37]. Additionally, cancer-affected persons may recall their weight in young adulthood differently compared with cancer-unaffected persons which may have introduced an over- or underestimation of the true effect.

A major strength of this study includes the large sample size, i.e. it is the largest study with data of both lifestyle factors and cancer diagnoses of persons with LS. We adjusted for ascertainment bias with weighted Cox proportional hazard regressions[25] and accounted for any dependency of observations within families with a robust sandwich-covariance estimate by clustering on family membership[27, 28]. In addition, we were able to adjust for several confounding covariates although, as in every observational study, residual confounding may not be eliminated.

In summary, a higher BMI at young adulthood is suggested to increase the risk of cancer at all sites for women, but not for men, with LS. Still, it is recommended to avoid excess body weight in young adulthood for all persons with LS, because it may be beneficial to decrease the cancer burden for women and will probably not be harmful for the cancer burden for men.

Acknowledgements

We thank all GEOLynch and CCFR study participants and staff for their cooperation and contribution to the study.

Funding

This work was supported by the Wereld Kanker Onderzoek Fonds Netherlands as part of the World Cancer Research Fund International grant program [2014/1184] and by the National Cancer Institute (NCI) of the U.S. National Institutes of Health (NIH) under Award Number U01CA167551 and through NCI/NIH U01/U24 cooperative agreements with the following CCFR centers: Australasian [CA074778 and CA097735], Mayo Clinic [CA074800], Ontario [CA074783], Seattle [CA074794], University of Hawaii [CA074806 and R01 CA104132 to L LeMarchand], USC Consortium [CA074799]. Additional support for case ascertainment was provided from the Surveillance, Epidemiology and End Results (SEER) Program of the NCI, the following U.S. state cancer registries: AZ, CO, MN, NC, NH, and by the Victorian Cancer Registry, Australia, and the Ontario Cancer Registry, Canada. The funding sources were not involved in the study design; in collection, analysis, and interpretation of data; in writing of the manuscript; or in the decision to submit the manuscript for publication.

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Supplemental tables

Supplemental table S1. Number of cancer cases at each site for each cancer category in the main analyses.[a]

	Cancer category				
	All sites		*Extra-CRC*		*Extra-CRCEC*
	Men (N=547)	Women (N=716)	Men (N=109)	Women (N=384)	Women (N=190)
Colorectal, *n* (%)	475 (86.8)	420 (58.7)	-	-	-
Upper gastrointestinal tract[b], *n* (%)	17 (3.1)	16 (2.2)	24 (22.0)	24 (6.3)	24 (12.6)
Urinary tract[c], *n* (%)	16 (2.9)	11 (1.5)	32 (29.4)	20 (5.2)	20 (10.5)
Breast, *n* (%)	-	29 (4.1)	-	54 (14.1)	54 (28.4)
Endometrium, *n* (%)	-	163 (22.8)	-	194 (50.5)	-
Ovary, *n* (%)	-	30 (4.2)	-	33 (8.6)	33 (17.4)
Prostate, *n* (%)	14 (2.6)	-	21 (19.3)	-	-
Other, *n* (%)	25 (4.6)	47 (6.6)	32 (29.4)	59 (15.4)	59 (31.1)

[a]Non-melanoma skin cancers were not considered as cancer. The events that end observation time varies per cancer category (see statistical analysis in methods). Therefore, the total number of extra-CRC and extra-CRCEC differs from the total number of extra-CRC or extra-CRCEC reported among cancers at all sites. [b]Upper gastrointestinal tract cancers include cancers in the biliary tract, stomach, pancreas and small bowel. [c]Urinary tract cancers include urothelial cancers and cancers in the kidney, ureter and bladder. Extra-CRC, cancer located outside the colorectum; extra-CRCEC, cancer located outside the colorectum and endometrium.

Supplemental table S2. Calculation of the weights applied to men with cancer at any site (affected) and without cancer (unaffected) for the analyses regarding body mass index at young adulthood and cancer at all sites.[a,b]

				Person years in age group				
Mutated gene	**Age group (years)**	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**External rate[c]**	**Affected weight**	**Unaffected weight**
MLH1	<35	57	68	1714	1874	0.0126	1.1541	0.8708
	35-40	45	21	1669	775	0.0176	0.4075	2.2697
	40-45	44	22	1832	916	0.0261	0.4110	2.1780
	45-50	33	15	1534	704	0.0307	0.4016	2.3164
	50-55	19	13	991	677	0.0263	0.3484	1.9523
	55-60	13	11	742	625	0.0347	0.2923	1.8363
	≥60	6	7	390	457	0.0240	0.2730	1.6232
MSH2/EPCAM[d]	<35	52	81	1558	2326	0.0080	1.0529	0.9660
	35-40	31	40	1152	1485	0.0110	0.5352	1.3602
	40-45	35	29	1483	1207	0.0179	0.5879	1.4973
	45-50	49	28	2307	1306	0.0205	0.3258	2.1799
	50-55	36	26	1872	1358	0.0403	0.5244	1.6586
	55-60	16	16	898	908	0.0545	0.7608	1.2392
	≥60	21	14	1413	915	0.0322	0.3062	2.0407
MSH6	<35	5	17	136	496	0.0051	2.0688	0.6856
	35-40	4	6	148	220	0.0053	0.7111	1.1926
	40-45	4	12	176	506	0.0050	0.5928	1.1357

Supplemental table S2 continued.

Mutated gene	Age group (years)	Affected (N)	Unaffected (N)	Person years in age group: Affected	Person years in age group: Unaffected	External rate[c]	Affected weight	Unaffected weight
MSH6	45-50	15	8	703	374	0.0122	0.2974	2.3174
	50-55	8	11	412	571	0.0074	0.2440	1.5498
	55-60	7	9	403	515	0.0197	0.5268	1.3680
	≥60	14	16	897	1056	0.0104	0.1308	1.7605
PMS2	<35	2	7	56	195	0.0006[e]	0.1713	1.2368
	35-40	3	2	112	71	0.0009[e]	0.0717	2.3924
	40-45	3	2	128	82	0.0015[e]	0.1055	2.3418
	45-50	10	3	467	142	0.0169	0.2856	3.3812
	50-55	2	2	101	105	0.0142	0.8966	1.1034
	≥55	13	11	810	744	0.0095	0.2114	1.9319

[a]The clinic-based enrolment of participants may have introduced an oversampling of cancer cases and thus ascertainment bias, because those participants were preferentially tested for Lynch syndrome since they originate from families with multiple cancer cases, mainly colorectal cancer and/or endometrial cancer, or because they had been diagnosed with cancer at a young age. Therefore, a weighted Cox model as proposed by Antoniou *et al.*[1] was used to adjust for such ascertainment bias in the risk estimates. [b]Weights for the model were calculated by comparing the incidence of cancer-affected and cancer-unaffected participants in our study sample with those of an external referent population. [c]The external rates are age-, sex- and mutated gene specific cancer risk incidences for persons with Lynch syndrome obtained from a publication by Dominguez-Valentin *et al.*[2]. [d]Numbers of *EPCAM* mutation carriers were small. Therefore, *EPCAM* mutation carriers were added to *MSH2* mutation carriers because the majority of the cancer diagnoses were located in the colorectum and Kempers *et al.* did not observe evidence for a different cumulative colorectal cancer risk before the age of 70 years for *EPCAM* vs. *MSH2* mutation carriers[3]. [e]An annual incidence ratio of 0 was observed for male *PMS2* mutation carriers. Therefore, age-specific incidences for men in the general population were used instead. Those were obtained from the *Cancer Incidence in Five Continents* report[4] and included the incidence in Australia, the Netherlands and the USA.

Supplemental table S3. Calculation of the weights applied to women with cancer at any site (affected) and without cancer (unaffected) for the analyses regarding body mass index at young adulthood and cancer at all sites.[a,b]

				Person years in age group				
Mutated gene	**Age group (years)**	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**External rate[c]**	**Affected weight**	**Unaffected weight**
MLH1	<35	59	103	1695	2783	0.0070	0.8332	1.0956
	35-40	33	39	1228	1460	0.0208	0.9934	1.0056
	40-45	59	25	2483	1044	0.0302	0.5882	1.9719
	45-50	51	30	2392	1404	0.0302	0.4341	1.9621
	50-55	26	28	1343	1459	0.0440	0.7114	1.2679
	55-60	20	13	1134	736	0.0415	0.4234	1.8872
	≥60	11	19	703	1322	0.0515	1.0421	0.9756
MSH2/EPCAM[d]	<35	68	127	1960	3449	0.0121	1.6299	0.6627
	35-40	42	49	1564	1812	0.0087	0.4212	1.4961
	40-45	86	45	3620	1894	0.0351	0.6038	1.7573
	45-50	71	36	3356	1699	0.0375	0.4792	2.0271
	50-55	40	28	2071	1435	0.0495	0.5271	1.6756
	55-60	14	17	791	965	0.0160	0.2621	1.6077
	≥60	12	23	799	1513	0.0522	0.9193	1.0421
MSH6	<35	6	32	161	945	0.0007[e]	0.3416	1.1234
	35-40	9	11	327	399	0.0054	0.4201	1.4745
	40-45	21	12	890	505	0.0130	0.3562	2.1266
	45-50	11	13	506	608	0.0214	0.8169	1.1549

Supplemental table S3 continued.

Mutated gene	Age group (years)	Affected (N)	Unaffected (N)	Person years in age group: Affected	Person years in age group: Unaffected	External rate[c]	Affected weight	Unaffected weight
MSH6	50-55	20	11	1030	572	0.0254	0.3665	2.1518
	55-60	13	10	743	569	0.0402	0.4962	1.6550
	≥60	6	17	392	1142	0.0330	0.8550	1.0512
PMS2	<35	5	8	152	238	0.0007[e]	0.1738	1.5163
	35-40	6	6	219	224	0.0018[e]	0.0906	1.9094
	40-45	6	5	251	211	0.0029[e]	0.1161	2.0607
	45-50	6	3	286	141	0.0169	0.5514	1.8971
	50-55	4	4	203	210	0.0142	0.5363	1.4637
	≥55	11	16	684	1044	0.0095	0.2328	1.5274

[a]The clinic-based enrolment of participants may have introduced an oversampling of cancer cases and thus ascertainment bias, because those participants were preferentially tested for Lynch syndrome since they originate from families with multiple cancer cases, mainly colorectal cancer and/or endometrial cancer, or because they had been diagnosed with cancer at a young age. Therefore, a weighted Cox model as proposed by Antoniou *et al.*[1] was used to adjust for such ascertainment bias in the risk estimates. [b]Weights for the model were calculated by comparing the incidence of cancer-affected and cancer-unaffected participants in our study sample with those of an external referent population. [c]The external rates are age-, sex- and mutated gene specific cancer risk incidences for persons with Lynch syndrome obtained from a publication by Dominguez-Valentin *et al.*[2]. [d]Numbers of *EPCAM* mutation carriers were small. Therefore, *EPCAM* mutation carriers were added to *MSH2* mutation carriers because the majority of the cancer diagnoses were located in the colorectum and Kempers *et al.* did not observe evidence for a different cumulative colorectal cancer risk before the age of 70 years for *EPCAM* vs. *MSH2* mutation carriers[3]. [e]An annual incidence ratio of 0 was observer female *PMS2* mutation carriers. Therefore, age-specific incidences for women in the general population were used instead. Those were obtained from the *Cancer Incidence in Five Continents* report[4] and included the incidence in Australia, the Netherlands and the USA.

Supplemental table S4. Calculation of the weights applied to men and women with extra-CRC (affected) and without extra-CRC (unaffected) for the analyses regarding body mass index at young adulthood and extra-CRC.[a,b]

				Person years in age group				
Sex	**Age group (years)**	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**External rate[c]**	**Affected weight**	**Unaffected weight**
Men	<35	9	159	261	4775	0.0005	0.8134	1.0106
	35-40	8	127	295	4733	0.0008	0.4160	1.0368
	40-45	7	135	292	5668	0.0013	0.6191	1.0198
	45-50	16	160	750	7507	0.0025	0.3823	1.0618
	50-55	18	130	934	6753	0.0049	0.4486	1.0763
	55-60	20	84	1141	4787	0.0084	0.4444	1.1323
	≥60	31	139	2068	9297	0.0197	0.7409	1.0578
Women	<35	41	183	1196	5356	0.0007	0.3672	1.1418
	35-40	37	137	1383	5073	0.0017	0.2579	1.2004
	40-45	78	190	3285	7973	0.0027	0.1528	1.3478
	45-50	79	167	3724	7837	0.0040	0.1598	1.3975
	50-55	67	119	3464	6177	0.0054	0.1673	1.4688
	55-60	39	100	2219	5699	0.0069	0.2337	1.2989
	≥60	43	166	2906	11246	0.0129	0.4846	1.1335

[a]The clinic-based enrolment of participants may have introduced an oversampling of cancer cases and thus ascertainment bias, because those participants were preferentially tested for Lynch syndrome since they originate from families with multiple cancer cases, mainly colorectal cancer and/or endometrial cancer, or because they had been diagnosed with cancer at a young age. Therefore, a weighted Cox model as proposed by Antoniou *et al.*[1] was used to adjust for such ascertainment bias in the risk estimates. [b]Weights for the model were calculated by comparing the incidence of extra-CRC-affected and extra-CRC-unaffected participants in our study sample with those of an external referent population. [c]The external rates are age- and sex- specific extra-CRC incidences for the general population since no reliable extra-CRC incidences could be

Supplemental table S4 continued.

obtained from literature for persons with Lynch syndrome. The incidences for the general population were obtained from the *Cancer Incidence in Five Continents* report[4] and included the incidence in Australia, the Netherlands and the USA. Extra-CRC, cancer located outside the colorectum.

Supplemental table S5. Calculation of the weights applied to women with extra-CRCEC (affected) and without extra-CRCEC (unaffected) for the analyses regarding body mass index at young adulthood and extra-CRCEC.[a,b]

			Person years in age group				
Age group (years)	**Affected (N)**	**Unaffected (N)**	**Affected**	**Unaffected**	**External rate[c]**	**Affected weight**	**Unaffected weight**
<35	30	194	851	5701	0.0007	0.4868	1.0794
35-40	15	159	561	5895	0.0016	0.6064	1.0371
40-45	38	230	1595	9663	0.0026	0.2986	1.1159
45-50	33	213	1563	9998	0.0038	0.3613	1.0989
50-55	25	161	1310	8331	0.0049	0.4080	1.0919
55-60	19	120	1082	6836	0.0062	0.4289	1.0904
≥60	30	179	2079	12073	0.0120	0.6391	1.0605

[a]The clinic-based enrolment of participants may have introduced an oversampling of cancer cases and thus ascertainment bias, because those participants were preferentially tested for Lynch syndrome since they originate from families with multiple cancer cases, mainly colorectal cancer and/or endometrial cancer, or because they had been diagnosed with cancer at a young age. Therefore, a weighted Cox model as proposed by Antoniou *et al.*[1] was used to adjust for such ascertainment bias in the risk estimates. [b]Weights for the model were calculated by comparing the incidence of extra-CRCEC-affected and extra-CRCEC-unaffected participants in our study sample with those of an external referent population. [c]The external rates are age-specific extra-CRCEC incidences for the general population since no reliable extra-CRCEC incidences could be obtained from literature for persons with Lynch syndrome. The incidences for the general population were obtained from the *Cancer Incidence in Five Continents* report[4] and included the incidence in Australia, the Netherlands and the USA. Extra-CRCEC, cancer located outside both the colorectum and endometrium.

References supplemental tables

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CHAPTER 5

IS A COLORECTAL NEOPLASM DIAGNOSIS A TRIGGER TO CHANGE DIETARY AND OTHER LIFESTYLE HABITS FOR PERSONS WITH LYNCH SYNDROME?

A PROSPECTIVE COHORT STUDY

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Familial Cancer 2020; https://doi.org/10.1007/s10689-020-00201-5

Abstract

Background A cancer diagnosis is suggested to be associated with changes in dietary and lifestyle habits. Whether this applies to persons with familial cancer, such as Lynch syndrome (LS) is unknown. We investigated whether a colorectal neoplasm (CRN) diagnosis in persons with LS is associated with changes in dietary and lifestyle habits over time.

Methods We used data of confirmed LS mutation carriers from the GEOLynch study, a prospective cohort study. Information on dietary intake and lifestyle habits was collected with a validated semi-quantitative food frequency questionnaire and a general questionnaire administered at baseline (2006-2008) and follow-up (2012-2017). Participants' medical records were used to identify CRN diagnoses. Changes in dietary and lifestyle habits in participants who developed a CRN between baseline and follow-up (CRN group) and participants who did not develop a CRN between baseline and follow-up (no-CRN group) were compared using multivariable linear regression models for continuous variables and cross-tables with percentage change at follow-up compared with baseline for categorical variables.

Results Of the 324 included participants, 146 developed a CRN between baseline and follow-up, while 178 did not. Smoking cessation was more often reported in the CRN than in the no-CRN group (41.4% vs. 35.0%). There were no differences in changes of energy intake, alcohol, red meat, processed meat, dairy, fruit, vegetables and dietary fiber consumption, body mass index, physical activity and non-steroidal anti-inflammatory drug use.

Conclusions Apart from a potentially higher likelihood of smoking cessation, we found no evidence that a CRN diagnosis is associated with changes in lifestyle habits in persons with LS.

Background

It is estimated that 1 in every 279 individuals living in a Western population has a germline mutation in one of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* or *PMS2* or a deletion in the *MSH2*-adjacent *EPCAM* gene[1]. These mutations and deletions lead to Lynch syndrome (LS)[2, 3], which is the most common cause of hereditary colorectal cancer (CRC)[4]. Persons with LS have an increased risk of colorectal adenomas (CRAs), and are at a high risk of developing cancer relatively early in life[3, 5-12]. For persons with LS, CRC is the most commonly diagnosed cancer type with cumulative risk estimates by the age of 70 years ranging from 11% to 98%[3, 11, 13-15], whereas lifetime risk in the Western population is 4-5%[16].

Apart from the mutated gene, most results of studies in persons with LS suggest that the risk of CRAs, precursor lesions of CRC[17], and CRC is increased in persons who smoke or who have a high body mass index (BMI)[18-25]. Additionally, a high alcohol consumption[23, 25, 26] and a high consumption of snack foods[27] are associated with increased risk of CRA and/or CRC. In contrast, regular physical activity[28, 29], aspirin intake[30, 31], higher fruit or fiber intakes[20], and long-term use of multivitamin and calcium supplements[32] seem to decrease CRC risk.

In the general population, it has been suggested that a cancer diagnosis may be a window of opportunity for healthy changes in diet and other lifestyle habits[33-36]. Several studies reported an increased fruit and vegetable intake, a decreased red meat intake and a high percentage of smoking cessation after a cancer diagnosis in persons diagnosed with several types of sporadic cancer [33, 35, 36]. Increases, decreases and no changes in alcohol intake, physical activity and BMI were observed[33-36]. However, not all changes in cancer-affected persons were different in comparison with changes observed in cancer-free persons[33, 35, 36].

Even though persons with LS are often diagnosed with CRAs and CRCs, i.e. colorectal neoplasms (CRNs), it is unknown if this triggers changes in their dietary and lifestyle habits. A better understanding of changes in dietary and lifestyle factors following CRN diagnosis in persons with LS is relevant since these changes may impact subsequent cancer risk. Therefore, our aim was to investigate whether a CRN diagnosis in persons with LS is associated with changes in dietary and lifestyle habits over time.

Methods

Study population

We used data of the GEOLynch study, a prospective cohort study established in the Netherlands in 2006 (ClinicalTrials.gov identifier NCT03303833)[18]. Carriers of a mutation in one of the DNA M or *EPCAM* genes – as confirmed by a clinical genetics center – were identified through

the Netherlands Foundation for the Detection of Hereditary Tumours, the Radboud University Medical Center Nijmegen and the University Medical Center Groningen, the Netherlands. Participants were between 18 and 80 years of age, Dutch-speaking, mentally competent to participate and underwent regular colonoscopy surveillance. Terminally ill patients, those living outside the Netherlands and those with familial adenomatous polyposis, inflammatory bowel diseases, and a history of proctocolectomy or colostomy were excluded.

A total of 686 presumed eligible subjects were invited to participate between July 2006 and July 2008 (Figure 1). All subjects had ever received a diagnosis of LS. Of the 686 invited, 501 (73.0%) agreed to participate. Nine participants appeared ineligible after signing informed consent, leaving 492 included participants. All participants completed questionnaires on demographics, dietary and lifestyle characteristics at study enrolment. Considering the observational design of the study, the completed questionnaires were not used to provide participants with any personal feedback to change lifestyle habits. Between January 2012 and December 2017, 447 (90.8%) of the 492 participants were invited to complete the questionnaires again for a follow-up measurement. The remaining 45 participants were not approached since they had not given researchers consent to contact them for follow-up measurements (n=9), were living abroad (n=1), could not be traced (n=9) or had died (n=26). Of the 447 participants invited, 324 (72.5%) completed the follow-up questionnaires and were included in the current analyses. All study participants provided written informed consent and the study was approved by the Institutional Review Board CMO Region Arnhem-Nijmegen.

Assessment of dietary intake

Habitual dietary intake of the previous month was assessed with a semi-quantitative 183-item food frequency questionnaire (FFQ). This FFQ was an updated version of two FFQ's previously developed and validated by the department of Human Nutrition and Health, Wageningen University & Research[37, 38]. The FFQ used at baseline and follow-up were similar in terms of type of food groups and number of items per food group recalled. However, the FFQ used at follow-up contained some additional questions for the dairy food items in order to distinguish between use of fermented and non-fermented dairy products. At both time points, participants were asked to report the frequency and amount of food items used. For all items, frequencies per day and standard portion sizes were multiplied to obtain intake in grams per day. Subsequently, intake of energy and nutrients was quantified by using the Dutch food composition table (NEVO) 2011[39]. We used the NEVO 2011 since most participants completed the follow-up FFQ around the same time period (2012). Moreover, the same (2011) version

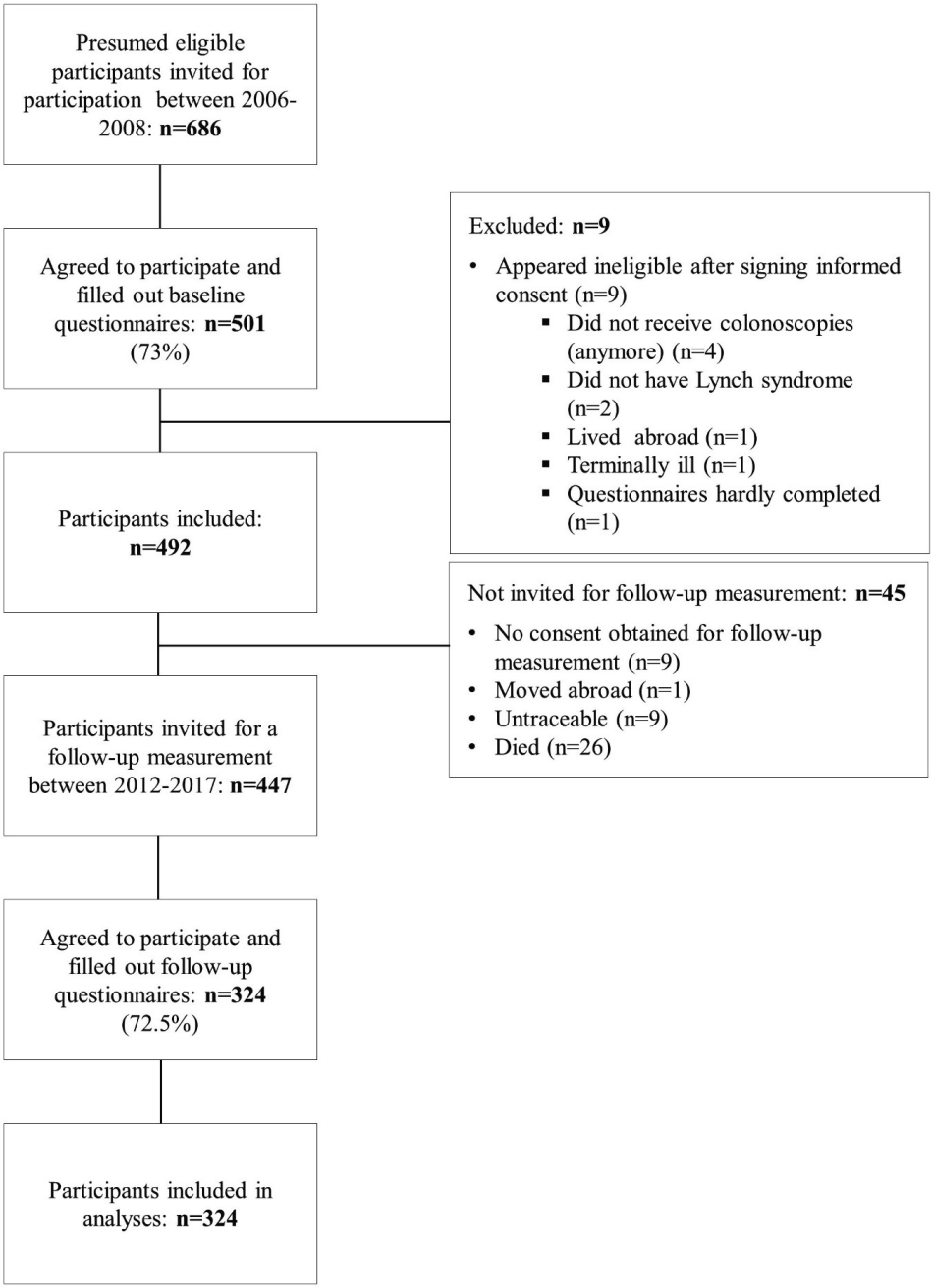

Figure 1. Flowchart of included study participants.

was used for both baseline and follow-up FFQ data to prevent any changes in dietary intake to be a result of using different food composition tables.

Assessment of demographic and lifestyle characteristics

Information on age, sex, education level [low (i.e., finished primary school or lower vocational or lower general secondary education); middle (i.e., finished general secondary school, pre-university education, or vocational education); and high (i.e., finished higher professional education or university)], current height and weight, smoking status [(current, former, never) smoking of tobacco products (cigarettes, cigar, pipe)] and non-steroidal anti-inflammatory drug (NSAID) use [never (i.e. less than once a month) vs. ever (i.e. equal to or more than once a month)] was collected through a standardized general questionnaire. Physical activity was assessed with a modified Baecke questionnaire consisting of 19 items which measures the level of physical activity in three domains: household, sports and non-sports leisure time activities[40, 41]. In accordance with the questionnaire protocol[41], each domain was scored between 1 and 5 points and domain scores were then summed to calculate the total activity score (ranging from 3 to 15), with a higher score reflecting a higher level of physical activity.

Identification of colorectal neoplasms

Participants' medical records were regularly reviewed (on average every 3 years) to obtain clinical information about performed colonoscopies, surgical interventions and CRAs, CRCs and all other cancer diagnoses (excluding non-melanoma skin cancers) before recruitment and during observation time (i.e. period between baseline and follow-up questionnaire completion).

Statistical analyses

Descriptive statistics were used to describe the characteristics at baseline for participants with and without a CRN diagnosis during observation time. Participants who were diagnosed with a CRN during observation time were included in the CRN group, while those who were not diagnosed with a CRN were included in the no-CRN group (both regardless of CRN diagnosis before baseline). Multivariable linear regression models with 95% confidence intervals (CI) were used to investigate whether changes in BMI, physical activity and each dietary variable were different for those with and without a CRN diagnosis during observation time. Analyses were adjusted for sex, age, education level, BMI (<25.0, 25.0-30.0 and ≥30 kg/m^2) and smoking status at baseline. To control for any imbalance at baseline and measurement error at baseline and follow-up, an additional adjustment for the average value of baseline and follow-up was

applied for each lifestyle factor and dietary variable. For analyses of the dietary variables, a comparison was made between estimates obtained from multivariable linear regression models with and without additional adjustment for energy intake based on the residual method[42]. Since both models yielded similar findings, only the results without adjustment for energy intake were presented. The assumptions underlying the multivariable linear regression models were investigated by inspecting the models' residuals. No violations of the assumptions were observed.

For categorical variables (smoking status, categorized BMI and NSAID use), cross-tables were created which showed the percentage of individuals in a category at follow-up for each category at baseline for the CRN and no-CRN group.

Since a CRN diagnosis before baseline may already have influenced current dietary and lifestyle habits, a sensitivity analysis was performed by repeating the analyses in participants without a CRN diagnosis before baseline only (n=164).

A two-sided p-value of 0.05 was considered statistically significant. Data analyses were performed with the use of SAS software version 9.4 of the SAS System for Windows (SAS Institute, Cary, NC, USA).

Results

Participant characteristics

Of the 324 participants who completed both baseline and follow-up questionnaires, 146 (45.1%) were diagnosed and 178 (54.9%) were not diagnosed with a CRN during observation time (Table 1). Participants who developed a CRN during observation time had a median age of 51.9 [interquartile range (IQR), i.e. quartile 1, quartile 3: 44.2, 57.5] years while participants without a CRN had a median age of 47.6 [IQR: 38.4, 56.2] years at baseline. The majority of the participants in the CRN and no-CRN group were women (52.1% vs. 58.4% respectively). Highly educated participants accounted for 29.5% and 41.6% in the CRN group and no-CRN group respectively. At baseline 29 (19.9%) participants in the CRN group and 22 (12.4%) in the no-CRN group smoked. Overweight or obesity was seen in 65 (44.5%) participants of the CRN group and 64 (36.0%) participants of the no-CRN group. A median energy intake of 2134.9 [IQR: 1731.0, 2622.0] kcal/day was reported in the CRN group and 2149.3 [IQR: 1780.2, 2587.8] kcal/day in the no-CRN group.

Follow-up measurements were performed after a median of 80.7 [IQR: 71.4, 86.1] months after baseline measurement in the CRN group vs. 82.5 [IQR: 71.4, 86.5] months in the no-CRN group (data not shown). In the CRN group, a median of 2 [IQR: 2, 2] CRNs per person

were diagnosed during observation time. Median time between the most recently diagnosed CRN and completion of the follow-up questionnaire was 27.5 [IQR: 16.7, 49.7] months. Cancer other than CRC during observation time was diagnosed in 13 (8.9%) participants of the CRN group and in 12 (6.7%) participants of the no-CRN group.

Differential changes in dietary and lifestyle factors

Energy intake decreased with a mean of 295.6 ± SD 534.0 kcal/day in the CRN group and 297.2 ± 481.5 kcal/day in the no-CRN group (Table 2). The change in energy intake was not different in the CRN group compared with the no-CRN group (adjusted difference in change of -7.5 (95% CI: -119.1, 104.0) kcal/day). Mean fruit intake decreased in the CRN group (-15.6 ± 119.4 g/day) while it increased (4.1 ± 113.3 g/day) in the no-CRN group, but the difference in fruit intake change was not statistically significant (adjusted difference in fruit intake change of -13.4 (95% CI: -39.7, 12.8) g/day). Changes in BMI, physical activity and other dietary intakes did not differ between the no-CRN and CRN group either.

Smoking cessation was reported by 41.4% of the smokers in the CRN group vs. 35.0% of the smokers in the no-CRN group (Table 3). A shift from normal weight to overweight was seen in 10 (12.7%) participants in the CRN group and 23 (21.1%) participants in the no-CRN group (Table 4). In the CRN group, 10.3% of the participants increased the use of NSAIDs from less than once a month to equal to or more than once a month against 12.1% of the participants in the no-CRN group (data not shown).

Table 1. Characteristics of the colorectal neoplasm and no colorectal neoplasm group at baseline.[a]

	Colorectal neoplasm[b]	**No colorectal neoplasm[b]**
N	146	178
Age (years), median [IQR]	51.9 [44.2-57.5]	47.6 [38.5-56.2]
Mutated gene, n (%)		
MLH1	55 (37.7)	72 (40.5)
MSH2	64 (43.8)	66 (37.1)
MSH6	26 (17.8)	38 (21.4)
PMS2	1 (0.7)	2 (1.1)
Sex (woman), n (%)	76 (52.1)	104 (58.4)
Education level[c], n (%)		
Low	47 (32.2)	43 (24.2)
Medium	56 (38.4)	61 (34.3)
High	43 (29.5)	74 (41.6)
Smoking status[d], n (%)		
Current	29 (19.9)	22 (12.4)
Pack-years current smokers, median [IQR]	15.4 [8.0-22.5]	10.0 [1.5-16.5]
Former	67 (45.9)	77 (43.3)
Pack-years former smokers, median [IQR]	6.9 [2.9-14.5]	6.0 [2.0-11.5]
Never	48 (32.9)	75 (42.1)
BMI (kg/m^2)[d], median [IQR], n (%)	24.7 [23.2-26.4]	24.1 [22.3-26.4]
<18.5	1 (0.7)	1 (0.6)
18.5-25.0	79 (54.1)	109 (61.2)
25.0-30.0	53 (36.3)	50 (28.1)
≥30.0	12 (8.2)	14 (7.9)
Physical activity level[e], mean ± SD	8.4 ± 1.1	8.3 ± 1.0
Energy intake (kcal/day), median [IQR]	2134.9 [1731.0-2622.0]	2149.3 [1780.2-2587.8]

Table 1 continued.

	Colorectal neoplasm[b]	No colorectal neoplasm[b]
Alcohol intake (g/day), median [IQR]	10.5 [2.3-21.0]	6.5 [1.1-16.2]
Red meat intake (g/day), median [IQR]	41.3 [23.7-55.7]	40.2 [24.8-53.8]
Processed meat intake (g/day), median [IQR]	18.2 [10.7-35.2]	18.7 [7.9-32.5]
Dairy intake (g/day), median [IQR]	322.0 [220.1-458.9]	332.5 [211.7-457.9]
Fruit intake (g/day), median [IQR]	216.5 [49.7-239.3]	151.9 [78.5-230.6]
Vegetable intake (g/day), median [IQR]	137.8 [78.7-193.9]	147.7 [97.6-202.4]
Fibre intake (g/day), mean ± SD	23.7 ± 7.4	24.3 ± 7.0
NSAID use[f], n (%)	23 (15.8)	29 (16.3)
CRN diagnosis before baseline, n (%)	78 (53.4)	82 (46.1)
Cancer other than CRC diagnosed before baseline, n (%)	23 (15.8)	27 (15.2)

[a]The numbers reflect the information collected at baseline, unless stated otherwise. Characteristics are expressed as mean ± SD for normally distributed variables, median [IQR, i.e. quartile 1–quartile 3] for variables deviating from normality or n (%) for categorical variables. [b]The CRN group includes participants with a CRN diagnosis between the baseline and follow-up measurement. If no CRN was diagnosed between baseline and follow-up, the participant was added to the no-CRN group. [c]Low reflects finishing primary school or lower vocational or lower general secondary education; middle reflects finishing general secondary school, pre-university education or vocational education; high reflects finishing higher professional education or university. [d]Percentages do not add up to 100 due to 6 missing values for smoking status and 5 for BMI. [e]Physical activity level is calculated with the Baecke questionnaire[40, 41]. [f]NSAID use equal to or more than once a month. BMI, body mass index; CRC, colorectal cancer; CRN, colorectal neoplasm; IQR: interquartile range: NSAID, non-steroidal anti-inflammatory drugs: SD, standard deviation.

Table 2. Changes in lifestyle characteristics and multivariable linear regression models for differences in change in lifestyle and dietary factors among persons with and without a colorectal neoplasm (CRN) diagnosis.[a]

	Change per group	Crude difference (95% CI) between groups	Adjusted[b] differences (95% CI) between groups
BMI (kg/m^2), mean ± SD			
No CRN[c]	0.5 ± 1.7	Reference	Reference
CRN[c]	0.7 ± 2.8	0.2 (-0.3, 0.7)	-0.2 (-0.5, 0.2)
Physical activity level[d], mean ± SD			
No CRN[c]	0.3 ± 1.2	Reference	Reference
CRN[c]	0.3 ± 1.2	-0.1 (-0.3, 0.2)	-0.1 (-0.3, 0.2)
Energy intake (kcal/day), mean ± SD			
No CRN[c]	-297.2 ± 481.5	Reference	Reference
CRN[c]	-295.6 ± 534.0	1.5 (-110.6, 113.7)	-7.5 (-119.1, 104.0)
Alcohol intake (g/day), mean ± SD			
No CRN[c]	-1.3 ± 7.8	Reference	Reference
CRN[c]	-1.5 ± 11.5	-0.2 (-2.3, 2.0)	0.3 (-1.9, 2.5)
Red meat intake (g/day), median [IQR]			
No CRN[c]	-9.7 [-22.5, 3.4]	Reference	Reference
CRN[c]	-8.1 [-27.6, 3.0]	-1.2 (-6.1, 3.7)	-0.9 (-5.9, 4.0)
Processed meat intake (g/day), mean ± SD			
No CRN[c]	3.9 ± 25.4	Reference	Reference
CRN[c]	3.4 ± 23.7	-0.4 (-5.9, 5.0)	-0.1 (-5.5, 5.3)
Dairy intake (g/day), mean ± SD			
No CRN[c]	-32.1 ± 212.8	Reference	Reference
CRN[c]	-26.2 ± 159.7	5.9 (-36.4, 48.1)	-0.2 (-43.3, 42.8)

Table 2 continued.

Fruit intake (g/day), mean ± SD			
No CRN[c]	4.1 ± 113.3	Reference	Reference
CRN[c]	-15.6 ± 119.4	-19.7 (-45.5, 6.0)	-13.4 (-39.7, 12.8)
Vegetable intake (g/day), median [IQR]			
No CRN[c]	-26.2 [-79.3, 30.5]	Reference	Reference
CRN[c]	-15.1 [-61.8, 14.4]	8.1 (-8.7, 25.0)	9.4 (-7.8, 26.7)
Fibre intake (g/day), median [IQR]			
No CRN[c]	-2.5 [-5.5, 1.0]	Reference	Reference
CRN[c]	-1.0 [-4.7, 1.3]	0.5 (-0.9, 1.8)	0.5 (-0.9, 1.8)

[a]Changes are calculated among those without a missing value at both baseline and follow-up i.e. among 319 for BMI, 298 for physical activity and 318 for all dietary intakes. Changes are expressed as mean ± SD for normally distributed variables and median [IQR, i.e. quartile 1 – quartile 3] for variables deviating from normality. [b]Adjusted for age, sex, education level, BMI and smoking status at baseline and the average of baseline and follow-up intake of the corresponding dietary or lifestyle factor. [c]The CRN group includes participants with a CRN diagnosis between the baseline and follow-up measurement. If no CRN was diagnosed between baseline and follow-up, the participant was added to the no-CRN group. [d]Physical activity level is calculated with the Baecke questionnaire[40, 41]. BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; CRN, colorectal neoplasm: IQR, interquartile range: NSAID, non-steroidal anti-inflammatory drugs: SD, standard deviation.

Sensitivity analyses

Participants diagnosed with a CRN before baseline (n=160) were excluded in the sensitivity analysis. Of the 164 participants without a CRN diagnosis before baseline, 68 (41.5%) participants developed a CRN during observation time while 96 (58.5%) participants did not. The difference in percentage of smoking cessation between the CRN and no-CRN group increased with smoking cessation reported by 6 (75.0%) of the 8 smokers at baseline in the CRN group and 3 (25.0%) of the 12 smokers at baseline in the no-CRN group (data not shown). Differences in changes in physical activity, BMI, dietary intakes and NSAID use between the CRN group and no-CRN group tended to increase for most habits but remained statistically non-significant for all (data not shown).

Table 3. Smoking behaviour at baseline and at follow-up time by subgroup.[a]

		Smoking status at follow-up		
No colorectal neoplasm[b]		Current	Former	Never
Smoking status at baseline	Current (N=20)	13 (65.0)	7 (35.0)	0 (0.0)
	Former (N=75)	5 (6.7)	70 (93.3)	0 (0.0)
	Never (N=75)	0 (0.0)	3 (4.0)	72 (96.0)
Colorectal neoplasm[b]		Current	Former	Never
Smoking status at baseline	Current (N=29)	17 (58.6)	12 (41.4)	0 (0.0)
	Former (N=64)	1 (1.6)	63 (98.4)	0 (0.0)
	Never (N=48)	0 (0.0)	3 (6.3)	45 (93.8)

[a]Percentages of those without missing values in smoking status. Reported values reflect n (%). Participants who reported to be current smoker at baseline and never smokers at follow-up (n=2) or to be former smoker at baseline and never at follow-up (n=5) were not taken into account. [b]Participants with no colorectal neoplasm (CRN) includes those who did not develop a CRN between the baseline and follow-up measurement. Participants with a CRN includes those who developed a CRN between the baseline and follow-up measurement. CRN, colorectal neoplasm.

Table 4. Body mass index (BMI) at baseline and at follow-up time by subgroup.[a]

		BMI (kg/m^2) at follow-up[b]			
No colorectal neoplasm[c]		Underweight	Normal weight	Overweight	Obese
BMI (kg/m^2) status at baseline[b]	Underweight (N=1)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
	Normal weight (N=109)	2 (1.8)	84 (77.1)	23 (21.1)	0 (0.0)
	Overweight (N=50)	0 (0.0)	7 (14.0)	37 (74.0)	6 (12.0)
	Obese (N=14)	0 (0.0)	0 (0.0)	2 (14.3)	12 (85.7)
Colorectal neoplasm[c]		Underweight	Normal weight	Overweight	Obese
BMI (kg/m^2) status at baseline[b]	Underweight (N=1)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Normal weight (N=79)	1 (1.3)	67 (84.8)	10 (12.7)	1 (1.3)
	Overweight (N=53)	0 (0.0)	6 (11.3)	40 (75.5)	7 (13.2)
	Obese (N=12)	0 (0.0)	0 (0.0)	2 (16.7)	10 (83.3)

[a]Percentages of those without missing values in BMI. Reported values reflect n (%). [b]Underweight reflects a BMI<18.5 kg/m^2, normal weight a BMI of 18.5-25.0 kg/m^2, overweight a BMI of 25.0-30.0 kg/m^2 and obese a BMI≥30 kg/m^2. [c]Participants with no colorectal neoplasm (CRN) includes those who did not develop a CRN between the baseline and follow-up measurement. Participants with a CRN includes those who developed a CRN between the baseline and follow-up measurement. BMI, body mass index; CRN, colorectal neoplasm.

Discussion

We investigated whether a CRN diagnosis is associated with changes in dietary and lifestyle habits in persons with LS. Apart from a potentially higher likelihood of smoking cessation, we found no evidence for an association between a CRN diagnosis and changes in dietary and lifestyle habits in persons with LS.

This is the first study on changes in diet and lifestyle following a CRN diagnosis in persons with LS. In the general population, it has been suggested that a cancer diagnosis may be a window of opportunity for healthy changes in diet and other lifestyle habits[33-36]. Several studies reported an increased fruit and vegetable intake, a decreased red meat intake and a decrease in BMI after a cancer diagnosis[34-36]. We did not observe this in our population. This may be explained by the high percentage of colorectal adenomas (89.0%) instead of carcinomas in the CRN group. Colorectal adenomas, precursor lesions of CRC, that are identified during surveillance colonoscopy are removed before they can progress into CRC. Therefore, it could be speculated that an adenoma, which is directly removed after identification without any additional treatment, will have less impact on diet and lifestyle as compared to a CRC or cancer diagnosis. However, due to the small numbers of CRC (n=16) and cancer cases (n=35) in our cohort, it was not possible to further study changes in dietary and lifestyle habits in these cancer-affected subgroups. Hence, a possible differential impact of a (colorectal) cancer diagnosis as compared with an adenoma diagnosis on changes in dietary and lifestyle habits in persons with LS could not be eliminated in this study.

Despite the absence of an association between CRN diagnosis and changes in most dietary and lifestyle habits in our population, we did observe a higher percentage of smoking cessation in those with a CRN than in those without a CRN. This result was even stronger when the analyses were repeated in participants without a CRN diagnosis before baseline only. Similar findings have been observed for cancer-affected persons vs. cancer-free persons in studies among the general population[33, 36]. It should however be mentioned that in our study the number of current smokers who quit smoking was too small to allow additional adjustments for other factors that may potentially influence a change in smoking behavior in the statistical analyses. Therefore, these results must be interpreted with caution. Still, our findings carefully suggest that a CRN diagnosis might trigger smoking cessation in persons with LS.

Our study has some limitations which should be considered. First, we relied on self-reported measures of dietary and lifestyle factors, which may be subject to recall bias to promote social desirability. However, if social desirable answers were given, it is not likely to have affected those with and without a CRN diagnosis differently. Second, information on dietary

and lifestyle habits was collected at a median of 27.5 months after the most recent CRN diagnosis during observation time. Hence, it is possible that in our study short-term changes in diet and lifestyle were missed but long-term changes could still be captured. Nevertheless, previous studies reporting on changes in diet and lifestyle after a cancer diagnosis in the general population had similar[33, 36], or even longer[34] lengths of follow-up since diagnosis. We therefore do not expect that time since CRN diagnosis has had much impact on our results. A third limitation is that, although all participants had been aware of their LS diagnosis before study inclusion, we do not know when participants became aware of their LS status. It could be hypothesized that a diagnosis of a genetically inherited syndrome may trigger a change in dietary and lifestyle habits and that this change already occurred before our study inclusion. A study by Ramsey *et al.*[43] found that hypothetical testing for a gene variant predisposing to CRC increased participants' motivation to adopt healthier diet and exercise behaviors. A similar finding was observed by Brodersen *et al.*[44]. In that study, first degree relatives of CRC patients at high risk of CRC, based on hypothetical genetic test results, more often anticipated leading a healthier lifestyle compared to those at low risk. Nevertheless, an increased motivation for behavioral change, as found in these studies, does not necessarily imply changes will occur. For instance, Kim *et al.*[45] found that LS mutation carriers who discovered their genetic predisposition to CRC were not more likely to quit smoking compared to LS carriers who did not obtain their genetic test results. Moreover, in a qualitative study among a population similar to ours, Visser *et al.*[46] found that receiving a LS diagnosis was not reported as an important determinant of adherence to lifestyle recommendations and was actually found to be a barrier in adapting to a more healthy lifestyle. We therefore expect that the LS diagnosis has had little to no effect on our results. A final consideration relates to the generalizability of our study sample. Participants were recruited via a hereditary cancer registry and hospitals and were therefore more likely to originate from LS families with the highest risk of cancer. It may hence not be a random sample of the total LS population. Generalizing the findings to all LS mutations carriers might therefore not hold.

Strengths of this study include the prospective and longitudinal design which enabled us to investigate changes in dietary and lifestyle habits over time in one of the largest cohorts including persons with LS worldwide. Moreover, we were able to collect detailed data on a wide range of modifiable risk factors which are associated with many cancer types in the general population.

In conclusion, apart from a potentially higher likelihood of smoking cessation, we found no evidence that a CRN diagnosis is associated with changes in dietary and lifestyle habits in

persons with LS. The growing evidence that a healthy diet and lifestyle may modify LS-associated cancer risk highlights the need to identify effective support for health behavior change in persons with LS.

Acknowledgements

We are indebted to all study participants for their cooperation. We thank all research assistants, nurse practitioners and physicians for their assistance with participant recruitment and data collection. The medical specialist of the participants are gratefully acknowledged for their collaboration.

Funding

Funding for grant 2014/1184 was obtained from Wereld Kanker Onderzoek Fonds (WCRF NL), as part of the WCRF International grant program. The funding body was not involved in the study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

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CHAPTER 6

GENERAL DISCUSSION

The overall aim of this thesis was to evaluate associations between lifestyle-related factors, i.e. the inflammatory potential of the diet, height and body mass index (BMI) at young adulthood, with various tumour types for persons with Lynch syndrome (LS). It was also explored whether a colorectal tumour diagnosis (i.e. colorectal adenoma or colorectal carcinoma) was associated with a change in lifestyle habits for persons with LS.

Below a summary of the results of this thesis is provided. These results are compared to results of previous publications for persons with LS and for persons with cancer in the general population. Subsequently, it is discussed whether inconsistent results or contradictions in results can be explained by suggested underlying biological mechanisms or by methodological considerations. Moreover, the generalizability of the study results is addressed. Finally, an overall conclusion is drawn, potential clinical implications are mentioned and suggestions for future research directions are provided.

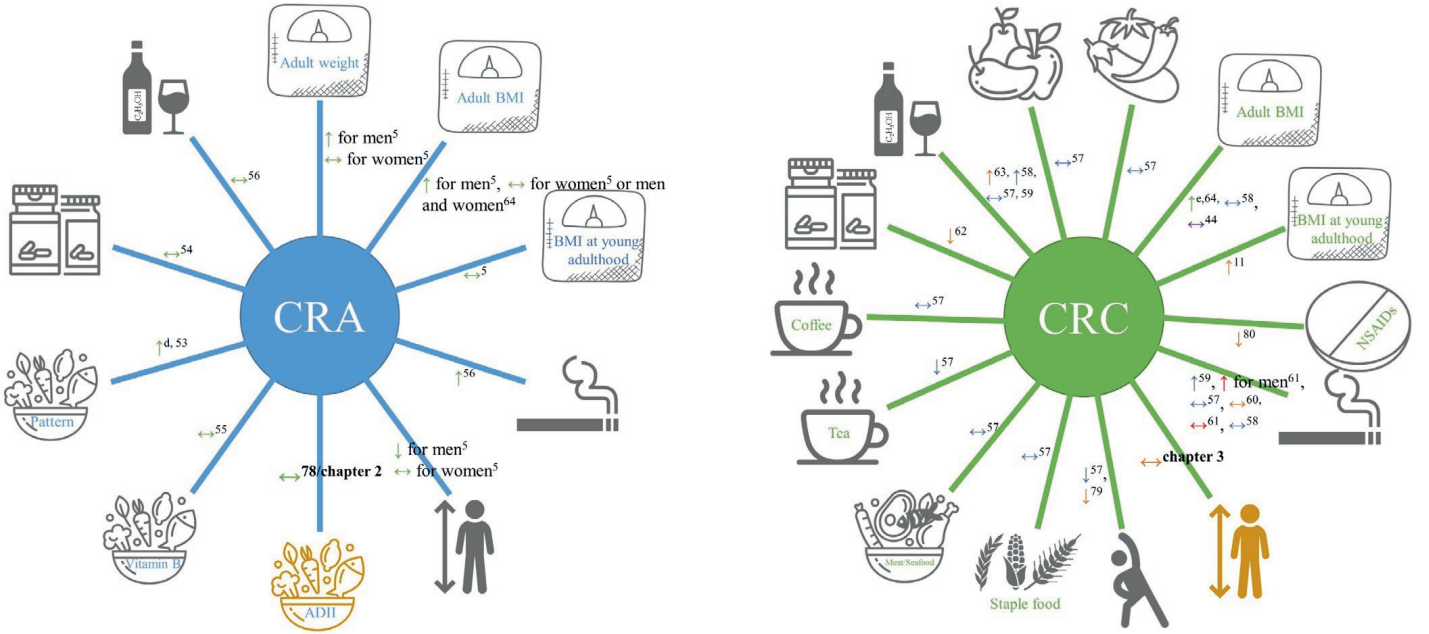
Summary of thesis results and comparison with previous publications

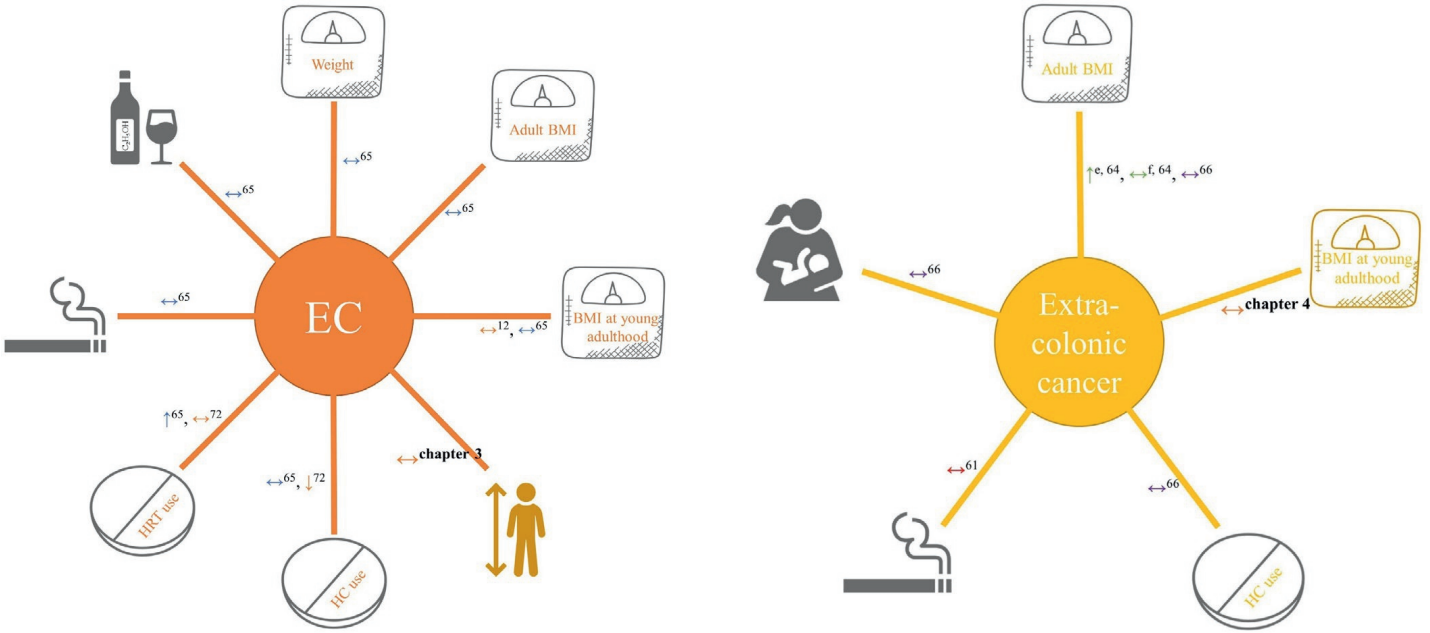
The inflammatory potential of the diet

In chapter 2, no evidence was observed for an association between the inflammatory potential of the diet and colorectal tumour risk, which means that a more pro-inflammatory potential of the diet did not increase or decrease the risk of colorectal tumours for persons with LS (Figure 1). Apart from this study, no other studies exist in which the association between the inflammatory potential of the diet and the risk of colorectal tumours has been investigated for persons with LS.

By now, a number of studies on the association between the inflammatory potential of the diet and colorectal cancer risk for the general population have been published. These studies were summarized in two meta-analyses[1, 2]. In both meta-analyses, the same nine studies were evaluated which included five case-control studies and four prospective cohorts. A 1-unit increment in the dietary inflammatory index increased the risk of colorectal cancer by 7% (relative risk [RR] 1.07, 95% confidence interval [CI] 1.04-1.10)[1] and 6% (RR 1.06, 95% CI 1.04-1.08)[2]. Within a large cross-sectional study of 23 788 men and 20 467 women, men with the most pro-inflammatory potential of the diet had a 41% (odds ratio [OR] 1.41, 95% CI 1.23-1.62) higher likelihood of distal colorectal adenomas compared with men with the most anti-inflammatory potential of the diet[3]. For women, a weaker statistically non-significant higher likelihood of distal colorectal adenomas was observed for those with the most pro-inflammatory vs. most anti-inflammatory potential of the diet (OR 1.08, 95% CI 0.91-1.29)[3].

Figure 1. Observed associations between lifestyle-related factors and several tumour types for persons with Lynch syndrome in previous publications (grey coloured icons) and this thesis (golden coloured icons).[a,b,c]





[a]Associations are only presented for studies that included persons with genetically confirmed Lynch syndrome (LS) and/or persons who were obligate/inferred for LS. Studies in persons suspected for LS based on a strong family history, i.e. based on agreement with the Amsterdam Criteria (II) or Revised Bethesda Guidelines, are not considered in this figure. [b]Observed associations were categorized as positive (i.e. increased risk [↑]), inverse (i.e. decreased risk [↓]), no association (i.e. no increased or decreased risk [↔]). Categorization of the association was based on statistically significant adjusted hazard ratios or odds ratios, or statistically significant differences in percentage (two-sided p-value<0.05). If no adjusted risk estimates were reported in the publication, the crude risk estimate was used to categorize the association. [c]The study design is reflected in the colour of the arrow. A green arrows reflects a prospective cohort study, orange arrows reflect a weighted cohort study, blue arrows reflect a retrospective cohort study, purple arrows reflect a case-control study and red arrows reflect a cross-sectional study. [d]Refers to adhering more to a dietary "Snack" pattern. [e]For *MLH1* gene mutation carriers. [f]For *MSH2* mutation carriers. ADII, adapted dietary inflammatory index; BMI, body mass index; C_2H_5OH, molecular formula of alcohol; CRA, colorectal adenoma; CRC, colorectal cancer; EC, endometrial cancer; extra-colonic cancer, cancer located outside the colorectum; HC, hormonal contraceptive; HRT, hormonal replacement therapy; NSAID, non-steroidal anti-inflammatory drug. All lifestyle-related icons were obtained from the Noun project (www.thenounproject.com).

Thus, no evidence was found for an association between the inflammatory potential of the diet and colorectal tumours risk for persons with LS whereas for the general population, a more pro-inflammatory potential of the diet seems to increase the risk of colorectal cancer, while little is known for colorectal adenomas. This might suggest a different influence of the inflammatory potential of the diet on LS-associated colorectal tumour risk versus colorectal tumour risk for the general population.

Height

No evidence was observed for an association between height and colorectal cancer for men and women with LS in chapter 3 of this thesis (Figure 1). Similarly, no evidence was observed for an association between height and endometrial cancer for women with LS in that chapter. Previously, conflicting results have been published regarding height and colorectal cancer for persons with LS. In a Canadian case-control study, women suspected to have LS based on their family history were found to have a 47% (OR 1.47, 95% CI 1.16-1.87), 53% (OR 1.53, 95% CI 1.20-1.96) or 127% (OR 2.27, 95% CI 1.46-3.59) higher likelihood of colorectal cancer with a height from 1.55 meter to 1.65 meter, 1.65 meter to 1.75 meter, or 1.75 meter and taller versus being shorter than 1.55 meter, respectively[4]. However, no association was observed for men[4]. In contrast, for colorectal adenomas instead of colorectal cancer, previous results with prospectively collected data of the GEOLynch study showed a 57% (hazard ratio [HR] 0.43, 95% CI 0.23-0.83) decreased risk for men with each 5 cm increment in height[5]. However, no association was observed for women[5]. In the general population, a meta-analyses of fourteen studies showed a 4% (RR 1.04, 95% CI 1.02-1.05) increased risk of colorectal cancer for each 5 cm increment in height[6] for both men and women, while in two studies no association was observed between height and colorectal adenomas for men[7] and men and women combined[8]. However, one study observed a 71% (OR 1.71, 95% CI 1.28-2.29) higher likelihood of distal colorectal adenomas for the tallest versus shortest women[9]. Overall, it is not clear if height is associated with colorectal tumour risk for persons with LS whereas for the general population, being taller seems to increase the risk of colorectal cancer, while studies on the association between height and colorectal adenomas are too limited for a conclusion.

No studies have been published in which the association between height and endometrial cancer for women with LS has been investigated. For the general population, a 15% (RR 1.15, 95% CI 1.09-1.22) increased risk of endometrial cancer for each 10 cm increment in height was presented in a meta-analysis of thirteen cohort studies[10].

BMI at young adulthood

In chapter 4 of this thesis it was evaluated whether BMI at young adulthood is associated with cancer at all sites and with cancers located outside the colorectum (extra-colonic) for men and women with LS separately, and with cancers located outside both the colorectum and endometrium for women with LS (Figure 1). Whereas no statistically significant sex difference in risk estimates was observed for the association between BMI at young adulthood and the risk of cancer at all sites, a positive association was found for women ($HR_{per\ 5\ kg/m2}$ 1.27, 95% CI 1.10-1.47), while no association was observed for men ($HR_{per\ 5\ kg/m2}$ 1.00, 95% CI 0.85-1.17). No association between BMI at young adulthood and the risk of extra-colonic cancer for men and women, and the risk of cancer located outside both the colorectum and endometrium for women was observed. Previously, a 5 kg/m^2 increment in BMI at young adulthood has been associated with a 30% (HR 1.30, 95% CI 1.08-1.58) increased risk of colorectal cancer for men and women with LS included in the Colon Cancer Family Registry (CCFR)[11]. A higher BMI at young adulthood was not associated with the risk of colorectal tumours in prospective analyses with data of the GEOLynch study[5]. CCFR data did not show an association between BMI at young adulthood and the risk of endometrial cancer[12]. In contrast, for the general population, a higher BMI at young adulthood was associated with a higher risk of colorectal and endometrial cancer, and several other types of cancer but, for women, an association with a decreased risk of pre- and postmenopausal breast cancer was observed[13]. In this meta-analysis of four case-cohort studies, 24 case-control studies and 29 prospective cohort studies, no sex-specific differences in risk estimates were observed[13]. Another prospective cohort study in the general population observed for women that being obese at young adulthood (BMI ≥27.5 kg/m^2) was associated with a 44% increased colorectal cancer risk compared to those with a BMI between 15 and 19 kg/m^2, while this was not observed for men (HR 1.44, 95% CI 1.06-1.95 for women and HR 1.18, 95% CI 0.84-1.65 for men)[14]. However, the sex-specific risk estimates were not statistically significantly different from each other. In summary, a higher BMI at young adulthood seems to be associated with an increased risk of colorectal cancer, but not colorectal tumours and endometrial cancer, for persons with LS. For the general population, a higher BMI at young adulthood is associated with an increased risk of several types of cancer including colorectal and endometrial cancer, while for women of the general population it is associated with a decreased risk of both pre- and postmenopausal breast cancer.

Colorectal tumours and a change in lifestyle habits

In chapter 5 of this thesis, it was explored whether a colorectal tumour diagnosis was associated with a change in lifestyle habits. No evidence was observed for a change in lifestyle habits after a colorectal tumour diagnosis apart from a potential higher likelihood of smoking cessation in those diagnosed with a colorectal tumour compared with those not diagnosed with a colorectal tumour. No previous observational quantitative studies have been published for persons with LS in which the association between a tumour diagnosis and a change in lifestyle habits has been investigated.

Generally, a change in lifestyle habits after a cancer diagnosis can be measured by identifying cancer cases and ask them about lifestyle habits before their cancer diagnosis and current lifestyle habits. With this retrospective approach, a (favourable) change in diet, physical activity, BMI and smoking after a cancer diagnosis is often reported for the general population[15-19]. In prospective studies in which changes in lifestyle habits are based on measurements before and after a cancer diagnosis, those diagnosed with cancer showed a decrease[20] or increase in BMI[21], increase or decrease in the intake of several dietary components[21, 22] and a tendency to quit smoking[20, 21]. A decrease in physical activity[19, 23] and an increase in sedentary time[23] after a cancer diagnosis is also observed. Not all studies included a cancer-free comparison group in their study to investigate if observed or reported changes in lifestyle habits are different in persons with cancer compared to those without cancer. Those who did compare changes in lifestyle habits between cancer-affected and cancer-free persons, show inconsistent results[20, 21, 24, 25]. Overall, for the general population, some changes in lifestyle habits are reported, but results are inconsistent and it is not clear if a cancer diagnosis is associated with such changes or if they follow secular trends.

Thus, in summary, the aim of this thesis was to evaluate associations between the inflammatory potential of the diet, height, BMI at young adulthood, and various tumour types for persons with LS. Moreover, it was also explored whether a colorectal tumour diagnosis was associated with a change in lifestyle habits for persons with LS. In this thesis, no evidence was found for an association between the inflammatory potential of the diet and the risk of colorectal tumours, and between height and the risk of colorectal or endometrial cancer for persons with LS. BMI at young adulthood was found to be positively associated with the risk of cancer at all sites for women, but not for men with LS. Moreover, besides a potential higher likelihood of smoking cessation after a colorectal tumour diagnosis, no evidence was found for an association between a colorectal tumour diagnosis and a change in lifestyle habits for persons with LS. Overall, the

observed associations between lifestyle-related factors and tumour risk for persons with LS and between a colorectal tumour diagnosis and a change in lifestyle habits in this thesis do not always agree with results of research on those associations for the general population. The inconsistent or conflicting results for associations between lifestyle-related factors and tumour risk for persons with LS versus the general population may be due to differences in biological mechanisms leading to tumour development in LS versus the general population. Moreover, methodological differences may explain why results are inconsistent, even if associations are investigated in groups of persons with LS only. Hence, below is discussed if the inconsistent or conflicting results can be explained by suggested biological mechanism or methodological considerations.

Biological mechanisms

In general, the results of the studies on lifestyle-related factors in relation to tumour risk described in this thesis (i.e. the inflammatory potential of the diet, height and BMI at young adulthood for men) are not in agreement with results observed for the general population. This could be due to differences in molecular pathways of tumour development for LS-associated tumours versus sporadic tumours diagnosed in the general population. The majority of LS-associated tumours, especially colorectal cancers, show loss of mismatch repair (MMR) protein expression and microsatellite instability (MSI) which is observed in >95% of the LS-associated colorectal cancers[26-32] versus in about 15% of the sporadic tumours[26, 33]. Moreover, a tumour-surrounding local inflammatory response that suppresses tumorigenesis is often presented in LS-associated colorectal tumours[33-35]. The molecular pathway by which LS-associated tumours arise hence appear to differ from sporadic cancers. The difference in molecular characteristics and pathways of cancer development for LS-associated cancer compared with sporadic cancers, may hamper the applicability of underlying mechanisms of cancer development in the general population to that in persons with LS. It may hence explain why no association was observed between the inflammatory potential of the diet, height and BMI at young adulthood, and LS-associated colorectal tumours, endometrial cancer, or extra-colonic cancers, while those lifestyle-related factors seem to be associated with cancer risk for the general population.

For colorectal cancer specifically, a multiple stage development of colorectal cancer has been proposed which would classify a colorectal adenoma as precursor lesion of colorectal cancer[36]. Recently, a new pathway for LS-associated colorectal cancer has been identified in which normal mucosa with MMR-deficient crypt foci directly progress to colorectal cancer without adenoma or polyp formation[32]. Studies on lifestyle-related factors with LS-associated

colorectal adenoma development can therefore not be directly extrapolated to studies that use colorectal cancer as endpoint. This may explain why in previous analyses with data of the GEOLynch study an increase in height for men was strongly associated with a decrease in colorectal adenoma risk, while such association was not observed for colorectal cancer with harmonized data of both the GEOLynch study and CCFR in chapter 3 of this thesis.

Methodological considerations

In research, the choice of, among others, study designs and data-analyses can threaten the internal validity of study results. Below, several methodological issues and their potential impact on the results of the chapters of this thesis are considered. Those considerations mainly concern combining different datasets including participant recruitment, harmonization of confounding covariates and power. Moreover, consequences of the applied data analyses to adjust for ascertainment bias and confounder selection are discussed. At the end of this section, the external validity is considered.

Participant recruitment

In chapter 3 and 4 of this thesis, a dataset with combined data from the GEOLynch study[5] and CCFR[37, 38] has been used. Combining datasets can introduce bias due to differences in, among others, participants recruitment, or due to data harmonization. Data of the CCFR has already been harmonized since the CCFR is a consortium of six centres in four countries and procedures for participants recruitment and data collection are not completely similar in each centre[37, 38]. However, all study protocols and procedures have been developed in close collaboration and hence it is not expected that strong biases have been introduced this way. On the other hand, study protocols and procedures of the GEOLynch study have been developed independently of those of the CCFR. As a consequence, recruitment of persons with LS differed between the GEOLynch and CCFR (Figure 2). For the GEOLynch study, included participants were known carriers of a LS-causing pathogenic variant before study inclusion, while in the CCFR included participants were newly identified persons with LS. Therefore, GEOLynch participants received colonoscopy surveillance before study inclusion while those of the CCFR did not. This may theoretically result in a lower percentage of colorectal cancer diagnosis for GEOLynch participants compared with CCFR participants because colonic surveillance is, among others, aimed at removing precursor lesions of colorectal cancer to prevent its progression to colorectal cancer. Indeed, in chapter 3 colorectal cancer was more often diagnosed in CCFR participants compared with GEOLynch participants (48.9% and 29.3% respectively [data not shown]).

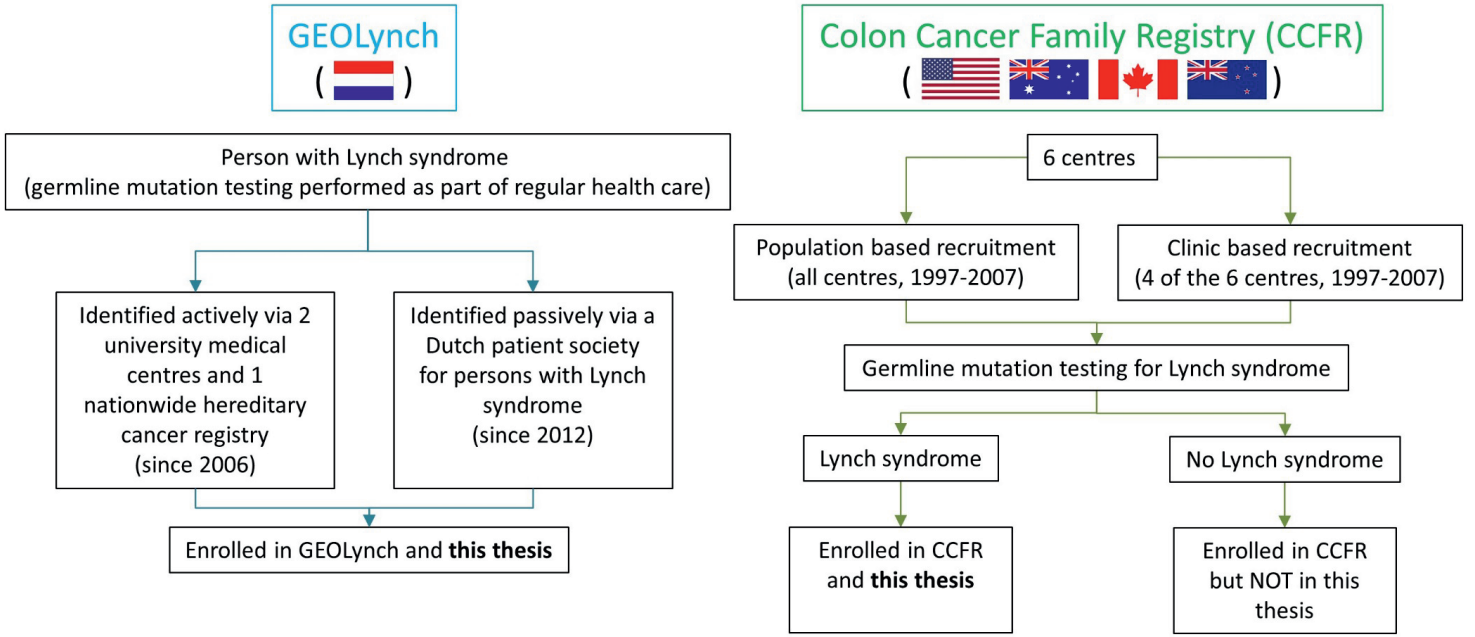


Figure 2. Schematic simplification of participant recruitment and enrolment for persons with Lynch syndrome in the GEOLynch study[5] and Colon Cancer Family Registry (CCFR)[37].

Combined with the fact that Dutch persons are among the tallest in the world[39], and therefore GEOLynch participants are taller than CCFR participants, this may introduce a dilution of any true positive association between height and colorectal cancer. Therefore, for data analyses with colorectal cancer as outcome, person time for GEOLynch participants ended at the first surveillance colonoscopy to increase comparability with CCFR participants. Since the subsequent observed risk estimates did not differ by cohort, it is not expected that the differences in participants recruitment will have introduced biases that may explain the observed results for the investigated associations with the combined data.

Harmonization of confounding covariates

Harmonizing data will increase the number of study participants but can also result in a loss of details of covariates because the cohort in which the least detailed information was requested will be leading. For the harmonized data of the GEOLynch and CCFR, harmonization of education level and level of physical activity could not be performed perfectly since educational systems differed between countries and questions about physical activity level were asked with a lot more details regarding specific sports activities and age-period of performed activities in CCFR participants compared with GEOLynch participants. In our combined analyses, cohort specific categories were used for education level. For physical activity, cohort specific tertiles were created based on adult physical activity levels to reflect physical activity levels at young adulthood for GEOLynch participants and physical activity level in the age period 20 to 30 years was used to reflect physical activity level at young adulthood for CCFR participants. As a consequence of using the above-mentioned harmonization, the lowest level of education or physical activity of GEOLynch participants may not be completely comparable to the lowest level of education or physical activity for CCFR participants. This may have introduced imperfect adjustment for education and physical activity in the analyses where these variables were considered as a confounder. For CCFR participants, tertiles of physical activity level in the age period 30 to 50 years and ≥50 years were moderately correlated with physical activity level in the age period 20 to 30 years (ρ of 0.56 and 0.44, respectively). Hence, the categorization of physical activity level at young adulthood based on adult physical activity level for GEOLynch may have classified some participants with a high physical activity level at young adulthood in reality as having a low physical activity level based on current level of physical activity. Since the GEOLynch participants had a lower BMI at young adulthood as compared with CCFR participants, those with a lower BMI may have been more likely to be misclassified regarding their physical activity level at young adulthood when the association

between BMI at young adulthood and cancer risk was evaluated. It is not known if and in which direction imperfect confounder adjustment as a consequence of the harmonization of the variables education level and physical activity level at young adulthood may have influenced the results observed in chapter 3 and 4 of this thesis.

Power

The international collaboration was established to increase power for investigating sex-specific associations between lifestyle-related factors and colorectal cancer, endometrial cancer and other less common extra-colonic cancer types for persons with LS. However, since colorectal cancer is often the first manifesting tumour in persons with LS, numbers of extra-colonic tumours remain relatively low when using Cox proportional hazard regression models in which persons are censored at their first event to analyse the data. This caused a suboptimal power for the associations between BMI at young adulthood and extra-colonic cancers for men, and between BMI at young adulthood and cancers located outside both the colorectum and endometrium for women (a power of 58% and 72%, respectively, if a 30% increased risk per 5 kg/m^2 increment in BMI at young adulthood was hypothesized[11]). In sensitivity analyses in which not the first diagnosed cancer was considered to end person time but in which the first diagnosed cancer of interest, i.e. extra-colonic cancers, ended person time, sufficient power was obtained. Nevertheless, no associations were observed then either. This may suggest that there is no association between BMI at young adulthood and extra-colonic cancer for men and women, and cancers located outside the colorectum and endometrium for women with LS.

Ascertainment bias

The GEOLynch participants were known carriers of a LS-causing pathogenic germline variant before study enrolment whereas CCFR participants were newly identified carriers of a LS-causing pathogenic germline variant (Figure 2). In the Netherlands, persons can be identified to carry a LS-causing pathogenic variant in regular health care via two ways. Firstly, cancer-affected or cancer-unaffected persons can be referred to a clinical geneticist for a family or personal history of young onset (colorectal) cancers (clinic-based). Subsequent germline mutation testing may identify their LS status. Secondly, more recently it is recommended that all colorectal cancers diagnosed in persons below the age of 70 years, irrespective of family history, in the Netherlands are immunohistochemically stained for MMR proteins or tested for MSI[40] (population-based). In case of a deficiency in MMR protein expression or a MSI-high tumour, referral to a clinical geneticist and subsequent germline mutation testing may reveal a

pathogenic variant in one of the MMR genes leading to LS. In the CCFR, participants with LS have also been identified both clinic- and population-based as part of the study protocol. Clinic-based identified participants are more likely to originate from families with many cancer cases. The selection of persons with LS for both studies is therefore based on a personal or family history of (mainly colorectal) cancer and not random with respect to the evaluated outcomes in this thesis, i.e. colorectal, endometrial, and other cancers. This may results in an oversampling of cancer-affected persons with LS and hence ascertainment bias when a retrospective approach is used to evaluate the associations between height and BMI at young adulthood and several types of cancer (chapter 3 and 4). As a consequence, if height or BMI at young adulthood would in reality be positively associated with cancer for persons with LS, the risk estimate might be biased to zero, i.e. no association, because too many (future) cancer cases are included in the comparison group. By differentially weighing the cancer-affected and cancer-unaffected participants, i.e. by using a weighted cohort approach[41], adjustments for this ascertainment bias were made. Weights were, where possible, calculated based on cancer incidences observed for the general population multiplied by the increased risk observed for persons with LS. Cancer risk estimates for persons with LS are not clearly delineated for all LS-associated cancer types. Therefore, to calculate weights for the associations between BMI at young adulthood and cancer risk, cancer incidences for the general population without multiplying by increased risk estimates were used for the association with extra-colonic cancers for men and women, and with cancers outside both the colorectum and endometrium for women. Using cancer incidences for the general population only, probably underestimates the true incidence for persons with LS and hence the used weights were not correctly specified in these analyses. As a consequence, the observed associations between BMI at young adulthood and extra-colonic cancer and both extra-colonic and extra-endometrial cancer may be biased. Nevertheless, associations are suggested to be less biased when using cancer incidences which are lower than the true incidence to calculate weights compared with using no weights to adjust for ascertainment bias[41]. To prevent ascertainment bias, it would be better to analyse data with a prospective approach instead of the retrospective approach used to evaluate the associations between height, BMI at young adulthood and several types of LS-associated cancers. However, for a prospective approach a large number of persons with LS should be followed for a long time to obtain sufficient power, which severely hampers the feasibility of such an approach.

Confounder selection

Within the articles of this thesis, adjustments for confounding covariates have been applied to prevent any inference with other variables in the studied association, i.e. to get as close to a causal inference as possible. Those confounding covariates have been identified in two ways in this thesis. A classical way of confounder selection based on statistical criteria has been used to evaluate associations between the inflammatory potential of the diet and colorectal tumour risk (chapter 2), between BMI at young adulthood and cancer risk (chapter 3), and between colorectal tumours and a change in lifestyle habits (chapter 5). For this classical way, potential confounding covariates were identified based on combinations of confounders used in the literature, a statistically significant univariate association between the covariate and both the exposure and the outcome, and/or a change in the risk estimate of the exposure by more than a pre-specified threshold if the potential confounder is added to or removed from the model. It is common to use such an approach, however, identifying confounding covariates based on statistical criteria only, ignores causality of the identified associations. Therefore, this classical approach may, besides adjusting for true confounding, also introduce confounding without noticing[42]. To prevent the accidental introduction of confounding with this classical approach, causal diagrams[43] were created to identify confounding covariates for the association between height and both colorectal and endometrial cancer risk (chapter 4). Causal diagrams are created before performing data analyses and represent underlying causal relationships of the studied association. Judgement of causality and directions of relationships between covariates for causal diagrams is based on existing studies. Using causal diagrams to identify confounding covariates is time-consuming but transparent and, if applied properly, will prevent introducing bias by over-adjusting or adjusting for non-confounding covariates. However, a proper application is challenging because results from research on associations between several covariates cannot always rule out non-causality or clarify in which direction an (causal) association runs. Consequently, using a causal diagram that is created on the basis of a wrong assumption of causality between two covariates may also introduce bias. Confounding covariates identified by a causal diagram for the association between height and colorectal and endometrial cancer overlapped with those identified by the classical approach used in previously published articles[44-46]. Due to the overlap in identified confounding covariates between the causal diagrams and classical approach, it is not expected that using the classical approach of confounder selection in chapter 2, 3 and 5 or using a causal diagram to identify confounding covariates in chapter 4 can explain the observed null results.

External validity

External validity refers to whether the results obtained in this thesis can be generalized to all persons with LS. The majority of study participants in this thesis are originating from families with an LS-causing pathogenic variant in either the *MLH1* or *MSH2* gene (percentages ranging from 36% to 39% and 40% to 44%, respectively). Characteristics of the global LS population are unknown, because if no germline mutation testing is performed, it is not known who is and who is not carrying a LS-causing pathogenic variant. The clinic-based approach to identify persons with LS will especially identify those originating from families with a highly penetrant phenotype. Nowadays, several (inter)national guidelines in Europe and the USA recommend to use a population-based approach to identify persons who may have LS in which tumour immunohistochemical staining for DNA MMR proteins and/or MSI testing is suggested for all diagnosed colorectal cancers and sometimes also for endometrial cancers[47-50]. If implemented properly, this approach may increase the population confirmed to have LS. Moreover, it will probably shift the current predominance of highly penetrant mutations in the *MLH1* and *MSH2* gene, such as often identified in persons who seek genetic counselling at clinics for a family cancer history, to a predominance of mutations in the less penetrant *MSH6* and *PMS2* gene[51, 52]. As previously mentioned, the majority of study participants in this thesis are originating from families with an LS-causing pathogenic variant in either the *MLH1* or *MSH2* gene. Therefore, results may not hold for all persons with LS.

Overall conclusion and clinical implications

The studies described in this thesis add to the scientifically gathered information regarding lifestyle-related factors and the risk of tumours for persons with LS. With regard to the evaluated lifestyle-related factors, i.e. the inflammatory potential of the diet, height and BMI at young adulthood, a positive association was only observed between BMI at young adulthood and the risk of cancer at all sites, and only for women. In addition, a colorectal tumour diagnosis did not seem to trigger a change in lifestyle factors. The question mark that ends the subtitle of this thesis, i.e. *Genes load the gun, lifestyle pulls the trigger?*, will therefore remain and cannot be replaced by a period (yet).

The limited number of studies combined with inconsistent or null results (Figure 1) do not allow for LS-specific lifestyle recommendations yet. However, based on this thesis and previous results[5, 11, 12, 44, 53-66], current cancer prevention recommendations do not seem to be harmful for the cancer burden of persons with LS either and hence, the recommendation to eat a healthy

diet, to maintain a healthy body weight - also at young adulthood -, to be physically active and to not start smoking or to quit smoking[67] may be suggested for persons with LS as well.

Future research

In this thesis, associations between lifestyle-related factors and cancer risk have been evaluated. A definite answer to the question *Genes load the gun, lifestyle pulls the trigger?*, cannot be provided yet based on the results of this thesis. Therefore, some suggestions for future research that may assist in answering this question for persons with LS are given below.

It may be questioned if research on lifestyle-related factors and tumour risk for persons with LS should be continued for several reasons. At first, the mostly inconsistent and null associations observed in this thesis may suggest little influence of lifestyle-related factors on tumour risk. Secondly, a lack of changes in lifestyle habits was observed after a colorectal tumour diagnosis in the prospective study described in chapter 4 of this thesis. Thirdly, persons with LS did not adhere more to cancer prevention guidelines after increasing knowledge of those guidelines in a randomized controlled trial[68]. Finally, only few persons with LS reported that having LS or having a cancer diagnosis would facilitate adherence to cancer prevention recommendations in a qualitative study[69]. However, research on lifestyle-related factors and cancer risk for persons with LS may not only aim at identifying lifestyle-related factors that may be changed to decrease someone's cancer risk, but it may also provide clues for mechanistic research on LS-associated cancer development. For example, height and BMI at young adulthood share similar underlying mechanisms in the general population, but in this thesis, only an association between BMI at young adulthood, and not height, and the risk of cancer was observed for women with LS. This may suggest that the small differences in underlying processes leading to height and BMI at young adulthood, e.g. adipose-tissue-derived hormones[70, 71], remain interesting for future (mechanistic) research on LS-associated cancer. Research on lifestyle-related factors and cancer risk for persons with LS should hence not be discontinued.

The lifestyle-related factors evaluated in this thesis were, similar to several previous studies on lifestyle-related factors and LS-associated tumour risk[5, 11, 12, 53-58, 61, 72], based on a single measurement. It is not known if a change in lifestyle factors can influence subsequent cancer risk for persons with LS. In one study was observed that an increase in risk of LS-associated colorectal adenomas was less high for former compared with current smokers[56], while in another study a lower risk of colorectal cancer was observed for former smokers compared with current smokers[60]. This may suggest that a change can influence subsequence

tumour risk. Either randomized controlled trials with lifestyle interventions or long term prospective follow-up studies with regular lifestyle-related measurements and identification of cancer diagnoses are required to evaluate whether a change in lifestyle factors changes subsequent cancer risk for persons with LS. Unfortunately, the high costs of such studies combined with the difficulties in changing lifestyle habits for the general population and likely also for persons with LS[68, 69], questions the feasibility of such studies.

As previously mentioned, mechanisms by which lifestyle-related factors are suggested to influence colorectal cancer risk are based on research for the general population, while the molecular pathway by which LS-associated tumours develop are suggested to differ from sporadic cancers[26-35]. The most important characteristic of LS-associated tumours includes a deficiency in MMR protein expression. MMR deficiency can be an early or a late event in LS-associated colorectal cancer development[32, 73]. A recent publication suggested one pathway in which MMR deficiency is a late event and two pathways in which MMR deficiency is an early event for LS-associated colorectal cancer development[32, 74]. For MMR deficiency as a late event, MMR proficient adenomas transform to MMR deficient adenomas after secondary MMR inactivation. The MMR deficient adenoma subsequently progresses to a carcinoma. The two pathways in which MMR deficiency is an early event in colorectal cancer development, start with MMR-deficient crypt foci. Those crypt foci may either progress to MMR-deficient adenomas and subsequently carcinomas or they progress directly into carcinomas without polypous formation. The latter pathway, in which the polypous formation appears to be skipped, is associated with the presence of somatically obtained variants in the *CTNBB1* gene[32, 75]. This pathway is suggested to be responsible for a small proportion of all LS-associated colorectal cancers[32, 75], but those cancers cannot be detected or removed at an early (polypous) state during colonoscopies. Therefore, colorectal cancers that are diagnosed despite colonoscopy surveillance may reflect those developed via the non-polypous pathway. Interestingly, persons with LS due to a pathogenic variant in the *PMS2* gene do not seem to develop colorectal cancer once under colonic surveillance[76]. Moreover, in a small study no variants in the *CTNBB1* gene were observed for colorectal cancers of *PMS2* mutation carriers whereas such variants were observed for colorectal cancers of *MLH1* mutation carriers[74, 77]. This may suggest that colorectal cancer for *PMS2* mutation carriers only develop via the adenoma-carcinoma pathway[36] and hence LS-associated colorectal cancer development may differ by mutated gene. It is not known if the influence of lifestyle-related factors differs by polypous or non-polypous LS-associated colorectal cancer development. The inconsistent results in associations between height and LS-associated colorectal adenomas[5] and colorectal cancer[4], and between BMI at young adulthood

and LS-associated colorectal adenomas[5] and colorectal cancer[11], may suggest that a differential influence of height and BMI at young adulthood on polypous and non-polypous colorectal cancer development exist. Future studies in which the molecular characteristics, e.g. *CTNBB1* gene variants, of diagnosed colorectal cancers are considered in research on lifestyle-related factors and LS-associated colorectal cancer or in which risk estimates are presented by mutated gene, may help to identify if the involvement of lifestyle-related factors in LS-associated cancer differs by developmental pathways and mutated gene.

In summary, future studies on lifestyle-related factors and tumour risk for persons with LS may focus on evaluating whether a change in lifestyle-related factors influences subsequent tumour risk. Preferably, such studies should consider molecular characteristics of the developed tumours and/or present results by LS-causing mutated gene.

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SUMMARY

Lynch syndrome (LS) is caused by a dominantly inherited pathogenic variant in one of the DNA mismatch repair genes. Persons with LS are predisposed to early onset cancer, mainly colorectal and endometrial cancer, and colorectal adenomas which are precursor lesions of colorectal cancer. Cancer risk estimates are variable within and between families with the same mutated gene which suggests that, similar to cancer in the general population, lifestyle-related factors may be involved in cancer development. A limited number of studies on the influence of lifestyle-related factors on cancer risk exist for persons with LS. This thesis aimed at evaluating the association between the inflammatory potential of the diet and the risk of colorectal tumours (i.e. colorectal adenomas and carcinomas), between height and the risk of both colorectal and endometrial cancer, and between body mass index (BMI) at young adulthood and the risk of cancer at all sites and cancer outside the colorectum and/or outside the endometrium for persons with LS. It was also explored whether a colorectal tumour diagnosis was associated with a change in lifestyle habits. For the research aims, data of persons with LS residing in the Netherlands and participating in the GEOLynch study has been used separately or has been used after harmonization with data of persons with LS residing in Australia, New Zealand, Canada and the USA from the Colon Cancer Family Registry (CCFR).

In **chapter 2**, dietary intake of 457 participants of the GEOLynch study was determined with a food frequency questionnaire (FFQ) and used to calculate the adapted dietary inflammatory index (ADII). A higher ADII score reflects a higher inflammatory potential of an individual's diet. After a median follow-up time of 59 months, 200 (43.8%) participants developed a colorectal adenoma or carcinoma (CRT). A higher inflammatory potential of the diet was not associated with the risk of CRTs for persons with LS.

Harmonized data of both the GEOLynch study and CCFR has been used to evaluate the association between height and colorectal cancer risk for men and women separately and between height and endometrial cancer risk for women in **chapter 3**. Self-reported height of 1155 men and 1553 women was used and cancer diagnoses were obtained from or confirmed, where possible, in medical records and/or pathology reports. After 28 279 and 37 090 person years for men and women respectively, colorectal cancer was diagnosed in 511 (44.2%) men and 436 (28.1%) women. For endometrial cancer, 1544 women were included of whom 171 (11.1%) were diagnosed with endometrial cancer after 39 227 person years. No evidence for an

association was observed between height and colorectal cancer for men and women, and between height and endometrial cancer for women with LS.

In **chapter 4**, the association between body fatness, as reflected by BMI, at young adulthood and cancer risk for persons with LS was evaluated. Harmonized data of 1044 men and 1446 women with LS from the GEOLynch and CCFR studies was used. BMI at young adulthood was calculated with self-reported height and recalled weight at the age of 18 or 20 years. Where possible, medical records and/or pathology reports were used to identify cancer diagnoses. A 5 kg/m^2 increment in BMI at young adulthood was associated with an increased risk of cancer at all sites for women, but not for men. No association was observed between BMI at young adulthood and cancer outside the colon for men and women with LS, and for cancers outside both the colorectum and endometrium for women with LS.

Data of the GEOLynch study was used to explore if a colorectal tumour diagnosis was associated with a change in lifestyle habits for persons with LS in **chapter 5**. A FFQ and a general questionnaire about lifestyle habits were completed by 324 participants at both baseline and after a median follow-up of 82.0 [interquartile range, 71.4-86.3] months. A CRT was diagnosed in 146 (45.1%) persons between baseline and follow-up. Apart from a potentially higher likelihood of smoking cessation for those with a CRT diagnosis compared to those without a CRT diagnosis, no evidence was observed for a difference in change in intake of energy, alcohol, red meat, processed meat, dairy, fruit, vegetables and dietary fibre, and in adult BMI, physical activity level and non-steroidal anti-inflammatory drugs use for persons with LS.

No previous research has been published in which the association between the inflammatory potential of the diet and colorectal tumour risk has been evaluated for persons with LS. Nor does another publication exist in which the association between a colorectal tumour diagnosis and a change in lifestyle habits has been investigated. For height and BMI at young adulthood, inconsistent results for the association between height and colorectal tumours were observed in previous research for persons with LS whereas a higher BMI at young adulthood was associated with an increased risk of colorectal cancer, but not for endometrial cancer. For the general population, a more pro-inflammatory potential of the diet seems to be associated with an increased risk of colorectal tumours, being taller or having a higher BMI at young adulthood increases the risk of colorectal and endometrial cancer, and inconsistent results are reported for

an association between a cancer diagnosis and a change in lifestyle habits. The observed contradiction in associations between lifestyle-related factors and tumour risk for persons with LS compared with the general population may be explained by differences in tumour development for persons with LS versus the general population. Moreover, the discovery of LS-associated colorectal cancer development without adenoma or polyp formation, may explain why associations between lifestyle-related factors and colorectal adenoma risk do not agree with studies in which LS-associated colorectal cancer was used as endpoint. Methodological issues that resulted from combining data of the GEOLynch study and CCFR, and consequences of the applied data analyses did not introduce biases to such an extent that they can explain the observed results of this thesis.

Overall, results of this thesis suggest that the inflammatory potential of the diet and height are not associated with tumour development for persons with LS. For BMI at young adulthood, a positive association with cancer at all sites is observed for women, but not for men. A colorectal tumour diagnosis does not seem to trigger a change in lifestyle factors. Current cancer prevention recommendations for the general population do not seem to be harmful for the cancer burden in persons with LS and hence, the cancer prevention recommendation to eat a healthy diet and maintain a healthy body weight – also at young adulthood - may be suggested for persons with LS as well. Future studies to lifestyle-related factors and tumour risk for persons with LS may focus on evaluating whether a change in lifestyle-related factors influences subsequent tumour risk. Preferably, such studies should consider molecular characteristics of the developed tumours and/or present results by LS-causing mutated gene. The question mark that ends the subtitle of this thesis, i.e. *Genes load the gun, lifestyle pulls the trigger?*, will currently remain and cannot be replaced by a period (yet).

ACKNOWLEDGEMENTS
DANKWOORD

Daar ligt het dan. Eindelijk! Het resultaat van een bijna vijf jaar durend uniek, interessant en leerzaam traject. Dit resultaat had er niet kunnen zijn zonder de hulp en steun van vele anderen. Iedereen die hieraan meegewerkt heeft, wil ik ontzettend bedanken en een aantal mensen in het bijzonder.

Allereerst dank aan alle deelnemers van de GEOLynch studie en de Colon Cancer Family Registry. De onderzoeksvragen in dit proefschrift hadden niet onderzocht kunnen worden als jullie de informatie niet gedeeld hadden.

Heel veel dank aan mijn begeleiders Ellen, Hans en Fränzel. Ellen, bedankt voor het vertrouwen en de kritische blik op artikelen en het delen van ideeën en ervaring met betrekking tot het onderwerp. Hans, jouw verhalen uit de praktijk en medische blik op het project heb ik erg gewaardeerd. Fränzel, fijn dat je mijn dagelijks begeleider was en dat ik altijd voor vragen bij je terecht kon. Dat een overleg van een half uur meestal zowel langer als korter mocht zijn, was altijd handig.

Professor de Groot, professor van Leeuwen, dr. de Wind en dr. Cats, bedankt dat jullie mijn proefschrift wilden lezen en aanwezig zijn bij de verdediging.

I would like to thank all co-authors of the articles of this thesis. I really appreciate the time and effort that you have put in data collection, considering the methodology and analyses, and in improving the manuscripts.

Zonder leuke collega's, geen leuk werk. Dank aan alle collega's van de afdeling Human Nutrition and Health van de Wageningen Universiteit die de afgelopen jaren hun kennis en ideeën wilden delen. Mariska en Merel S., bedankt voor jullie inzet voor de GEOLynch studie als onderzoekmedewerksters. Zonder jullie (praktische) hulp had het promotietraject een stuk langer geduurd. Joeri, bedankt voor de hulp bij het (her)structureren van de verzamelde GEOLynch data. Ook de lunchwandelingen met diverse mensen waaronder Vera, Moniek, Elly, Korrie, Anniek, Esther S., Annick, Lenneke, Esther C. en Charlotte sloeg ik niet graag over. Dank ook aan Moniek, Anne, Elly, Vera, Harm, Kamalita, Liangzi, Maria, Iris, Carlijn, Laura, Anniek, Merel van V. en Suzanne voor het delen, lezen en verbeteren van elkaars manuscripten tijdens de NAD-paperclub.

Zonder leuke kantoorgenoten natuurlijk ook geen leuke werkdag. Dus Harm, Marije, Elbrich, Amy, Novita, Inge en Anniek, bedankt dat ik met jullie het kantoor heb mogen delen!

Eefje, Lisanne, Sylvia, Eggi (Ginanjar), Manette, Eline, Hermien, Liza en Anouk, bedankt dat ik jullie mocht begeleiden tijdens jullie scriptie. Dit was voor mij ook erg leerzaam.

Een bijzonder bedankje voor mijn paranimfen Harm en Else. Harm, we zijn tegelijkertijd begonnen met ons PhD-avontuur en we hebben altijd een kantoor gedeeld (op het thuiswerken na). Nog even en we kunnen beide de titel dr. voor onze naam zetten. Bedankt voor de gedeelde lunch-time hardlooprondjes, ook dank aan Rieneke daarvoor, en de koffie/chocolademelk-momentjes. Else, bedankt dat je zo'n fijne vriendin bent en dat je voor de lay-out van het proefschrift de laatste puntjes op de 'i' wilde zetten. Super dat jullie mijn paranimfen willen zijn.

Naast fantastische collega's waren er de afgelopen jaren gelukkig ook mensen om mij heen die ervoor zorgden dat de balans tussen werken en niet werken behouden bleef.

Lieve schoonfamilie, bedankt dat ik voor de afleiding de afgelopen jaren een nieuwe 'taal' heb mogen leren. Mijn begrip van de Twentse 'taal' is inmiddels bijna net zo goed als dat van de Engelse taal.

Lieve meiden, Else, Mellanie, Sara, Willemijn, Sandra, Mariska, Mirrin, Karin en Yvonne, het is altijd fijn om niet-werk-gerelateerde activiteiten met jullie te ondernemen. Bedankt daarvoor.

Lieve papa, hoe verdrietig ook, uw situatie heeft al op jonge leeftijd mijn interesse in oncologie gevoed en daardoor indirect geleid tot dit proefschrift. *We shall meet again.*

Lieve mama en Siem, bedankt dat ik altijd kan binnenvallen voor een kop thee, het plunderen van de koekjestrommel, chocoladerozijnen of appelvoorraad en om gewoon even niets te moeten.

En wat moet ik zonder de sceptici van, hilariteit en ontspanning met m'n zussen Marit en Elke. Jullie karakters, de uitjes met jullie, jullie betere wederhelften en/of kinderen hebben de afgelopen jaren extra kleur gegeven.

Lieve Fender, de laatste loodjes met het schrijven van mijn proefschrift maakte je gezellig met jouw enthousiaste getrappel in mijn buik. Het was bijzonder om dit mee te maken en het is nog leuker om nu ook in jouw stralende oogjes te kunnen kijken.

Lieve Bastiaan, bedankt voor je liefde, inzet, steun, geduld en de benodigde 'en nu is het tijd om de computer uit te zetten en niet te werken'-momenten. "Ik ben er klaar mee" heb je vaak genoeg van me moeten horen de afgelopen tijd. Deze keer is het ook echt klaar.

Jesca

ABOUT THE AUTHOR

Curriculum vitae

Jesca Geertruida Maria Brouwer was born on February 2, 1989 in Purmerend, the Netherlands. In 2007, she completed secondary school at the Da Vinci College in Purmerend. Thereafter, she started with her bachelor in Biomedical Science at the Radboud University in Nijmegen, the Netherlands. After obtaining her BSc degree in 2010, this was followed by a master in Biomedical Science at the same university with a specialization in Epidemiology. During her MSc training, Jesca completed a minor in oncology, drug research and international public health. For the latter minor, she followed a three month course at the Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania. She completed her MSc internship at the Canisius Wilhelmina Hospital in Nijmegen in which she looked at guideline adherence to prevent contrast-induced nephropathy in hospitalized patients who received iodinated contrast media. For her MSc thesis, she investigated the association between the use of anti-epileptic drugs and the risk of suicide attempts in young adults with data obtained from The Health Improvement Network at the University College London, United Kingdom. At the same time, she performed a literature study in which she investigated whether androgenic alopecia was associated with the risk of prostate cancer for men. She achieved her MSc degree in 2013. In 2015, she was appointed as PhD candidate at the division of Human Nutrition and Health of the Wageningen University. During her PhD project, the association between lifestyle-related factors and the risk of tumours for persons with Lynch syndrome was investigated. The results of this project are described in this thesis. Jesca collected and analysed observational data for the GEOLynch study and she was involved in an international collaboration. She joined the educational programme of the graduate school VLAG, and attended courses and several (international) conferences to present the results. She was involved in teaching and supervising BSc and MSc students, and she was a member of the organizing committee of the 3rd Wageningen PhD symposium.

List of publications

Publications in peer-reviewed journals

Brouwer JGM*, Snellen M*, Bisseling TM, Koornstra JJ, Vasen HFA, Kampman E, van Duijnhoven FJB. *Is a colorectal neoplasm diagnosis a trigger to change dietary and other lifestyle habits for persons with Lynch syndrome? A prospective cohort study*. Accepted for publication in Fam Cancer.
*Shared first authorship.

Brouwer JGM, Newcomb PA, Bisseling TM, Figueiredo JC, Hopper JH, Jenkins MA, Koornstra JJ, Lindor NM, Vasen HFA, Win AK, Kampman E, van Duijnhoven FJB. *Height and colorectal and endometrial cancer risk for persons with Lynch syndrome.* Accepted for publication in the Am J of Epidemiol.

Van Baar H, Winkels RM, **Brouwer JGM**, Posthuma L, Bours MJL, Weijenberg MP, Boshuizen HC, van Zutphen M, van Duijnhoven FJB, Kok DE, Wesselink E, Slooter GD, Spillenaar Bilgen EJ, Hansson BME, de Wilt JHW, Kampman E, Beijer S. *Associations of skeletal muscle mass, fat mass and mortality among men and among women with stage I-III colorectal cancer.* CEBP 2020; 29(5): 956-965.

Van Duijnhoven FJB, **Brouwer JGM**, van Woudenbergh GJ, Kampman E, Feskens EJM. *Comment on "Perspective: The Dietary Inflammatory Index (DII)—Lessons Learned, Improvements Made, and Future Directions"*. Adv Nutr 2020; 11(1): 177-178.

Brouwer JGM, Makama M, van Woudenbergh GJ, Vasen HFA, Nagengast FM, Kleibeuker JH, Kampman E, van Duijnhoven FJB. *Inflammatory potential of the diet and colorectal tumor risk in persons with Lynch syndrome.* Am J Clin Nutr 2017; 106: 1287–94.

Submitted publications

Brouwer JGM, Vasen HFA, Win AK, Figueiredo JC, Koornstra JJ, Buchanan DD, Le Marchand L, Bisseling TM, Jenkins MA, Hopper JL, Newcomb PA, Kampman E, van Duijnhoven FJB. *A higher body mass index at young adulthood is associated with an increased risk of cancer at all sites for women with Lynch syndrome.*

Eijkelboom AH, **Brouwer JGM**, Koornstra JJ, Vasen HFA, Bisseling TM, Kampman E, van Duijnhoven FJB. *Diet quality and colorectal tumor risk in persons with Lynch syndrome*.

Overview of completed training activities

Discipline specific activities	Organiser and location	Year
Courses		
NutriScience course: A multifaceted approach to nutrition research	VLAG, Wageningen, NL	2015
Masterclass 'Mixed models'	VLAG, Wageningen, NL	2017
Masterclass 'Nutrition and Cancer: from Bench to Bed to Behaviour'	VLAG/WUR Division of Human Nutrition & Health, Wageningen, NL	2019
Conferences and meetings		
WEON 2016 'Fit for the Future'	VvE, Wageningen, NL	2016
Life course influences and mechanisms: Obesity, physical activity and cancer	WCRF/World Obesity Federation, London, UK	2016
WEON 2017 'Epidemiological methods for implementation research'	VvE, Antwerp, BE	2017
InSiGHT biennial meeting	InSiGHT, Florence, IT	2017
Symposium 'Gene-specific epidemiological and molecular aspects of Lynch syndrome'	LUMC, Leiden, NL	2018
Annual meeting of the European Hereditary Tumour Group	EHTG, Barcelona, ES	2019
General courses and activities		
VLAG PhD week	VLAG, Baarlo, NL	2015
Project & time management	WGS, Wageningen, NL	2015
Good Clinical Practice	Profess Medical Consultancy, Wageningen, NL	2016
Wageningen PhD symposium	WPC, Wageningen, NL	2016
Teaching and supervising thesis students	ESD, Wageningen, NL	2016
Presenting with impact	Wageningen in'to Languages, Wageningen, NL	2017
Effective behaviour in your professional surroundings	WGS, Wageningen, NL	2017
Reviewing a scientific paper	WGS, Wageningen, NL	2018
Career perspectives	WGS, Wageningen, NL	2019
Optional courses and activities		
Preparing PhD research proposal	WUR, Wageningen, NL	2015
PhD study tour UK	WUR, UK	2017
Staff seminars and chair group meetings	WUR, Wageningen, NL	2015-2019

Colophon

The research described in this thesis was financially supported by Wereld Kanker Onderzoek Fonds (WKOF), as part of the World Cancer Research Fund International grant programme, grant number 2014/1184.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design	Stefanie van den Herik
Lay out	Jesca G.M. Brouwer
Printing	Proefschriftmaken.nl