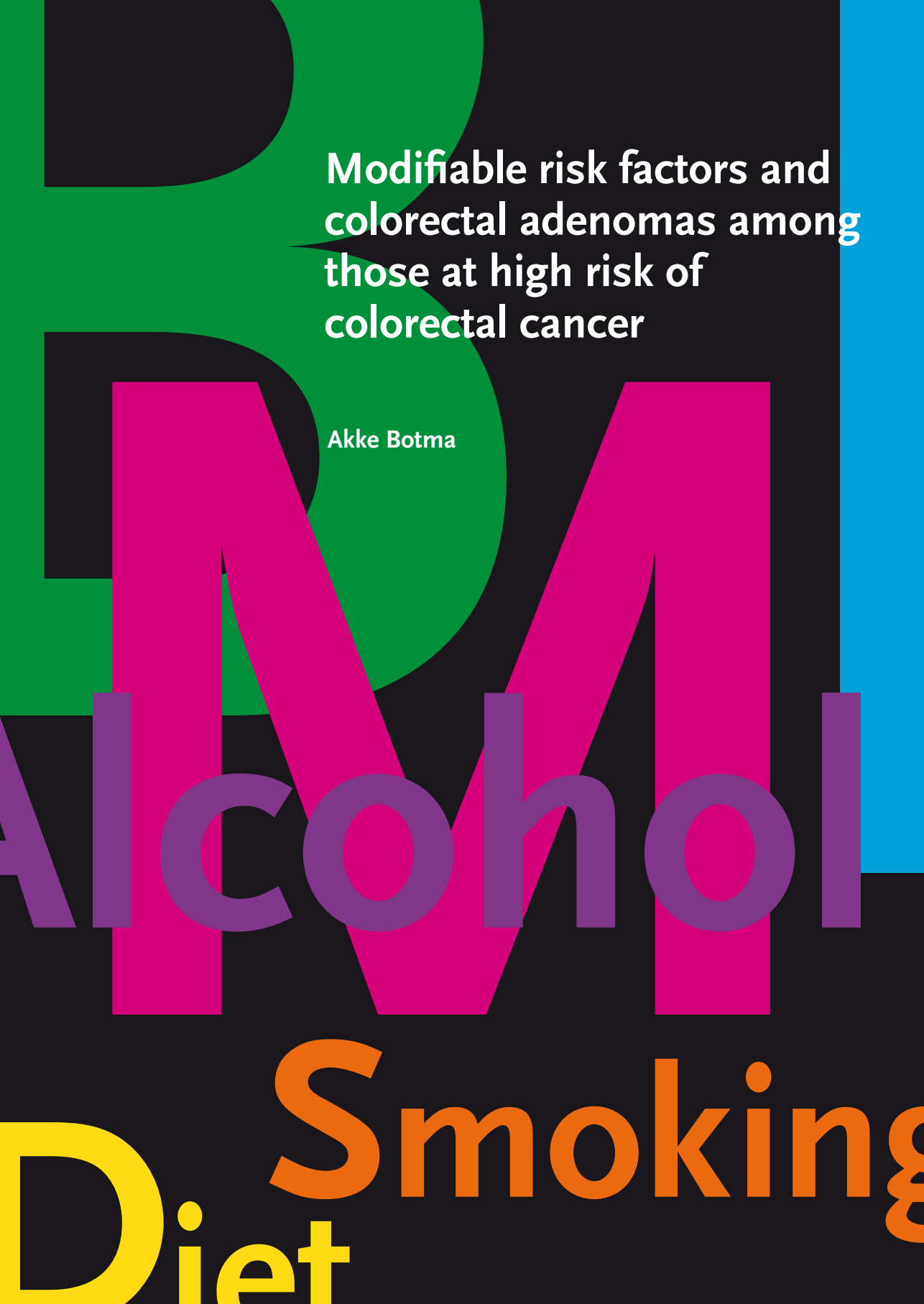
Modifiable risk factors and colorectal adenomas among those at high risk of colorectal cancer

Akke Botma



Modifiable risk factors and colorectal adenomas among those at high risk of colorectal cancer

Akke Botma

Thesis committee

Thesis supervisors
Prof. dr. ir. E. Kampman
Personal chair at the Division of Human Nutrition, Wageningen University

Prof. dr. H.F.A. Vasen
Professor of Prevention of Hereditary Tumours, Leiden University Medical Centre

Thesis co-supervisor
Dr. F.M. Nagengast
Associate professor, Radboud University Nijmegen Medical Centre

Other members
Prof. dr. P.A. Newcomb
Fred Hutchinson Cancer Research Center, Seattle, USA
University of Washington, Seattle, USA

Dr. A. Cats
Netherlands Cancer Institute, Amsterdam, the Netherlands

Dr. M.A. Rookus
Netherlands Cancer Institute, Amsterdam, the Netherlands

Prof. dr. R.F. Witkamp
Wageningen University, Wageningen, the Netherlands

This research was conducted under the auspices of the Graduate School of Food Technology, Agrobiotechnology, Nutrition and Health Sciences (VLAG)

Modifiable risk factors and colorectal adenomas among those at high risk of colorectal cancer

Akke Botma

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday November 2nd, 2011
at 1:30 p.m. in the Aula

Akke Botma

Modifiable risk factors and colorectal adenomas
among those at high risk of colorectal cancer
128 pages

Thesis Wageningen University, Wageningen, the Netherlands (2011)
With abstract in English and summary in Dutch

ISBN 978-94-6173-043-5

Abstract

Epidemiological studies have identified several modifiable risk factors for colorectal neoplasms in the general population. However, associations between modifiable risk factors, including body mass index (BMI), smoking, alcohol consumption and dietary patterns, and colorectal neoplasms in two groups at high risk of colorectal cancer, Lynch syndrome patients and sporadic adenoma patients, have been sparsely studied.

This thesis presents two cohort studies, one of 486 Lynch syndrome patients (the GEOLynch cohort study) and one including data from 565 persons with sporadic adenomas (the POLIEP follow-up study), in which we assessed whether a high BMI, smoking, high alcohol consumption and specific dietary patterns influenced colorectal adenoma development. We also assessed whether the association between BMI and recurrence of sporadic adenomas was modified by polymorphisms in the insulin-like growth factor (IGF) genes.

First, we observed that excess body weight increased the risk of incident colorectal adenomas in men with Lynch syndrome. Secondly, we showed that current smoking increased the risk of colorectal adenomas in Lynch syndrome in both sexes. Former smokers still showed an elevated risk, but lower than current smokers. Number of years smoked, among ever smokers, was positively associated with colorectal adenomas. A clear association with alcohol consumption was not observed. Thirdly, we identified four dietary patterns in the Lynch syndrome cohort; i) 'Prudent', ii) 'Meat', iii) 'Snack', vi) 'Cosmopolitan'. The 'Snack' pattern was associated with increased adenoma occurrence. The other patterns showed Hazard Ratios in the expected directions based on similar studies in the general population but these were not statistically significantly associated with adenoma occurrence. Additionally, among 565 sporadic adenoma patients, we found that BMI was not associated with adenoma recurrence (n=165), nor with recurrence of advanced adenomas (n=37) after a median of 4.7 years of follow-up. Variation in IGF-axis genes (rs1520220 in *IGF1* and rs3213221 in *IGF2*) influenced the likelihood of colorectal adenoma recurrence. Furthermore, we observed that the association between BMI and adenoma recurrence was modified by variation in the *IGF2* gene (rs1004446 and rs1003483). Finally, the three dietary patterns identified ('Low meat', 'Cosmopolitan', or 'Refined foods') among the sporadic adenoma patients did not show marked associations with adenoma recurrence, although the 'Low meat' pattern might reduce the risk of advanced recurrences. No significant associations were seen for smoking and alcohol consumption.

Overall, the results of our Lynch syndrome cohort suggest that modifiable risk factors, e.g. high BMI and smoking, influence colorectal adenoma development in Lynch syndrome patients. On the other hand, these risk factors do not appear to influence recurrence of sporadic colorectal adenomas.

Contents

Risk factors

1

General introduction

Modifiable risk factors for colorectal cancer

Epidemiological studies have identified several modifiable risk factors for colorectal cancer in the general population. The World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) published a comprehensive review on food, nutrition, physical activity and cancer prevention in 2007 with an update for colorectal cancer in 2011 [1,2]. These reviews and meta-analyses indicated that it is convincing that body fatness (expressed as body mass index [BMI]), abdominal fatness (waist circumference and waist-hip ratio), and greater adult attained height increase risk of colorectal cancer. Associations with BMI were slightly stronger among men than women, which is also reported by several other meta-analyses [3-7]. It is also convincing that higher physical activity protects against colorectal cancer. In addition, red meat, processed meat and alcoholic drinks, the latter in men, are convincingly found to increase colorectal cancer risk. Probably, alcohol drinks are risk factors of colorectal cancer in women as well. In the WCRF/AICR report, no conclusions were drawn on any possible relationship between dietary patterns and risk of colorectal cancer, as there was too much variation within the studied patterns to compare results. Two recent literature reviews about dietary patterns and colorectal cancer both conclude that the 'less healthy' patterns, characterized by higher intakes of red and processed meat, potatoes and refined grains or refined carbohydrates, may increase colorectal cancer risk, while patterns that consist of greater intakes of fruits, vegetables, fish and poultry may protect against colorectal cancer [8,9] Although smoking was not evaluated in the WCRF/AICR report, two systematic reviews provide evidence that smoking is associated with an increased risk of colorectal cancer [10,11]. Since the publication of these two systematic reviews, additional cohort studies have been published that support these conclusions [12-16]

Who are at high risk for colorectal cancer? Defining high risk groups

A strong family history of colorectal cancer has been shown to increase personal colorectal cancer risk (systematically reviewed in Johns *et al.* 2001 [17]). The risk depends on the number of first-degree relatives with colorectal cancer and the age of diagnosis. Lynch syndrome is one of the inherited cancer syndromes. Around 1-3% of all colorectal cancers are due to Lynch syndrome [18-24] Estimates for colorectal cancer risk to age 70 in Lynch syndrome patients range from 25-70% [25-31] compared to a risk up to 2.5% in the general population [32]. Besides hereditary factors or a positive family history of colorectal cancer, a personal history of colorectal adenomas also increases colorectal cancer risk, particularly when adenomas have villous structures, are large (≥1 cm) or have a high grade of dysplasia (the so-called advanced adenomas) [33]. Most colorectal carcinomas are thought to develop from adenomas, which is supported by the observation that removal of adenomas by colonoscopic polypectomy is associated with lower colorectal cancer risk [34,35]

Lynch syndrome

Patients with Lynch syndrome have an increased risk not only for colorectal cancer but also for cancer of the endometrium, stomach, small bowel, upper urinary tract, the ovaries and the brain. Pathogenic germ line mutations in genes involved in mismatch repair, *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*, are responsible for the high cancer risks. The germ line mutations in the *MLH1* and *MSH2* gene account for the vast majority of the known mismatch repair gene mutations in Lynch syndrome (see InsiGht mutation database at www.insight-group.org/mutations/). Mean age of diagnosis of colorectal cancer in Lynch syndrome is estimated to be around 45 years, although one study has estimated a higher mean age of around 55-60 years [27], while in sporadic colorectal cancer the mean age is around 65-70 years. Also in Lynch syndrome, carcinomas develop from adenomas. Surveillance and removal of adenomas reduces colorectal cancer risk and increases survival [36]. Mismatch repair gene mutation carriers probably have an accelerated carcinogenesis as cancers develop already within 3 years after a negative colonoscopic screening [37]. In addition, adenomas found in Lynch syndrome patients more often have a villous growth and high grade dysplasia compared to sporadic adenomas in the general population [38,39]

Sporadic colorectal adenoma

Colorectal adenomas are considered precursor lesions for colorectal cancer. In the general population, prevalence of adenomas is estimated to be around 20% at age 50 years up to 50% by age 70 [40,41]. The risk of progression of adenomas into cancer is positively associated with number [33,42], size and histological type of the adenoma [33]. Recurrence of adenomas is fairly common, with recurrence rates of 22 to 50% after 2 or 4 years [43-47]. Also, adenoma recurrences are associated with characteristics of the initial adenoma, being at increased risk of recurrence with multiple adenoma [48] or when the initial adenoma had villous structures, high grade dysplasia or was large (≥1 cm) [49-54]. This was also seen for advanced adenoma recurrences [55]. Furthermore, the observation that a personal history of colorectal adenomas increases risk of advanced recurrences, is especially seen with an advanced adenoma history [56].

Modifiable risk factors for colorectal cancer in high risk groups

Lynch syndrome

Not all patients with Lynch syndrome develop colorectal cancer. Within Lynch syndrome affected-families, the expression of the syndrome varies: some patients develop CRC at a young age, others at an advanced age (e.g. >60 years). Also the spectrum of tumours has changed over time. In the first family with Lynch syndrome reported by Warthin [57], gastric cancer was one of the most common cancers whereas in families nowadays it is colorectal cancer that is most frequent [58]. The observation that outcome of a mismatch repair defect is not the same for all persons, suggests the influence of environmental and/or lifestyle factors. Which modifiable factors may influence colorectal tumour development in Lynch syndrome patients? To date only a few studies have examined relationships of diet and or lifestyle factors in mismatch repair gene mutation carriers. One retrospective study investigated the relation between BMI and colorectal cancer in Lynch syndrome [59]. This cohort consisted of MMR mutation carriers recruited via the Colon Cancer Family Registry, a consortium of centres in the United States, Australia, Canada and New Zealand. The study showed that higher BMI might be a risk factor for colorectal cancer in Lynch syndrome, because MMR mutation carriers who were obese at age 20 had an increased colorectal cancer risk compared with carriers of normal weight at age 20 [59]. Furthermore, smoking has been associated with an increased risk of colorectal cancer in persons with Lynch syndrome, also only in retrospective studies [60,61]. A previously reported case-control study of our group, including both MMR gene mutation carriers and untested individuals who were suspected of Lynch syndrome, found that smoking increased the risk of colorectal tumours in Lynch syndrome families [62] Likewise, for alcohol intake associations with colorectal cancer in Lynch syndrome have only been studied retrospectively in one case-control and in one cohort study [60,62]. Both studies did not detect a significant association between alcohol intake and colorectal cancer risk. The only studies reporting on dietary factors were from our group showing that increased fruit consumption and dietary fibre intake possibly decrease the risk of colorectal tumours in Lynch syndrome affected-families [62,63].

Sporadic colorectal adenoma recurrence

Epidemiological studies about modifiable risk factors and their associations with colorectal adenoma incidence are abundant and have revealed similar associations as with colorectal cancer [64]. The number of studies that have investigated associations with adenoma recurrences, however, is limited, and many of the observations were done in chemoprevention trials. A pooling study [65] of seven trials (n=8,213) from the USA showed a positive association between being obese (≥30 kg/m^2) and recurrent adenomas (OR, 1.29; 95% CI, 1.14-1.45). This association was only apparent in men [65]. One of the ways in which obesity may have an effect on colorectal adenomas is through differences in insulin-like growth factor signalling. Insulin-like growth factor signalling is associated with cell proliferation and cell survival (reviewed by Pollack *et al*. [66]), which are important processes in the development of neoplasms. Several studies investigated associations between polymorphisms in insulin-like growth factor genes and colorectal neoplasms, but results are inconclusive [67-72].

To our knowledge only one study investigated if polymorphisms in insuline-like growth factors genes can modify the association between a high body mass index and colorectal cancer [69].

The four studies on smoking habits and risk of colorectal adenoma recurrence are inconclusive. Two of the four studies did not show clear associations [73,74] while two observed an increased risk [75,76]. Although this last study showed that this increased risk was only apparent after long duration of smoking [76]. Two different studies investigated the association of alcohol intake and recurrence [73,75]. A case-control study found no association between alcohol intake and risk of recurrences [75]. However, in the Polyp prevention study they found an increased risk with seven or more drinks per week [73].
While individual nutrients [45,46,77-85] and foods [77,84,86-89] have been studied in relation to the recurrence of adenomas, both in trials and observational studies, not many studies have addressed the whole diet. In a European fibre intervention study [90] a Mediterranean diet pattern was associated with a reduced colorectal adenoma recurrence risk in women, while none of the dietary patterns seemed to influence recurrence of adenomas in men. An intervention study [44] in which the intervention group was assigned to a diet low in fat (20% of calories), high in fibre (18 g per 1000 kcal) and high in fruits and vegetables (3.5 servings per 1000 kcal) did only show a decreased risk of recurrence among participants who reported to meet the three dietary goals at all annual visits [44,91].

Outline of this thesis

This thesis studies both Lynch syndrome patients and persons with a personal history of colorectal adenomas, since only limited literature is available on the association between modifiable risk factors and colorectal neoplasms within these two high-risk groups. The influence of modifiable risk factors on Lynch syndrome associated colorectal carcinogenesis has only be addressed in retrospective studies. Furthermore, associations between modifiable risk factors and colorectal adenoma recurrence are studied mainly as secondary analysis in recurrence trials, which showed no or moderate associations with recurrence. This thesis was performed to obtain more information on four modifiable risk factors, namely body fatness (expressed as BMI), smoking, alcohol consumption and overall diet (dietary patterns), and colorectal adenoma development in two high risk groups: Lynch syndrome patients and patients with sporadic colorectal adenomas.

The main objective of the studies presented in this thesis was to provide further insight in possible associations between modifiable risk factors and risk of colorectal tumours in those at high risk of colorectal cancer. Chapter 2 describes whether body mass index is associated with colorectal adenomas in our cohort of Lynch syndrome patients, the GEOLynch cohort study. The association of smoking habits and alcohol consumption with colorectal adenoma development in the GEOLynch study is described in chapter 3. In chapter 4 we studied the association between dietary patterns and colorectal adenomas in the GEOLynch study. Chapter 5 evaluates the association between body mass index and sporadic colorectal adenoma recurrence, plus possible effect modification by polymorphisms in the insulin-like growth factor-axis genes in the POLIEP-follow-up study. Within the POLIEP follow-up study sporadic adenoma recurrence in association with diet, alcohol consumption and smoking was studied in chapter 6. In the last chapter (chapter 7) the studies and results are discussed.

References

1 WCRF/AICR: Food, nutrition, physical activity, and the prevention of cancer: a global perspective. (ed 2nd). Washington DC, AICR, 2007
2 WCRF/AICR: Continuous update project interim report summary: food, nutrition and physical activity and the prevention of colorectal cancer, 2011
3 Ning Y, Wang L, Giovannucci EL. A quantitative analysis of body mass index and colorectal cancer: findings from 56 observational studies. Obes Rev 11:19-30, 2010
4 Renehan AG, Tyson M, Egger M, *et al.* Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371:569-578, 2008
5 Moghaddam AA, Woodward M, Huxley R. Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. Cancer Epidemiol Biomarkers Prev 16:2533-2547, 2007
6 Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr 86:556-565, 2007
7 Dai Z, Xu YC, Niu L. Obesity and colorectal cancer risk: a meta-analysis of cohort studies. World J Gastroenterol 13:4199-4206, 2007
8 Miller PE, Lesko SM, Muscat JE, *et al.* Dietary patterns and colorectal adenoma and cancer risk: a review of the epidemiological evidence. Nutr Cancer 62:413-424, 2010
9 Randi G, Edefonti V, Ferraroni M, *et al.* Dietary patterns and the risk of colorectal cancer and adenomas. Nutr Rev 68:389-408, 2010
10 Botteri E, Iodice S, Bagnardi V, *et al.* Smoking and colorectal cancer: a meta-analysis. JAMA 300:2765-2778, 2008
11 Liang PS, Chen T-Y, Giovannucci E. Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. Int J Cancer 124:2406-2415, 2009
12 Leufkens AM, Van Duijnhoven FJB, Siersema PD, *et al.* Cigarette smoking and colorectal cancer risk in the European Prospective Investigation into Cancer and nutrition study. Clin Gastroenterol Hepatol 9:137-144, 2011
13 Limsui D, Vierkant RA, Tillmans LS, *et al.* Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst 102:1012-1022, 2010
14 Nordenvall C, Nilsson PJ, Ye W, *et al.* Smoking, snus use and risk of right- and left-sided colon, rectal and anal cancer: a 37-year follow-up study. Int J Cancer 128:157-165, 2011
15 Hannan LM, Jacobs EJ, Thun MJ. The association between cigarette smoking and risk of colorectal cancer in a large prospective cohort from the United States. Cancer Epidemiol Biomarkers Prev 18:3362-3367, 2009
16 Gram IT, Braaten T, Lund E, *et al.* Cigarette smoking and risk of colorectal cancer among Norwegian women. Cancer Causes Control 20:895-903, 2009
17 Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancer risk. Am J Gastroenterol 96:2992-3003, 2001
18 Aaltonen LA, Salovaara R, Kristo P, *et al.* Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. New Engl J Med 338:1481 - 1487, 1998
19 Salovaara R, Loukola A, Kristo P, *et al.* Population-based molecular detection of hereditary nonpolyposis colorectal cancer. J Clin Oncol 18:2193-2200, 2000
20 Cunningham JM, Kim C-Y, Christensen ER, *et al.* The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet 69:780-790, 2001
21 Samowitz W, Curtin K, Lin HH, *et al.* The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. Gastroenterology 121:830-838, 2001
22 Hampel H, Frankel WL, Martin E, *et al.* Screening for the Lynch syndrome (Hereditary Nonpolyposis Colorectal Cancer). N Engl J Med 352:1851-1860, 2005
23 Piñol V, Castells A, Andreu M, *et al.* Accuracy of revised bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA 293:1986-1994, 2005
24 Lamberti C, Mangold E, Pagenstecher C, *et al.* Frequency of hereditary non-polyposis colorectal cancer among unselected patients with colorectal cancer in Germany. Digestion 74:58-67, 2006
25 Dunlop MG, Farrington SM, Carothers AD, *et al.* Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 6:105-110, 1997
26 Quehenberger F, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the hMLH1 and hMSH2 gene: correction for ascertainment. J Med Genet 42:491-496, 2005
27 Hampel H, Stephens JA, Pukkala E, *et al.* Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 129:415-421, 2005
28 Jenkins MA, Baglietto L, Dowty JG, *et al.* Cancer risks for mismatch repair gene mutation carriers: a population-based early onset case-family study. Clin Gastroenterol Hepatol 4:489-498, 2006

29 Senter L, Clendenning M, Sotamaa K, *et al.* The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 135:419-428, 2008
30 Barrow E, Alduaij W, Robinson L, *et al.* Colorectal cancer in HNPCC: cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. Clin Genet 74:233-242, 2008
31 Stoffel EM, Mukherjee B, Raymond VM, *et al.* Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology 137:1621-1627, 2009
32 Signaleringscommissie-Kanker-van-KWF-kankerbestrijding: Signaleringsrapport 'De kans op kanker. Bewerking van cijfers NKR & CBS 1999-2003'. Amsterdam, 2007
33 Atkin WS, Morson BC, Cuzick J. Long-term risk of colorectal cancer after excision of rectosigmoid adenomas. N Engl J Med 326:658-662, 1992
34 Winawer SJ, Zauber AG, Ho MN, *et al.* Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study workgroup. N Engl J Med 329:1977-1981, 1993
35 Loeve F, van Ballegooijen M, Snel P, *et al.* Colorectal cancer risk after colonoscopic polypectomy: a population-based study and literature search. Eur J Cancer 41:416-422, 2005
36 Järvinen HJ, Aarnio M, Mustonen H, *et al.* Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 118:829-834, 2000
37 Vasen HFA, Nagengast FM, Meera Khan P. Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome). Lancet 345:1183-1184, 1995
38 Jass JR, Stewart SM, Stewart J, *et al.* Hereditary non-polyposis colorectal cancer -- morphologies, genes and mutations. Mutat Res 310:125-133, 1994
39 de Jong AE, Morreau H, Van Puijenbroek M, *et al.* The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 126:42-48, 2004
40 O'Brien MJ, Winawer SJ, Zauber AG, *et al.* The National Polyp Study: Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. Gastroenterology 98:371-379, 1990
41 Thoma M, Castro F, Golawala M, *et al.* Detection of colorectal neoplasia by colonoscopy in average-risk patients age 40–49 versus 50–59 years. Dig Dis Sci 56:1503-1508, 2011
42 Lotfi AM, Spencer RJ, Ilstrup DM, *et al.* Colorectal polyps and the risk of subsequent carcinoma. Mayo Clin Proc 61:337-343, 1986
43 Neugut AI, Jacobson JS, Ahsan H, *et al.* Incidence and recurrence rates of colorectal adenomas: a prospective study. Gastroenterology 108:402-408, 1995
44 Schatzkin A, Lanza E, Corle D, *et al.* Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. N Engl J Med 342:1149-1155, 2000
45 Alberts DS, Martinez ME, Roe DJ, *et al.* Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. N Engl J Med 342:1156-1162, 2000
46 Bonithon-Kopp C, Kronberg O, Giacosa A, *et al.* Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. Lancet 356:1300-1306, 2000
47 Amonkar MM, Hunt TL, Zhou Z, *et al.* Surveillance patterns and polyp recurrence following diagnosis and excision of colorectal polyps in a medicare population. Cancer Epidemiol Biomarkers Prev 14:417-421, 2005
48 Winawer SJ, Zauber AG, O'Brien MJ, *et al.* Randomized comparison of surveillance intervals after colonoscopic removal of newly diagnosed adenomatous polyps. The National Polyp Study workgroup. N Engl J Med 328:901-906, 1993
49 van Stolk RU, Beck GJ, Baron JA, *et al.* Adenoma characteristics at first colonoscopy as predictors of adenoma recurrence and characteristics at follow-up. Gastroenterology 115:13-18, 1998
50 Martinez ME, Sampliner RE, Marshall JR, *et al.* Adenoma characteristics as risk factors for recurrence of advanced adenomas. Gastroenterology 120:1077-1083, 2001
51 Bonithon-Kopp C, Piard F, Fenger C, *et al.* Colorectal adenoma characteristics as predictors of recurrence. Dis Colon Rectum 47:323-333, 2004
52 Noshirwani KC, van Stolk RU, Rybicki LA, *et al.* Adenoma size and number are predictive of adenoma recurrence: implications for surveillance colonoscopy. Gastrointest Endosc 51:433-437, 2000
53 Keku TO, Amin A, Galanko J, *et al.* Apoptosis in normal rectal mucosa, baseline adenoma characteristics, and risk of future adenomas. Cancer Epidemiol Biomarkers Prev 17:306-310, 2008
54 Laiyemo AO, Murphy G, Albert PS, *et al.* Postpolypectomy colonoscopy surveillance guidelines: predictive accuracy for advanced adenoma at 4 years. Ann Intern Med 148:419-426, 2008
55 Martínez ME, Baron JA, Lieberman DA, *et al.* A pooled analysis of advanced colorectal neoplasia diagnoses after colonoscopic polypectomy. Gastroenterology 136:832-841, 2009
56 Saini SD, Kim HM, Schoenfeld P. Incidence of advanced adenomas at surveillance colonoscopy in patients with a personal history of colon adenomas: a meta-analysis and systematic review. Gastrointest Endosc 64:614-626, 2006
57 Warthin AS. Heredity with reference to carcinoma: as shown by the study of the cases examined in the pathological laboratory of the University of Michigan. Arch Intern Med 12:546-555, 1913
58 Lynch HT, Krush AJ. Cancer family "G" revisited: 1895-1970. Cancer 27:1505-1511, 1971

59 Win AK, Dowty JG, English DR, *et al.* Body mass index in early adulthood and colorectal cancer risk for carriers and non-carriers of germline mutations in DNA mismatch repair genes. Br J Cancer 105:162-169, 2011
60 Watson P, Ashwathnarayan R, Lynch HT, *et al.* Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). Arch Intern Med 164:2429-2431, 2004
61 Pande M, Lynch PM, Hopper JL, *et al.* Smoking and colorectal cancer in Lynch syndrome: results from the Colon Cancer Family Registry and the University of Texas M.D. Anderson Cancer Center. Clin Cancer Res 16:1331-1339, 2010
62 Diergaarde B, Braam H, Vasen HF, *et al.* Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 5:736-742, 2007
63 Voskuil DW, Kampman E, Grubben MJAL, *et al.* Meat consumption and meat preparation in relation to colorectal adenomas among sporadic and HNPCC family patients in the Netherlands. Eur J Cancer 38:2300-2308, 2002
64 Peipins LA, Sandler RS. Epidemiology of colorectal adenomas. Epidemiol Rev 16:273-297, 1994
65 Jacobs ET, Ahnen DJ, Ashbeck EL, *et al.* Association between body mass index and colorectal neoplasia at follow-up colonoscopy: a pooling study. Am J Epidemiol 169:657-666, 2009
66 Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 4:505-518, 2004
67 Giovannucci E, Haiman CA, Platz EA, *et al.* Dinucleotide repeat in the insulin-like growth factor-I gene is not related to risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 11:1509-1510, 2002
68 Slattery ML, Samowitz W, Curtin K, *et al.* Associations among IRS1, IRS2, IGF1, and IGFBP3 genetic polymorphisms and colorectal cancer. Cancer Epidemiol Biomarkers Prev 13:1206-1214, 2004
69 Morimoto LM, Newcomb PA, White E, *et al.* Insulin-like growth factor polymorphisms and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 14:1204-1211, 2005
70 Le Marchand L, Kolonel LN, Henderson BE, *et al.* Association of an exon 1 polymorphism in the IGFBP3 gene with circulating IGFBP-3 levels and colorectal cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 14:1319-1321, 2005
71 Pechlivanis S, Wagner K, Chang-Claude J, *et al.* Polymorphisms in the insulin like growth factor 1 and IGF binding protein 3 genes and risk of colorectal cancer. Cancer Detect Prev 31:408-416, 2007
72 Feik E, Baierl A, Hieger B, *et al.* Association of IGF1 and IGFBP3 polymorphisms with colorectal polyps and colorectal cancer risk. Cancer Causes Control 21:91-97, 2010
73 Baron JA, Sandler RS, Haile RW, *et al.* Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. J Natl Cancer Inst 90:57-62, 1998
74 Paskett ED, Reeves K, Pineau B, *et al.* The association between cigarette smoking and colorectal polyp recurrence (United States). Cancer Causes Control 16:1021-1033, 2005
75 Jacobson JS, Neugut AI, Murray T, *et al.* Cigarette smoking and other behavioral risk factors for recurrence of colorectal adenomatous polyps (New York City, NY, USA). Cancer Causes Control 5:215-220, 1994
76 Reid ME, Marshall JR, Roe D, *et al.* Smoking exposure as a risk factor for prevalent and recurrent colorectal adenomas. Cancer Epidemiol Biomarkers Prev 12:1006-1011, 2003
77 Neugut AI, Garbowski GC, Lee WC, *et al.* Dietary risk factors for the incidence and recurrence of colorectal adenomatous polyps: a case-control study. Ann Intern Med 118:91-95, 1993
78 Greenberg ER, Baron JA, Tosteson TD, *et al.* A clinical trial of antioxidant vitamins to prevent colorectal adenoma. N Engl J Med 331:141-147, 1994
79 Hofstad B, Almendingen K, Vatn M, *et al.* Growth and recurrence of colorectal polyps: a double-blind 3-year intervention with calcium and antioxidants. Digestion 59:148-156, 1998
80 Whelan RL, Horvath KD, Gleason NR, *et al.* Vitamin and calcium supplement use is associated with decreased adenoma recurrence in patients with a previous history of neoplasia. Dis Colon Rectum 42:212-217, 1999
81 Baron JA, Beach M, Mandel JS, *et al.* Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. N Engl J Med 340:101-107, 1999
82 Martinez ME, Henning SM, Alberts DS. Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence. 79:691-697, 2004
83 Steck-Scott S, Forman MR, Sowell A, *et al.* Carotenoids, vitamin A and risk of adenomatous polyp recurrence in the polyp prevention trial. Int J Cancer 112:295-305, 2004
84 Robertson DJ, Sandler RS, Haile R, *et al.* Fat, fiber, meat and the risk of colorectal adenomas. Am J Gastroenterol 100:2789-2795, 2005
85 Cole BF, Baron JA, Sandler RS, *et al.* Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 297:2351-2359, 2007
86 Almendingen K, Hofstad B, Vatn MH. Dietary habits and growth and recurrence of colorectal adenomas: results from a three-year endoscopic follow-up study. Nutr Cancer 49:131-138, 2004
87 Mathew A, Sinha R, Burt R, *et al.* Meat intake and the recurrence of colorectal adenomas. Eur J Cancer Prev 13:159-164, 2004

88 Martínez ME, Jacobs ET, Ashbeck EL, *et al*. Meat intake, preparation methods, mutagens and colorectal adenoma recurrence. Carcinogenesis 28:2019-2027, 2007
89 Hubner RA, Muir KR, Liu J-F, *et al*. Dairy products, polymorphisms in the vitamin D receptor gene and colorectal adenoma recurrence. Int J Cancer 123:586-593, 2008
90 Cottet V, Bonithon-Kopp C, Kronborg O, *et al*. Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. Eur J Cancer Prev 14:21-29, 2005
91 Sansbury LB, Wanke KL, Albert PS, *et al*. The effect of strict adherence to a high-fiber, high-fruit and -vegetable, and low-fat eating pattern on adenoma recurrence. Am J Epidemiol 170:576-584, 2009

2

Body Mass Index increases risk of Colorectal Adenomas in men with Lynch sydrome

The GEOLynch cohort study

mass index

Akke Botma
Fokko M. Nagengast
Marieke G.M. Braem
Jan C.M. Hendriks
Jan H. Kleibeuker
Hans F.A. Vasen
Ellen Kampman

Botma, A *et al*: J Clin Oncol 28(28), 2010:4346-53

Abstract

Purpose
High body mass index (BMI) is an established risk factor for sporadic colorectal cancer. Still, the influence of BMI on hereditary colorectal cancer (e.g., Lynch syndrome [LS]), is unknown. The objective of this study was to assess whether BMI is associated with colorectal adenoma occurrence in persons with LS.

Patients and methods
A prospective cohort study of 486 patients with LS was conducted. Cox regression models with robust sandwich estimates controlling for age, sex, extent of colon surgery, smoking, and alcohol intake were used to evaluate associations between BMI, height, weight, weight change, and risk of colorectal adenoma. Analyses were performed separately for those without (incident cohort; n = 243) and those with (prevalent cohort; n = 243) a history of colorectal neoplasms at baseline.

Results
A statistically significant association between current overweight (≥25 kg/m²) and developing colorectal adenomas was seen among men in the incident cohort (overweight vs normal weight hazard ratio [HR]: 8.72; 95% CI, 2.06 to 36.96). This association was not observed among women (overweight vs normal weight HR, 0.75; 95% CI, 0.19 to 3.07), nor was it observed in the prevalent cohort. In the incident cohort, height was statistically significantly associated with a decreased risk of adenomatous polyps among men (per 5 cm HR, 0.43; 95% CI, 0.23 to 0.83), but the association between weight and adenomatous polyps among men was of marginal significance (per 5 kg HR, 1.17; 95% CI, 1.00 to 1.37). No statistically significant associations were observed among women in either the incident cohort or the prevalent cohort.

Conclusion
Excess body weight increased the risk of incident colorectal adenomas in people with LS. This increased risk was seen only in men.

Supported by Grant No. UW-2005-3275 from the Dutch Cancer Society

Introduction

Lynch syndrome (LS) is a common form of hereditary colorectal cancer (CRC) and is thought to be responsible for 1% to 3% of the total CRC burden [1]. The syndrome is inherited in an autosomal dominant manner and is caused by germline mutations in one of the DNA mismatch repair (MMR) genes -*MLH1*, *MSH2*, *MSH6*, or *PMS2* [2]. Carriers of the germline mutations have a substantially higher CRC risk to age 70 years (22% to 69%) than is seen in the general population (2.1% to 2.5%) [3-7]. In addition, carriers have an increased risk of developing colorectal adenomas at a younger age compared with noncarriers [8]. Their MMR gene defects most probably accelerates their tumour progression. Carriers also have higher lifetime risks for other types of cancer, especially endometrial cancer [5,9].

Several lifestyle factors have been found to increase colorectal adenoma and cancer risk in the general population. One factor for which this is convincingly seen is overweight [10-20]. An important ongoing area of research is to determine whether factors that alter colorectal adenoma and cancer risk in the general population also affect risk in people with LS, because this may influence prevention strategies for those with a high risk of CRC. To our knowledge, there are no studies that primarily examined the association between obesity and colorectal adenomas or carcinomas in MMR gene carriers only. One case-control study [21] evaluated this association in persons who were thought to have LS on the basis of family history. They found that obesity was associated with an increased risk among men, but not among women, comparing this high-risk group with population-based controls. However, the MMR gene mutation status of the cases was unknown. To determine whether obesity and other lifestyle factors influence colorectal neoplasm development in patients with LS, we have to prospectively study these associations in carriers. Our objective was to evaluate whether weight, height, body mass index (BMI), and weight change were associated with colorectal adenoma development for incident as well as recurrent adenomatous polyps in persons with LS.

Patients and Methods

Study population

Eligible MMR gene mutation carriers for this prospective cohort study -the GEOLynch study- were identified via the Netherlands Foundation for the Detection of Hereditary Tumours (NFDHT) in Leiden, the Radboud University Nijmegen Medical Centre (RUNMC) in Nijmegen, and the University Medical Centre Groningen (UMCG) in Groningen (all in the Netherlands). Information on MMR gene mutation carrier status was collected at the NFDHT where this information is gathered to adequately control screening of carriers. Mutation diagnostics were done in one of the clinical genetics centres in the Netherlands, and the techniques used have been previously reported [22]. Between July 2006 and July 2008, a total of 713 known carriers of a germline mutation in at least one of the MMR genes were, with approval of their medical specialist, invited to participate in this study. Eligible patients were Dutch-speaking, white, mentally competent to participate men and women between 18 and 80 years of age who were screened regularly by colonoscopy. Terminally ill patients, and those with familial adenomatous polyposis, inflammatory bowel diseases, a proctocolectomy, or colostomy were excluded. We were able to contact 695 people of whom nine were ineligible. Seventy-three percent (499 of 686) of the eligible patients agreed to participate. Eight participants did not return one (n = 1) or both (n = 7) questionnaires, and we were not able to collect medical information for five carriers. Therefore, a total of 486 participants were included. Approval for this study was obtained from the medical ethical committee of the RUNMC. All participants provided written informed consent.

Data collection

Information on lifestyle factors, including current height (cm) and weight (kg), weight at age 18 years, weight at age 40 years, weight 2 years before study entry, medical history, and physical activity [23], was collected by using a standardized self-administered questionnaire. Usual dietary intake was assessed by using a 183-item self-administered food frequency questionnaire that was developed and validated by the Division of Human Nutrition of Wageningen University [24,25].

Follow-up colonoscopies were ascertained via the NFDHT [8] and from the medical records at the RUNMC, and UMCG hospitals. Information about all previous performed colonoscopies, colon surgeries, and cancer and adenomatous polyp occurrences was also gathered. For each colonoscopy, the number of neoplasms, location, size, and histology were ascertained. Of all lesions (130) removed during follow-up colonoscopies, seven were not sent to pathology, while for five polyps, we were not able to retrieve the pathology reports.

Statistical analyses

Risk of developing colorectal adenomatous polyps (International Classification of Diseases, Third Revision [ICD-03] codes C18-C20 M8140/0) was estimated by calculating hazard ratios (HR's) and 95% CI's using a Cox regression. Because some participants were members of the same family, standard errors were calculated by computing the robust sandwich estimates of the covariance matrix clustering on family membership to account for dependence of observations. Follow-up started at the time of questionnaire completion and ended at the date of first adenomatous polyp diagnosis, date of colorectal or extracolonic cancer diagnosis, date of diagnosis of metastasis, or date of death, whichever occurred first. Neoplasm-free carriers

were censored on January 31 2009, or on the date of their last known colonoscopy if later than January 31, 2009.

Current BMI, BMI at age 18 years, and BMI at age 40 years were calculated as weight divided by the square of height in meters (kg/m²). Obesity categories were created on the basis of WHO classifications (overweight: ≥25 kg/m²; obese: ≥30 kg/m²) [26]. Adult weight change and 2-year weight change were calculated by subtracting weight at age 18 and weight 2 years before study entry from current weight. Two-year weight change was grouped into three categories: weight loss, ≥2 kg; stable weight, <2 kg loss or gain; and weight gain, ≥2 kg. Adult weight change was grouped on the basis of the median in the total cohort. If more than 5% of a variable was unknown, these missing values were coded as a separate category. Univariate comparisons between patients with adenomatous polyps and patients without adenomatous polyps for baseline characteristics were evaluated with Chi-square or Fisher's exact tests for categoric and Wilcoxon rank sum test for continuous variables.

Carriers with one or more colorectal neoplasms before the start of the study and those without were analysed separately (i.e., the prevalent and incident cohorts, respectively). Associations were different for men and women, so we stratified all analyses by sex, except for the analysis in which we stratified by MMR gene because numbers were not large enough to stratify any further. In the basic model, we adjusted for age and sex. The fully adjusted model included age, sex, smoking habits (never, current, former), and alcohol intake (g/d). In addition, the analyses in the prevalent cohort were adjusted for the extent of colon resection (none, partial colectomy, subtotal colectomy). Analyses for height were adjusted for age, sex, extent of colon resection, and current weight; smoking habits and alcohol intake were not associated with current height. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC).

Results

Our cohort comprised 243 MMR gene mutation carriers in the incident cohort and 243 in the prevalent cohort. These carriers were from at least 161 families. Table 2.1 summarizes the baseline characteristics of both cohorts. The median age at study entry was 44.2 years (interquartile [IQ] range, 36.8 to 53.3 years) and 55.8 years (IQ range, 46.5 to 61.6 years) for the incident and prevalent cohorts, respectively. Sixty-five percent of the carriers from the incident cohort and 54% from the prevalent cohort were women. In both cohorts, more than 70% carried mutations in the *MLH1* or *MSH2* gene. Thirty-seven percent of the carriers from the incident cohort and 49% of those from the prevalent cohort were overweight or obese at start of the study.
During a median follow-up of 20.0 months, 22 MMR gene mutation carriers in the incident cohort and 36 in the prevalent cohort developed histologically confirmed colorectal adenomas. Compared with the total incident cohort, patients in the incident adenomatous polyp group were slightly older, more likely to be male, less often had a college or university education, were less likely to have an *MSH6* mutation, had a higher median current BMI, were more often smokers, and had a higher median alcohol intake. Compared with the total prevalent cohort, adenomatous polyp patients were more often male, had less education, were less often diagnosed with CRC before the start of this study, had been diagnosed more often with adenomatous polyps, and less often had a subtotal colectomy. No differences were seen for current BMI but patients were more often current smokers and drank slightly more alcohol than those in the total cohort.

Table 2.1 Baseline characteristics of the Mismatch Repair gene mutation carriers, stratified by history of colorectal neoplasms

Characteristic	Incident cohort Adenomatous Polyp cases (n=22)	Total cohort (n=243)	Prevalent cohort Adenomatous Polyp cases (n=36)	Total cohort (n=243)
Person months [median (IQR [a])]	7.5 (3.3-16.9)	20.0 (15.1-21.2) [b]	6.7 (4.2-11.9)	19.7 (10.2-21.2) [b]
Demographic characteristics				
Age at study entry, years [median (IQR [a])]	53.8 (44.3-56.4)	44.2 (36.8-53.3) [b]	54.8 (49.1-60.4)	55.8 (46.5-61.6)
Sex, female [n (%)]	12 (54.6)	158 (65.0)	15 (41.7)	130 (53.5)
Education, higher [n (%)] [c]	4 (18.2)	94 (38.7)	8 (22.2)	71 (29.2)
Medical characteristics				
MMR gene mutation [n (%)]				
MLH1	8 (36.4)	80 (32.9)	18 (50.0)	107 (44.0)
MSH2	11 (50.0)	102 (42.0)	14 (38.9)	92 (37.9)
MSH6	3 (13.6)	59 (24.3)	3 (8.3)	41 (16.9)
PMS2	0 (0.0)	1 (0.4)	1 (2.8)	2 (0.8)
History of cancer [n (%)]				
Colorectal cancer	0 (0.0)	0 (0.0)	12 (33.3)	127 (52.3) [b]
Other cancer	3 (13.6)	31 (12.8)	8 (22.2)	58 (23.9)
History of adenomatous polyps [n (%)]	0 (0.0)	0 (0.0)	32 (88.9)	156 (64.2) [b]
Time between colonoscopies [n (%)] [d]				
≤24 month	9 (40.9)	119 (49.0)	22 (61.1)	163 (67.1)
>24 month	12 (54.5)	117 (48.1)	14 (38.9)	80 (32.9)
No. of colonoscopies during study [median (IQR [a])]	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	1.0 (1.0-1.0)

Table 2.1 Continued

Characteristic	Incident cohort Adenomatous Polyp cases (n=22)	Total cohort (n=243)	Prevalent cohort Adenomatous Polyp cases (n=36)	Total cohort (n=243)
Colon surgery [n (%)]				
None	22 (100.0)	242 (99.6)	22 (61.1)	105 (43.2)
Partial colon resection	0 (0.0)	0 (0.0)	11 (30.6)	84 (34.6)
Subtotal colectomy	0 (0.0)	1 (0.4)	3 (8.3)	41 (16.9)
Anthropometric characteristics				
Height, cm [median (IQR [a])]	174 (170-178)	174 (168-180)	176 (167-184)	174 (167-181)
Weight, kg [median (IQR [a])]	78 (64-82)	74 (65-83)	78 (68-89)	76 (68-86)
BMI, kg/m² [median (IQR [a])]				
Current	25.7 (23.7-27.0)	24.1 (22.0-26.4)	24.9 (23.4-28.5)	24.9 (23.2-27.5)
At age 18 years [e]	21.7 (20.3-23.3)	20.8 (19.5-22.6)	20.8 (19.0-22.6)	21.5 (20.0-23.1)
At age 40 years [e]	24.4 (23.4-26.4)	23.7 (21.7-25.6)	23.4 (22.1-25.1)	23.9 (22.0-25.7)
2 years before study entry [e]	24.7 (23.4-27.1)	24.2 (21.7-26.7)	25.2 (23.3-27.1)	25.1 (23.1-27.8)
Other lifestyle factors				
Physical activity, high [n (%)] [f]	7 (31.8)	78 (32.1)	8 (22.2)	73 (30.0)
Total energy intake, kcal/d [median] (IQR [a])	2114.9 (1737.1-2341.6)	2109.2 (1747.5-2595.3)	1892.2 (1531.3-2423.7)	1998.8 (1660.6-2524.5)
Smokers, current [n (%)]	6 (27.3)	36 (14.8) [b]	15 (41.7)	55 (22.6) [b]
Alcohol intake, g/d [median (IQR [a])]	10.9 (3.5-21.0)	6.6 (1.3-17.1)	8.4 (3.8-28.0)	7.4 (1.6-16.1) [b]
NSAID use ≥1 times per month [n (%)]	5 (22.7)	42 (17.3)	5 (13.9)	41 (16.9)
Red meat intake, g/d [median (IQR [a])]	51.9 (44.3-66.6)	46.1 (29.5-64.7)	42.6 (28.8-61.2)	46.1 (29.7-62.8)
Poultry intake, g/d [median (IQR [a])]	10 (6.0-14.3)	12.1 (8.0-19.2)	7.9 (0.0-12.5)	11.1 (5.5-16.6) [b]
Fish intake, g/d [median (IQR [a])]	12.2 (4.7-18.1)	13.7 (5.1-16.4)	12.2 (6.0-15.8)	11.7 (7.9-16.7)
Vegetable intake, g/d [median (IQR [a])]	117.5 (68.0-184.9)	131.4 (82.4-181.5)	100.8 (58.5-156.2)	115.4 (72.1-161.4)
Fruit intake, g/d [median (IQR [a])]	218.6 (77.7-234.0)	151.9 (74.2-232.8)	82,5 (26.7-271.8)	164.7 (74.9-236.1) [b]

Abbreviation

n, number; IQR, inter quartile-range; MMR, mismatch repair; cm, centimeter; kg, kilo gram; BMI, body mass index; m, meter; kcal, kilocalories; g, gram; d, day; NSAID, non-steroidal anti-inflammatory drugs

Notes

[a] IQR inter quartile-range is the 25th-75th percentile

[b] P value <0.05, difference tested between cases and non-cases with Chi-square or Fisher's Exact Test for categorical and with Wilcoxon rank-sum test for continuous variables

[c] Higher education is a college or university education

[d] Time between the last colonoscopy before and the first colonoscopy after baseline, seven carriers did not have a colonoscopy before baseline

[e] Data for BMI at age 18, BMI at age 40, BMI 2 years before study entry were available for 360, 319, and 469 patients respecively

[f] High physical activity is the highest tertile of the physical activity score

In the total LS group, combining both cohorts, a two-fold increased risk for adenomatous polyps was observed for obese carriers (current BMI ≥30 kg/m²) versus normal-weight carriers (HR adjusted for age, sex, extent of colon surgery, smoking habits, and alcohol intake, 2.04; 95% CI, 1.08-3.86). This association was solely driven by the association among men (obese vs normal-weight men: HR, 2.94; 95% CI, 1.11 to 7.78; obese vs normal-weight women: HR, 0.99; 95% CI, 0.27-3.57; data not shown). Excluding seven carriers with no known colonoscopies before baseline did not change these results.

The adjusted HR's for the association between current overweight, height, and weight and the risk of adenomatous polyp development for the incident and prevalent cohort stratified by sex are presented in table 2.2. We observed a statistically significant 8.7-fold increased risk of adenomatous polyps for overweight men (current BMI ≥25 kg/m²) compared with normal weight men (current BMI <25 kg/m²) among

Table 2.2

Adjusted Hazard Ratios for BMI, height and weight and developing colorectal adenomatous polyps stratified by sex for both incident and prevalent Lynch syndrome cohorts

	Incident				
	No. of cases (n=22)	**Total cohort (n=243)**	**Follow-up time (months)**	**Age adjusted HR (95% CI)**	**Fully adjusted HR (95% CI) [a) b)]**
Women					
BMI, current					
normal weight (<25 kg/m²)	8	106	2026.2	1.0	1.0
overweight or obese (≥25 kg/m²)	4	52	969.8	0.78 (0.20-3.05)	0.75 (0.19-3.07)
per 5 kg/m²	12	158	2995.9	1.07 (0.53-2.16)	1.06 (0.51-2.20)
Height, per 5 cm	12	158	2995.9	1.10 (0.78-1.55)	1.09 (0.70-1.68)
Weight, per 5 kg	12	158	2995.9	1.03 (0.85-1.26)	1.00 (0.76-1.31)
Men					
BMI, current					
normal weight (<25 kg/m²)	2	47	832.6	1.0	1.0
overweight or obese (≥25 kg/m²)	8	38	564.1	5.19 (1.30-20.80)	8.72 (2.06-36.96)
per 5 kg/m²	10	85	1396.7	2.68 (1.16-6.19)	1.84 (1.13-3.02)
Height, per 5 cm	10	85	1396.7	0.49 (0.32-0.76)	0.43 (0.23-0.83)
Weight, per 5 kg	10	85	1396.7	1.03 (0.81-1.30)	1.17 (1.00-1.37)

Abbreviation: BMI, body mass index; n, number; HR, hazard ratio; CI, confidence interval; kg, kilo gram; m, meter; cm, centimeter

Notes:
a) Adjusted for age, smoking habits & alcohol intake, height was adjusted for weight & vice versa, the 'prevalent' cohort is also adjusted for the extent of colon resection
b) Height analysis were adjusted for age, extent of colon resection and weight

Table 2.3

Adjusted Hazard Ratios for current BMI and developing colorectal adenomatous polyps stratified by MMR gene for incident and prevalent Lynch syndrome cohorts

	Incident				
	No. of cases (n=22)	**Total cohort (n=243)**	**Follow-up time (months)**	**Age & sex adjusted HR (95% CI)**	**Fully adjusted HR (95% CI) [a)]**
MLH1					
BMI, current					
normal weight (<25 kg/m²)	5	61	1214.0	1,0	1,0
overweight or obese (≥25 kg/m²)	3	19	326.5	2.60 (0.50-13.40)	2.64 (0.47-14.89)
per 5 kg/m²	8	80	1540.5	1.37 (0.68-2.76)	1.39 (0.70-2.76)
MSH2					
BMI, current					
normal weight (<25 kg/m²)	4	53	981.3	1,0	1,0
overweight or obese (≥25 kg/m²)	7	49	872.4	1.38 (0.38-5.07)	1.08 (0.21-5.73)
per 5 kg/m²	11	102	1853.7	1.43 (0.56-3.63)	1.14 (0.47-2.74)
MSH6					
BMI, current					
normal weight (<25 kg/m²)	1	38	639.8	1,0	1,0
overweight or obese (≥25 kg/m²)	2	21	315.4	3.19 (0.40-25.43)	4.69 (0.62-35.61)
per 5 kg/m²	3	59	955.1	1.31 (0.52-3.30)	2.77 (0.19-40.27)

Abbreviation: BMI, body mass index; MMR, mismatch repair; n, number; HR, hazard ratio; CI, confidence interval; kg, kilo gram; m, meter; cm, centimeter

Notes:
a) Adjusted for age, smoking habits, and alcohol intake, the prevalent cohort is also adjusted for the extent of colon resection

Prevalent

No. of cases (n=36)	Total cohort (n=243)	Follow-up time (months)	Age adjusted HR (95% CI)	Fully adjusted HR (95% CI) [a) b)]
9	69	1161.5	1,0	1,0
6	61	1058.9	0.84 (0.31-2.27)	0.91 (0.29-2.91)
15	130	2220.4	0.78 (0.48-1.26)	0.87 (0.49-1.52)
15	130	2220.4	0.79 (0.54-1.16)	0.85 (0.59-1.22)
15	130	2220.4	0.88 (0.71-1.09)	0.92 (0.70-1.23)
10	55	859.6	1,0	1,0
11	58	933.3	1.00 (0.42-2.38)	0.87 (0.36-2.12)
21	113	1792.8	1.23 (0.76-1.97)	1.47 (0.71-3.06)
21	113	1792.8	1.24 (0.90-1.69)	1.16 (0.87-1.54)
21	113	1792.8	1.11 (0.97-1.27)	1.12 (0.91-1.38)

Prevalent

No. of cases (n=36)	Total cohort (n=243)	Follow-up time (months)	Age & sex adjusted HR (95% CI)	Fully adjusted HR (95% CI) [a)]
11	58	921.3	1,0	1,0
7	49	812.2	0.78 (0.37-1.67)	0.61 (0.26-1.41)
18	107	1733.6	0.82 (0.50-1.33)	0.80 (0.51-1.23)
6	44	752.8	1,0	1,0
8	48	812.5	1.23 (0.34-4.48)	1.06 (0.35-3.23)
14	92	1565.2	1.20 (0.71-2.04)	1.24 (0.72-2.15)
2	21	325.9	1,0	1,0
1	20	332.4	0.32 (0.02-4.65)	0.29 (0.03-2.88)
3	41	658.3	0.93 (0.27-3.20)	0.73 (0.14-3.86)

MMR gene mutation carriers in the incident cohort. No association was seen among women. Furthermore, for men within the incident cohort, but not for women, a 5 cm increase in height was associated with a statistically significant decrease in adenomatous polyp risk (men, per 5 cm HR, 0.43; 95% CI, 0.23-0.83; women, per 5 cm HR, 1.09; 95% CI, 0.70-1.68). In addition, we observed a borderline significant increase in risk of adenomatous polyps with 5 kg increase in weight among men (per 5 kg HR 1.17; 95% CI, 1.00-1.37), but again did not see an association between weight and risk of adenomatous polyps among women (per 5 kg HR, 1.0; 95% CI, 0.75-1.31).

Among men and women in the prevalent cohort, we did not observe statistically significant associations with current overweight, height, and weight (table 2.2). In both cohorts, there were no statistically significant associations with overweight or obesity at age 40 years compared with normal weight for men and women. Additionally, in both cohorts, BMI at age 18 years was not statistically significantly associated with risk of adenomatous polyps (data not shown).

A 2-year weight gain of >2 kg was associated with an increased risk of adenomatous polyps (HR, 4.09; 95% CI, 1.04-16.19) among women in the prevalent cohort. This association was not seen in women in the incident cohort (HR, 0.74; 95% CI, 0.10 -5.64) nor in men (incident cohort HR, 1.71; 95% CI, 0.30-9.78; prevalent cohort: HR, 1.73; 95% CI, 0.67-4.45; data not shown). In both the incident and prevalent cohorts, adult weight gain was not statistically significantly associated with risk of adenomatous polyps in men (incident cohort: HR, 3.60; 95% CI, 0.38-34.28; prevalent cohort: HR, 2.29; 95% CI, 0.54-9.67) or women (incident cohort: HR, 0.83; 95% CI, 0.20-3.48; prevalent cohort: HR, 1.53; 95% CI, 0.37-6.29; data not shown).

Associations between current BMI and risk of adenomatous polyps stratified by MMR gene in which the mutation did occur (i.e., *MLH1*, *MSH2*, and *MSH6*) are provided in table 2.3. No marked differences were observed. The groups were too small to further stratify by sex.

Discussion

This prospective study among MMR gene mutation carriers shows a statistically significant positive association between current overweight and adenomatous polyps in men without a history of colorectal neoplasms. For men within this incident cohort, a 5 cm increase in height was associated with a decrease in adenomatous polyps risk, while an increase in risk was seen with a 5 kg increase in weight. No association with current overweight was observed among men with a history of colorectal neoplasms, and no associations were observed among women.

BMI influences CRC incidence in the general population, especially among men [10,11] For incident adenomatous polyps, most studies also show positive associations [12-20], but some do not [27-29]. Four of the six studies that investigated the association between BMI and adenomatous polyps among men and women separately also indicate a stronger association for men than for women [12,15,17,18]. Using waist circumference may be more informative for risk of colorectal neoplasms in women than measurements of BMI, because men and women have different distribution of fat [30]. For a given BMI, greater amounts of visceral and hepatic adipose tissue are often seen in men compared with women, which could partly explain the differences between sexes [31]. We were not able to investigate this association in this study.

The effects of BMI on secondary primary adenomas or recurrences in the population at large are less frequently studied and inconsistent; two studies did find statistically significant associations [32,33], two studies did not [34,35], and one observed associations for growth of adenomas but not for recurrences [36]. This variation in findings could be explained by the fact that some studies did not stratify for sex [35,36].

The differences observed for incident and recurrent adenomas in our study could be explained by differences in baseline characteristics of both populations. First, the median age in the prevalent cohort was 10 years higher than that in the incident cohort, which most probably influences the number of recurrences rather than the association. In addition, the differences between both cohorts in the number of carriers with a (partial) colon resection may contribute, because complaints after resection could theoretically influence eating habits, energy intake, and therefore adenoma recurrences. However, in the prevalent cohort, current BMI was not different between those with and without partial colon resection (median BMI, 24.8; IQ range, 23.4 to 27.7; and median BMI, 25.1; IQ range, 23.5 to 27.2, respectively). It is possible that colorectal polyp incidence is influenced by factors different from those that influence recurrence.

A recent case-control study examined the association between BMI and microsatellite instability-defined CRC. A stratified analysis of BMI, microsatellite instability and MMR gene mutation status also showed a nonsignificant increased risk (odds ratio, 3.96; 95% CI, 0.59-26.48) for obese MMR gene mutation carriers [37]. However, the sample size in this stratified analysis was small, limiting the statistical power to draw firm conclusions. Two case-control studies [21,38] assessed the association between BMI and the development of colorectal neoplasms in people who were thought to have LS on the basis of family history. The retrospective study by Campbell *et al.* [21] reported an increased CRC risk of 25% for overweight men and 83% for obese men, but not for women, which is consistent with our results. A previous retrospective case-control study [38] with partly the same population as was used in our analyses but also including those who were thought to be LS carriers did not observe an association between colorectal neoplasm occurrence and BMI,.one of the potential reasons being that the analysis was not stratified by sex. Moreover, this latter

study included patients (18%) who filled out the questionnaires 5 years after being diagnosed with colorectal neoplasms. BMI at time of study entry could be influenced by this diagnosis, instead of vice versa. Furthermore, the cases in this study were thought to be LS carriers with incident and recurrent neoplasms. These issues could have resulted in an attenuation of the association. In contrast to our findings, Campbell *et al*. [21] observed a positive association between height and CRC among women, but not among men. We found a decreased risk per 5 cm increase in height among men but not for women. This decreased risk is not only in contrast to the study of Campbell *et al*. [21], but also in contrast with studies evaluating height and sporadic CRC in the general European population [30]. Our population is much taller than that in the studies of Campbell *et al*. [21] and Pischon *et al*. [30]: men (median, 182 cm; IQ range, 177 to 187 cm) and women (median, 169 cm; IQ range, 163 to 174 cm). The positive association between height and colorectal neoplasms could have a threshold, which might be a reason for not finding an association among women. This, however, does not explain the inverse association in men found in this study.

Our study failed to show associations for adult weight change, 2-year weight change, BMI at age 18 years, and BMI at age 40 years. This might be because of missing data in these variables and, thus, limited power to detect associations.

Limitations of this study were the use of self-reported height and weight data to calculate BMI. Studies that examined the validity of self-reported weight and height have shown that people tend to under-report their body weight, especially those with increased adiposity, and over-report their height, especially those with a higher BMI and those who are older (>60 years of age) [39,40]. Nonetheless, McAdams *et al*. [41] show that self-reported and measured anthropometric data are highly correlated (range r, 0.88 to 0.97). Self-reporting can lead to some underestimation of obesity prevalence, which introduced minimal bias in the measures of association in that study. Another limitation was that, although this is the largest prospective cohort study in MMR gene mutation carriers to date, it is a small prospective cohort study. As a result, we had limited power to evaluate some subgroups (e.g. high and low physical activity).

Strengths of this study include a prospective cohort of only MMR gene mutation carriers and a high participation rate of 73%, which reduces the chance of recall and selection bias and makes these results generalizable to persons with LS in other clinical series. The median values for BMI in this study (43% BMI ≥25 kg/m^2) were comparable with those of the general Dutch population measured in 2007 (45.5% BMI ≥25 kg/m^2). This shows that these patients with LS, according to their BMI, reflect the average Dutch population and makes us believe that this group is not overly health conscious [42]. We included covariates in the analysis to control for confounding factors, although, as in every observational study, residual confounding may still exist.

In summary, our results suggest that BMI is associated with the incidence of colorectal adenomas in men with LS. We did not observe an association between BMI and the development of recurrent or new primary colorectal adenomas. If confirmed, overweight may be an important modifiable risk factor for colorectal adenoma incidence in men with LS. Future studies should also examine the association between waist circumference and development of colorectal neoplasms, because this might better reflect the risks associated with excess body weight in women.

Acknowledgement

We are indebted to all study participants for their cooperation. We thank Mary Velthuizen and Alice Donselaar, Netherlands Foundation for Detection of Hereditary Tumours, Maria van Vugt, Radboud University Nijmegen Medical Centre, and Leontien Witjes, Wageningen University, for assistance with participant recruitment and data collection. The medical specialists of the participants are gratefully acknowledged for their collaboration.

References

1 de la Chapelle A. The incidence of Lynch syndrome. Fam Cancer 4:233-237, 2005
2 Abdel-Rahman WM, Mecklin JP, Peltomaki P. The genetics of HNPCC: application to diagnosis and screening. Crit Rev Oncol Hematol 58:208-220, 2006
3 Barrow E, Alduaij W, Robinson L, *et al.* Colorectal cancer in HNPCC: cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. Clin Genet 74:233-242, 2008
4 Jenkins MA, Baglietto L, Dowty JG, *et al.* Cancer risks for mismatch repair gene mutation carriers: a population-based early onset case-family study. Clin Gastroenterol Hepatol 4:489-498, 2006
5 Quehenberger F, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the *HMLH1* and *HMSH2* gene: correction for ascertainment. J Med Genet 42:491-496, 2005
6 Hampel H, Stephens JA, Pukkala E, *et al.* Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 129:415-421, 2005
7 Signaleringscommissie-Kanker-van-KWF-kankerbestrijding: Signaleringsrapport 'De kans op kanker. Bewerking van cijfers NKR & CBS 1999-2003'. Amsterdam, 2007
8 de Jong AE, Morreau H, Van Puijenbroek M, *et al.* The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 126:42-48, 2004
9 Watson P, Vasen HF, Mecklin JP, *et al.* The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. Int J Cancer 123:444-449, 2008
10 WCRF/AICR: Food, nutrition, physical activity, and the prevention of cancer: a global perspective. (ed 2nd). Washington DC, AICR, 2007
11 Renehan AG, Tyson M, Egger M, *et al.* Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371:569-578, 2008
12 Giovannucci E, Ascherio A, Rimm EB, *et al.* Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 122:327-334, 1995
13 Giovannucci E, Colditz GA, Stampfer MJ, *et al.* Physical activity, obesity, and risk of colorectal adenoma in women (United States). Cancer Causes Control 7:253-263, 1996
14 Neugut AI, Lee WC, Garbowski GC, *et al.* Obesity and colorectal adenomatous polyps. J Natl Cancer Inst 83:359-361, 1991
15 Kahn HS, Tatham LM, Thun MJ, *et al.* Risk factors for self-reported colon polyps. J Gen Intern Med 13:303-310, 1998
16 Bird CL, Frankl HD, Lee ER, *et al.* Obesity, weight gain, large weight changes, and adenomatous polyps of the left colon and rectum. Am J Epidemiol 147:670-680, 1998
17 Boutron-Ruault M-C, Senesse P, Meance S, *et al.* Energy intake, body mass index, physical activity, and the colorectal adenoma-carcinoma sequence. Nutr Cancer 39:50-57, 2001
18 Morimoto LM, Newcomb PA, Ulrich CM, *et al.* Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential. Cancer Epidemiol Biomarkers Prev 11:1012-1018, 2002
19 Terry MB, Neugut AI, Bostick RM, *et al.* Risk factors for advanced colorectal adenomas: a pooled analysis. Cancer Epidemiol Biomarkers Prev 11:622-629, 2002
20 Guilera M, Connely-Frost A, Keku TO, *et al.* Does physical activity modify the association between body mass index and colorectal adenomas. Nutr Cancer 51:140-145, 2005
21 Campbell PT, Cotterchio M, Dicks E, *et al.* Excess body weight and colorectal cancer risk in Canada: associations in subgroups of clinically defined familial risk of cancer. Cancer Epidemiol Biomarkers Prev 16:1735-1744, 2007
22 Wijnen JT, Vasen HFA, Khan PM, *et al.* Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med 339:511-518, 1998
23 Baecke J, Burema J, Frijters J. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 36:936-942, 1982
24 Feunekes G, Van Staveren W, De Vries J, *et al.* Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr 58:489-496, 1993
25 Verkleij-Hagoort AC, de Vries JHM, Stegers MPG, *et al.* Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. Eur J Clin Nutr 61:610-615, 2006
26 WHO: Obesity: preventing and managing the global epidemic. Geneva, World Health Organization, 2000
27 Olsen J, Kronborg O, Lynggaard J, *et al.* Dietary risk factors for cancer and adenomas of the large intestine. A case-control study within a screening trial in Denmark. Eur J Cancer 30A:53-60, 1994
28 Erhardt JG, Kreichgauer HP, Meisner C, *et al.* Alcohol, cigarette smoking, dietary factors and the risk of colorectal adenomas and hyperplastic polyps – a case control study. Eur J Nutr 41:35-43, 2002
29 Otake S, Takeda H, Suzuki Y, *et al.* Association of visceral fat accumulation and plasma adiponectin with colorectal adenoma: evidence for participation of insulin resistance. Clin Cancer Res 11:3642-3646, 2005
30 Pischon T, Lahmann PH, Boeing H, *et al.* Body size and risk of colon and rectal cancer in the European Prospective Investigation Into Cancer and nutrition (EPIC). J Natl Cancer Inst 98:920-931, 2006

31 Geer EB, Shen W. Gender differences in insulin resistance, body composition, and energy balance. Gend Med 6:60-75, 2009
32 Jacobs ET, Martinez ME, Alberts DS, *et al*. Association between body size and colorectal adenoma recurrence. Clin Gastroenterol Hepatol 5:982-990, 2007
33 Jacobs ET, Ahnen DJ, Ashbeck EL, *et al*. Association between body mass index and colorectal neoplasia at follow-up colonoscopy: a pooling study. Am J Epidemiol 169:657-666, 2009
34 Davidow AL, Neugut AI, Jacobson JS, *et al*. Recurrent adenomatous polyps and body mass index. Cancer Epidemiol Biomarkers Prev 5:313-315, 1996
35 Sass DA, Schoen RE, Weissfeld JL, *et al*. Relationship of visceral adipose tissue to recurrence of adenomatous polyps. Am J Gastroenterol 99:687-693, 2004
36 Almendingen K, Hofstad B, Vatn MH. Does high body fatness increase the risk of presence and growth of colorectal adenomas followed up in situ for 3 years? Am J Gastroenterol 96:2238-2246, 2001
37 Campbell PT, Jacobs ET, Ulrich CM, *et al*. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. J Natl Cancer Inst 102:391-400, 2010
38 Diergaarde B, Braam H, Vasen HF, *et al*. Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 5:736-742, 2007
39 Engstrom JL, Paterson SA, Doherty A, *et al*. Accuracy of self-reported height and weight in women: an integrative review of the literature. J Midwifery Womens Health 48:338-345, 2003
40 Gorber SC, Tremblay M, Moher D, *et al*. A comparison of direct vs. self-report measures for assessing height, weight and body mass index: a systematic review. Obes Rev 8:307-326, 2007
41 McAdams MA, Van Dam RM, Hu FB. Comparison of self-reported and measured BMI as correlates of disease markers in U.S. adults. Obes Res 15:188-196, 2007
42 CBS. Zelfgerapporteerde medische consumptie, gezondheid en leefstijl: Lengte en gewicht (20+) & Onder- en overgewicht (20+). Centraal Bureau voor de Statistiek, 2009

3

Smoking and alcohol intake and the risk of colorectal adenomas in persons with Lynch syndrome

Renate M. Winkels [1]
Akke Botma [1]
Fränzel J.B. van Duijnhoven
Fokko M. Nagengast
Jan H. Kleibeuker
Hans F.A. Vasen
Ellen Kampman
[1] authors contributed equally to this work

Submitted

Abstract

Purpose
Individuals with Lynch syndrome have a high risk of developing colorectal carcinomas and adenomas at a young age, due to inherited mutations in mismatch repair genes. Modifiable lifestyle factors, such as smoking and alcohol intake, may unfavourably influence this risk.

Patients and methods
Using data from the GEOLynch cohort study, a prospective study of 486 persons with Lynch syndrome, we calculated hazard ratios (HR's) for the association between smoking and alcohol intake and development of colorectal adenoma. We used robust variance estimates in the calculation of 95% confidence intervals (CI) to account for dependency within families and adjusted for confounding by age, sex, smoking (in the analyses of alcohol intake), number of colonoscopies during follow-up, colonic resection and body mass index.

Results
During a median follow-up of 20 months, 58 persons developed a histologically confirmed colorectal adenoma. The HR for current smokers was 7.06 (95% CI 3.11-16.04) and for former smokers was 2.69 (1.23-5.90) compared with never smokers. Among ever smokers, a higher number of pack years was associated with an increased risk of colorectal adenoma (p for trend: 0.03). Alcohol intake slightly increased the risk of colorectal adenoma, although this was not statistically significant; HR for the highest tertile of intake (median 22 g/day) versus the lowest tertile (0.4 g/day) was 1.33 (0.58-3.05).

Conclusion
Among persons with Lynch syndrome, current smokers have an increased risk of colorectal adenomas. Former smokers have a lower risk than current smokers, but greater risk than never smokers. Persons with Lynch syndrome should be encouraged to avoid smoking.

Financially supported by the Dutch Cancer Society (grant no. UW-2005-3275); Wereld Kanker Onderzoek Fonds (WCRF NL), and World Cancer Research Fund International (WCRF International).

Introduction

Persons with Lynch syndrome have a 20-70% risk of developing colorectal cancer before the age of 70 [1-4], have a higher risk for developing colorectal adenomas at a younger age [5] and manifest a rapid progression from colorectal adenoma to carcinoma [6]. It is estimated that 1-3% of all colorectal cancer is caused by Lynch syndrome [7]. The increased risk of colorectal cancer in Lynch syndrome is caused by pathogenic germline mutations in genes involved in DNA mismatch repair: *MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM* [7-10].

Considering the high life-time risk of developing colorectal cancer in individuals with Lynch syndrome, it is very relevant to study whether modifiable lifestyle factors can affect the risk of developing this hereditary cancer. Several modifiable lifestyle factors affect the risk of sporadic colorectal cancer among which smoking [11] and alcohol consumption [12]. However, this association has only sparsely been studied in Lynch syndrome [13-15].

So far, only retrospective studies on smoking and colorectal cancer risk in persons with Lynch syndrome were performed [13-15], these cohort [14,15] and case-control studies [13] showed that smoking was associated with an increased risk of colorectal cancer. Due to the retrospective nature, the information on smoking history was limited in one of these studies [15] as it had to be obtained partly from medical records or family reports. Moreover, the case-control study [13] included persons who were not all confirmed carriers of a mismatch repair gene mutation. Likewise for alcohol intake, the association with colorectal cancer in Lynch syndrome has only been studied retrospectively in these studies [13-15]. Both studies did not detect a significant association between alcohol intake and colorectal cancer risk.

The aim of this study was to prospectively assess the association between smoking and/or alcohol intake and colorectal adenoma development in a cohort study of persons with Lynch syndrome. To our best knowledge, the association with colorectal adenomas has not been studied before as previous studies in Lynch syndrome focused on colorectal carcinoma risk [14,15]. In the general population smoking shows a stronger association with development of colorectal adenomas than with carcinoma [11,16]. For persons with Lynch syndrome who undergo regular surveillance colonoscopies, colorectal adenoma are removed which lowers these persons' risks of colorectal carcinoma [17,18]. Therefore, it is very relevant to study whether the risk of developing colorectal adenomas is modifiable.

Patients and Methods

Population

Details of the prospective cohort of persons with Lynch syndrome (the GEOLynch study) were described earlier [19]. In short, we identified persons known to have a pathogenic mutation in one of the mismatch repair genes - as confirmed by a clinical genetics centre - through the Netherlands Foundation for the Detection of Hereditary Tumours in Leiden, the Radboud University Nijmegen Medical Centre and the University Medical Centre in Groningen, the Netherlands. Persons had to be Dutch-speaking, Caucasian, mentally competent to participate in the study and between 18 and 80 years of age to be eligible for our study. Additionally, persons with familial adenomatous polyposis, inflammatory bowel disease, a personal history of a complete proctocolectomy or colostomy and persons who were terminally ill were excluded.

A total of 713 eligible mutation carriers were invited to participate between July 2006 and July 2008 – with approval of their medical specialist. Of these, 73% (499 out of 713) persons agreed to participate. The final cohort consisted of 486 out of 499 persons, as retrieval of medical and personal information was not complete for 13 persons. These 486 came from at least 161 families. Approval for this study was obtained from the Medical Ethical committee of the Radboud University Nijmegen Medical Centre. All participants provided written informed consent.

Exposure assessment

Using structured questionnaires, we collected detailed self-reported information on smoking and possible confounding factors, e.g. physical activity level, height and weight. Smoking information included smoking status at recruitment (current, former, ever), duration of smoking, type of tobacco product (cigarettes, pipe, cigar) and number of cigarettes smoked per day. Since 287 out of the 301 ever smokers in the cohort smoked cigarettes, we did not distinguish between type of tobacco in our analyses.

Information on alcohol intake was extracted from a self-administered, validated food frequency questionnaire [20,21]. In this questionnaire, persons reported type and frequency of intake of alcoholic drinks during the past month. From this information in combination with data from the Dutch food composition table [22], we calculated intake of alcohol in grams per day. We used the data of all persons in the cohort to create tertiles of alcohol intake. Moreover, we evaluated whether the alcohol intake was below the recommendations set by the World Cancer Research Fund [12] of ≤1 glass per day for women (≤10 g of alcohol) and of ≤2 glasses per day for men (≤20 g of alcohol).

Outcome data

Medical information was gathered via the participating centres. From the medical records, we extracted information on date and number of colonoscopies, colon surgeries, incidence of cancer and adenomatous polyps before recruitment and during follow-up. We ascertained detailed information about location, size and histology for all documented polyps that occurred during follow-up from pathology records.

Data analysis

The outcome of our analysis was the time to diagnosis of the first pathology-confirmed colorectal adenomatous polyp. Person-time started at the date participants

had completed the questionnaires. For the persons without an adenoma diagnosis, we censored the person-time at the date of diagnosis of colorectal cancer, metastasis or death, the date of the last colonoscopy during follow-up, or January 31 2009.

We used Cox proportional hazard regression to assess hazard ratios (HR's) for the association between smoking and/or alcohol intake and development of colorectal adenomas. We used robust estimates of variance in the calculation of the 95% CI to account for dependency of observations within families. The proportional hazard assumption was not violated, as evaluated by the goodness-of-fit test using Schoenfeld residuals (p-value >0.05).

In additional analyses on number of cigarettes smoked per day, number of years smoked and pack years of smoking, we combined current and former smokers and excluded the never smokers. In test for trend analyses on those smoking variables, we assigned the median score for each tertile of the different smoking variables to each individual in this tertile. This new variable was included in the Cox model as a continuous variable.

To assess whether associations differed for prevalent versus incident colorectal adenoma cases, we stratified our analysis for history of colorectal adenomas or carcinomas. To assess multiplicative interaction between smoking and alcohol intake, we created categories based on both smoking (never, current, former smokers) and alcohol intake (low versus high intake, based on median split). We assessed the HR for the development of colorectal adenomas within each category versus "never smokers & low alcohol intake" as reference category. To test for multiplicative interaction, we used a log likelihood ratio test that compared a model with interaction terms of alcohol and smoking to a model without these interaction terms.

We assessed whether the following variables affected the associations between smoking, alcohol and colorectal adenomas: age (continuous), sex, history of colorectal adenomas or carcinomas (yes/no), number of colonoscopies during follow-up (categorical: 0, 1, 2 or 3), colonic resection (yes/no), BMI (continuous), NSAID-use (more or less than 1 time/week), education (categorical: high versus lower educated), type of gene-mutation, physical activity level (categorical: high versus lower physically active), energy intake (continuous), red and processed meat intake (continuous), smoking (categorical: never/former/current, in the analyses of alcohol), alcohol (continuous, in the analyses of smoking). Covariates were included in multivariate models if correlated with the exposure (smoking or alcohol) and the outcome (colorectal adenomas) in univariate analyses; using backward elimination, covariates remained in the final models if they produced changes in the HR of ≥10%, while age, sex and number of colonoscopies were always included in the models. All analyses were performed using Stata (Stata/SE 11.0 for Windows).

Results

During a median follow-up of 20 months, 58 out of 486 persons in our cohort developed a histologically confirmed colorectal adenoma. Table 3.1 shows that cases were slightly older, slightly lower educated and more often men compared to the total cohort. In addition, cases were more likely to have had colorectal adenomas in the past and to have had at least one colonoscopy during follow-up compared with the total cohort. There were more smokers and former smokers and alcohol intake appeared to be slightly higher among cases than among the total cohort (table 3.1).
Former and current smokers more often had a history of colorectal adenomas, were slightly lower educated and had slightly higher alcohol intake than never smokers. In addition, former and current smokers were more likely to have had at least one colonoscopy during follow-up and to have had a partial or subtotal colonic resection. Persons with a higher intake of alcohol were more often male, had more often a history of colorectal adenomas, were slightly higher educated, were more often current or former smoker and had more often had a colonoscopy during follow-up (data not shown).
Current and former smoking was associated with an increased risk of colorectal adenoma development during follow-up; the adjusted HR for current smokers was 7.06 (95% CI 3.11-16.04) and for former smokers 2.69 (1.23-5.90), compared with never smokers (table 3.2). By adjusting for the number of colonoscopies during follow-up, we accounted for the fact that not all persons in the cohort had a colonoscopy during follow-up.

Table 3.2 Hazard ratios for smoking status, intensity, and duration of smoking and development of colorectal adenomas in a cohort of 486 persons with Lynch syndrome

Smoking status [a]	**Never**	**Former**	**Current**	
Cases/cohort	8/184	29/210	21/91	
HR, crude (95% CI)	1.0	3.30 (1.55-7.04)	6.12 (2.71-13.85)	
HR, adjusted (95% CI) [b]	1.0	2.69 (1.23-5.90)	7.06 (3.11-16.04)	
Intensity and duration of smoking [a,c]	**Tertile 1**	**Tertile 2**	**Tertile 3**	**P for trend**
Cigarettes smoked, n/d [median (IQR [d])]	5 (3-6)	10 (10-15)	20 (20-25)	
Cases/cohort e)	15/104	20/116	15/70	
HR, adjusted (95% CI) [b]	1.0	1.37 (0.68-2.75)	1.33 (0.63-2.80)	0,52
No. of years smoked [median (IQR [d])]	7 (5-10)	17 (15-20)	30 (25-38)	
Cases/cohort	14/95	15/97	20/92	
HR, adjusted (95% CI) [b]	1.0	1.01 (0.48-2.14)	1.64 (0.76-3.55)	0,17
Pack years [median (IQR [d])]	2 (1-4)	9 (8-11)	23 (17-32)	
Cases/cohort	13/95	12/94	24/94	
HR, adjusted (95% CI) [b]	1.0	0.84 (0.37-1.93)	1.77 (0.91-3.41)	0,03

Abbreviation HR, hazard ratio; CI, confidence interval; n/d, no. per day; IQR, inter quartile-range

Notes
a) Data for smoking status is missing for 1 person, for no. of cigarettes per day 12 missings , for no. of years smoked 18 missings, and for pack years 19 missings
b) adjusted for age, sex, no. of colonoscopies during follow-up (categorical: 0, 1, 2 or 3), colonic resection (yes/no) and BMI (continuous)
c) never smokers are excluded from these analyses
d) IQR inter quartile-range is the 25th-75th percentile
e) no. of persons in each tertile is unequal because of the high number of ties on this variable

Table 3.1 Characteristics of colorectal adenoma cases in comparison to the total cohort of 486 persons with Lynch syndrome

Characteristic	Adenomatous Polyp cases (n=58)		Total cohort (n=486)	
Person months [median (IQR [a])]	7 (4-12)		20 (14-21)	
Demographic factors				
Age, years [median (IQR [a])]	54 (47-59)		50 (41-59)	
Sex, male [n (%)]	31 (53)		198 (40)	
BMI, kg/m² [median (IQR [a])]	25.0 (23.5-27.1)		24.5 (22.5-27.0)	
Education, higher [n (%)] [b]	12 (21)		165 (34)	
Lifestyle factors				
Alcohol intake, g/d [n (%)]				
Tertile 1, range 0-2.7	11 (19)		162 (33)	
Tertile 2, range 2.8-12.5	22 (38)		163 (34)	
Tertile 3, range 13.0-94.0	25 (43)		161 (33)	
Smoking status [n (%)] [c]				
Never	8 (14)		184 (38)	
Former	29 (50)		210 (43)	
Current	21 (36)		91 (19)	
Selected smoking variables [median (IQR [a])]	**Former smokers**	**Current smokers**	**Former smokers**	**Current smokers**
Pack years	8 (2-15)	18 (10-28)	8 (3-14)	15 (8-25)
Cigarettes smoked n/d	15 (5-20)	10 (10-18)	10 (6-15)	10 (7-18)
No. of years smoked	15 (8-21)	30 (20-39)	14 (9-21)	25 (19-35)
Clinical factors [n (%)]				
History of colorectal cancer	12 (21)		127 (26)	
History of colorectal adenomas	32 (55)		156 (32)	
History of other cancers	11 (19)		89 (18)	
Time (months) between colonoscopies				
≤24	26 (45)		197 (41)	
>24	31 (53)		282 (58)	
No. of colonoscopies during person time				
0	0 (0)		102 (21)	
1	53 (91)		329 (68)	
2	4 (7)		51 (10)	
3	1 (2)		4 (1)	
Colonic resection, partial or subtotal colectomy	14 (24)		137 (28)	
Gene mutated				
MLH1	26 (45)		187 (38)	
MSH2	25 (43)		194 (40)	
MSH6	6 (10)		100 (21)	
PMS2	1 (2)		3 (1)	

Abbreviation: n, number; IQR, inter quartile-range; BMI, body mass index; kg, kilo gram; m, meter; n/d, no. per day

Notes
[a] IQR inter quartile-range is the 25th-75th percentile
[b] Higher education is a college or university education
[c] Data for smoking status was missing for 1 person

Among current and former smokers, a higher number of pack years was associated with an increased risk of colorectal adenomas: p for trend was 0.03. A higher number of cigarettes smoked per day and a longer duration of smoking were also associated with an increased risk of colorectal adenomas compared to a low number of cigarettes or a shorter duration, although not statistically significant (table 3.2).
Alcohol intake was associated with a slightly increased risk of development of colorectal adenomas, although this was not statistically significant after adjustment for smoking status and other confounding factors; the adjusted HR for the highest tertile of alcohol intake (median 22 g/day) versus the lowest tertile of alcohol intake (0.4 g/day) was 1.33 (0.58-3.05) (table 3.3). Similarly, in the continuous model, the association of alcohol intake and risk of colorectal adenomas was not statistically significant after adjustment for smoking status and other factors. Alcohol intake was also evaluated according to the recommendations of the WCRF; no more than 1 glass/day for women and 2 glasses/day for men [12]. Alcohol intake above the recommendation was associated with a not statistically significantly increased risk of colorectal adenomas: adjusted HR for persons who had an intake above the recommendations versus persons who met the recommendation was 1.38 (0.72-2.65).
There was no interaction between alcohol intake (low or high, based on a median split) and smoking status (never, former, current smokers): p for interaction was 0.98 (table 3.4). Stratified analyses showed that associations for alcohol intake or smoking did not substantially differ between persons with or without a history of colorectal adenomas or carcinomas (data not shown).

Table 3.3 Hazard ratios for alcohol intake and development of colorectal adenomas in a cohort of 486 persons with Lynch syndrome

	Alcohol intake			
	Tertile 1	**Tertile 2**	**Tertile 3**	**per 10 gram**
Intake g/d [median (range)]	0.4 (0-2.7)	7 (2.8-12.5)	22 (13.0-94)	
Cases/cohort	11/162	22/163	25/161	58/486
HR, crude (95% CI)	1.0	1.93 (0.93-4.05)	2.40 (1.17-4.91)	1.20 (1.03-1.46)
HR, smoking-adjusted (95% CI)	1.0	1.75 (0.82-3.73)	1.93 (0.90-4.13)	1.13 (0.96-1.34)
HR, fully adjusted (95% CI) [a)]	1.0	1.70 (0.77-3.75)	1.33 (0.58-3.05)	1.02 (0.82-1.27)

Abbreviation g, gram; d, day; HR, hazard ratio; CI, confidence interval;
Notes a) adjusted for smoking (never, former, current smoker), age, sex, number of colonoscopies (categorical: 0, 1, 2 or 3), colonic resection (yes/no) and BMI (continuous)

Table 3.4 Hazard ratios for smoking and development of colorectal adenomas within sub-categories of low or high alcohol intake in a cohort of 486 persons with Lynch syndrome

	Smoking status			
Alcohol intake, g/d [median (range)] [a]	**Never**	**Former**	**Current**	
Low [1.3 (0-7)]				
Cases/cohort	4/115	11/85	9/42	
HR (95% CI) [b]	1.0	3.36 (1.05-10.72)	8.43 (2.83-25.03)	
High [17 (7-14)]				
Cases/cohort	4/69	18/125	12/49	
HR (95% CI) [b]	1.45 (0.34-6.16)	3.07 (1.08-8.71)	8.19 (2.82-23.79)	
P for interaction				0,98

Abbreviation: g, gram; d, day; HR, hazard ratio; CI, confidence interval;

Notes:
[a] persons were classified as low or high alcohol consumers based on the median of the total population
[b] adjusted for age, sex, number of colonoscopies (categorical: 0, 1, 2 or 3), colonic resection (yes/no) and BMI (continuous)

Discussion

In persons with Lynch syndrome, current smoking was associated with a more than 7-fold increased risk, whereas former smoking was associated with a 2-fold increased risk compared with never smoking. Although there was a trend for alcohol increasing the risk of colorectal adenomas in our cohort of persons with Lynch syndrome, this was not statistically significant after adjustment for smoking.

Two retrospective cohort studies found that smoking was associated with increased risk of colorectal carcinomas in Lynch syndrome [14,15], although their risk estimates for colorectal carcinomas are lower than the estimates for adenomas in this study. Similarly, in studies in the general population, smoking appears to be more strongly associated with sporadic colorectal adenoma than with sporadic colorectal carcinoma occurrence [11,16]. This difference in strength of the association may partly be related to the fact that in colorectal carcinoma studies, reference groups usually did not undergo a colonoscopy [11], while in many adenoma studies reference groups were adenoma-free as ascertained by colonoscopy [16]. Undiagnosed adenoma or (early stage) carcinoma cases in reference groups may attenuate any association between smoking and colorectal carcinomas. This attenuation may also appear in studies on Lynch syndrome [14,15], as not all the persons without colorectal cancer in those studies had undergone colonoscopies to confirm that they were really tumour-free.

The strength of the association between smoking and colorectal adenoma development appears to be stronger in persons with Lynch syndrome than in sporadic adenoma cases. The underlying explanation for this is unknown, but may be that smoking is involved in epigenetic modification of mismatch repair genes [23]. Smoking has been found to be associated with an increased risk of sporadic colorectal carcinomas that show hypermethylation in the promoter region of *MLH1* [23] and with an increased risk of carcinomas that show microsatellite instability [23,24]. Microsatellite instability is one of the features of colorectal adenomas and carcinomas in Lynch syndrome [5,25,26]. As persons with Lynch syndrome have a germ-line mutation in one allele of a mismatch repair gene, disruption of the unaffected copy can result in microsatellite instability and ultimately into colorectal carcinoma [27]. It has been suggested that hypermethylation of the promoter of the *MLH1* [28] and *MSH2* [29] genes can serve as a second hit in Lynch syndrome.

Our findings suggest that smoking cessation may be beneficial for persons with Lynch syndrome who smoke, as it will lower their risk of colorectal adenomas compared with persons who continue to smoke. Our findings contrast with the findings from an earlier study on colorectal carcinomas in persons with Lynch syndrome from the Colon Cancer Family registry and the Texas MD Anderson Cancer Centre [14] that found a decreased risk for former smokers compared with never smokers. The authors of that paper acknowledged that their findings could be a result of bias, as a large body of evidence for several types of cancer shows that although smoking cessation decreases the risk of cancer, the risk usually remains elevated, compared with persons who have never smoked [30,31]. In our own cohort, due to a lack of power, it was not possible to further study the association of smoking history in former smokers - e.g. by stratifying for the numbers of years since quitting.

Alcohol intake was not statistically significantly associated with increased risk of colorectal adenoma occurrence in Lynch syndrome after adjustment for smoking. In the general population, alcohol intake is associated with a modest increased risk of colorectal adenomas. For instance, in the EPIC-Heidelberg cohort [32], alcohol intake

between 15-30 g/day was associated with increased risk for colorectal adenomas (OR for 1.55 (95% CI 1.19, 2.02) compared with the lowest intake group (<5 g/day). Although our findings are in agreement with earlier findings in persons with Lynch syndrome [13,15], the absence of a statistically significant association may merely be a result of the limited size of our cohort. While this is one of the largest prospective cohorts of confirmed cases of Lynch syndrome, its size narrows the extent to which we can observe associations. The power to observe associations with alcohol intake could be further diminished by the fact that alcohol intake usually varies extensively within-persons, particularly when alcohol intake is high [33]. Moreover, although self-reported frequency questionnaires are considered to give valid and reliable estimations of alcohol intake over short reference periods, this short period may not be representative for long-term intake of alcohol [34].

An important strength of our study in comparison to other studies is the prospective design, which has the advantage that information on smoking and alcohol was collected before the events of interest. The extensive baseline questionnaires allowed us to explore confounding of our results by several demographic, clinical and lifestyle factors and to perform multivariate adjusted analyses. Nevertheless, residual confounding by other lifestyle factors may still partly explain our findings. Other strengths are the high participation rate in our cohort and the inclusion of only confirmed mismatch repair-gene mutation carriers. These factors make our findings generalizable to regularly screened persons with Lynch syndrome in other clinical series.

Our findings can help to formulate recommendations on smoking and alcohol intake to lower the risk of colorectal carcinomas for Lynch syndrome affected persons. However, such recommendations are only valid if modification of colorectal adenoma risk translates into a change of colorectal carcinoma risk and thus only when colorectal carcinomas develop through the adenoma-carcinoma pathway. That this pathway is indeed important, is supported by the fact that previous surveillance studies showed that through polypectomy the incidence of colorectal carcinomas was lowered in persons with Lynch syndrome [17,18]. Concluding, our results suggest that modifiable lifestyle factors clearly affect colorectal adenoma risk in persons with Lynch syndrome. As these persons have a high risk of developing colorectal cancer, any lifestyle modification that could help to lower this risk is vital for these persons. Lifestyle advice on smoking cessation should become standard care during the clinical screening of these persons.

Acknowledgements

We are indebted to all study participants for their cooperation. We thank Mary Velthuizen and Alice Donselaar (Netherlands Foundation for Detection of Hereditary Tumours), Maria van Vugt (Radboud University Nijmegen Medical Centre), and Leontien Witjes (Wageningen University), for assistance with participant recruitment and data collection. The medical specialists of the participants are gratefully acknowledged for their collaboration.

References

1 Barrow E, Alduaij W, Robinson L, *et al.* Colorectal cancer in HNPCC: cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. Clin Genet 74:233-242, 2008
2 Hampel H, Stephens JA, Pukkala E, *et al.* Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 129:415-421, 2005
3 Jenkins MA, Baglietto L, Dowty JG, *et al.* Cancer risks for mismatch repair gene mutation carriers: a population-based early onset case-family study. Clin Gastroenterol Hepatol 4:489-498, 2006
4 Quehenberger F, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the *hMLH1* and *hMSH2* gene: correction for ascertainment. J Med Genet 42:491-496, 2005
5 de Jong AE, Morreau H, Van Puijenbroek M, *et al.* The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 126:42-48, 2004
6 Rijcken FEM, Hollema H, Kleibeuker JH. Proximal adenomas in hereditary non-polyposis colorectal cancer are prone to rapid malignant transformation. Gut 50:382-386, 2002
7 de la Chapelle A. The incidence of Lynch syndrome. Fam Cancer 4:233-237, 2005
8 Lynch HT, Lynch PM, Lanspa SJ, *et al.* Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet 76:1-18, 2009
9 Kempers MJE, Kuiper RP, Ockeloen CW, *et al.* Risk of colorectal and endometrial cancers in EPCAM deletion-positive Lynch syndrome: a cohort study. Lancet Oncol 12:49-55, 2011
10 Kuiper RP, Vissers LELM, Venkatachalam R, *et al.* Recurrence and variability of germline EPCAM deletions in Lynch syndrome. Hum Mutat 32:407-414, 2011
11 Botteri E, Iodice S, Bagnardi V, *et al.* Smoking and colorectal cancer: a meta-analysis. JAMA 300:2765-2778, 2008
12 WCRF/AICR: Food, nutrition, physical activity, and the prevention of cancer: a global perspective. (ed 2nd). Washington DC, AICR, 2007
13 Diergaarde B, Braam H, Vasen HF, *et al.* Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 5:736-742, 2007
14 Pande M, Lynch PM, Hopper JL, *et al.* Smoking and colorectal cancer in Lynch syndrome: results from the Colon Cancer Family Registry and the University of Texas M.D. Anderson Cancer Center. Clin Cancer Res 16:1331-1339, 2010
15 Watson P, Ashwathnarayan R, Lynch HT, *et al.* Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). Arch Intern Med 164:2429-2431, 2004
16 Botteri E, Iodice S, Raimondi S, *et al.* Cigarette smoking and adenomatous polyps: a meta-analysis. Gastroenterology 134:388-395, 2008
17 Järvinen HJ, Aarnio M, Mustonen H, *et al.* Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 118:829-834, 2000
18 Vasen HFA, Abdirahman M, Brohet R, *et al.* One to 2-year surveillance intervals reduce risk of colorectal cancer in families with Lynch syndrome. Gastroenterology 138:2300-2306, 2010
19 Botma A, Nagengast FM, Braem MGM, *et al.* Body mass index increases risk of colorectal adenomas in men with Lynch syndrome: the GEOLynch cohort study. J Clin Oncol 28:4346-4353, 2010
20 Feunekes G, Van Staveren W, De Vries J, *et al.* Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr 58:489-496, 1993
21 Verkleij-Hagoort AC, de Vries JHM, Stegers MPG, *et al.* Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. Eur J Clin Nutr 61:610-615, 2006
22 Netherlands Nutrition Center: NEVO: Dutch Food Composition Table 2001. The Hague, Netherlands Nutrition Center, 2001
23 Limsui D, Vierkant RA, Tillmans LS, *et al.* Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst 102:1012-1022, 2010
24 Slattery ML, Curtin K, Anderson K, *et al.* Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J Natl Cancer Inst 92:1831-1836, 2000
25 Iino H, Simms L, Young J, *et al.* DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut 47:37-42, 2000
26 Peltomäki P, Lothe RA, Aaltonen LA, *et al.* Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res 53:5853-5855, 1993
27 Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 138:2073-2087, 2010
28 Herman JG, Graff JR, Myohanen S, *et al.* Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 93:9821-9826, 1996
29 Nagasaka T, Rhees J, Kloor M, *et al.* Somatic hypermethylation of *MSH2* is a frequent event in Lynch syndrome colorectal cancers. Cancer Res 70:3098-3108, 2010
30 Alberg AJ, Samet JM. Epidemiology of lung cancer. Chest 123:21S-49S, 2003

31 Peto R, Darby S, Deo H, *et al.* Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. BMJ 321:323-329, 2000
32 Hermann S, Rohrmann S, Linseisen J. Lifestyle factors, obesity and the risk of colorectal adenomas in EPIC-Heidelberg. Cancer Causes Control 20:1397-1408, 2009
33 Greenfield TK. Ways of measuring drinking patterns and the difference they make: experience with graduated frequencies. J Subst Abuse 12:33-49, 2000
34 Del Boca FK, Darkes J. The validity of self-reports of alcohol consumption: state of the science and challenges for research. Addiction 98:1-12, 2003

Cosmop

Prudent

S

Mea

4

Dietary patterns and colorectal adenomas in Lynch syndrome

The GEOLynch cohort study

Akke Botma
Hans F.A. Vasen
Fränzel J.B. van Duijnhoven
Fokko M. Nagengast
Ellen Kampman

Submitted

Abstract

Purpose
Lynch syndrome (LS) patients have a high risk of developing colorectal cancer due to mutations in mismatch repair genes. Because dietary factors, alone and in combination, influence sporadic colorectal carcinogenesis, we assessed whether dietary patterns are associated with colorectal adenomas in LS patients.

Patients and methods
In the GEOLynch cohort of 486 persons with LS, dietary information was collected using a food frequency questionnaire. Dietary pattern scores were obtained by principal components analysis. Hazard ratio's (HR) between dietary patterns and colorectal adenomas were calculated using Cox regression models. Robust sandwich variance estimates were used to control for dependency within families. Models were adjusted for age, sex, smoking habits, colorectal adenoma history, and extent of colon resection.

Results
During a median follow-up of 20 months, colorectal adenomas were detected in 58 persons. Four dietary patterns were identified: (i) a 'Prudent', (ii) 'Meat', (iii) 'Snack', and (vi) 'Cosmopolitan' pattern. Individuals within the highest tertile of the 'Prudent' pattern had a hazard ratio (HR) of 0.73 (95% CI, 0.32-1.66) for colorectal adenomas, compared with the lowest tertile. Those with high 'Meat' pattern scores had a HR of 1.70 (95% CI, 0.83-3.52). A high 'Snack' pattern was associated with an increased risk of colorectal adenomas (HR, 2.16; 95% CI, 1.03-4.49). A HR of 1.25 (95% CI, 0.61-2.55) was observed for persons in the highest tertile of the 'Cosmopolitan' pattern.

Conclusion
In conclusion our findings suggest that dietary patterns may be associated with risk of colorectal adenomas in Lynch syndrome patients. The directions of these findings are corroborative with those observed in cohorts investigating sporadic CRC.

This work was supported by the Dutch Cancer Society (grant number UW-2005-3275).

Introduction

Lynch syndrome (LS) is a dominantly inherited syndrome characterized by development of colorectal cancer (CRC), endometrial cancer and other cancers at an early age [1-5]. The syndrome is caused by germline mutations in genes involved in DNA mismatch repair (MMR), *MLH1*, *MSH2*, *MSH6*, *PMS2*, or EPCAM [6,7]. The risk to age 70 years of developing CRC in LS lies between 22 and 69% [1-4]. In addition, MMR gene mutation carriers have an increased risk of developing colorectal adenomas at a younger age compared with noncarriers from Lynch syndrome families [8]. The clinical expression of LS patients varies between geographic regions [9]. Moreover, the risk of CRC varies in and between families [10]. Possible explanations for these differences are the influence of modifier genes, lifestyle or dietary factors.

Numerous studies have investigated the association between single foods and sporadic CRC. There is general agreement that red and processed meat and alcohol increase the risk of sporadic colorectal neoplasms [11]. The influence of other foods or food groups is less convincing [11]. Recognition of the interactive and synergistic effects between foods, explain the increased research focus on the effect of dietary patterns. Several epidemiological studies show that dietary patterns indeed influence the risk of sporadic colorectal adenomas and cancer [12-16]. Two cohort studies associate increasing consumption of a 'Western' diet with an increased risk of colon adenomas, colon cancer [14,15], or colon cancer recurrence [12]. Another cohort showed both an increased risk of CRC for a higher intake of the 'Meat and potato' pattern and a decreased risk for persons with higher consumption of the 'Fruit and vegetable' pattern [13].

Only few studies evaluated lifestyle and dietary factors and colorectal neoplasms in patients with LS [17-21]. The only studies [17,19] reporting on dietary factors were from our group showing that increased fruit consumption and dietary fibre intake possibly decrease the risk of colorectal neoplasms [17,19]. This case-control study included both MMR gene mutation carriers and untested individuals who were suspected of LS. The current study examined dietary patterns in a prospective cohort restricted to MMR gene mutation carriers. The aim was to evaluate the association between dietary patterns, identified by using principal component analysis, and colorectal adenoma development.

Patients and Methods

Study population

Eligible MMR gene mutation carriers for this prospective cohort study [21], the GEOLynch cohort study, were identified from families registered at the Netherlands Foundation for the Detection of Hereditary Tumours (NFDHT) in Leiden, the Radboud University Nijmegen Medical Centre (RUNMC) in Nijmegen, and the University Medical Centre Groningen (UMCG) in Groningen (all in the Netherlands). Eligible subjects were Dutch-speaking, Caucasian, mentally competent to participate, men and women between 18 - 80 years of age who were screened regularly by colonoscopy. Terminally ill patients, and those with familial adenomatous polyposis, inflammatory bowel diseases, proctocolectomy, or colostomy were excluded. Between July 2006 and July 2008, 713 MMR gene mutation carriers were identified. The medical specialist of these subjects approved to contact their patients and 499 (73%) agreed to participate. The final study included 486 participants, because necessary questionnaire or medical data was incomplete for 13 participants. Approval for this study was obtained from the Medical Ethical committee of the RUNMC. All participants provided written informed consent.

Dietary assessment and determination of dietary patterns

At baseline, we collected information on diet and lifestyle habits, medication use, physical activity [22] and relevant medical history. Dietary intake information was collected using a self-administered food frequency questionnaire (FFQ), i.e., a 183-item questionnaire developed to asses habitual food intake of the previous month. This FFQ was an updated version of previously validated FFQ's [23,24]. The questionnaire asked for frequency of use on a scale of frequency categories: not this month, once per month, 2-3 times per month, once per week, 2-3 times per week, 4-5 times per week, 6-7 times per week. The number of servings per time point was asked in natural (e.g. orange, slice) or household units (e.g. glass, spoon). Questions on vegetables and fruits were specified with respect to season. Frequencies per day and standard portion sizes were multiplied to obtain grams per day for each food item. Energy intake was calculated using the Dutch food composition table [25]. When questionnaires were returned incomplete, participants were contacted by phone.

To identify dietary patterns, we used principal component analysis (PCA) to aggregate the dietary variables. First, the 183 food items of the FFQ were grouped into 87 food groups. Foods were grouped according to type of food (e.g., broccoli, cauliflower and cabbage were combined into cruciferous vegetables). Per person the intake of every food group (grams per day) was divided by the total daily energy intake (kcal) and multiplied by 1,000. This was done because we were interested in the composition of the diet, independent of the kilocalories consumed per day. These intake variables (grams/day per 1000 kcal) were used in the PCA to construct dietary patterns. Eventually, we retained 4 dietary patterns. First, components with an eigenvalue greater than one (33 of 87) were selected. Second, inspection of the Scree plot, a plot of the eigenvalues by number of components, indicated a final number of four or six dietary patterns. The Scree plot levelled off after the fourth and sixth component. Finally, we ran the PCA three times with a defined number of components, i.e. 4, 5 or 6, and selected four patterns based on the interpretability of all components retained with these runs. To achieve a simpler structure and easier interpretability, components were rotated by an orthogonal transformation, Varimax rotation in SAS. The four dietary patterns were labeled as the 'Prudent', 'Meat', 'Snack' and 'Cosmopolitan' pat-

tern. We calculated dietary pattern scores by summing a persons' food group intake, multiplied by its component (dietary pattern) loading for each food group (i.e. correlations with the patterns). The influence of food grouping on the patterns retained was checked by repeating the PCA with all 183 original food items. The same patterns emerged.

Identification of colorectal adenoma cases

Colonoscopy follow-up data was collected at the LS family registry at the NFDHT [8], and from medical records at the two hospitals, RUNMC, and UMCG up to at least January 31 2009. Also, information about all previous performed colonoscopies, surgical interventions, and cancer and adenoma occurrences was gathered. For each colonoscopy, information on number of neoplasms, plus location, size and histology of these was collected.

Statistical analyses

Risk of developing colorectal adenomas was estimated by calculating hazard ratios (HR's) and 95% confidence intervals (95% CI) using Cox regression. Because some participants were members of the same family, standard errors were calculated by computing robust sandwich estimates of the covariance matrix clustering on family membership to account for dependence of observations. Person-time started at the time of questionnaire completion and ended at the date of first adenomatous polyp diagnosis, date of colorectal cancer diagnosis, date of diagnosis of metastasis or date of death, whichever occurred first. Colorectal tumour-free carriers were censored at January 31 2009 or at the date of their last known colonoscopy if later than January 31 2009 to June 30 2009 at the latest.

Dietary pattern scores were grouped into tertiles based on the total cohort, with the lowest tertile being the reference group. The following variables were evaluated for confounding using backward selection: age (continuous), sex, smoking habits (current, former, never), regular use of NSAID's (more or less than once 1 week), physical activity (tertiles), colorectal adenoma history (yes/no), extent of colorectal resection (none, partial, or subtotal colectomy), and number of endoscopies during follow-up (continuous). Variables remained in the model if removing them changed a dietary pattern score tertile HR by 10% or more. Extra adjustment for body mass index (BMI) was performed to see whether the influence of dietary patterns on colorectal adenomas was (partly) explained by BMI. Energy intake was not considered as confounder, because the amount of energy consumed is interwoven within the dietary patterns. Part of the cohort did not yet receive a colonoscopy during study follow-up. Therefore sensitivity analyses were done, one analysis in which we assumed that all these persons had an adenoma, and an analysis in which the cohort was restricted to those persons who did have a colonoscopy. To test linear trends we entered dietary pattern scores continuously in the models. All statistical tests were two-sided. Cox regression models were tested for and met the proportional hazard assumption. All analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

Dietary patterns

PCA identified 4 dietary patterns in this LS patients cohort. The component loadings, which are correlations between foods and dietary patterns, are shown in table 4.1. Positive loadings indicate positive correlations between foods and dietary patterns, and negative loadings indicate inverse correlations with a dietary pattern. The 'Prudent' pattern heavily loaded (≥0.30) on several types of fruits and vegetables, whole grains, non-fat yoghurt and curd, low-fat cheese, poultry, fish, dressings, green and herbal tea, and added sweets. The 'Meat' pattern heavily loaded on poultry, beef, pork, minced meat, processed meat, and coffee and negatively loaded on whole grains, peanut butter, cakes and cookies, vegetarian products, and soy-based desserts. The 'Snack' pattern heavily loaded on chips, fried snacks, fast food snacks, spring rolls, mayonnaise based sauces, cooking fat and butter, peanut sauce, ketchup, sweets and diet sodas. The 'Cosmopolitan' pattern heavily loaded on leafy vegetables, tomatoes, and allium vegetables, refined grains, fish, dressings, tomato sauce, cream, low fat margarine, sweet sandwich spread, and wine.

Table 4.1 Rotated component loadings for the 4 major principal components of 87 food items/groups from the food-frequency questionnaire of the Lynch syndrome cohort

Foods	Factor 1 'Prudent'	Factor 2 'Meat'	Factor 3 'Snack'	Factor 4 'Cosmopolitan'
Legumes	**0.45**	...	...	...
Cruciferous vegetables	**0.63**	...	...	...
Leafy vegetables	**0.54**	...	...	**0.30**
Carrots	**0.63**	**- 0.30**	...	...
Tomatoes	**0.53**	...	...	**0.39**
Allium vegetables	**0.37**	- 0.21	...	**0.34**
Other vegetables	0.21	0.17	...	...
Citrus & kiwi fruit	**0.48**	...	- 0.25	...
Bananas	0.25	- 0.22	...	...
Apples and pears	**0.56**	...	- 0.16	- 0.18
Other fruits	**0.49**	...	- 0.16	...
Potatoes cooked	...	0.28	...	- 0.25
Chips	- 0.22	...	**0.51**	...
Refined pasta, noodles and rice	...	...	0.26	**0.30**
Wholegrain pasta, noodles and rice	**0.32**	**- 0.38**	...	...
Pizza	...	...	0.24	0.29
Pancakes	...	- 0.23	...	- 0.19
Breakfast cereals, low/medium fibre	0.17	...	...	...
Cereals high in fibre	0.21	...	...	...
white rusk, matza, cream crackers	- 0.19	...	...	...
White bread	- 0.26	...	...	...
Wholewheat rusk, wholewheat cracker	0.26	- 0.20	...	...
Brown bread	- 0.17	...	...	- 0.27
Wholewheat bread, rye bread	0.19	...	- 0.24	...
Milk and fruit juice based breakfast	...	...	0.16	...
Fat milk	...	...	- 0.19	...
Non-fat milk	0.28	...	...	...
Low fat yoghurt and curd	...	...	...	- 0.21
Non-fat yoghurt, custard and curd	**0.54**	...	...	...
Curd, pudding and mousse, Ice cream	...	...	0.21	...

Table 4.1 Continued

Foods	Factor 1 'Prudent'	Factor 2 'Meat'	Factor 3 'Snack'	Factor 4 'Cosmopolitan'
Cream	...	...	...	**0.32**
Coffeemilk (fat)	...	0.20	...	...
Coffeemilk (low fat)	...	0.17	...	- 0.20
Low fat cheese	**0.40**	...	...	...
Cheese (fat)	...	...	- 0.27	0.28
Cheese (luxury & fat)	...	...	...	0.23
Organ meat	...	0.17	...	...
Poultry	**0.38**	**0.37**	0.17	...
Beef	0.21	**0.54**	...	...
Pork	...	**0.48**	0.15	- 0.25
Other meat	...	...	...	0.16
Minced meat	...	**0.43**	0.21	...
Processed meat	...	**0.40**	...	- 0.17
Fish	**0.40**	...	...	**0.34**
Eggs	...	0.18	...	...
Cooking fat and butter	- 0.16	...	**- 0.34**	0.21
Low fat margarine	...	...	0.20	**- 0.37**
Margarine	- 0.27	0.16	- 0.22	...
Oils	0.28	...	...	0.23
Dressings	**0.34**	...	...	**0.49**
Ketchup	...	...	**0.54**	...
Mayonaise based sauces	...	...	**0.46**	...
Tomato sauce	...	...	...	**0.39**
Peanutsauce	- 0.16	...	**0.37**	...
Mushroom cream sauce	...	...	0.19	0.22
Fried snacks	- 0.21	0.22	**0.49**	...
Fast food snacks	- 0.20	...	**0.44**	...
Spring rolls	...	...	**0.33**	...
Kebab (snack)	...	...	0.25	...
Crisps	- 0.26	- 0.19	0.25	...
Cream cracker with spread	...	0.17	...	...
Nuts and seeds	...	...	...	0.24
Sandwich spread	...	...	0.18	...
Peanut butter	...	**- 0.44**	...	...
Sweet sandwich spread	...	- 0.25	...	**- 0.39**
Cakes and cookies	...	**- 0.32**	...	- 0.27
Added sweet	**- 0.45**	...	- 0.19	...
Chocolates, milk & white	- 0.19	- 0.29	0.25	...
Sweets	...	- 0.18	**0.30**	...
Coffee	...	**0.40**	...	...
Black tea	...	...	- 0.16	...
Green & herbal tea	**0.39**	- 0.25	...	...
Vegetable juice	0.17	- 0.19	- 0.17	...
Soda	- 0.23	...	0.19	...
Non-sugar soda	...	...	**0.36**	...
Beer	- 0.25	...	...	...
Wine	...	...	- 0.20	**0.38**
Spirits	...	0.22	...	...
Vegetarian products	0.22	**- 0.54**	...	...
Soy dessert	...	**- 0.31**	...	...
Soymilk	0.16	...	...	...
Soup	...	0.22	...	...

Notes
Factor loading less than |0.15| were omitted for simplicity
Loadings greater than |0.29| are bold
Foods for which all loadings were less than |0.15| were not shown; Dark chocolate, Bread (multicorn), Low fat milk Yoghurt and custard and Fruit juice

Baseline characteristics

Cases were more often men, slightly older and lower educated compared to the total cohort. There were more current smokers among the cases, and alcohol intake appeared to be slightly higher. In addition, cases were more likely to have had colorectal adenomas in the past (data not shown). Table 4.2 show baseline characteristics of the cohort by tertiles of each dietary pattern score. Participants with higher 'Prudent' pattern scores were older, more likely to be women, more physically active, less likely to be current smokers and had lower energy intakes compared with those with low 'Prudent' pattern scores. Participants with high 'Meat' pattern scores were older, more likely to be men, less likely to have higher education, more often current smokers, had a higher BMI, slightly lower energy intakes, and slightly higher median alcohol intakes compared with participants in the lowest tertile of the 'Meat' pattern scores. Participants with higher 'Snack' pattern scores tended to be younger, were having a higher BMI and had slightly higher median alcohol intakes, than those with low 'Snack' pattern scores. Persons in the highest tertile fo the 'Snack' patterns were more likely to be women compared to those in the lowest tertile. Participants with higher 'Cosmopolitan' pattern scores were more likely to be women, slightly younger, more likely to have college or university education, to be less physically active, had higher median alcohol intakes, lower BMI's, and used NSAID's more regularly than those with low 'Cosmopolitan' pattern scores.

Table 4.2 Baseline characteristics of the Lynch syndrome cohort stratified by tertile of dietary pattern scores

		Dietary pattern		
	Unit	Tertile 1 (Low)	Tertile 2	Tertile 3 (High)
Factor 1, 'Prudent' pattern				
Total cohort	n	161	165	160
Adenomatous polyp cases	n	23	19	16
Person-months	median	19.7	19.8	20.1
Age, years	median	45.2	49.2	53.7
Sex, female	%	40.4	63.0	74.4
Education, higher [a)]	%	31.1	36.4	34.4
BMI, kg/m²	median	24.6	24.5	24.5
Energy intake, kcal/d	median	2423.1	2103.1	1786.1
Physical activity, high [b)]	%	22.0	37.1	40.5
Smoking status				
Current	%	26.7	18.8	10.6
Former	%	37.9	40.0	51.9
Alcohol intake, g/d	median	8.5	7.3	5.5
NSAID use, regular [c)]	%	13.7	13.3	10.6
MMR gene mutation				
MLH1	%	39.8	33.3	42.5
MSH2	%	41.0	41.8	36.9
MSH6	%	18.0	24.2	19.4
History of colorectal cancer	%	21.1	23.6	33.8
History of other cancer	%	8.7	21.8	24.7
History of colorectal adenoma	%	31.1	32.1	33.1
Colon surgery				
partial colon resection	%	14.9	17.0	25.0
Subtotal colectomy	%	8.7	9.1	10.0
No. of colonoscopies during follow-up	median	1.0	1.0	1.0
Time (months) between colonoscopies [d)]				
≤24	%	58.4	57.6	58.1

Tabel 4.2 Continued

	Unit	Dietary pattern		
		Tertile 1 (Low)	Tertile 2	Tertile 3 (High)
Factor 2, 'Meat' pattern				
Total cohort	n	160	165	161
Adenomatous polyp cases	n	12	16	30
Person-months	median	20.0	20.1	19.2
Age, years	median	44.6	51.2	54.1
Sex, female	%	65.6	59.4	52.8
Education, higher [a)]	%	48.1	27.3	26.7
BMI, kg/m²	median	24.1	24.4	25.6
Energy intake, kcal/d	median	2201.4	2053.1	1909.9
Physical activity, high [b)]	%	34.4	37.8	27.1
Smoking status				
Current	%	9.4	21.2	25.5
Former	%	45.6	36.4	47.8
Alcohol intake, g/d	median	4.5	8.1	9.2
NSAID use, regular [c)]	%	13.1	13.9	10.6
MMR gene mutation				
MLH1	%	35.6	35.2	44.7
MSH2	%	45.0	43.6	31.1
MSH6	%	18.8	19.4	23.6
History of colorectal cancer	%	29.3	24.8	24.2
History of other cancer	%	13.8	23.6	17.4
History of colorectal adenoma	%	28.1	29.1	39.1
Colon surgery				
partial colon resection	%	30.2	18.8	18.6
Subtotal colectomy	%	11.3	7.3	9.3
No. of colonoscopies during follow-up	median	1.0	1.0	1.0
Time (months) between colonoscopies [d)]				
≤24	%	60.6	59.4	54.0
Factor 3, 'Snack' pattern				
Total cohort	n	160	166	160
Adenomatous polyp cases	n	17	23	18
Person-months	median	20.1	19.7	19.8
Age, years	median	57.3	50.0	41.9
Sex, female	%	57.5	53.0	67.5
Education, higher [a)]	%	38.1	28.9	35.0
BMI, kg/m²	median	24.0	24.5	24.9
Energy intake, kcal/d	median	2111.8	2079.9	2006.1
Physical activity, high [b)]	%	34.4	36.0	29.1
Smoking status				
Current	%	17.5	16.9	21.9
Former	%	46.9	47.0	35.6
Alcohol intake, g/d	median	9.5	7.0	4.7
NSAID use, regular [c)]	%	10.0	10.8	16.9
MMR gene mutation				
MLH1	%	36.3	41.6	37.5
MSH2	%	39.4	37.3	43.1
MSH6	%	24.4	18.7	18.8
History of colorectal cancer	%	35.0	27.1	16.3
History of other cancer	%	28.8	17.5	8.8
History of colorectal adenoma	%	37.5	34.3	24.4
Colon surgery				
partial colon resection	%	29.4	16.9	10.6
Subtotal colectomy	%	8.1	12.7	6.9
No. of colonoscopies during follow-up	median	1.0	1.0	1.0
Time (months) between colonoscopies [d)]				
≤24	%	61.3	60.2	52.5

Tabel 4.2 Continued

	Unit	Dietary pattern		
		Tertile 1 (Low)	Tertile 2	Tertile 3 (High)
Factor 4, 'Cosmopolitan' pattern				
Total cohort	n	160	165	161
Adenomatous polyp cases	n	20	16	22
Person-months	median	19.9	20.1	19.7
Age, years	median	52.8	49.5	48.1
Sex, female	%	57.5	59.4	60.9
Education, higher [a)]	%	21.3	35.8	44.7
BMI, kg/m²	median	25.1	24.4	24.2
Energy intake, kcal/d	median	2092.5	2026.1	2060.6
Physical activity, high [b)]	%	43.0	30.9	26.0
Smoking status				
Current	%	16.3	14.6	25.5
Former	%	35.6	51.5	42.2
Alcohol intake, g/d	median	2.7	8.5	11.3
NSAID use, regular [c)]	%	6.9	15.8	14.9
MMR gene mutation				
MLH1	%	40.0	38.2	37.3
MSH2	%	39.4	40.0	40.4
MSH6	%	18.8	21.2	21.7
History of colorectal cancer	%	23.8	27.3	27.3
History of other cancer	%	18.1	19.4	28.0
History of colorectal adenoma	%	37.5	27.3	31.7
Colon surgery				
partial colon resection	%	19.4	30	31
Subtotal colectomy	%	7.5	10.3	9.9
No. of colonoscopies during follow-up	median	1.0	1.0	1.0
Time (months) between colonoscopies [d)]				
≤24	%	58.1	55.8	60.2

Abbreviation: n, number; BMI, body mass index; kg, kilo gram; m, meter; kcal, kilocalories; d, day; g, gram; NSAID, non-steroidal anti-inflammatory drugs

Notes:
a) Higher education is a college or university education
b) High physical activity is the highest tertile of the physical activity score
c) Regular NSAID use is one or more times per month
d) Time between the last colonoscopy before and the first colonoscopy after baseline, seven carriers did not have a colonoscopy before baseline

Influence of dietary patterns on adenoma development

During a median follow-up of 20 months, 58 out of 486 (12%) MMR gene mutation carriers developed histologically confirmed colorectal adenomas. Thirteen of these adenomas had an advanced adenoma pathology (i.e. larger than 1cm, with villous architecture, or high grade dysplasia).

In table 4.3 associations between the four dietary patterns and colorectal adenomas are presented. Persons with the highest 'Prudent' pattern scores (third tertile) had a HR of 0.61 (95% CI, 0.28-1.32) of developing colorectal adenomas, comparing them with the lowest tertile of intake and adjusted for age and sex. With additional adjustment for smoking, colorectal adenoma history, and extent of colon resection the HR of developing colorectal adenoma for the highest tertile of Prudent pattern scores was 0.73 (95% CI, 0.32-1.66) compared with the lowest tertile. For the 'Meat' pattern the HR for the highest tertile was 2.48 (95% CI, 1.22-5.02). After additional adjustment for smoking, colorectal adenoma history, and extent of colon resection, a statistically non-significant HR of 1.70 (95% CI, 0.83-3.52) was observed for the highest tertile of the 'Meat' pattern scores versus the lowest tertile. Those within the high-

est tertile of 'Snack' pattern scores had an increased risk of developing colorectal adenomas ('Snack' pattern: HR, 2.13; 95% CI, 0.99-4.60) compared with the lowest tertile, adjusted for age and sex. With additional adjustment for smoking, colorectal adenoma history, and extent of colon resection the HR of developing colorectal adenoma for the highest tertile was 2.16 (95% CI, 1.03-4.49). The highest tertile of 'Cosmopolitan' pattern scores had a higher HR (age and sex-adjusted HR, 1.25; 95% CI, 0.64-2.43) of colorectal adenoma development than the lowest tertile. No change in HR was observed after adjustment for smoking, colorectal adenoma history, and extent of colon resection (HR 'Cosmopolitan' pattern highest versus lowest tertile, 1.25; 95% CI, 0.61-2.55).

Including BMI in the models did not substantially change the HR's of all dietary patterns. Extra adjustment for energy intake, which might be considered as part of the dietary patterns or as intermediate variable of the associations between dietary patterns and colorectal adenoma development, changed the HR's in 3 of the 4 dietary patterns with more than 10% ('Prudent' pattern HR, 0.51; 95% CI, 0.21-1.20; 'Meat' pattern HR, 1.44; 95% CI, 0.66-3.11; 'Snack' pattern HR, 1.62; 95% CI, 0.79-3.76; 'Cosmopolitan' pattern HR, 1.35; 95% CI, 0.66-2.76; data not shown).

Table 4.3 Hazard Ratios (95% Confidence Interval) of colorectal adenomas occurrence according to tertiles of dietary pattern scores of the Lynch syndrome cohort

	Dietary pattern			
	Tertile 1 (Low) HR	Tertile 2 HR (95% CI)	Tertile 3 (High) HR (95% CI)	P for trend
Factor 1: 'Prudent' pattern				
cases | total cohort	23 | 161	19 | 165	16 | 160	
person-months	19.7	19.8	20.1	
age & sex-adjusted	1.0	0.77 (0.41-1.45)	0.61 (0.28-1.32)	0.39
multivariate-adjusted [a)]	1.0	0.85 (0.47-1.54)	0.73 (0.32-1.66)	0.78
Factor 2: 'Meat' pattern				
cases | total cohort	12 | 160	16 | 165	30 | 161	
person-months	20.0	20.1	19.2	
age & sex-adjusted	1.0	1.29 (0.61-2.75)	2.48 (1.22-5.02)	0.02
multivariate-adjusted [a)]	1.0	1.05 (0.49-2.28)	1.70 (0.83-3.52)	0.21
Factor 3: 'Snack' pattern				
cases | total cohort	17 | 160	23 | 166	18 | 160	
person-months	20.1	19.7	19.8	
age & sex-adjusted	1.0	1.80 (0.96-3.40)	2.13 (0.99-4.60)	0.08
multivariate-adjusted [a)]	1.0	1.93 (1.04-3.60)	2.16 (1.03-4.49)	0.12
Factor 4: 'Cosmopolitan' pattern				
cases | total cohort	20 | 160	16 | 165	22 | 161	
person-months	19.9	20.1	19.7	
age & sex-adjusted	1.0	0.74 (0.43-1.27)	1.25 (0.64-2.43)	0.49
multivariate-adjusted [a)]	1.0	0.79 (0.45-1.38)	1.25 (0.61-2.55)	0.56

Abbreviation HR, hazard ratio; CI, confidence interval

Notes [a)] adjusted for age, sex, smoking habits, colorectal adenoma history and extent of colon resection

Sensitivity analysis showed that restricting the analyses to persons with at least one colonoscopy during follow-up (n=384) did not markedly changed associations ('Prudent' pattern HR, 0.73; 95% CI, 0.33-1.64; 'Meat' pattern HR, 1.61; 95% CI, 0.77-3.33; 'Snack' pattern HR, 2.40; 95% CI, 1.15-5.03; 'Cosmopolitan' pattern HR, 1.35; 95% CI, 0.65-2.79; data not shown). In an additional sensitivity analysis, we assumed that all persons without an colonoscopy would have colorectal adenomas. In this situation, the association between the 'Snack' pattern and colorectal adenomas also was statistically significant (HR high versus low, 2.02; 95% CI, 1.30-3.13; data not shown).

Discussion

We identified four dietary patterns, referred to as the 'Prudent', 'Meat', 'Snack' and 'Cosmopolitan' pattern and observed a statistically significant increased risk of adenomas for the 'Snack' pattern. For the 'Prudent' pattern a modest non-statistically significant inverse association with colorectal adenomas was observed. The 'Meat' and 'Cosmopolitan' patterns showed non-statistically significant positive associations.
Previous studies from our group observed that fruit and possibly dietary fibre influenced risk of developing colorectal neoplasms in LS families [17,19]. No other studies on LS and diet or dietary patterns have been conducted so far. Findings observed in the current study were consistent with the associations between dietary patterns and colorectal adenomas in general population cohorts [14-16]. In a cohort of US men [14] two major dietary patterns were obtained, i.e. 'Prudent' and 'Western', that were comparable to the 'Prudent' and the 'Snack' and 'Meat' patterns in our cohort. In that study an increased risk of distal colorectal adenomas was observed with higher 'Western' pattern scores. However, a substantial inverse association was not observed for the 'Prudent' pattern [14]. There are several differences between the two 'Prudent' patterns. Possibly the most important difference is the median amount of alcohol consumed in the highest tertile or quintile. The median alcohol intake in the highest tertile of the 'Prudent' pattern in our cohort (5.5 g/d) was half of the alcohol intake in the highest quintile of the cohort of US men (10.0 g/d). Because alcohol is a risk factor for the development of adenomas [11], differences in alcohol intake might explain differences in observed findings between studies. In a cohort of French women [15], four patterns were identified, i.e. 'Healthy', 'Western', 'Drinker', and 'Meat eaters' pattern. The high loading foods of the 'Healthy' and 'Meat eaters' pattern were largely comparable with the 'Prudent' and 'Meat' pattern in our cohort. The 'Healthy' pattern showed a statistically non-significant inverse association with colorectal adenomas and an increased colorectal cancer risk was seen with high 'Meat' pattern scores. The 'Western' pattern (potatoes, pizza and pie, sandwiches, legumes, sweets, cakes, bread, rice, pasta, processed meat, butter, cheese, and eggs), a combination of high loading foods from our 'Meat', 'Snack' and 'Cosmopolitan' patterns, and the 'Drinker' pattern (snacks, coffee, processed meat, wine, low-alcohol beverages, and high-alcohol beverages), a pattern we did not find, were both associated with an increased risk of colorectal adenomas in this French cohort. In an European study [16] on adenoma recurrence, patterns were derived for men and women separately. For both groups three patterns were obtained (men; 'Mediterranean', 'Sweets and snacks', and 'High fat and proteins'; women; 'Mediterranean', 'Western', and 'Snacks'). Components of the 'Mediterranean' pattern (high olive oil, fresh fruit, vegetables, legumes, lean meat and fresh fish consumption) were mostly comparable to our 'Prudent' diet. High pattern scores were associated with a decreased risk of adenoma recurrence in women only. The 'Snacks' and 'Sweet and snacks' patterns were partly comparable with our 'Snack' pattern, but ours included more fried and fast food snacks, and the 'High fat and protein' pattern included high loading foods from both our 'Meat and Snack' patterns. No associations were seen between the 'Snacks', 'Sweets and Snacks', 'High fat and proteins' or 'Western' patterns and recurrence of colorectal adenomas.
In our study as well as in the studies mentioned above PCA was used to identify dietary patterns. A criticism of this data-driven approach is that the components validity is dependent on the study population. The identified patterns reflect actual

existing dietary behaviour within the studied population. In different populations, or in the same population at a different time, another set of components might have been observed [13]. This limits the interpretation of these dietary patterns and may explain differences between studies, especially differences between studies from different countries with different eating and lifestyle habits. All mentioned studies [14-16], including ours identified a vegetable and fruit pattern ('Prudent', 'Healthy', or 'Mediterranean' patterns) indicating that this pattern does exist in several populations. Furthermore, comparison of our dietary patterns with those from a general Dutch population cohort, indicated that our 'Meat', 'Snack' and 'Cosmopolitan' patterns were similar to the other cohorts 'Traditional', 'Refined foods' and 'Cosmopolitan' patterns [26]. This suggests that our patterns reflect existing dietary patterns in the general Dutch population.

Using PCA requires subjective decisions about the grouping of input variables, the number of retained components, the method of rotation and the labelling of patterns. To study the influence of the food grouping on the PCA results, we performed a PCA with the 183 originally food items from the FFQ. This PCA gave us essentially the same dietary patterns, indicating minimal influence of the grouping. Ideally, performing a PCA in two random samples of the cohort, should have validated these patterns. However, splitting the cohort made the number of participants too small (n=243) to perform a PCA with 87 variables. To make all choices in retaining the number of components as objective and transparent as possible, we described all steps in the method section. The labelling of the identified patterns is subjective, but can be judged from the presented factor loadings (table 4.1).

The dietary patterns were associated with other lifestyle factors, e.g. smoking confounded the association between dietary patterns and colorectal adenoma development. To control for this, we performed a multivariate analysis with adjustments for these lifestyle factors. Still, we cannot completely rule out residual confounding effects because of the possibility of unmeasured confounding variables or variables measured with error. Although thus far this is the largest cohort of MMR gene mutation carriers, the power to detect statistically significant associations may have been limited. In addition, because of insufficient power it was not possible to perform subgroup analysis by sex, history of colorectal tumours or by MMR genes.

An important strength of the study is the dietary pattern approach, in which not only individual foods are considered but the whole diet. This approach takes possible interactions between foods into account and reduces the number of dietary variables, using correlations between these variables, and as such diminishes problems of multicollinearity. Other strengths of this study are the prospective design, the large cohort of MMR gene mutation carriers and high participation rate. This makes these results generalizable to regularly screened LS patients in other clinical series.

Our study provides information on dietary risk factors for the development of adenomas in LS patients. The final purpose is to develop lifestyle and dietary recommendations in order to decrease the risk of developing CRC in this group. However, such recommendations are only valid if CRC associated with LS develops via the adenoma-carcinoma sequence. The fact that the risk of CRC development substantially decreases by removal of adenomas in prospective surveillance studies suggests that the adenoma-carcinoma sequence is also applicable in LS and that decreasing the risk of adenoma by adjusting dietary and lifestyle factors, will also decrease the risk of CRC [27].

In conclusion, our findings suggest that dietary patterns may be associated with risk of colorectal adenomas in MMR gene mutation carriers. The directions of these find-

ings were corroborative with those observed in cohorts investigating sporadic CRC. Although more research is needed to estimate the exact influence of dietary patterns on LS colorectal carcinogenesis, modifiable factors, such as diet, could influence the development of colorectal neoplasms in LS.

Acknowledgements

We are indebted to all study participants for their cooperation. We thank Mary Velthuizen and Alice Donselaar (Netherlands Foundation for Detection of Hereditary Tumours), Maria van Vugt (Radboud University Nijmegen Medical Centre), and Leontien Witjes (Wageningen University), for assistance with participant recruitment and data collection. The medical specialists of the participants are gratefully acknowledged for their collaboration.

References

1 Barrow E, Alduaij W, Robinson L, *et al.* Colorectal cancer in HNPCC: cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. Clin Genet 74:233-242, 2008
2 Jenkins MA, Baglietto L, Dowty JG, *et al.* Cancer risks for mismatch repair gene mutation carriers: a population-based early onset case-family study. Clin Gastroenterol Hepatol 4:489-498, 2006
3 Quehenberger F, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the *hMLH1* and *hMSH2* gene: correction for ascertainment. J Med Genet 42:491-496, 2005
4 Hampel H, Stephens JA, Pukkala E, *et al.* Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 129:415-421, 2005
5 Watson P, Vasen HF, Mecklin JP, *et al.* The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. Int J Cancer 123:444-449, 2008
6 Abdel-Rahman WM, Mecklin JP, Peltomaki P. The genetics of HNPCC: application to diagnosis and screening. Crit Rev Oncol Hematol 58:208-220, 2006
7 Ligtenberg MJL, Kuiper RP, Chan TL, *et al.* Heritable somatic methylation and inactivation of *MSH2* in families with Lynch syndrome due to deletion of the 3[prime] exons of *TACSTD1*. Nat Genet 41:112-117, 2009
8 de Jong AE, Morreau H, Van Puijenbroek M, *et al.* The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 126:42-48, 2004
9 Park JG, Park YJ, Wijnen JT, *et al.* Gene-environment interaction in hereditary nonpolyposis colorectal cancer with implications for diagnosis and genetic testing. Int J Cancer 82:516-519, 1999
10 Mitchell RJ, Farrington SM, Dunlop MG, *et al.* Mismatch repair genes *HMLH1* and *HMSH2* and colorectal cancer: a HuGE review. Am J Epidemiol 156:885-902, 2002
11 WCRF/AICR: Food, nutrition, physical activity, and the prevention of cancer: a global perspective. (ed 2nd). Washington DC, AICR, 2007
12 Meyerhardt JA, Niedzwiecki D, Hollis D, *et al.* Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. JAMA 298:754-764, 2007
13 Flood A, Rastogi T, Wirfalt E, *et al.* Dietary patterns as identified by factor analysis and colorectal cancer among middle-aged Americans. Am J Clin Nutr 88:176-184, 2008
14 Wu K, Hu FB, Fuchs CS, *et al.* Dietary patterns and risk of colon cancer and adenoma in a cohort of men (United States). Cancer Causes Control 15:853-862, 2004
15 Kesse E, Clavel-Chapelon F, Boutron-Ruault MC. Dietary patterns and risk of colorectal tumors: a cohort of French women of the National Education System (E3N). Am J Epidemiol 164:1085-1093, 2006
16 Cottet V, Bonithon-Kopp C, Kronborg O, *et al.* Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. Eur J Cancer Prev 14:21-29, 2005
17 Voskuil DW, Kampman E, Grubben MJAL, *et al.* Meat consumption and meat preparation in relation to colorectal adenomas among sporadic and HNPCC family patients in the Netherlands. Eur J Cancer 38:2300-2308, 2002
18 Watson P, Ashwathnarayan R, Lynch HT, *et al.* Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). Arch Intern Med 164:2429-2431, 2004
19 Diergaarde B, Braam H, Vasen HF, *et al.* Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 5:736-742, 2007
20 Pande M, Lynch PM, Hopper JL, *et al.* Smoking and colorectal cancer in Lynch syndrome: results from the Colon Cancer Family Registry and the University of Texas M.D. Anderson Cancer Center. Clin Cancer Res 16:1331-1339, 2010
21 Botma A, Nagengast FM, Braem MGM, *et al.* Body mass index increases risk of colorectal adenomas in men with Lynch syndrome: the GEOLynch cohort study. J Clin Oncol 28:4346-4353, 2010
22 Baecke J, Burema J, Frijters J. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 36:936-942, 1982
23 Feunekes G, Van Staveren W, De Vries J, *et al.* Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr 58:489-496, 1993
24 Verkleij-Hagoort AC, de Vries JHM, Stegers MPG, *et al.* Validation of the assessment of folate and vitamin B12 intake in women of reproductive age: the method of triads. Eur J Clin Nutr 61:610-615, 2006
25 Netherlands Nutrition Center: NEVO: Dutch food composition table 2001. The Hague, Netherlands Nutrition Center, 2001
26 van Dam RM, Grievink L, Ocke MC, *et al.* Patterns of food consumption and risk factors for cardiovascular disease in the general Dutch population. Am J Clin Nutr 77:1156-1163, 2003
27 Järvinen HJ, Mecklin J-P, Sistonen P. Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 108:1405-1411, 1995

5

BMI, polymorphisms in insulin-like growth factor axis genes and colorectal adenoma recurrence

normal overw obe

Akke Botma
Brenda Diergaarde
Fränzel J.B. van Duijnhoven
Fokko M. Nagengast
Annekatrien C.T.M. Depla
P.A.M. van Hees
Ben J.M Witteman
Hans F.A. Vasen
Ellen Kampman

Abstract

Purpose
Higher body mass index (BMI) is an established risk factor for colorectal adenomas and cancer. Overweight may alter the amount of free insulin-like growth factors (IGF's) via their binding proteins (IGFBP's). These free IGF's can increase colorectal carcinogenesis. Common variation in IGF-axis genes also influence IGF-levels, this influence might be different for overweight and normal weight individuals. We evaluated associations of BMI, common polymorphisms in IGF-axis genes, and their interactions with recurrence of colorectal adenomas in a prospective study.

Patients and methods
Adenoma cases (n=565) from a case-control study (the POLIEP-study), were prospectively followed for adenoma recurrence. Median person-time was 4.7 years. We estimated hazard ratios (HR) and 95% confidence intervals (95% CI) for the associations between overweight (≥25 kg/m²), common single nucleotide polymorphisms (SNP's) in 8 IGF-axis genes and adenoma recurrence.

Results
BMI was not associated with any colorectal adenoma recurrence, nor with recurrence of advanced adenomas. Two of the evaluated SNP's, rs1520220 in *IGF1* and rs3213221 in *IGF2* were statistically significantly associated with risk of developing recurrent advanced adenomas (heterozygotes + minor allele homozygotes versus common allele homozygotes; rs1520220, HR: 2.23, 95% CI: 1.12-4.44; rs3213221, HR: 2.44 95% CI: 1.06-5.63). A *IGF2* gene x overweight interaction was observed for rs1003483 and rs1004446 in *IGF2* (P-interaction=0.03 and 0.001, respectively). For both these SNP's, risk of any adenoma recurrence among normal weight individuals was higher for those with at least one minor allele, while among overweight individuals the risk of recurrence was higher for common allele homozygotes.

Conclusion
Our results suggest that common variation in IGF-axis genes influence the likelihood of colorectal adenoma recurrence and may modify the association between BMI and colorectal adenoma recurrence.

This work was supported by the Dutch Cancer Society (grant UW-2005-3275)

Introduction

Being overweight or obese is positively associated with risk of colorectal cancer. Recent meta-analyses showed that a higher body mass index (BMI) is a convincing risk factor for colon (per five-unit increment RR, 1.21; 95% CI, 1.17-1.26) and rectal cancer (per five-unit increment RR, 1.11; 95% CI, 1.06-1.16), especially among men [1-3]. Also occurrence of the precursor lesion of colorectal cancer, colorectal adenoma, is positively associated with BMI [4-6]. Furthermore, a pooling study of US trials showed an association between BMI and risk of colorectal adenoma recurrence as well, again mainly in men [7].

Overweight and obesity may affect development of colorectal neoplasms through changes in insulin-like growth factor (IGF) signalling. Insulin-like growth factors (IGF's) are proposed to be associated with colorectal neoplasms and IGF blood levels have been reported to vary depending on BMI [8-10]. Activating the signalling of the IGF-pathway probably leads to increased cell proliferation and increased cell survival [11]. IGF signalling is determined by the possibility of the two IGF types, IGF1 and IGF2, to bind with the IGF type 1 receptor (IGF1R). The binding to the IGF1R is not only dependent on the concentrations of IGF1 and IGF2, but also on the concentrations of the six IGF-binding proteins (IGFBP's) and the acid-labile subunit (ALS). When IGF1 is bound in a complex with IGFBP3 and IGFALS it is too large to be transported out of the blood stream and is not available for the receptors. In addition, binding of IGF2 with the IGF type 2 receptor (IGF2R) reduces IGF2 signalling activity by keeping it away from the IGF1R.

A twin study indicated that variation in circulating IGF1, IGF2 and IGFBP3 levels are determined by genetic factors [12]. In line with this, a case-control study based in the United Kingdom [13], using tagging polymorphisms, showed that common variants in *IGF1* (rs1520220) and *IGFBP3* (rs2854744) are associated with IGF1 and IGFBP3 levels. Polymorphisms in *IGF1* (CA dinucleotide repeat [14] and rs6214 [15]) and *IGFBP3* (rs2854746) genes were associated with colorectal cancer [14-16]. Indeed suggesting that SNP's in IGF genes can influence colorectal cancer risk. However, three other studies did not observe associations between *IGF1* (CA dinucleotide repeat) [17-19] or *IGFBP3* variants (whole gene covered) [19,20] and colorectal cancer or adenomas. This inconsistency in results could be due to not taking BMI into account. The effect of genetic variants on colorectal cancer risk may become detectable only in the presence of specific environmental factors. In a case-control study from Seattle [14], the CA dinucleotide repeat polymorphism in *IGF1* as well as the G => C single nucleotide polymorphism (rs2854746) in *IGFBP3* modified the association between BMI and colorectal cancer.

To date, no studies have investigated associations between BMI, IGF polymorphisms and colorectal adenoma recurrence. The objective of this prospective study among colorectal adenoma cases was to assess the association between BMI and colorectal adenoma recurrences. We additionally evaluated whether common polymorphisms in IGF-axis genes modified the association between BMI and recurrence.

Methods

Study population

For this study, colorectal adenoma cases were included, who were recruited for a endoscopy-based case-control study in the Netherlands. Details of this case-control study have been described elsewhere [21,22]. Briefly, participants were recruited among those undergoing endoscopy in 10 outpatient clinics between June 1997 and June 2002. Participants were informed of the study by endoscopy staff at the time of colonoscopy or by mail at 3-month intervals using colonoscopy reports of all patients who had undergone colonoscopy. Eligible participants were Dutch-speaking, Caucasian, between 18 and 75 years of age at time of enrolment, had no hereditary colorectal cancer syndromes, did not suffer from inflammatory bowel diseases, had no personal history of colorectal cancer and had not had a (partial) bowel resection. In addition, we used complete information of 42 adenoma cases meeting our criteria, recruited between December 1995 and June 1997, from a preceding similar study in one of the ten hospitals [23]. In total 768 participants were diagnosed with at least one histologically confirmed colorectal adenomatous polyp ever in their life and eligible for this study. After inclusion in the case-control study ten persons became ineligible due to surgeries for colorectal neoplasms detected at recruitment (n=7), diagnosed proctitis ulcerosa, having a histological unconfirmed adenoma only, and one person was recruited twice into the POLIEP-(case-control) study, leaving 758 subjects eligible. Adenoma recurrence will only be detected through large bowel examinations, therefore persons who did not have a documented colonic examination after recruitment in the hospital of their recruitment endoscopy (n=143) were excluded from this follow-up study. Subjects who could not be traced in the hospital records (n=50) were not included in the follow-up. In total, 565 participants were included in this prospective study.

Data collection

Body Mass Index, diet and lifestyle factors

Self-administered questionnaires were filled out by participants at recruitment according to habits in the year preceding their endoscopy. Height in centimetres and weight in kilograms were assessed using a structured questionnaire also including questions on smoking behaviour, NSAID's usage, hormone replacement therapy usage, physical activity (assessed according to Baecke *et al.* [24]), number of first- and second degree family members with colorectal cancer and highest completed level of education. Usual dietary intake was assessed with a food frequency questionnaire (FFQ) described by Ocke *et al.* [25]. Of the foods reported in the present study (table 5.1) relative validity was lowest for vegetables (r=0.31 among women and r=0.38 among men) and highest for alcoholic beverages (r=0.87 among women and r=0.74 among men).

Medical (follow-up) information

Participants were prospectively followed via medical records in the recruitment hospitals, until 2009. Information on all performed colonic examinations, colon surgeries, cancer and adenomatous polyp occurrences and other relevant medical information was gathered. For each colonic examination, the number of neoplasms, location, size, and histology was ascertained. Any histological confirmed colorectal adenoma detected at least one year after recruitment was counted as a recurrent adenoma. In the Netherlands, colonoscopies performed within a year are mainly

done to check if the initial adenoma is adequately removed, rather than check for recurrences [26]. Advanced adenomas were those with a diameter of 1 cm or more and/or tubulovillous or villous histology and/or with high grade dysplasia and/or 3 or more adenomas detected at the same colonic examination. When more than 1 adenoma was diagnosed, size, histology and dysplasia of the largest and/or most advanced adenoma was used to characterize the adenomas. If the size, the dysplasia or histology of the adenoma was not mentioned in the colonoscopy and/or pathology reports, we assumed that the adenoma was not advanced.

SNP selection and genotyping

Our SNP selection strategy consisted of a literature search on IGF's, IGFBP's, IGF-Receptors and IGFALS polymorphisms. We chose polymorphisms in the *IGF1*, *IGF2*, *IGFBP1-6*, *IGF-receptor* and *IGFALS* genes which were associated with either IGF(BP)'s blood levels or with breast, prostate and/or colorectal cancer risk and could be genotyped using the iPLEX Gold assay (Sequenom; San Diego, CA). SNP's with a minor allele frequency of less than 5% were not selected because our study would not have the power to detect any associations. In total, 40 single nucleotide polymorphisms (SNP's) in IGF-axis genes were genotyped (See Supplementary table 5-I).

DNA was extracted from buffy coats of EDTA treated blood using a QIAamp 96 DNA blood kit (Qiagen, Inc). Genotyping was performed at the University of Pittsburgh Genomics and Proteomics Core Laboratories using MassARRAY® iPLEX Gold (Sequenom, Inc., San Diego, CA). Repeats were included in the genotyping analyses. Three SNP's (rs9658194 [*IGFBP1*], rs2854746, and rs2453840 [both *IGFBP3*]) were not successfully genotyped, genotyping rate was 0%, 0% or 45% respectively. These SNP's were excluded from the analyses. For all other SNP's at least 98% of the samples were successfully genotyped. Eighteen samples were excluded because of a low (less than 85%) genotyping success rate of the SNP's. All but one SNP (rs6214, P-value 0.02) were in Hardy-Weinberg equilibrium as tested by a Chi-square test (P-value ≥0.05).

Statistical analysis

Cox regression models were used to evaluate the association between BMI, IGF-axis SNP's and development of the first colorectal adenoma recurrence. Person-time started at the date of recruitment sigmoido- or colonoscopy, and ended at the date of diagnosis of the first recurrent (advanced) adenoma, date of colorectal cancer diagnosis, date of colonic surgery, date of death, or date of last known colonic examination whichever occurred first. Persons who deceased with unknown date of death were censored at the date of their last large bowel examination.

BMI was divided into categories based on the WHO criteria: underweight (<18.5 kg/m²), normal (18.5 to 25 kg/m²), overweight (25 to 30 kg/m²), and obese (≥30 kg/m²). SNP's were grouped according to genotype, homozygous major (0 minor alleles), heterozygous (1 minor allele), and homozygous minor (2 minor alleles). The number of minor-allele homozygotes was relatively small, therefore heterozygotes and minor-allele homozygotes were combined in the regression analyses; using common-allele homozygotes as reference group.

To assess possible modification of the association between BMI and colorectal adenoma recurrence we stratified our analysis for genotypes (heterozygotes and minor-allele homozygotes versus common-allele homozygotes) and estimated HR's with normal weight common allele homozygotes as reference group. The P value for interaction was calculated by Chi-square test of the likelihood ratio test, comparing the models with and without overweight-by-genotype interaction term(s).

Co-variables were evaluated as confounder if associated with the exposure (BMI in categories) in univariable analysis and known to be associated with outcome (colorectal adenoma recurrence or advanced adenoma recurrence) from literature. Evaluated as possible confounders of the relation between BMI and colorectal adenoma or advanced adenoma recurrence were age (continuous), sex, family history of colorectal cancer (yes/no), number of large bowel examinations during follow-up (continuous), personal history of adenomas before index scopy (yes/no), size of last adenoma (at or before index colonoscopy) (≥1 cm, yes/no/missing), histology of last adenoma (villous structures, yes/no), dysplasia of last adenoma (high grade, yes/no), number of polyps at last positive colonoscopy (<3, ≥3, missing), smoking habits (never, current, former), alcohol intake (continuous), red meat (continuous), and processed meat (continuous). Co-variables were considered confounding the association if they changed the estimate by 10% or more. Including physical activity (continuous and in tertiles), and energy intake (tertiles), both variables in the same pathway as BMI, in the model did not change the estimate. $P<0.05$ was considered statistically significant. Statistical analysis were done using SAS software, version 9.2 (SAS Institute, Cary, NC)

Results

During a median person-time of 4.7 years, 165 of the 565 adenoma patients (29.2%), developed at least one adenoma, of these 37 (6.5%) developed an adenoma with advanced adenoma pathology (AAP). Baseline characteristics of the total study population and of those with an adenoma recurrence are shown in table 5.1. Recurrent adenoma cases were more often male, and had more often right-sided colon adenomas at the last positive colonoscopy than was seen in the total population.
Table 5.2 shows the associations between BMI and adenoma recurrence. No association was seen for overweight (≥25 kg/m²) and adenoma recurrence (HR, 1.04; 95% CI, 0.75-1.44), adjusted for age, sex, number of large bowel examination during follow-up, red meat intake and number of polyps at the last positive endoscopy. Exploring the association between overweight and advanced adenoma recurrences did not show an association either (HR, 1.06; 95% CI, 0.52-2.18). Sex stratified analysis showed no differences in association between overweight and any adenoma recurrence among men and women, (overweight men HR, 0.89; 95% CI, 0.57-1.40; overweight women HR, 1.21; 95% CI, 0.75-1.95).

Table 5.1 Baseline characteristics of the POLIEP-follow up cohort

Characteristics	Units	Total cohort n=565	Any adenoma n=165	Advanced adenoma n=37
Person years [median (P10-P90)]		4.7 (1.7-8.0)	3.6 (1.4-7.2)	4.1 (1.1-7.7)
No. of large bowel examinations [a] [median (P10-P90)]		1 (1-3)	1 (1-2)	1 (1-2)
General				
Sex, female	%	270 (47.8)	71 (43.0)	16 (43.2)
Age at study entry (mean ±SD)	years	58.9 (9.7)	59.4 (9.7)	59.8 (11.3)
Smoking status, current	%	147 (26.0)	45 (27.3)	9 (24.3)
Smoking status, former	%	223 (39.5)	65 (39.4)	16 (43.2)
Physical activity, low [b]	%	208 (36.8)	62 (37.6)	13 (35.1)
Regular NSAID use (≥12/y)	%	148 (26.2)	44 (26.7)	6 (16.2)
Hormone replacement therapy use [c]	%	45 (21.7)	8 (13.1)	3 (21.4)
Education, high [d]	%	129 (22.8)	37 (22.4)	6 (16.2)
BMI				
Normal (<25 kg/m²)	%	234 (41.4)	67 (40.6)	14 (37.8)
Overweight (25 to 30 kg/m²)	%	258 (45.7)	79 (47.9)	19 (51.4)
Obese (≥30 kg/m²)	%	70 (12.4)	18 (10.9)	4 (10.8)
Family history of colorectal cancer	%	141 (24.9)	45 (27.3)	10 (27.0)
Personal history of adenomas [e]	%	112 (19.8)	51 (30.9)	17 (46.0)
Characteristics last adenoma at baseline				
Type of last adenoma				
adenoma of unknown histology	%	96 (17.0)	29 (17.6)	6 (16.2)
tubular	%	285 (50.4)	83 (50.3)	13 (35.1)
tubulovillous	%	118 (20.9)	35 (21.2)	14 (37.8)
villous	%	64 (11.3)	18 (10.9)	4 (10.8)
missing	%	2 (0.4)	0	0
Amount of dysplasia of last adenoma				
high grade dysplasia [f]	%	43 (7.6)	9 (5.5)	2 (5.4)
missing	%	142 (25.1)	40 (24.2)	9 (24.3)
Size of last adenoma				
≥1cm	%	220 (38.9)	59 (35.8)	10 (27.0)
missing	%	69 (12.2)	23 (13.9)	4 (10.8)

Table 5.1 Continued

Characteristics	Units	Total cohort n=565	Any adenoma n=165	Advanced adenoma n=37
No of polyps last pos scopie [g)]				
3 or more	%	122 (21.6)	42 (25.5)	11 (29.7)
missing	%	40 (7.1)	18 (10.9)	4 (10.8)
Dietary intake				
Energy [median (P10-P90)]	mJ/d	8.4 (5.9-11.8)	8.1 (5.8-12.0)	8.6 (5.7-13.9)
Fibre [median (P10-P90)]	g/d	23.7 (15.2-32.3)	23.2 (15.0-33.4)	24.8 (14.7-34.6)
Vegetables [h)] [median (P10-P90)]	g/d	113.6 (67.1-177.0)	111.5 (65.4-182.3)	116.6 (75.0-189.9)
Fruit [i)] [median (P10-P90)]	g/d	129.0 (24.5-374.1)	126.4 (25.8-374.1)	142.3 (17.5-374.1)
Fresh red meat [median (P10-P90)]	g/d	58.8 (13.8-99.0)	62.5 (15.5-102.9)	70.6 (22.1-107.5)
Processed meat [median (P10-P90)]	g/d	26.9 (3.4-66.0)	28.6 (1.7-63.9)	31.6 (4.6-73.1)
Alcohol [median (P10-P90)]	g/d	8.8 (0.02-41.7)	8.9 (0.02-44.8)	5.9 (0.28-45.4)
Supplementary multivitamin use	%	91 (16.1)	28 (17.0)	6 (16.2)

Abbreviation: P10-P90, 10-90th percentile; n, number; SD, standard deviation; NSAID, non-steroida anti-inflammatory drugs; y, year; BMI, body mass index; kg, kilo gram; m, meter; cm, centimeter; mJ, mega Joules; d, day; g, gram

Notes:
a) number of large bowel examinations counting from 1 year after recruitment until end of personal follow-up
b) Low physical activity is the lowest tertile of the physical activity score
c) among postmenopausal women only, n=207 (76.7%)
d) Higher education, missing for 51 persons
e) Personal history of adenomas before index adenoma
f) defined as adenomas with severe dysplasia
g) number of polyps at last endoscopy with adenomas
h) Definition of total vegetables includes nonstarch legumes and excludes potatoes and vegetables juice
i) Definition of total fruits excludes fruit juices

Main effect of Polymorphisms

Results of analyses conducted to assess main SNP effects on adenoma recurrence are presented in table 5.3. None of the evaluated SNP's were associated with any adenoma recurrence. Two of the evaluated SNP's, rs1520220 in *IGF1* and rs3213221 in IGF2 were statistically significantly associated with risk of developing recurrent advanced adenomas. Having at least one minor rs1520220 allele was associated with a 2.2 fold (95% CI, 1.12-4.44) increased risk of developing an advanced adenoma recurrence, while having at least one minor rs3213221 allele increased the risk of an advanced adenoma recurrence 2.4 fold (95% CI, 1.06-5.63), adjusted for age and sex. Additionally, in crude analysis a borderline statistically significant association was observed for rs5742678 in *IGF1*, which was not statistically significant after adjustment for age and sex. None of the SNP's in *IGF1R*, *IGFBP1*, *IGFBP2*, *IGFBP3*, *IGFBP5* and *IGFALS* were associated with advanced adenoma recurrence (table 5.3)

Effect modification by SNP in IGF2

There was evidence of effect modification by SNP's rs1003483 and rs1004446 in *IGF2* of the association between BMI and any adenoma recurrence (p interaction 0.03 and 0.001 respectively), see table 5.4. For both SNP's common-allele homozygotes had an increased risk of any adenoma recurrences when being overweight, while having at least one minor allele showed an more increased risk of developing any adenomas for normal weight persons None of the other SNP's showed effect modification of the association between BMI and (advanced) adenoma recurrence.

Table 5.2 Hazard ratios for BMI and colorectal adenoma recurrence of the POLIEP-follow up cohort

	Any adenoma (n=165)		Advanced adenoma (n=37)	
	BMI <25 kg/m² HR	**BMI ≥25 kg/m² HR (95% CI)**	**BMI <25 kg/m² HR**	**BMI ≥25 kg/m² HR (95% CI)**
total cohort (n=565)				
n	67	97	14	23
HR, age & sex adjusted	1.0	1.04 (0.75-1.43)	1.0	1.23 (0.61-2.45)
HR, fully adjusted [a)]	1.0	1.04 (0.75-1.44)	1.0	1.06 (0.52-2.18)
Men (n=295)				
n	34	60	7	14
HR, age & sex adjusted	1.0	0.95 (0.62-1.48)	1.0	1.20 (0.46-3.14)
HR, fully adjusted [a)]	1.0	0.89 (0.57-1.40)	1.0	0.92 (0.33-2.56)
Women (n=270)				
n	33	37	7	9
HR, age & sex adjusted	1.0	1.20 (0.75-1.92)	1.0	1.39 (0.52-3.73)
HR, fully adjusted [a)]	1.0	1.21 (0.75-1.95)	1.0	1.25 (0.45-3.45)
No history of adenoma (n=453)				
n	49	64		
HR, age & sex adjusted	1.0	0.97 (0.66-1.43)		
HR, fully adjusted [a)]	1.0	0.98 (0.66-1.46)		
With history of adenoma (n=112)				
n	18	33		
HR, age & sex adjusted	1.0	1.26 (0.70-2.28)		
HR, fully adjusted [a)]	1.0	1.47 (0.78-2.76)		

Abbreviation n, number; BMI, body mass index; kg, kilo gram; m, meter; HR, hazard ratio; CI, confidence interval

[a)] age, sex, number of bowel examinations, red meat intake & number of polyps at last postive scopy adjusted

Table 5.3 Distribution of genotypes for SNP's in *IGF1R*, *IGF1*, *IGF2*, *IGFBP1*, *IGFBP2*, *IGFBP3*, *IGFBP5* and *IGFALS* genes in a cohort of colorectal adenoma patients and associations with adenoma recurrence

				Genotype distribution					
				AA [a]			Aa + aa [a]		
Gene	SNP	Major allele	Minor allele	Adeno cases	AAP [b] cases	total cohort	Adeno cases	AAP [b] cases	total cohort
IGF1R	rs2229765	G	A	42	7	140	101	26	368
	rs8038415	C	T	42	11	139	101	22	369
IGF1	rs1520220	C	G	95	16	338	48	17	170
	rs1549593	C	A	115	27	389	28	6	119
	rs2195239	G	C	88	16	300	55	17	208
	rs2946834	C	T	67	11	234	76	22	274
	rs35765	C	A	113	28	393	30	5	115
	rs35767	C	T	104	25	358	39	8	149
	rs4764876	G	C	75	13	262	68	20	246
	rs5742625	A	Del	91	18	311	52	15	196
	rs5742678	C	G	85	14	287	64	19	221
	rs6214	G	A	44	11	166	99	22	342
	rs6219	G	A	108	22	398	35	11	106
	rs7136446	T	C	50	9	176	93	24	332
	rs7965399	T	C	135	30	473	8	3	35
	rs9989002	G	A	85	16	276	58	17	231
IGF2	rs1003483	T	G	38	12	142	105	21	366
	rs1004446	C	T	44	8	180	99	25	328
	rs3213221	C	G	52	7	192	91	26	315
	rs3213223	C	T	82	19	311	61	14	197
	rs680	G	A	76	15	273	67	19	234
IGFBP1	rs10228265	A	G	65	14	244	78	19	264
	rs3763497	C	T	61	14	223	81	19	280
IGFBP2	rs9341134	A	T	122	26	448	21	7	59
	rs9341145	C	T	124	26	439	19	7	69
IGFBP3	rs2132571	G	A	71	16	240	72	17	268
	rs2270628	C	T	85	16	315	56	15	191
	rs2471551	G	C	85	19	310	58	14	198
	rs2854744	C	A	36	8	155	107	25	352
	rs2960436	G	A	34	8	150	109	25	356
	rs3110697	G	A	40	7	153	103	26	355
	rs6670	A	T	84	23	300	59	10	208
	rs903889	A	C	87	19	309	56	14	199
	rs924140	G	A	36	8	155	107	25	351
IGFBP5	rs2241193	G	A	99	25	376	44	8	131
IGFALS	rs17559	C	T	117	25	419	26	8	88
influencing *IGFBP2* and/or *IGFBP5*	rs13387042	G	A	34	7	132	109	26	376

Abbreviation: Statistically significant hazard ratios are bold; SNP, single nucleotide polymorphism; AAP = advanced adenoma pathology or advanced adenoma recurrence; HR, hazard ratio; CI, confidence interval

Notes:
a) AA = homozygous major, Aa = heterozygous, aa = homozygous minor
b) AAP = advanced adenoma pathology or advanced adenoma recurrence
c) age & sex adjusted

Any adenoma		Advanced adenoma	
HR [c)]	95% CI	HR [c)]	95% CI
0.78	(0.54-1.12)	1.23	(0.53-2.84)
0.86	(0.60-1.23)	0.73	(0.35-1.50)
1.12	(0.79-1.60)	**2.23**	**(1.12-4.44)**
0.82	(0.54-1.24)	0.73	(0.30-1.77)
0.98	(0.70-1.37)	1.62	(0.82-3.23)
0.94	(0.67-1.30)	1.73	(0.83-3.58)
1.05	(0.70-1.58)	0.69	(0.27-1.80)
1.03	(0.71-1.49)	0.88	(0.39-1.95)
0.93	(0.67-1.30)	1.62	(0.80-3.27)
0.96	(0.68-1.35)	1.36	(0.68-2.71)
1.07	(0.76-1.49)	1.86	(0.93-3.74)
0.96	(0.67-1.37)	0.96	(0.46-1.98)
1.18	(0.80-1.73)	1.68	(0.81-3.48)
0.91	(0.65-1.29)	1.37	(0.64-2.96)
0.78	(0.38-1.60)	1.41	(0.43-4.66)
0.88	(0.62-1.23)	1.35	(0.68-2.68)
1.10	(0.75-1.60)	0.64	(0.31-1.30)
1.15	(0.81-1.65)	1.74	(0.79-3.88)
1.14	(0.81-1.60)	**2.44**	**(1.06-5.63)**
1.21	(0.87-1.69)	1.18	(0.59-2.36)
1.01	(0.72-1.40)	1.41	(0.71-2.81)
1.13	(0.81-1.58)	1.32	(0.66-2.63)
1.22	(0.88-1.71)	1.17	(0.59-2.34)
1.27	(0.80-2.02)	1.99	(0.86-4.60)
0.94	(0.58-1.53)	1.55	(0.67-3.59)
0.83	(0.60-1.16)	0.87	(0.44-1.72)
1.13	(0.81-1.59)	1.56	(0.77-3.16)
1.05	(0.75-1.47)	1.10	(0.55-2.21)
1.22	(0.83-1.78)	1.30	(0.59-2.88)
1.24	(0.84-1.82)	1.23	(0.56-2.73)
1.20	(0.83-1.73)	1.64	(0.71-3.79)
1.05	(0.75-1.46)	0.60	(0.29-1.26)
1.11	(0.79-1.56)	1.24	(0.62-2.48)
1.23	(0.84-1.80)	1.31	(0.59-2.91)
1.34	(0.93-1.92)	0.95	(0.43-2.11)
1.20	(0.78-1.84)	1.63	(0.74-3.63)
1.10	(0.75-1.62)	1.35	(0.58-3.11)

Table 5.4 Interplay between body mass index (categories, <25 kg/m² & ≥25 kg/m²), *IGF1*, *IGF2*, *IGFBP1*, *IGFBP2*, *IGFBP3*, *IGFBP5* and *IGFALS* genotypes and risk of colorectal adenoma recurrence

Gene SNP	genotype	BMI (<25 kg/m²) (67 cases, 234 total cohort) HR [a)]	(95% CI)	BMI (≥25 kg/m²) (97 cases, 328 total cohort) HR [a)]	(95% CI)	P for interaction
IGF1r						
rs2229765	GG	1.0		1.20	(0.65-2.22)	
	GA + AA	0.91	(0.52-1.60)	0.82	(0.481.40)	0.44
rs8038415	CC	1.0		1.11	(0.59-2.07)	
	CT + TT	0.97	(0.54-1.73)	0.88	(0.51-1.55)	0.61
IGF1						
rs1520220	CC	1.0		0.88	(0.58-1.33)	
	CG + GG	0.95	(0.54-1.68)	1.11	(0.69-1.78)	0.44
rs1549593	CC	1.0		1.13	(0.76-1.66)	
	CA + AA	1.20	(0.67-2.16)	0.64	(0.34-1.22)	0.08
rs2195239	GG	1.0		0.91	(0.59-1.40)	
	GC + CC	0.89	(0.52-1.53)	0.95	(0.59-1.52)	0.67
rs2946834	CC	1.0		0.81	(0.50-1.32)	
	CT + TT	0.76	(0.45-1.29)	0.87	(0.55-1.38)	0.32
rs35765	CC	1.0		0.95	(0.65-1.39)	
	CA + AA	1.02	(0.54-1.94)	1.05	(0.61-1.82)	0.85
rs35767	CC	1.0		0.89	(0.60-1.33)	
	TC + TT	0.87	(0.47-1.63)	1.04	(0.64-1.70)	0.54
rs4764876	GG	1.0		0.83	(0.52-1.33)	
	GC + CC	0.76	(0.45-1.30)	0.87	(0.55-1.39)	0.36
rs5742625	AA	1.0		0.92	(0.60-1.40)	
	A.DEL + DEL	0.87	(0.51-1.50)	0.94	(0.58-1.51)	0.66
rs5742678	CC	1.0		0.95	(0.61-1.48)	
	CG + GG	1.05	(0.62-1.79)	1.04	(0.65-1.67)	0.91
rs6214	GG	1.0		0.94	(0.51-1.72)	
	AG + AA	0.94	(0.53-1.66)	0.92	(0.53-1.58)	0.90
rs6219	GG	1.0		0.86	(0.58-1.28)	
	AG + AA	0.87	(0.46-1.65)	1.22	(0.74-2.03)	0.23
rs7136446	TT	1.0		0.96	(0.55-1.69)	
	CT + CC	0.91	(0.53-1.57)	0.89	(0.53-1.48)	0.95
rs7965399	TT	1.0		0.96	(0.68-1.37)	
	CT + CC	0.66	(0.16-2.73)	0.80	(0.34-1.87)	0.78
rs9989002	GG	1.0		0.91	(0.59-1.41)	
	CA + AA	0.81	(0.48-1.38)	0.85	(0.53-1.37)	0.68
IGF2						
rs1003483	TT	1.0		1.82	(0.90-3.70)	
	GT + GG	1.86	(0.96-3.59)	1.44	(0.75-2.75)	0.03
rs1004446	CC	1.0		2.41	(1.19-4.90)	
	TC + TT	2.65	(1.34-5.24)	1.79	(0.90-3.54)	0.00
rs3213221	CC	1.0		1.03	(0.59-1.79)	
	CG + GG	1.21	(0.71-2.06)	1.12	(0.68-1.84)	0.77
rs3213223	CC	1.0		0.96	(0.62-1.50)	
	CT + TT	1.22	(0.72-2.06)	1.17	(0.73-1.87)	.
rs680	GG	1.0		0.78	(0.50-1.23)	
	GA + AA	0.75	(0.44-1.28)	0.96	(0.61-1.50)	0.16
IGFBP1						
rs10228265	AA	1.0		1.13	(0.69-1.86)	
	GA + GG	1.39	(0.82-2.33)	1.14	(0.70-1.85)	0.35
rs3763497	CC	1.0		0.87	(0.52-1.46)	
	CT + TT	1.14	(0.68-1.91)	1.13	(0.71-1.81)	0.71
IGFBP2						
rs9341134	AA	1.0		0.90	(0.62-1.30)	
	AT	0.90	(0.41-1.99)	1.41	(0.77-2.56)	0.26

Table 5.4 Continued

Gene SNP	genotype	BMI (<25 kg/m²) (67 cases, 234 total cohort) HR [a]	((95% CI))	BMI (≥25 kg/m²) (97 cases, 328 total cohort) HR [a]	(95% CI)	P for interaction
rs9341145	CC	1.0		0.94	(0.65-1.35)	
	TC + TT	0.77	(0.31-1.92)	0.96	(0.53-1.74)	0.59
IGFBP3						
rs2132571	GG	1.0		0.84	(0.52-1.35)	
	AG + AA	0.71	(0.42-1.19)	0.79	(0.50-1.26)	0.40
rs2270628	CC	1.0		1.15	(0.74-1.78)	
	TC + TT	1.58	(0.93-2.70)	1.06	(0.65-1.72)	0.13
rs2471551	GG	1.0		0.81	(0.52-1.24)	
	CG + CC	0.79	(0.46-1.37)	1.03	(0.65-1.63)	0.18
rs2854744	CC	1.0		1.36	(0.68-2.74)	
	CA + AA	1.57	(0.83-2.96)	1.37	(0.74-2.57)	0.28
rs2960436	GG	1.0		1.30	(0.64-2.64)	
	GA + AA	1.54	(0.81-2.91)	1.36	(0.73-2.53)	0.34
rs3110697	GG	1.0		0.92	(0.49-1.73)	
	GA + AA	1.14	(0.64-2.04)	1.14	(0.65-2.00)	0.83
rs6670	AA	1.0		0.99	(0.63-1.54)	
	AT + TT	1.07	(0.63-1.80)	1.00	(0.62-1.62)	0.88
rs903889	AA	1.0		1.07	(0.69-1.67)	
	CA + CC	1.30	(0.77-2.19)	1.08	(0.65-1.78)	0.47
rs924140	GG	1.0		1.33	(0.66-2.68)	
	AG + AA	1.58	(0.83-2.98)	1.37	(0.73-2.56)	0.29
IGFBP5						
rs2241193	GG	1.0		0.92	(0.61-1.40)	
	GA + AA	1.14	(0.66-1.96)	1.41	(0.83-2.40)	0.43
IGFALS						
rs17559	CC	1.0		0.95	(0.65-1.38)	
	CT + TT	1.13	(0.55-2.30)	1.21	(0.69-2.12)	0.78
influencing *IGFBP2* and/or *IGFBP5*						
rs13387042	GG	1.0		1.41	(0.69-2.85)	
	GA + AA	1.50	(0.79-2.83)	1.28	(0.69-2.38)	0.22

Abbreviation: BMI, body mass index; kg, kilo gram; m, meter; HR, hazard ratio; CI, confidence interval; SNP, single nucleotide polymorphism

Notes: [a] age & sex adjusted

Supplementary table 5-I Selected SNP's for genotyping in the POLIEP-follo up cohort

Gene SNP-id	Chromosome	Chromosome position	Local loci	Update build-id	Ref. genome	Other SNP's captured
IGF-1R						
rs2229765	15	99478225	IGF1R	132	GRCh37	
rs8038415	15	99499434	IGF1R	132	GRCh37	
IGF1						
rs1520220	12	102796522	IGF1	132	GRCh37	
rs1549593	12	102796791	IGF1	132	GRCh37	
rs2195239	12	102856702	IGF1	132	GRCh37	
rs2946834	12	102787814		132	GRCh37	
rs35767	12	102875569	IGF1	132	GRCh37	rs855228, rs2162679
rs35765	12	102881696		132	GRCh37	
rs4764876	12	102758702		132	GRCh37	
rs5742625	12	102858089	IGF1	130	GRCh37	
rs5742678	12	102814332	IGF1	132	GRCh37	rs6220, rs978458, rs5742694
rs6214	12	102793569	IGF1	132	GRCh37	
rs6219	12	102790192	IGF1	132	GRCh37	
rs7136446	12	102838515	IGF1	132	GRCh37	
rs7965399	12	102891686		132	GRCh37	rs11111285
rs9989002	12	102850223	IGF1	132	GRCh37	rs10735380
IGF2						
rs1003483	11	2167543	IGF2, IGF2AS, INS-IGF2	132	GRCh37	
rs1004446	11	2170143	IGF2, INS-IGF2	132	GRCh37	
rs3213221	11	2157044	IGF2	132	GRCh37	
rs3213223	11	2156930	IGF2	132	GRCh37	
rs680	11	2153634	IGF2	132	GRCh37	
IGFBP1						
rs10228265	7	45908915		132	GRCh37	
rs3763497	7	45925348		132	GRCh37	
rs9658194	7	45928787	IGFBP1	132	GRCh37	
IGFBP2						
rs9341134	2	217507926	IGFBP2	132	GRCh37	
rs9341145	2	217511100	IGFBP2	132	GRCh37	
IGFBP3						
rs2132571	7	45961674	IGFBP3	132	GRCh37	
rs2270628	7	45949570		132	GRCh37	
rs2453840	7	45953812	IGFBP3	132	GRCh37	
rs2471551	7	45957055	IGFBP3	132	GRCh37	
rs2854744	7	45961075	IGFBP3	132	GRCh37	
rs2854746	7	45960645	IGFBP3, LOC100129619	132	GRCh37	
rs2960436	7	45977282		132	GRCh37	
rs3110697	7	45955029	IGFBP3	132	GRCh37	
rs6670	7	45952254	IGFBP3	132	GRCh37	
rs903889	7	45964995		132	GRCh37	rs2132570, rs2132572
rs924140	7	45963114		132	GRCh37	rs2854744, rs2854746
IGFBP5						
rs2241193	2	217554213	IGFBP5	132	GRCh37	
IGFALS						
rs17559	16	1841033	IGFALS	132	GRCh37	
-						
rs13387042	2	217905832		132	GRCh37	

Supplementary table 5-II Baseline characteristics of the POLIEP-follow up-cohort by category of BMI

Characteristics	Unit	Normal weight n=234	Overweight n=258	Obese n=70
Person time [median (P10-P90)]	years	4.8 (2.0-8.0)	4.7 (1.5-7.8)	5.1 (1.7-8.1)
No. of large bowel examinations [a] [median (P10-P90)]		1 (1-3)	1 (1-3)	1 (1-3)
Adenoma recurrence	%	67 (28.6)	79 (30.6)	18 (25.7)
Advanced adenoma recurrence	%	14 (6.0)	19 (7.4)	4 (5.7)
General				
Sex, female	%	139 (59.4)	88 (34.1)	40 (57.1)
Age at study entry (mean ±SD)	years	58.1 (10.6)	59.0 (9.2)	60.6 (7.9)
Smoking status, current	%	73 (31.2)	63 (24.4)	11 (15.7)
Smoking status, former	%	70 (29.9)	119 (46.1)	34 (48.6)
Physical activity, low [b]	%	73 (31.2)	106 (41.1)	28 (40.0)
Regular NSAID use (≥12/y)	%	64 (27.4)	61 (23.6)	22 (31.4)
Hormone replacement therapy use [c]	%	24 (23.5)	12 (17.7)	8 (23.5)
Education, high [d]	%	62 (26.5)	55 (21.3)	11 (15.7)
BMI [median (P10-P90)]	kg/m²	23.2 (21.0-24.7)	27 (25.0-29.0)	32.7 (30.0-37.0)
Family history of colorectal cancer	%	70 (29.9)	58 (22.5)	12 (17.1)
Personal history of adenomas [e]	%	40 (17.1)	54 (20.9)	17 (24.3)
Characteristics last adenoma at baseline				
Type of last adenoma				
adenoma of unknown histology	%	44 (18.8)	44 (17.1)	8 (11.4)
tubular	%	120 (51.3)	128 (49.6)	35 (50.0)
tubulovillous	%	42 (18.0)	59 (22.9)	17 (24.3)
villous	%	26 (11.1)	27 (10.5)	10 (14.3)
missing	%	2 (0.9)	0	0
Amount of dysplasia of last adenoma				
high grade dysplasia [f]	%	18 (7.7)	18 (7.0)	6 (8.6)
missing	%	55 (23.5)	64 (24.8)	22 (31.4)
Size of last adenoma				
≥1cm	%	90 (38.5)	100 (38.8)	28 (40.0)
missing	%	20 (8.6)	41 (15.9)	7 (10.0)
No of polyps last pos scopie [g]				
3 or more	%	52 (22.2)	53 (20.5)	15 (21.4)
missing	%	11 (4.7)	21 (8.1)	8 (11.4)
Dietary intake				
Energy [median (P10-P90)]	mJ/d	8.2 (5.8-11.6)	8.6 (6.1-12.4)	8.2 (5.8-11.1)
Fibre [median (P10-P90)]	g/d	23.3 (14.5-31.8)	23.8 (15.2-33.1)	24.1 (16-33)
Vegetables [h]	g/d	112 (69.4-170.0)	115.3 (66.2-177.0)	117 (70.7-218.0)
Fruit [i] [median (P10-P90)]	g/d	153 (18.4-374.0)	125.1 (17.8-361)	181 (35.8-411.0)
Fresh red meat [median (P10-P90)]	g/d	49 (9.5-94.5)	67.8 (22.4-109.0)	65.8 (20.3-98.8)
Processed meat [median (P10-P90)]	g/d	21.5 (2.4-56.5)	29.6 (3.5-77.0)	33.4 (6.9-76.2)
Alcohol [median (P10-P90)]	g/d	10.2 (0.03-40.2)	8.6 (0.03-45.3)	4.1 (0.00-30.4)
Supplementary multivitamin use	%	46 (19.7)	38 (14.7)	7 (10)

Abbreviation P10-P90, 10-90th percentile; n, number; SD, standard deviation; NSAID, non-steroida anti-inflammatory drugs; y, year; BMI, body mass index; kg, kilo gram; m, meter; cm, centimeter; mJ, mega Joules; d, day; g, gram

Notes
[a] number of large bowel examinations counting from 1 year after recruitment until end of personal follow-up
[b] Low physical activity is the lowest tertile of the physical activity score
[c] among postmenopausal women only, n=207 (76.7%)
[d] Higher education, missing for 51 persons
[e] Personal history of adenomas before index adenoma
[f] defined as adenomas with severe dysplasia
[g] number of polyps at last endoscopy with adenomas
[h] Def. of total vegetables incl. nonstarch legumes and excl. potatoes & vegetables juice
[i] Definition of total fruits excludes fruit juices

Discussion

In this study, we examined associations of BMI, polymorphisms in IGF-axis genes and colorectal adenoma recurrence in a cohort of sporadic adenoma cases. Furthermore, we evaluated whether SNP's in IGF-axis genes modified the association between BMI and adenoma recurrence. We did not find evidence for an association between BMI and colorectal adenoma recurrence, nor for advanced adenoma recurrence. Two polymorphisms, one in the *IGF1* gene, SNP rs1520220, and one in the *IGF2* gene, SNP rs3213221 were associated with an increased risk of advanced adenoma recurrence. Furthermore, two SNP's, rs1003483 and rs1004446, in the *IGF2* gene modified the associations between BMI and any colorectal adenoma recurrence.

In contrast to our study, a pooling study of 7 prospective USA based trials (n=8,213) showed a positive association between being obese (≥30 kg/m²) and recurrent adenomas, but only among men (OR, 1.36; 95% CI, 1.17-1.58) [7]. A possible reason why our study failed to show an association with overweight is, the lower percentage of obese persons in our study (12.1%) compared with the pooling study (25.7%) and thus a smaller exposure range. A case-control study [27] (n=539), which grouped BMI into quartiles, also found no association. Among men, they observed an effect for the second quartile (BMI-range 24.42 to 26.63) compared to the first, but not for the third and fourth [27]. Additionally, a very small study28 (n=62) from Norway did not find any association with BMI and adenoma recurrence, but indicated that BMI influenced growth of adenomas [28].

No other studies have investigated associations between IGF-axis SNP's and colorectal adenoma recurrence. Several studies (14-17,19,20,29-32) evaluated the association between IGF-axis SNP's and incident CRC or colorectal adenoma risk. None of these specifically studied rs1520220 in the *IGF1* gene, which was associated with advanced colorectal adenoma recurrence in our study. However, one case-control study [19] used tagging SNP's and studied a *IGF1* gene variant, which was in LD with rs1520220, in relation to colorectal cancer. In contrast to our study, they did not observe an association with the SNP's in the *IGF1* gene and colorectal cancer incidence. Further research is needed to confirm this finding.

The minor allele of SNP rs1520220 in the *IGF1* gene has been associated with elevated circulating IGF1 levels [33-35]. The observed positive association between the minor allele and risk of colorectal adenoma recurrence in our study, might be caused by increased IGF1 levels. Positive associations between higher IGF1 levels and colorectal cancer incidence have been reported in several studies [36-39]. Two studies [40,41] examined associations between IGF1 levels and recurrence of colorectal adenomas. To their own surprise they observed lower risks for higher IGF1 levels. In both studies high IGF1 levels were associated with lower BMI, suggesting that these IGF1 levels and BMI could confound one another, although the models in one of the studies [41] are adjusted for BMI.

To our knowledge no other studies examined SNP's in the *IGF2* gene in relation to colorectal neoplasms. Our initial findings need to be confirmed by others. Consequently no other studies have observed effect modification of the BMI-colorectal adenoma association by SNP's in the *IGF2* gene, like we observed for SNP's rs1003483 and rs1004446. Effect modification of the association between BMI and colorectal cancer by a SNP in *IGFBP3* gene was observed in one study in the USA [42], but not in another [18].

We did not observe associations between common variants in IGFBP1, IGBP2, IGFBP3, IGFBP5, IGF1R, or IGFALS genes and adenoma recurrence. Most earlier studies did not observe associations between SNP's in the *IGFBP3* gene and colorectal cancer incidence [15,19,29] or colorectal adenomas [32] and the associations seen were modest [14,16]. SNP's in *IGFBP1* were also not associated with colorectal cancer [31], while *IGF1R* was not associated with colorectal polyps risk [32].

The study is not without limitations. The sample size limited the possibility to study gene-environment interactions for advanced adenoma recurrences. Furthermore, we had no coverage of the complete IGF-axis genes. However studying common variants that were associated in earlier studies with protein levels or specific tumours could result in relevant findings. We used self-reported height and weight data to calculate BMI. It should be noted that persons tend to under-report their body weight, especially those with increased adiposity, and over-report their height, especially those with an higher BMI and those who are older (>60 years of age) [43]. Nonetheless, validation studies [44] have shown that self-reported and measured anthropometric data are highly correlated (range r: 0.88-0.97). Also, not all participants had an adenoma at the recruitment endoscopy, these patients had an adenoma detected before this exam. Having had an adenoma several years before the recruitment colonoscopy will not change the risk of a recurrence, but it may influence the time to a new adenoma. Furthermore, BMI measured at recruitment might have influenced later stages of adenoma development. One hundred and forty three (18.6%) patients did not have a large bowel examination after the recruitment endoscopy. This could result in bias due to loss to follow-up. The whole study population was insured for health costs, which is obligatory in the Netherlands. Therefore it is unlikely that social class influenced the loss and it is less likely to be associated with the exposures under study

Strengths of this study were the extensive modifiable risk factor data, standardized collection of medical information from medical records and collection of pathology reports.

These findings suggest that common variation in IGF-axis genes influence the likelihood of colorectal adenoma recurrence and may modify the association between BMI and colorectal adenoma recurrence.

Acknowledgements

We are grateful to all study participants for their willingness to participate. We thank Maria van Vugt and Ursula Oldenhof (Radboud University Medical Centre), Leontien Witjes, and Marlieke Visser (Wageningen University) for assistance with participant recruitment and collection of follow-up information. The medical specialists of the participants and research nurses at the participating hospitals are gratefully acknowledged for their collaboration.

References

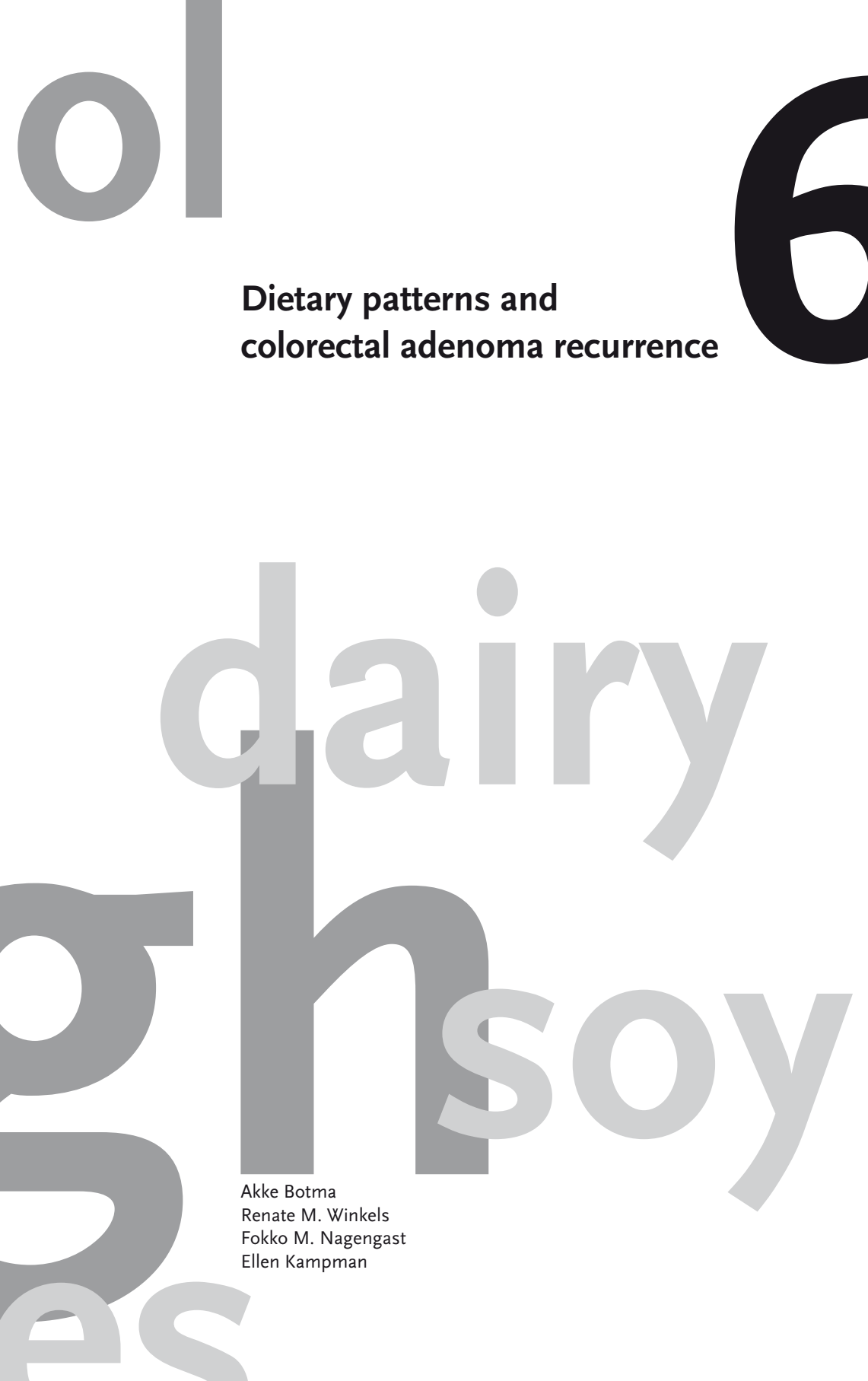
1 Ning Y, Wang L, Giovannucci EL. A quantitative analysis of body mass index and colorectal cancer: findings from 56 observational studies. Obes Rev 11:19-30, 2010.
2 WCRF/AICR. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. 2nd ed. Washington DC: AICR; 2007.
3 WCRF/AICR. Continuous update project interim report summary: Food, nutrition and physical activity and the prevention of colorectal cancer, 2011.
4 Morois S, Mesrine S, Besemer F, *et al.* Risks of colon and rectal adenomas are differentially associated with anthropometry throughout life: the French E3N prospective cohort. Int J Epidemiol Epub:doi: 10.1093/ije/dyr097, 2011.
5 Giovannucci E, Ascherio A, Rimm EB, *et al.* Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 122:327-334, 1995
6 Giovannucci E, Colditz GA, Stampfer MJ, *et al.* Physical activity, obesity, and risk of colorectal adenoma in women (United States). Cancer Causes Control 7:253-263, 1996
7 Jacobs ET, Ahnen DJ, Ashbeck EL, *et al.* Association between body mass index and colorectal neoplasia at follow-up colonoscopy: a pooling study. Am J Epidemiol 169:657-666, 2009
8 Voskuil DW, Bueno de Mesquita HB, Kaaks R, *et al.* Determinants of circulating insulin-like growth factor (IGF)-I and IGF binding proteins 1–3 in premenopausal women: physical activity and anthropometry (Netherlands). Cancer Causes Control 12:951-958, 2001
9 Gram IT, Norat T, Rinaldi S, *et al.* Body mass index, waist circumference and waist-hip ratio and serum levels of IGF-I and IGFBP-3 in European women. Int J Obes 30:1623-1631, 2006
10 DeLellis K, Rinaldi S, Kaaks RJ, *et al.* Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 13:1444-1451, 2004
11 Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 4:505-518, 2004
12 Harrela M, Koistinen H, Kaprio J, *et al.* Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. J Clin Invest 98:2612-2615, 1996
13 Al-Zahrani A, Sandhu MS, Luben RN, *et al.* IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. Hum Mol Genet 15:1-10, 2006
14 Morimoto LM, Newcomb PA, White E, *et al.* Insulin-like growth factor polymorphisms and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 14:1204-1211, 2005
15 Feik E, Baierl A, Hieger B, *et al.* Association of IGF1 and IGFBP3 polymorphisms with colorectal polyps and colorectal cancer risk. Cancer Causes Control 21:91-97, 2010
16 Le Marchand L, Kolonel LN, Henderson BE, *et al.* Association of an exon 1 polymorphism in the IGFBP3 gene with circulating IGFBP-3 levels and colorectal cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 14:1319-1321, 2005
17 Giovannucci E, Haiman CA, Platz EA, *et al.* Dinucleotide repeat in the insulin-like growth factor-I gene is not related to risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 11:1509-1510, 2002
18 Slattery ML, Murtaugh M, Caan B, *et al.* Energy balance, insulin-related genes and risk of colon and rectal cancer. Int J Cancer 115:148-154, 2005
19 Pechlivanis S, Wagner K, Chang-Claude J, *et al.* Polymorphisms in the insulin like growth factor 1 and IGF binding protein 3 genes and risk of colorectal cancer. Cancer Detect Prev 31:408-416, 2007
20 Slattery ML, Samowitz W, Curtin K, *et al.* Associations among IRS1, IRS2, IGF1, and IGFBP3 genetic polymorphisms and colorectal cancer. Cancer Epidemiol Biomarkers Prev 13:1206-1214, 2004
21 Tiemersma EW, Wark PA, Ocke MC, *et al.* Alcohol consumption, alcohol dehydrogenase 3 polymorphism, and colorectal adenomas. Cancer Epidemiol Biomarkers Prev 12:419-425, 2003
22 Tijhuis MJ, Wark PA, Aarts JM, *et al.* GSTP1 and GSTA1 polymorphisms interact with cruciferous vegetable intake in colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 14:2943-2951, 2005
23 Voskuil DW, Kampman E, van Geloof W, *et al.* No major difference in K-ras and p53 abnormalities in sporadic and hereditary nonpolyposis colorectal adenomas. Dig Dis Sci 45:2187-2194, 2000
24 Baecke J, Burema J, Frijters J. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 36:936-942, 1982
25 Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, *et al.* The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 26:S37-S48, 1997
26 Nagengast FM, Kaandorp CJE, namens Werkgroep Herziening Consensus Follow-up na poliepectomie. Herziene CBO-richtlijn 'Follow-up na poliepectomie'. Ned Tijdschr Geneeskd 145:2022-2025, 2001
27 Davidow AL, Neugut AI, Jacobson JS, *et al.* Recurrent adenomatous polyps and body mass index. Cancer Epidemiol Biomarkers Prev 5:313-315, 1996

28 Almendingen K, Hofstad B, Vatn MH. Does high body fatness increase the risk of presence and growth of colorectal adenomas followed up in situ for 3 years?. Am J Gastroenterol 96:2238-2246, 2001
29 Deal C, Ma J, Wilkin F, *et al*. Novel promotor polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. J Clin Endocrinol Metab 86:1274-1280, 2001
30 Samowitz WS, Wolff RK, Ma KN, *et al*. Polymorphisms in insulin-related genes predispose to specific KRAS2 and TP53 mutations in colon cancer. Mutat Res 595:117-124, 2006
31 Pechlivanis S, Pardini B, Bermejo JL, *et al*. Insulin pathway related genes and risk of colorectal cancer: INSR promoter polymorphism shows a protective effect. Endocr Relat Cancer 14(3):733-740, 2007
32 Wernli KJ, Newcomb PA, Wang Y, *et al*. Body size, IGF and growth hormone polymorphisms, and colorectal adenomas and hyperplastic polyps. Growth Horm IGF Res 20:305-309, 2010
33. Gu F, Schumacher FR, Canzian F, *et al*. Eighteen insulin-like growth factor pathway genes, circulating levels of IGF-I and its binding protein, and risk of prostate and breast cancer. Cancer Epidemiol Biomarkers Prev 19:2877-2887, 2010
34. Patel AV, Cheng I, Canzian F, *et al*. IGF-1, IGFBP-1, and IGFBP-3 polymorphisms predict circulating IGF levels but not breast cancer risk: findings from the Breast and Prostate Cancer Cohort Consortium (BPC3). PLOS ONE 3:e2578, 2008
35 Johansson M, McKay JD, Wiklund F, *et al*. Implications for prostate cancer of insulin-like growth factor-I (IGF-I) genetic variation and circulating IGF-I levels. J Clin Endocrinol Metab 92:4820-4826, 2007
36 Kaaks R, Toniolo P, Akhmedkhanov A, *et al*. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J Natl Cancer Inst 92:1592-1600, 2000
37 Palmqvist R, Hallmans G, Rinaldi S, *et al*. Plasma insulin-like growth factor 1, insulin-like growth factor binding protein 3, and risk of colorectal cancer: a prospective study in northern Sweden. Gut 50:642-646, 2002
38 Ma J, Pollak MN, Giovannucci E, *et al*. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 91:620-625, 1999
39 Gunter MJ, Hoover DR, Yu H, *et al*. Insulin, insulin-like growth factor-I, endogenous estradiol, and risk of colorectal cancer in postmenopausal women. Cancer Res 68:329-337, 2008
40 Jacobs ET, Martinez ME, Alberts DS, *et al*. Plasma insulin-like growth factor I is inversely associated with colorectal adenoma recurrence: a novel hypothesis. Cancer Epidemiol Biomarkers Prev 17:300-305, 2008
41 Flood A, Mai V, Pfeiffer R, *et al*. Serum concentrations of insulin-like growth factor and insulin-like growth factor binding protein 3 and recurrent colorectal adenomas. Cancer Epidemiol Biomarkers Prev 17:1493-1498, 2008
42 Morimoto LM, Newcomb PA, White E, *et al*. Variation in plasma insulin-like growth factor-1 and insulin-like growth factor binding protein-3: genetic factors. Cancer Epidemiol Biomarkers Prev 14:1394-1401, 2005
43 Gorber SC, Tremblay M, Moher D, *et al*. A comparison of direct vs. self-report measures for assessing height, weight and body mass index: a systematic review. Obes Rev 8:307-326, 2007
44 McAdams MA, Van Dam RM, Hu FB. Comparison of self-reported and measured BMI as correlates of disease markers in U.S. adults. Obesity 15:188-196, 2007


alcoh
LOW
meat
fruit
Hi
vegetabl

6

Dietary patterns and colorectal adenoma recurrence



Akke Botma
Renate M. Winkels
Fokko M. Nagengast
Ellen Kampman

Abstract

Purpose
Although associations between diet and colorectal cancers have been studied quite extensively, few studies assessed whether dietary patterns are associated with adenomas, especially with recurrence of colorectal adenomas.

Patients and methods
Adenoma cases (n=565) recruited between 1995 and 2002 were followed up till 2008. Principal components analysis was used to identify dietary patterns from 45 food groups. Associations between these patterns and colorectal adenoma recurrence were examined using Cox regression models.

Results
Within a median person-time of 4.6 years, 165 patients had an adenoma recurrence. Three dietary patterns were identified, referred to as the 'Cosmopolitan', 'Low-meat', and 'Refined foods' patterns. None of these dietary patterns showed clear associations with colorectal adenoma recurrence; hazard ratio (HR) for the highest versus lowest tertile was 1.12 (95% CI, 0.76-1.64), 0.93 (95% CI, 0.60-1.44), and 0.99 (95% CI, 0.66-1.47) respectively. The HR of advanced adenoma recurrence for the highest tertile of the 'Low-meat' pattern was 0.58 (95% CI, 0.23-1.47). Persons in the highest tertile of the 'Refined foods' pattern scores had a HR of advanced adenoma recurrences which was 0.56 (95% CI, 0.22-1.44).

Conclusion
In conclusion, none of the dietary patterns were associated with colorectal adenoma recurrence. Exploratory findings indicated possible associations between patterns and advanced adenomas. Larger studies should specifically focus on advanced colorectal adenoma recurrences.

This work was supported by the Dutch Cancer Society (grant UW-2005-3275)

Introduction

Dietary factors and foods have been thoroughly investigated in relation to colorectal cancer (CRC) and there is convincing evidence that red and processed meat, and alcohol intake increase the risk of CRC, while foods containing dietary fibre protect against CRC [1]. For all other foods and food groups, although not less thoroughly investigated, the influence on CRC is not convincing [1]. One of the possible reasons for these non-convincing results is the difficulty to study separate effects of specific foods, because dietary exposures are highly interrelated [2]. Also analyses of individual foods do ignore the possible interactions between foods and their association with disease. Recognition of the interactive and synergistic effects between foods, explain the increased research focus on the effect of dietary patterns.

In epidemiological studies, dietary patterns have been shown to influence the risk of CRC [3,4]. For example, increasing consumption of a Western-type of diet is associated with an increased risk of colon cancer in most cohorts in the US [5-7], although this is not always seen in European studies [8,9]. Up until now, few prospective studies from Europe have investigated associations between dietary patterns and colorectal tumours.

Colorectal adenomatous polyps are considered precursors for colorectal cancer. Furthermore, persons with a history of colorectal adenomas have an increased risk of colorectal cancer [10]. Identifying dietary factors that prevent colorectal adenoma development could lead to targets for cancer prevention. Few observational studies have investigated effects of dietary patterns on colorectal adenoma recurrence. In a calcium and fibre intervention trial, secondary analyses of dietary patterns show a decreased colorectal adenoma recurrence for those within the highest tertile of Mediterranean dietary pattern scores [11]. The observed dietary intake, and constructed dietary patterns, in this intervention with food supplements might be influenced by the intervention.

The aim of the present study was to examine whether dietary patterns are associated with colorectal adenoma recurrence in a prospective cohort study.

Methods

Study population

For this study, colorectal adenoma patients were included, who were recruited for a endoscopy-based case-control study in the Netherlands. Details of this case-control study have been described elsewhere [12,13]. Briefly, participants were recruited among those undergoing endoscopy in 10 outpatient clinics in the Netherlands between June 1997 and June 2002. Participants were informed of the study by endoscopy staff at the time of colonoscopy or by mail at 3-month intervals using colonoscopy reports of all patients who had undergone colonoscopy. Eligible participants were Dutch-speaking, Caucasian, between 18 and 75 years of age at time of enrolment, had no hereditary colorectal cancer syndromes, did not suffer from inflammatory bowel diseases, had no personal history of colorectal cancer and had not had a (partial) bowel resection. In addition, we used complete information of 42 adenoma cases meeting our criteria, recruited between December 1995 and June 1997, from a preceding study in one of the ten hospitals [14]. Recruitment procedures as well as the questionnaires used were essentially the same as those for the main study. In total 768 participants were diagnosed with at least one histologically confirmed colorectal adenomatous polyp ever in their life and eligible for this study. After inclusion in the case-control study ten persons became ineligible due to surgeries for colorectal neoplasms detected at recruitment (n=7), diagnosed proctitis ulcerosa, having a histological unconfirmed adenoma only, and one person was recruited twice into the POLIEP-(case-control) study, leaving 758 subjects eligible. Adenoma recurrence will only be detected through large bowel examinations, therefore persons who did not have a documented colonic examination after recruitment in the hospital of their recruitment endoscopy (n=143) were excluded from this follow-up study. Subjects who could not be traced in the hospital records (n=50) were not included in the follow-up. In total, 565 participants were included in this prospective study.

Data collection

Dietary assessment and determination of dietary patterns

Habitual food and beverage intake, consumed during the year preceding recruitment colonoscopy, was collected using a self-administered food frequency questionnaire (FFQ). This FFQ was developed for the Dutch European Prospective Investigation Into Cancer cohort [15,16]. The frequency of consumption of 79 main food items could be indicated per day, week, month or year. Colour photographs were included for 21 food items and used to estimate portion sizes. For other foods, a commonly used unit or portion size was specified. Frequencies and portion sizes were multiplied to obtain the amount (in grams) per day for each food item. Total energy intake for each participant was calculated using the Dutch food composition table [17].

To identify dietary patterns, we used principal component analysis (PCA) to aggregate the dietary variables. First, the food items of the FFQ were grouped into 45 food groups, according to type of food (e.g., apples, strawberries and bananas were combined into fruit). Per person the intake of every food group (grams per day) was divided by the total daily energy intake (kcal) and multiplied by 1,000. This was done because we were interested in the composition of the diet, independent of the kilocalories consumed per day. Second, these intake variables (grams/day per 1000 kcal) were used in the PCA to construct dietary patterns. Varimax rotation was applied to obtain orthogonal factors. Eventually, we retained 3 dietary patterns. First, components with an eigenvalue greater than one [19 of 45] were selected. Second, inspec-

tion of the Scree plot, a plot of the eigenvalues by number of components, indicated a final number of three dietary patterns. The Scree plot levelled off after the third component. We ran the PCA three times with a defined number of components, i.e. 2, 3 or 4, and selected three patterns based on the interpretability of all components retained with these runs. The three dietary patterns were labelled as the 'Cosmopolitan', 'Low meat', and 'Refined foods' patterns. We calculated dietary pattern scores by summing a persons' food group intake, multiplied by its component (dietary pattern) loading for each food group (i.e. correlations with the patterns). The influence of food grouping on the patterns retained was checked by repeating the PCA with all original food items. The same patterns emerged.
General life-style factors and disease-related issues, such as smoking, self-reported family history of cancer, medication use, and physical activity were assessed by a self-administered questionnaire. Both FFQ and general questionnaire were handed at the time of endoscopy or sent within 3 months after endoscopy.

Medical (follow-up) information
Participants were prospectively followed via medical records in the recruitment hospitals. Information about all performed colonoscopies, other colonic examinations, colon surgeries, cancer and adenomatous polyp occurrences was abstracted from the records. For each colonic examination, the number of neoplasms, location, size, and histology was ascertained. Any histological confirmed colorectal adenoma detected at least one year after recruitment was counted as a recurrent adenoma. In the Netherlands, colonoscopies performed within a year are mainly done to check if the initial adenoma is adequately removed, rather than check for recurrences [18]. Advanced adenomas were those with a diameter of 1 cm or more and/or tubulovillous or villous histology and/or with high grade dysplasia and/or 3 or more adenomas detected at the same colonic examination. When more than 1 adenoma was diagnosed, size, histology and dysplasia of the largest and/or most advanced adenoma was used to characterize the adenomas. If the size, the dysplasia or histology of the adenoma was not mentioned in the colonoscopy and/or pathology reports, we assumed that the adenoma was not advanced.

Statistical analysis
Cox regression models were used to evaluate the association between the dietary pattern scores and development of the first colorectal adenoma recurrence. Hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated. Person-time started at the date of recruitment sigmoido- or colonoscopy, and ended at date of diagnosis of the first recurrent (advanced) adenoma, date of colorectal cancer diagnosis, date of colonic surgery, date of death, or date of last known colonic examination whichever occurred first. Persons who deceased with unknown date of death were censored at the date of their last hospital visit.
Dietary pattern scores were grouped into tertiles, with the lowest tertile being the reference. Evaluated as possible confounders of the relation between the dietary patterns and colorectal adenoma recurrence were age (continuous), sex, number of large bowel examinations during follow-up (continuous), smoking habits (current, former, never), physical activity (tertiles), regular use of NSAID's (more or less than once a week), family history of CRC (yes/no), personal history of adenomas (yes/no), size of last adenoma (≥1 cm, yes/no/missing), histology of last adenoma (villous structure, yes/no/missing), dysplasia of last adenoma (high grade, yes/no/missing), number of polyps at last colonoscopy with adenomas (≥3, yes/no/missing). Sex and age were the only variables which substantially changed the estimate. Smoking hab-

its and a personal history of adenomas were added to the model to enhance comparability with analysis in chapter 4. Energy intake was not considered as confounder, because the amount of energy consumed is interwoven within the dietary patterns. Extra adjustment for body mass index (BMI) was performed to see whether the influence of dietary patterns on colorectal adenomas was (partly) explained by BMI. Statistical analysis were done using SAS software, version 9.2 (SAS Institute, Cary, NC).

Table 6.3 Hazard ratios (95% Confidence Intervals) of colorectal adenoma recurrence and tertiles of dietary pattern scores of the POLIEP follow-up study

	Dietary pattern, factor 1 'Cosmopolitan' pattern		
	T1 (Low)	**T2**	**T3 (High)**
Total cohort, n	187	195	187
Person time, median	4.9	5	4.3
	HR	**HR (95% CI)**	**HR (95% CI)**
Any adenoma recurrence			
n	53	59	53
HR, age & sex adjusted	1.0	1.02 (0.70-1.48)	1.10 (0.75-1.62)
HR, fully [a)] adjusted	1.0	1.01 (0.69-1.48)	1.12 (0.76-1.64)
Advanced adenoma recurrence			
n	14	12	11
HR, age & sex adjusted	1.0	0.79 (0.36-1.73)	0.85 (0.38-1.90)
HR, fully [a)] adjusted	1.0	0.75 (0.34-1.68)	0.83 (0.37-1.85)

Abbreviation T, tertile; n, number; HR, hazard ratio; CI, confidence interval

Notes [a)] adjusted for age, sex, smoking habits (never, former, current), personal history of adenomas (yes/no), and number of large bowel examination during follow-up

Results

Dietary patterns

We identified three dietary patterns among 565 persons who were previously diagnosed with colorectal adenomas. The component loadings, which are correlations between foods and dietary patterns, of the 3 dietary patterns, are shown in table 6.1. Positive loadings indicate positive associations between foods and dietary patterns, and negative loadings indicate inverse associations with a dietary pattern. The first pattern, labelled 'Cosmopolitan' pattern characterized by high consumption of fried vegetables, salad vegetables, garlic, vegetable oil, chicken, fish, pasta and wine, and lesser consumption of high fat dairy products, and added sugar. The second, labelled 'low-meat' pattern, was characterized by greater consumption of salad vegetables, fruits, low fat dairy products, soy products, pastries, tea and water and lesser consumption of processed meat, red meat, and beer. The third pattern labelled as 'refined foods' pattern characterized by French fries, rice (white & brown), fried vegetables, high-sugar beverages, mayonnaise, salty snacks, candy, and lesser consumption of boiled vegetables, whole-grain bread, potatoes, red meat.

Lifestyle characteristics by dietary pattern

Table 6.2 shows lifestyle and other baseline characteristics of this cohort by tertile of each dietary pattern score. Participants with higher 'cosmopolitan' pattern scores had a higher educational level, were slightly less often current smokers, were more often obese, and had higher alcohol intakes. Participants with a higher 'low-meat' pattern score were more often women, slightly older, less often current smokers, less

Dietary pattern, factor 2 'Low-meat' pattern			Dietary pattern, factor 3 'Refined foods' pattern		
T1 (Low)	**T2**	**T3 (High)**	**T1 (Low)**	**T2**	**T3 (High)**
187	195	187	187	194	188
4.5	4.9	4.8	4.2	5	5
HR	**HR (95% CI)**	**HR (95% CI)**	**HR**	**HR (95% CI)**	**HR (95% CI)**
53	66	46	60	49 56	
1.0	1.20 (0.82-1.76)	0.93 (0.60-1.44)	1.0	0.72 (0.49-1.05)	0.97 (0.66-1.43)
1.0	1.31 (0.89-1.94)	0.93 (0.60-1.44)	1.0	0.67 (0.45-0.98)	0.99 (0.66-1.47)
15	13	9	17	11	9
1.0	0.78 (0.35-1.71)	0.59 (0.23-1.50)	1.0	0.60 (0.28-1.29)	0.55 (0.23-1.33)
1.0	0.87 (0.39-1.94)	0.58 (0.23-1.47)	1.0	0.59 (0.27-1.28)	0.56 (0.22-1.44)

often overweight or obese, more physically active, had a lower alcohol intake, and reported more often a family history of CRC. Participants with higher 'refined food' pattern scores were younger, higher educated, more often current smokers, and less often obese.

Influence of dietary patterns on adenoma recurrence

During a median follow-up of 4.7 years, 165 participants developed an adenoma, 37 participants developed an advanced adenoma. Table 6.3 shows associations between the three dietary patterns and colorectal adenoma recurrences. No associations were observed for high dietary pattern scores and recurrence of any colorectal adenoma, HR 1.12 (95% CI, 0.76-1.64) 0.93 (95% CI, 0.60-1.44), and 0.99 (95% CI, 0.66-1.47) for 'Cosmopolitan', 'Low-meat', and 'Refined foods' patterns respectively. The 'Cosmopolitan' patterns scores were not associated with advanced adenoma recurrences, HR 0.83 (0.37-1.85). The highest tertile of the 'Low-meat' pattern scores was inversely associated with advanced adenoma recurrences (HR, 0.58; 95% CI, 0.23-1.47), but this finding was not statistically significant. Also the 'Refined foods' was not statistically significant inversely associated with advanced adenoma recurrence (HR, 0.56; 95% CI, 0.22-1.44).

Including BMI in the models did not substantially change the HR's of all dietary patterns (data not shown). Extra adjustment for energy intake, which might be considered as part of the dietary patterns or as intermediate variable of the associations between dietary patterns and colorectal adenoma recurrence, did not markedly change the HR's for any adenoma recurrence. However, adding energy (kJ, continuous) to the model changed the HR's of advanced adenoma recurrence in 1 of the 3 dietary patterns with more than 10% ('Cosmopolitan' pattern HR, 0.94; 95% CI, 0.41-2.16; 'Low-meat' pattern HR, 0.62; 95% CI, 0.25-1.54; 'Refined foods' pattern HR, 0.51; 95% CI, 0.21-1.25; data not shown).

Table 6.1 Rotated component loadings for the 3 major principal components of 45 food items/groups from the food frequency questionnaire of participants of the POLIEP-follow-up study

Foods	Factor 1 'Cosmopolitan'	Factor 2 'Low meat'	Factor 3 'Refined foods'
Salad vegetables	**0.55**	**0.36**	...
Fried vegetables	0.54	...	0.29
Legumes	0.23	...	...
Boiled vegetables	...	0.27	**- 0.45**
Garlic	**0.54**	...	...
Juice	...	0.15	...
Soy products	0.17	**0.48**	...
Nuts	...	...	0.17
Fruit	...	**0.57**	- 0.17
Eggs	0.17	...	...
Red meat	...	**- 0.46**	- 0.29
Organ meat	...	- 0.20	...
Processed meat	...	**- 0.54**	...
Chicken	**0.33**	...	...
Fish	**0.31**	0.20	- 0.21
Cheese	0.29	0.16	- 0.22
Dairy products			
>2% fat	**- 0.38**	**0.33**	...
<2% fat	...	**0.42**	...
Vegetable oil	**0.55**	...	...
Added fat			
<0.35 SFA/g fat	- 0.26	- 0.15	- 0.21
>0.35 SFA/g fat	- 0.27	- 0.28	- 0.17
Warm sauces	...	...	0.22
Mayonaises	0.28	...	**0.33**
Salty snacks	...	- 0.18	**0.44**
Added sugar	**- 0.42**	...	...
Candy	...	...	**0.32**
Pastries	- 0.19	**0.38**	...
Coffee	0.20	...	...
Tea	...	**0.43**	...
High-sugar beverages	...	...	**0.47**
Low-sugar beverages	...	...	**0.35**
Beer	...	**- 0.38**	...
Wine	**0.37**	...	...
Spirits	...	- 0.29	...
Water	0.18	**0.40**	...
Pasta	**0.34**	- 0.17	0.24
White rice	**0.31**	...	**0.41**
Brown rice	**0.31**	0.19	**0.30**
Potatoes	- 0.21	- 0.19	**- 0.33**
French fries	...	- 0.28	**0.47**
Breakfast cereals	...	**0.33**	...
White bread	- 0.17	...	**0.30**
Whole-grain bread	...	...	**- 0.51**
Soup	...	...	...
Pizza	0.23	...	0.22

Notes
Factor loading less than |0.15| were omitted for simplicity
Factor loadings greater than |0.29| are bold

Table 6.2 Baseline characteristics of the POLIEP-follow up cohort by tertiles of the 3 dietary patterns

Characteristics	Unit	Factor 1, 'Cosmopolitan' pattern T1, n=186	T2, n=193	T3, n=186
Person years [median (P10-P90)]		4.9 (2.0-8.0)	5.0 (1.6-8.1)	4.3 (1.6-7.9)
No. of large bowel examination [c] [median (P10-P90)]		1 (1-3)	1 (1-3)	1 (1-3)
Adenoma recurrence	%	28.5	30.6	28.5
Advanced adenoma recurrence [a]	%	7.5	6.2	5.9
General				
Sex. female	%	47.3	46.1	50.0
Age at study entry (mean ±SD)	years	60.5 (10.4)	58.9 (9.3)	57.2 (9.1)
Smoking status, current	%	26.9	25.4	25.8
Smoking status, former	%	37.6	40.4	40.3
Physical activity, low [b]	%	33.9	37.8	38.7
Regular NSAID use (≥12/y)	%	22.6	26.4	29.6
Hormone replacement therapy use [j]	%	23.0	19.7	22.6
Education, high [d]	%	12.4	23.3	32.8
BMI				
Normal (<25 kg/m²)	%	44.1	40.9	39.2
Overweight (25 to 30 kg/m²)	%	45.2	45.6	46.2
Obese (≥30 kg/m²)	%	10.2	12.4	14.5
Family history of colorectal cancer	%	23.1	23.8	28.0
Personal history of adenomas [e]	%	21.5	18.1	19.9
Characteristics last adenoma at baseline				
Type of last adenoma				
adenoma of unknown histology	%	14.5	15.5	21.0
tubular	%	52.7	50.3	48.4
tubulovillous	%	19.9	23.3	19.4
villous	%	12.4	10.4	11.3
missing	%	0.5	0.5	0.0
Amount of dysplasia of last adenoma				
high grade dysplasia [f]	%	6.5	8.8	7.5
missing	%	24.2	23.8	27.4
Size of last adenoma [d]				
≥1cm	%	39.8	43.5	33.3
missing	%	14.5	8.8	13.4
No of polyps last pos scopie [g]				
3 or more	%	22.0	23.3	19.4
missing	%	9.1	4.2	8.1
Dietary intake				
Energy intake [median (P10-P90)]	mJ/d	8.9 (6.6-12.0)	8.8 (6.3-12.0)	7.7 (5.6-11.3)
Fibre [median (P10-P90)]	g/d	23.9 (15.4-32.3)	24.2 (15.6-33.4)	22.4 (14.8-31.2)
Vegetables [h] [median (P10-P90)]	g/d	102.6 (64.2-148.3)	116.0 (66.9-170.3)	126.0 (76.2-206.0)
Fruit [i] [median (P10-P90)]	g/d	125.3 (27.3-366.0)	152.9 (17.5-369.4)	136.0 (26.3-404.0)
Fresh red meat [median (P10-P90)]	g/d	56.3 (21.7-102.9)	60.7 (16.0-100.0)	59.7 (9.3-97.5)
Processed meat [median (P10-P90)]	g/d	28.0 (4.0-73.9)	29.5 (6.3-66.4)	20.8 (1.6-62.9)
Alcohol [median (P10-P90)]	g/d	2.6 (0.01-30.3)	11.9 (0.06-42.7)	14.3 (0.06-45.8)
Supplementary multivitamin use	%	10.2	18.1	19.9

Abbreviation: P10-P90, 10-90th percentile; T, tertile; n, number; SD, standard deviation; NSAID, non-steroidal anti-inflammatory drugs; y, year; BMI, body mass index; kg, kilo gram; m, meter; cm, centimeter; mJ, mega Joules; d, day; g, gram

Notes:
[a] Advanced adenomas were those with a diameter of 1 cm or more, and/or a tubulovillous or villous histology, and/or with high grade dysplasia, and/or 3 or more adenomas detected at the same colonic examination
[b] Low physical activity is the lowest tertile of the physical activity score
[c] number of large bowel examinations counting from 1 year after recruitment until end of personal follow-up

Factor 2, 'Low meat' pattern			Factor 3, 'Refined foods' pattern		
T1, n=187	**T2, n=192**	**T3, n=186**	**T1, n=186**	**T2, n=193**	**T3, n=186**
4.5 (1.4-8.1)	4.9 (1.9-7.9)	4.8 (1.9-7.8)	4.2 (1.3-8.1)	5 (2.1-7.8)	5 (1.9-7.9)
1 (1-3)	1 (1-3)	1 (1-3)	1 (1-3)	1 (1-3)	1 (1-3)
28.3	34.4	24.7	32.3	25.4	30.1
8	6.8	4.8	9.1	5.7	4.8
18.2	53.7	71.5	48.9	43.5	51.1
57.1 (9.4)	58.7 (10.4)	60.9 (8.8)	63.0 (7.8)	59.8 (8.7)	53.8 (10.1)
35.8	22.9	19.4	22.0	24.9	31.2
44.4	34.9	39.3	37.6	42.5	38.2
51.9	30.2	28.5	37.1	38.9	34.4
23.5	28.1	26.9	24.2	25.9	28.5
31.8	19.5	11.1	16.7	21.5	29.3
25.1	19.8	23.7	20.4	23.8	24.2
28.9	45.3	50.0	40.3	42.5	41.4
55.6	41.7	39.8	43	49.2	44.6
15.0	12.5	9.7	16.1	8.3	12.9
21.4	25.5	28.0	24.2	26.4	24.2
19.8	17.7	22.0	23.1	19.2	17.2
19.3	19.8	11.8	16.1	20.7	14.0
45.5	50.5	55.4	48.9	48.7	53.8
25.1	17.2	20.4	19.9	19.7	23.1
10.2	12.0	11.8	14.5	10.4	9.1
0.0	0.5	0.5	0.5	0.5	0.0
8	8.3	6.5	8.1	8.8	5.9
24.1	25	26.3	22.6	30.6	22.0
40.1	39.1	37.6	43	36.8	37.1
12.8	14.1	9.7	11.3	9.3	16.1
27.3	20.3	17.2	21.5	24.4	18.8
8.6	7.3	5.4	6.5	7.8	7.0
9.1 (6.3-12.5)	8.4 (6.2-11.7)	7.7 (5.4-11.0)	7.6 (5.4-11.0)	8.6 (6.0-11.8)	9.0 (6.6-13.1)
22.5 (13.7-32.4)	24.1 (15.9-32.4)	23.9 (16.8-31.8)	24.3 (15.4-33.4)	23.6 (15.1-32.2)	22.6 (14.5-31.3)
103.6 (79.2-155.3)	115 (66.2-179.5)	125.5 (79.5-191.7)	123.1 (82.9-193.1)	116 (64.9-179.0)	102.3 (62.6-164.1)
81.6 (8.4-249.1)	173 (43.0-310.0)	238 (105.1-468.4)	175.6 (17.8-389.5)	142 (35.9-375.0)	123 (17.7-354.0)
74.9 (40.5-118.5)	61.5 (20.1-93.8)	33.6 (16.6-80.0)	66.3 (18.5-107.5)	63.5 (12.5-99.0)	51.9 (13.1-97.5)
41.1 (10.9-91.5)	27.3 (7.3-60.2)	11.1 (0.8-35.8)	22.7 (4.4-72.4)	30.2 (3.3-64.5)	24.4 (3.0-64.0)
23.4 (0.2-58.9)	6.9 (0.02-28.7)	2.9 (0.06-24.7)	8.8 (0.00-35.3)	10.1 (0.02-40.9)	8.0 (0.1-46.8)
14.4	14.6	19.4	15.1	17.6	15.6

Notes

d) Higher education, missing for 51 persons
e) Personal history of adenomas before index adenoma
f) defined as adenomas with severe dysplasia
g) number of polyps at last endoscopy with adenomas
h) Definition of total vegetables includes nonstarch legumes and excludes potatoes and vegetables juice
i) Definition of total fruits excludes fruit juices
j) among postmenopausal women only, n=207 (76.7%)

Additional table 6-I

Hazard ratios of colorectal adenoma recurrence and smoking status in a cohort of 565 sporadic adenoma patients

	Smoking status		
	Never smokers	**Former smokers**	**Current smokers**
Total cohort, n	195	223	147
Person time, median	5.0	4.4	4.7
	HR	**HR (95% CI)**	**HR (95% CI)**
Any adenoma recurrence			
n	55	65	45
HR, age & sex adjusted	1.0	1.09 (0.75-1.59)	1.21 (0.80-1.82)
HR, fully [a)] adjusted	1.0	1.05 (0.72-1.54)	1.18 (0.77-1.81)
Advanced adenoma recurrence			
n	12	16	9
HR, age & sex adjusted	1.0	1.19 (0.55-2.60)	1.10 (0.44-2.75)
HR, fully [a)] adjusted	1.0	1.37 (0.61-3.07)	1.12 (0.43-2.91)

Abbreviation: T, tertile; n, number; HR, hazard ratio; CI, confidence interval

Notes: a) adjusted for age, sex, personal history of adenomas (yes/no), no. of large bowel eamination during persontime, alcohol intake (continous)

Additional table 6-II

Hazard ratios of colorectal adenoma recurrence and tertile of alcohol intake in a cohort of 565 sporadic adenoma patients

	Alcohol intake		
	T1 (Low)	**T2**	**T3 (High)**
Total cohort, n	186	193	186
Person time, median	4,7	5,0	4,4
	HR	**HR (95% CI)**	**HR (95% CI)**
Any adenoma recurrence			
n	57	52	56
HR, age & sex adjusted	1.0	0.80 (0.55-1.17)	0.89 (0.61-1.32)
HR, fully [a)] adjusted	1.0	0.84 (0.58-1.24)	0.90 (0.60-1.34)
Advanced adenoma recurrence			
n	11	15	11
HR, age & sex adjusted	1.0	1.20 (0.54-2.63)	0.84 (0.35-2.06)
HR, fully [a)] adjusted	1.0	1.23 (0.55-2.76)	0.81 (0.32-2.06)

Abbreviation: T, tertile; n, number; HR, hazard ratio; CI, confidence interval

Notes: a) adjusted for age, sex, personal history of adenomas (yes/no), no. of large bowel eamination during person time, smoking habits (never, former, current)

Discussion

Three dietary patterns, referred to as the 'Cosmopolitan', 'Low-meat', and 'Refined foods' pattern were identified among those with previous colorectal adenomas. None of the dietary patterns were associated with colorectal adenoma recurrence. Exploratory findings suggest that the 'Low-meat' and 'Refined foods' patterns are both inversely associated with advanced colorectal adenoma recurrences.

In contrast to our null findings, PCA analyses in a calcium and fibre supplementation trial [11], derived a so called 'Mediterranean' pattern (high olive oil, fresh fruit, vegetables, legumes, lean meat and fresh fish consumption) which was associated with a decreased risk of adenoma recurrence in women. No associations were seen in men, nor were associations observed between the other two patterns, 'Western' and 'Snacks' and colorectal adenoma recurrence among women. Patterns in this study were obtained separately for men and women. A dietary intervention trial in which the intervention group had to adhere to a 'Low-fat, high-fibre, and high fruit and vegetable' eating pattern was associated with reduced recurrence of colorectal adenomas only for those who adhered strictly to this diet intervention [19].

More studies looked at associations between dietary patterns and colorectal adenoma incidence. In a cohort of US men [6] two major dietary patterns were obtained, i.e. 'Prudent' and 'Western'. In that study an increased risk of distal colorectal adenomas was observed with higher 'Western' pattern scores. However, an statistically significant inverse association was not observed for the 'Prudent' pattern [6]. In a cohort of French women [20], four patterns were identified, i.e. 'Healthy', 'Western', 'Drinker', and 'Meat eaters' pattern. The 'Healthy' pattern showed a statistically non-significant inverse association with colorectal adenomas and an increased colorectal cancer risk was seen with high 'Meat' pattern scores. The 'Western' pattern (potatoes, pizza and pie, sandwiches, legumes, sweets, cakes, bread, rice, pasta, processed meat, butter, cheese, and eggs), and the 'Drinker' pattern (snacks, coffee, processed meat, wine, low-alcohol beverages, and high-alcohol beverages), were both associated with an increased risk of colorectal adenomas in this French cohort. In our study as well as in several studies [6,11,20] mentioned above PCA was used to identify dietary patterns. A criticism of this data-driven approach is that the components validity is dependent on the study population. The identified patterns reflect actual existing dietary behaviour within the studied population. In different populations, or in the same population at a different time, another set of components might have been observed [14]. This limits the interpretation of these dietary patterns and may explain differences between studies, especially differences between studies from different countries with different eating and lifestyle habits. All mentioned studies [6,11,20], including ours, identified a vegetable and fruit pattern ('Prudent', 'Healthy', or 'Mediterranean' patterns) indicating that this pattern does exist in several populations.

Using PCA requires subjective decisions about the grouping of input variables, the number of retained components, the method of rotation and the labelling of patterns. To study the influence of the food grouping on the PCA results, we performed a PCA with the originally food items from the FFQ. This PCA gave us essentially the same dietary patterns, indicating minimal influence of the grouping. Ideally, performing a PCA in two random samples of the cohort, should have validated these patterns. However, splitting the cohort made the number of participants too small to perform a PCA with 45 variables. To make all choices in retaining the number of components as objective and transparent as possible, we described all steps in the method sec-

tion. The labelling of the identified patterns is subjective, but can be judged from the presented factor loadings (table 6.1).

We used food frequency questionnaires (FFQ) to estimate dietary intake. These questionnaires are measuring true intake with error, but are able to rank participants according to their intake. It should be noted that persons with increased adiposity tend to under-report their dietary intake [21]. Also, not all participants had an adenoma at the recruitment endoscopy, these patients had an adenoma detected before this exam. Having had an adenoma several years before the recruitment colonoscopy will not change the risk of a recurrence, but it may influence the time to a new adenoma. Not all participants (6.5%), could be traced in the recruitment hospitals. This is not likely to be influenced by the exposures understudy, therefore it may not have a large influence on the estimates. One hundred and forty three (18.6%) patients did not have a large bowel examination after the recruitment endoscopy. This could result in bias due to loss to follow-up. The whole study population was insured for health costs, which is obligatory in the Netherlands. Therefore it is unlikely that social class influenced the loss and it is less likely to be associated with the exposures under study

Besides of its prospective design, other strengths of this study were the extensive modifiable risk factor data, standardized collection of medical information from medical records and collection of pathology reports. An important strength of the study is the dietary pattern approach, in which not only individual foods are considered but the whole diet. This approach takes possible interactions between foods into account and reduces the number of dietary variables, using correlations between these variables, and as such diminishes problems of multicollinearity.

In conclusion, none of the dietary patterns were associated with colorectal adenoma recurrence. Larger studies should specifically focus on advanced colorectal adenoma recurrences.

References

1 WCRF/AICR: Continuous update project interim report summary: food, nutrition and physical activity and the prevention of colorectal cancer, 2011
2 Jacques PF, Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? Am J Clin Nutr 73:1-2, 2001
3 Miller PE, Lesko SM, Muscat JE, et al. Dietary patterns and colorectal adenoma and cancer risk: a review of the epidemiological evidence. Nutr Cancer 62:413-424, 2010
4 Randi G, Edefonti V, Ferraroni M, et al. Dietary patterns and the risk of colorectal cancer and adenomas. Nutr Rev 68:389-408, 2010
5 Flood A, Rastogi T, Wirfalt E, et al. Dietary patterns as identified by factor analysis and colorectal cancer among middle-aged Americans. Am J Clin Nutr 88:176-184, 2008
6 Wu K, Hu FB, Fuchs CS, et al. Dietary patterns and risk of colon cancer and adenoma in a cohort of men (United States). Cancer Causes Control 15:853-862, 2004
7 Fung T, Hu FB, Fuchs C, et al. Major dietary patterns and the risk of colorectal cancer in women. Arch Intern Med 163:309-314, 2003
8 Terry P, Hu FB, Hansen H, et al. Prospective study of major dietary patterns and colorectal cancer risk in women. Am J Epidemiol 154:1143-1149, 2001
9 Dixon LB, Balder HF, Virtanen MJ, et al. Dietary patterns associated with colon and rectal cancer: results from the Dietary Patterns and Cancer (DIETSCAN) Project. Am J Clin Nutr 80:1003-1011, 2004
10 Atkin WS, Morson BC, Cuzick J. Long-term risk of colorectal cancer after excision of rectosigmoid adenomas. N Engl J Med 326:658-662, 1992
11 Cottet V, Bonithon-Kopp C, Kronborg O, et al. Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. Eur J Cancer Prev 14:21-29, 2005
12 Tiemersma EW, Wark PA, Ocke MC, et al. Alcohol consumption, alcohol dehydrogenase 3 polymorphism, and colorectal adenomas. Cancer Epidemiol Biomarkers Prev 12:419-425, 2003
13 Tijhuis MJ, Wark PA, Aarts JM, et al. *GSTP1* and *GSTA1* polymorphisms interact with cruciferous vegetable intake in colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 14:2943-2951, 2005
14 Voskuil DW, Kampman E, van Geloof W, et al. No major difference in K-ras and p53 abnormalities in sporadic and hereditary nonpolyposis colorectal adenomas. Dig Dis Sci 45:2187-2194, 2000
15 Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 26:S37-S48, 1997
16 Ocke MC, Bueno-de-Mesquita HB, Pols MA, et al. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 26:S49-S58, 1997
17 Netherlands Nutrition Center: NEVO: Dutch food composition table 2001. The Hague, Netherlands Nutrition Center, 2001
18 Nagengast FM, Kaandorp CJE, namens Werkgroep Herziening Consensus Follow-up na poliepectomie. Herziene CBO-richtlijn 'Follow-up na poliepectomie'. Ned Tijdschr Geneeskd 145:2022-2025, 2001
19 Sansbury LB, Wanke KL, Albert PS, et al. The effect of strict adherence to a high-fiber, high-fruit and -vegetable, and low-fat eating pattern on adenoma recurrence. Am J Epidemiol 170:576-584, 2009
20 Kesse E, Clavel-Chapelon F, Boutron-Ruault MC. Dietary patterns and risk of colorectal tumors: a cohort of French women of the National Education System (E3N). Am J Epidemiol 164:1085-1093, 2006
21 Gorber SC, Schofield-Hurwitz S, Hardt J, et al. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. Nicotine Tob Res 11:12-24, 2009


BMI
Alcohol
Smoking
Dietary
GEO Lynch
POLIEP TO

7

General discussion

This thesis aimed to investigate whether modifiable risk factors influence the risk of colorectal adenomas among populations at high risk of developing colorectal cancer. To meet this aim we have set up two cohort studies, one among persons with Lynch syndrome (the GEOLynch cohort study) and one among former sporadic colorectal adenoma patients (the POLIEP follow-up study). This chapter summarizes the main results, and considers remaining issues about the quality of the studies. Furthermore, it discusses the main findings in light of the existing literature. The chapter ends with concluding remarks, implications and suggestions for future research.

Table 7.1 Summary of main results described in this thesis regarding the associations between modifiable risk factors and colorectal adenomas in high risk populations.

Modifiable risk factor		HR	95% CI	Chapter
Lynch syndrome				
BMI [a] women	≥25 vs <25 kg/m²	0.8	(0.2-3.1)	2
BMI [a] men		8.7	(2.1-37.0)	
Smoking [b]	former vs never	2.7	(1.2-5.9)	3
	current vs never	7.1	(3.1-16.0)	
Alcohol [b]	≥13.0 vs <2.7 g/d (HvL [c])	1.3	(0.6-3.1)	3
'Prudent' pattern [b]	HvL [c]	0.7	(0.3-1.7)	4
'Meat' pattern [b]	HvL [c]	1.7	(0.8-3.5)	
'Snack' pattern [b]	HvL [c]	2.2	(1.0-4.5)	
'Cosmopolitan' pattern [b]	HvL [c]	1.3	(0.6-2.6)	
Sporadic colorectal adenoma				
BMI [d]	≥25 vs <25 kg/m²	1.0	(0.8-1.4)	5
Smoking [d]	former vs never	1.1	(0.7-1.5)	6
	current vs never	1.2	(0.8-1.8)	
Alcohol [d]	≥17.3 vs <2.3 g/d (HvL [c])	0.9	(0.6-1.3)	6
'Cosmopolitan' pattern [d]	HvL [c]	1.1	(0.8-1.6)	6
'Low meat' pattern [d]	HvL [c]	0.9	(0.6-1.4)	
'Refined foods' pattern [d]	HvL [c]	1.0	(0.7-1.5)	

Notes
[a] outcome is adenoma incidence (no association was observed among those with a history of colorectal neoplasms)
[b] outcome is adenoma occurrence in total cohort
[c] HvL= high versus low
[d] outcome is adenoma recurrence

Summarizing the main findings

Table 7.1 summarizes the main findings per high risk group. In the Lynch syndrome cohort (n=486), higher body mass index (BMI) increased risk of incident colorectal adenomas, among men only. In addition, current smokers had an increased risk of colorectal adenomas among both sexes in the Lynch syndrome cohort. Former smokers also had an elevated risk. An association between alcohol consumption and adenoma occurrence could not be detected. Among the Lynch syndrome cohort we identified four dietary patterns: i) 'Prudent', ii) 'Meat', iii) 'Snack', vi) 'Cosmopolitan'. The 'Snack' pattern was associated with increased adenoma occurrence. The other three patterns showed associations into the expected directions, based on findings in general population cohorts, but were not statistically significantly associated with adenoma occurrence.

Whereas BMI showed a positive association with adenoma occurrence in the Lynch syndrome cohort, BMI was not associated with adenoma recurrence (n=165) among 565 sporadic adenoma patients in the POLIEP follow-up study. Nor was it associated with recurrence of advanced adenomas (n=37) in the latter population. Also, no associations were seen for smoking, alcohol consumption and recurrence of adenomas among sporadic adenoma patients. The three dietary patterns ('Cosmopolitan', 'Low meat', or 'Refined foods') did not reveal associations with adenoma recurrence.

In conclusion, the results of our Lynch syndrome cohort suggests that certain modifiable risk factors, e.g. high BMI and smoking, indeed influence colorectal adenoma development. In sporadic colorectal adenoma patients, no statistically significant associations were seen between modifiable risk factors and adenoma recurrence.

Considerations of study quality

The research described in this thesis focused on four different exposures: high body fat measured as BMI, smoking, alcohol consumption and overall diet measured as dietary patterns. Before answering the research question, quality issues of the cohort studies used will be reflected on. Strengths and limitations of these studies have already been addressed in the relevant chapters. This chapter addresses additional issues related to the internal and external validity of the studies, e.g. issues about the study populations, exposure and outcome measurements, and co-variables.

Study populations

Ascertainment bias

Mismatch repair (MMR) mutation carriers in the GEOLynch cohort were identified mainly through a hereditary cancer registry which initially registered families based on their family history of cancer. Therefore larger families and families with young cancer cases are more likely to be in this registry. Consequently, patients, and their families, in this cohort might not be a selection of all MMR mutation carriers in the Netherlands. This selection is problematic when the exposures under study are associated with reasons for identification by the registry. Studying the exposure cancer association might than be biased. As the registry's aim is to promote and coordinate cancer screening in high risk families, and identification is based on cancer history, BMI, smoking habits, alcohol consumption and dietary patterns are therefore not likely to be associated with this selection of families.

Index-event bias

Index-event bias may occur when recurrence is studied and patients are selected because of having had a first event [1,2]. Determinants related to selection of patients with disease, for example factors that are risk factors for disease, which are also related to disease recurrence, can influence the association between one of the determinants and recurrence, via the other factors that influence disease. Patients in the POLIEP follow-up study were ever diagnosed with at least one histologically confirmed colorectal adenoma. Adenoma development is a multifactorial process, which means that more than one risk factor is needed to develop adenomas. When a person has one relatively strong risk factor for developing adenomas, the contribution of the other risk factors needed to develop adenomas is smaller. Patients with colorectal adenomas may have different combinations of risk factors (i.e. risk factor profiles) than the general population. By recruiting colorectal adenoma patients, selected persons are more likely to have a strong risk factor, and therefore might have a more favourable profile of other risk factors. When studying the association between a strong risk factor and recurrence, the more favourable combination of other factors may lead to a null result or even a protective effect of this strong risk factor in relation to recurrence. In the POLIEP follow-up study more patients (25%) reported a family history of colorectal cancer than observed in a population based study (11%) of subjects aged 45 to 70 years [3]. Among these patients with a family history were, among other things, more women, and more patients with BMI <25 kg/m^2. This illustrates possible differences in risk factor profiles, which could have influenced our findings. Adjustment of known risk factors can help in standardizing risk profiles. Still, unknown or unmeasured factors may influence associations between the risk factor under study and recurrence.

Follow-up bias

Loss to follow-up in the GEOLynch study is nearly null. In the POLIEP-follow-up study 6.5% of patients could not be traced in the hospital records and another 18.6% did not have a documented colonic examination after recruitment. The validity of the association estimate may be effected when a percentage of participants is not analysed due to loss to follow-up [4]. Study losses will tend to bias the estimate when the exposure variable is an effect modifier for the association between study participation and disease. BMI, smoking habits, alcohol intake or dietary patterns most probably only modify the association between participation and disease when other morbidities, associated with these factors, like cardiovascular diseases, are a reason to stop colorectal screening. Ending follow-up screening is based mainly on age in combination with number of ever detected colorectal adenomas, as current guidelines [5] indicate end of follow-up at age 65 years with one detected adenoma ever or at age 75 years when two adenomas were ever detected. With three or more adenomas, the age to end screening depends on overall health, which could be influenced by the factors under study.

Heterogeneity of the study population

In both, the GEOLynch cohort and the POLIEP follow-up study, part of the study population had colorectal neoplasms before recruitment. Theoretically, other risk factors might be important for colorectal neoplasm recurrence than for first colorectal neoplasms. On the other hand most colorectal neoplasms develop via the adenoma-carcinoma sequence and probably the same set of risk factors may contribute to development of every adenoma. In the GEOLynch cohort as well as in the POLIEP follow-up study more adenomas developed among participants with former neoplasms. A higher risk of colorectal adenomas on its own will not influence the association between risk factors and disease. However, in the GEOLynch cohort study a different association between overweight (≥25 kg/m²) and adenomas was observed among men without earlier neoplasms than among men with former colorectal neoplasms. Differences in associations between persons with or without colorectal neoplasms were not observed for BMI in the POLIEP follow-up study, nor for smoking, alcohol consumption or dietary patterns in the GEOLynch cohort. Is the different association between BMI and colorectal adenomas in those with and without former colorectal neoplasms a real difference? In chapter 2 it is discussed that a higher median age among those with a history of colorectal neoplasms probably influences the number of recurrences rather than the association between BMI and colorectal adenomas. Furthermore, the higher number of partial colon resections among Lynch syndrome patients with a history of colorectal neoplasms might not have a large influence on the association, because current BMI was not different between patients with or without a partial colon resection. Although, the population of the GEOLynch cohort is too small to draw firm conclusions, the different associations for BMI in the separate subgroups indicate that other risk factors could be relevant for recurrence of adenomas than for adenoma incidence.

Generalizability

In the GEOLynch cohort patients were not a random sample of the whole Lynch syndrome population in the Netherlands, because of recruitment via a hereditary cancer registry and hospitals. Generalizing to all Lynch syndrome carriers might therefore not hold. Furthermore not all family members of the identified families are registered. Possibly, family members in the registry might be more health conscious, higher educated and more aware of their cancer risks than those who are not.

This will probably not bias associations between exposure and risk of adenomas but might diminish the variation or range of exposure and therefore decrease the possibility to detect relevant associations. However, as shown in chapter 3, a statistically significant increased risk of adenoma development was observed for current smokers despite the lower percentage of current smokers in the GEOLynch cohort (18%) compared to the general Dutch population (28% above 12 years) [6].
In the POLIEP follow-up study patients were recruited at outpatient clinics. To get a colonoscopy at an outpatient clinic in the Netherlands one must be referred, which is mainly done with an indication only. Because, patients were recruited at clinics, the study might not be representative for all persons with sporadic colorectal adenomas among the general population. However, generalization to those adenoma patients detected at clinics might hold. While BMI, smoking and alcohol intake are probably not associated with referral for endoscopy, a low-fibre diet might result in complaints needed for referral.

Measurement of modifiable risk factors
The modifiable risk factors in both cohorts are measured with comparable quality as is done in other studies. All risk factor assessments rely on self-report of the participants, which may be inaccurate due to, for example, incomplete recall. Inaccurate exposure assessment may lead to misclassification which could either be differential or non-differential, as has been discussed in detail elsewhere [7]. Differential misclassification might occur when patients believe that an exposure could have influenced their tumour. They might report exposures more accurately or at least differently than persons who have not yet experienced adenomas or cancer. Within the Lynch syndrome cohort half of the persons had colorectal neoplasms (20% had colorectal cancer) before the start of the study. In addition, in our sporadic colorectal adenoma cohort around 25% had adenomas before the baseline adenoma. While the way exposures are reported could not be different for future adenomas, having experienced cancer or adenomas, might lead to differential reporting. A recent article among persons considering genetic testing for Lynch syndrome revealed that around 76% of participants believed that dietary behaviours could influence cancer risk [8]. While their risk of adenomas might be higher because of having had colorectal neoplasms these patients might live healthier and report their habits more precise due to their beliefs. This may result in a biased overall result, because the association in persons with a history of neoplasms will be different. Stratifying for having had neoplasms in the Lynch syndrome cohort did, however, show similar associations for persons with and without former neoplasms, except for BMI. This suggests that potential differential reporting did not influence most associations.
The beliefs on lifestyle factors influencing adenoma risk might be the same as with cancer. However, having had a colorectal adenoma probably has less impact than having cancer and the resulting impact on reporting lifestyle factors might, therefore, not be large. Furthermore, in the POLIEP follow-up study everybody had at least one adenoma ever in their life. Differential reporting will thus not be an issue in the sporadic adenoma cohort.

Assessing body mass index
It is known from literature that reported and measured weight are highly correlated. Studies on validity of self-reported weight and height indicate that people tend to under-report their body weight, especially those with increased adiposity, and over-report their height, especially those with a higher BMI and those who are older [9,10]. In the studies described in this thesis, BMI was grouped into two categories. Under-

reporting might have resulted in persons who should be classified as overweight but end up in the normal weight category. This could result in underestimation of the estimate.

Smoking and alcohol intake
Socially undesirable behaviours, such as smoking, are particularly prone to under-reporting. A recent review found that self-reports underestimate true smoking prevalence [11]. Some current smokers might have classified themselves as former smokers. This could have overestimated the risk for former smokers. An earlier study found that the discrepancy between actual and reported smoking is larger when individuals have smoking-related diseases [12]. Whereas smoking is a known risk factor for cancer, it is more likely to be linked with other cancer types, like lung or head and neck cancer than with colorectal adenomas or colorectal cancer. We hypothesize that having had a history of adenomas or cancer, might not have led to larger underestimations of actual smoking in our cohorts than those without such a history. In the Lynch syndrome cohort, for example, the percentage of current smokers was higher among those with a history of neoplasms. Also we did not find differences in the association between smoking and colorectal adenomas in Lynch syndrome for those with and without a history of colorectal neoplasms.

A systematic review on alcohol intake assessment concluded that self-reported alcohol intake is systematically underreported [13]. Furthermore, the capacity of questionnaires to rank individuals according to their alcohol intake was satisfactory [13]. Alcohol intake in our populations was estimated with food frequency questionnaires (FFQ's). These were validated against 24-hour recalls showing high correlations of 0.83 (men) and 0.90 (women) for ethanol intake. Also correlations for ranking subjects on alcoholic drinks was high, 0.74 and 0.87 for men and women respectively [14,15]. The review did not reveal that underreporting was proportional to the level of intake, although they did not rule this out either. So, those with high alcohol intakes might possibly underreport more than others and this can influence the ranking. We studied alcohol intake continuously as well as grouped in tertiles. The continuous estimates may be attenuated if misreporting was present.

Assessing dietary intake
Assessing dietary intake by self-report in food frequency questionnaires (FFQ) is probably done with less accuracy (i.e. with more error) than assessment for the other exposures. In both our cohorts usual dietary habits were assessed using a FFQ. As with smoking, reported food intakes might be influenced by social desirable answering. In addition, participants may find it difficult to recall and average their long term intake, and using food composition data and average portion sizes may provide imperfectly estimated amounts consumed (discussed in Willett [16]). When studying diet-disease associations, underreporting is not a problem, if it is not influencing the ranking of the participants. The problem with dietary intake is that for example obese persons tend to underreport more, which might influence the ranking. This is a topic which is debated at large. Recently, a commentary [17] was written to stress the importance of correcting associations between diet and disease for measurement error, because these errors can attenuate associations. A single mismeasured variable will result in an attenuated but valid association, however for a multivariable model with two or more mismeasured exposures the estimated risks may become attenuated, inflated, or change direction. Based on the OPEN study Freedman *et al.* [17] concluded that there is no concern over false-positive results, but false-negative

results can be present. In chapters 4 and 6, we used food intake data (grams per day / 1000 kcal) from FFQ's to construct dietary patterns. We did not estimate measurement errors and correct the associations between the dietary patterns and colorectal adenomas for this. So far there are no studies addressing possible effects of measurement error in dietary pattern construction and the subsequent association with disease. The dietary patterns are combinations of multiple foods entered in a model as a single dietary exposure, which according to Freedman *et al*. [17] may result in valid but possible attenuated associations.

Multiple measurements of the exposure under study will reduce random variation and might therefore be more efficient when having a relatively small sample size. Measuring the same exposure with different instruments, such as questionnaires, or blood markers, could enhance the validity of the measurement. These issues were recently addressed by Freedman *et al*. [17] Possibly this can be done in a random sample of the population under study. While the self-report of height, weight and smoking are quite valid, dietary intake as discussed is often poor. When studying diet, we should consider the measurement errors of questionnaire data. Therefore, we need to estimate these errors in our own study populations so that we can calibrate the measurement errors of the questionnaires.

As said before, we made use of the highly correlated foods to construct dietary patterns. The order of the foods in the questionnaires is based on the way foods are traditionally consumed in the Netherlands. These foods could be correlated just by the way they are ordered or grouped in the questionnaire. Because the correlations of the foods are used in the principal component analysis (PCA) to construct dietary patterns, artificial correlations influenced by the way the FFQ is constructed might influence corresponding dietary patterns. Ideally, to grasp the dietary patterns that exist within the population, it is preferable to use a dietary assessment method that has no artificial correlations. However, it is unlikely that such a method exists as ordering and grouping foods makes it easier for participants to remember their food intake and probably increase correct recall. To check if dietary patterns emerging from a PCA are there because of the ordering of questions the PCA should be run with the questions about the main groups only. When we performed such analyses in our cohorts the same patterns were found.

Timing of exposure

Misclassification of exposure can also happen because the exposure under study is measured within a time period that is not relevant to the outcome of interest. The normal adenoma-carcinoma sequence is estimated to take 10 to 12 years [18,19]. The exposures might influence all the stages of this sequence, early as well as later stages in carcinogenesis. Patients with Lynch syndrome probably have a more rapid colorectal carcinogenesis than in the general population [20-22]. Exposures that influence early stages of an adenoma might act shortly before an adenoma is detected. So we think, although we only had a median follow-up of 20 months, that the exposures at the period measured indeed can have had an influence on the detected adenomas in the study. In addition, we assessed habitual dietary intake with a FFQ and assessed current as well as past smoking habits, giving us insight in longer term and habitual exposures.

In the sporadic colorectal adenoma cohort all participants had an endoscopy at recruitment. They were at risk for recurrence from their last endoscopy with an adenoma which in some cases happened a couple of years before recruitment. The

questionnaires were filled out around or several months after the recruitment colonoscopy. In some cases the early development of a new adenoma might have already started before the questionnaires were filled out. Thus the effects that we see might be a combined effect on early and later stages of adenoma formation. As with the Lynch syndrome population, longer term and habitual exposures were assessed. Therefore we think that we did have exposure information in the time window relevant for possible adenoma recurrences.

Outcome measurement

Hereditary cancer registry and Medical records

We have collected outcome information via the Netherlands Foundation for the Detection of Hereditary Tumours (NFDHT) and via medical records at two University Medical Centres. The NFDHT contacts specialists, whom perform colorectal screening of the patients, for follow-up information. The extra step between NFDHT and specialist makes it more vulnerable for mistakes in data collection than retrieving info from hospital records directly, although it is the same data. The relevant question is, do all colonoscopies end-up in this registry? Because the registry requests information about colonoscopic screenings repeatedly from specialists, information is delayed rather than missed. Even if we have missed some adenomas at the NFDHT due to a delay in retrieving the information from the specialists, we have no reason to believe that this is associated with the exposures under study. Therefore it seems unlikely that this has influenced our results.

In the POLIEP follow-up study the recurrence of colorectal adenomas was assessed using medical records. Medical record data are considered the gold standard when compared with registry info or self-report. Nevertheless, records could not be found for 6.5% of the participants. It is unlikely that this is associated with either the exposure under study or the recurrence of adenomas. Therefore it is not expected that this has biased our findings. Furthermore, recurrent adenoma cases were defined as those diagnosed with a histologically confirmed colorectal adenoma. However, in some hospitals not all lesions went to pathology. It seemed that based on the judgement of the endoscopist some were not thought relevant to send in. It might be that some of these polyps were small nonadvanced adenomas, but not classified as adenomas, because there was no histological confirmation.

Screening practices

Colorectal adenomas (having the outcome of interest) are detected only when bowel examinations are performed, cause disease is asymptomatic. Both studies were observational and therefore we had no influence on the periods between the large bowel examinations. Ideally you want that everybody has the same time between colonoscopies because the time can also introduce differences in finding an adenoma or not.

The GEOLynch cohort population is under constant surveillance, and around sixty per cent had its last two colonoscopies according to the guidelines [23] within two years. In addition, all participants are screened with full colonoscopies at standard intervals. Because of this standardized screening, it is possible to study the association between the modifiable risk factors and adenomas accurately. In contrast to the Lynch syndrome cohort, larger variation in the time between large bowel examinations was present between persons in the POLIEP follow-up study. During the follow-up of the POLIEP study no colorectal cancer screening programme existed for those individuals without a personal or family history of colorectal neoplasms in the

Netherlands. However, individuals with a history of adenomas were advised by the specialist according to the Dutch guidelines for follow-up after polypectomy of adenomas, which depend on the number of polyps that are found. Currently, a follow-up colonoscopy is indicated after 6 years, while if 3 or more adenomas are detected a follow-up colonoscopy is advised after 3 years [5].
The possibility of missing adenomas might be higher in the sporadic adenoma population, because screening is done less often. This outcome misclassification will only influence the associations studied if it is related to one of the exposures under study. We have no indication that the exposures under study, having a higher BMI, current or former smokers, drinking alcohol or having a specific dietary pattern, influenced screening practices. Lifestyle characteristics are not included in the guidelines for follow-up after polypectomy. They are not in indication for different screening practices in the Netherlands. However, it might be that more health conscious people adhere more to their follow-up screening than those who are less health conscious. This might influence the associations detected because when people who are healthy go for screening more often, this may result in detection of more adenomas in this group. Possibly this can result in biased estimates.

Confounding or intermediate variables

High body fatness, in our cohort measured by BMI, generally is the result of an energy intake exceeding energy expenditure, by either eating too much, or exercising too little or a combination of both. BMI, energy intake, and physical activity are variables within the pathway of energy balance and colorectal neoplasms. Adjusting for variables in the same pathway, might reduce associations towards null because it is part of the effect [24,25]. In our cohorts we were interested in the total effect of BMI on colorectal adenomas. Therefore, we did not adjust for energy intake and physical activity in our analysis. Within the same reasoning one could argue that BMI is an intermediate variable for the association between dietary patterns and colorectal adenomas. We chose to study the total effect of dietary patterns, including the effects it might have on BMI and show associations unadjusted for BMI. Also, most modifiable risk factors are associated with social economic status, or its proxy, educational level. Furthermore, educational level has been associated with colorectal cancer albeit in different directions [26,27]. Because education is a contributor to the variety of lifestyles and we wanted to study the total effects unconditional of educational level, we did not adjust for education.

Main findings, what do others find?

Body mass index

Lynch syndrome

The association between BMI and colorectal neoplasms in Lynch syndrome patients (chapter 2) has only been investigated by one other study so far [28]. This study also suggest that a high BMI increases the risk of colorectal neoplasms in Lynch syndrome patients. The association observed by Win *et al.* [28] was not different between men and women, while the positive association in the GEOLynch cohort was only seen among men. Furthermore, a much stronger association was observed with colorectal adenomas in the GEOLynch cohort study than with colorectal cancer in the study by Win *et al.* [28]. There are several differences between the studies that could explain these slightly different findings. First of all, the outcome studied is different, with colorectal adenomas being precursor lesions which might not all develop into cancer. A stronger association with adenomas might suggest that BMI is more associated with the early stages of colorectal carcinogenesis in Lynch syndrome. However, difference could also be observed because the GEOLynch cohort study is a prospective cohort, while Win *et al.* [28] was studying the association with BMI retrospectively. Participants had to recall their BMI at age 20, which might have resulted in larger measurement errors. Furthermore, patients in our cohort were regularly screened, while the retrospective cohort studied the association in an unscreened Lynch syndrome population, ending person-time with the first colonoscopy.

Sporadic Adenoma recurrence

Besides the prospective study described in chapter 5, two case-control [29,30] and four prospective studies [31-34], investigated the association between BMI and adenoma recurrence. These studies show inconsistent results, but one of these is a pooling study [34] of seven prospective USA based trials It shows a statistically significant moderate increased risk for those being obese compared to normal weight. Contrary to the findings of the pooling study [34], we did not observe a statistically significant increased risk of adenoma recurrence with being overweight. Possibly this association exists especially for persons who are obese, of which we had only a few in our cohort.

Smoking and alcohol consumption

Lynch syndrome

Besides the prospective cohort described in this thesis (chapter 3), two retrospective cohort studies assessed the association between smoking and colorectal cancer in Lynch syndrome [35,36]. In agreement to our findings the retrospective studies found increased colorectal cancer risks for carriers who smoked compared to never smokers.

Two studies investigated associations between alcohol intake and colorectal neoplasms in Lynch associated carcinogenesis [35,37]. In agreement with our results (chapter 3), both studies did not observe statistically significant associations.

Sporadic adenoma recurrence

The findings in chapter 6 did not reveal increased sporadic adenoma recurrences for former and current smokers. In addition, no positive associations were seen between smoking, current or former, and advanced recurrences. Other studies [38-41] observe inconsistent results. A case-control study in New York [38] and the Wheat-Bran-Fiber

trial [39], both saw a positive association between smoking and recurrence of any adenoma [38,39], while two other studies [40,41] did not find statistically significant associations between smoking and adenoma recurrences. Case-case analyses in the Wheat-Bran-Fiber trial, showed an increased risk of multiple adenomas with longer smoking duration. They also observed positive associations between smoking duration, number of cigarettes smoked per day and development of large (≥1 cm) adenomas [39]. The other three studies did not investigate the association with multiple, large or advanced adenomas.

Diet and dietary patterns

Lynch syndrome

The findings from chapter 4 suggest that dietary patterns may be associated with risk of colorectal adenomas in MMR gene mutation carriers. We observed a borderline significant risk of colorectal adenomas for the highest tertile of 'Snack' pattern scores. Besides the prospective cohort study described in this chapter, no other cohorts have studied the associations between diet and colorectal neoplasms in Lynch syndrome. Two reports from our earlier case-control study in families suspected for Lynch [37,42] revealed increased risks of colorectal neoplasms for fruit and possibly for dietary fibre as well. Our 'Prudent' dietary pattern, with high intakes of fruit, was inversely associated with colorectal adenomas, but this was not statistically significant.

Sporadic Adenoma recurrence

In chapter 6, we were unable to show a statistically significant decrease in risk of any adenoma recurrence with our 'Low-meat' pattern, which seems the healthiest pattern in our cohort. However, findings suggest a decreased risk of advanced recurrences for high consumption of this 'Low-meat' pattern. Within an randomized controlled trial of calcium and fibre supplementation in Europe, principal component analysis was used to derive dietary patterns [43] to study associations with adenoma recurrence. Of the three patterns seen in both men and women, only the Mediterranean pattern, with high consumption of olive oil, vegetables, fruit, and lean meat, was associated with a decrease in risk of adenoma recurrence among women. In a low-fat, high-fibre, high-fruit and -vegetable trial from the USA, strict adherence to the intervention, which are persons who consistently reported meeting the 3 dietary goals at all 4 annual visits, is associated with lower adenoma recurrence, especially with advanced recurrences [44]. Overall, the low-fat, high-fibre, high-fruit and -vegetable did not find an effect on the recurrence of colorectal adenomas [45,46].

Potential underlying mechanisms

Associations between BMI, smoking and colorectal adenomas appear to be much stronger in our Lynch syndrome population than is generally seen in the general population. Associations may be stronger among Lynch syndrome patients because the first hit, a germline mutation in a MMR gene, is already present from birth. Modifiable risk factors that can cause the second hit, can thus show their effect at an earlier age. Because only one hit is needed for the beginning of the accumulation of short repetitive sequences resulting in high microsatellites, the association with the risk factors capable of causing the hit seems stronger than when two alleles need to be silenced. Silencing two alleles need more time and will not necessarily be the result of the same factor. Influence of one of the multiple factors will be weaker than when one cause is needed to start an event.

In chapter 3 we discussed the possibility that smoking might influence hypermethylation of the mismatch repair genes, disrupting DNA mismatch repair in mutation carriers by silencing the normal allele, inherited from the unaffected parent [47,48]. Thygesen *et al*. [49] showed that alcohol is stronger associated with distal colorectal cancer as compared to right sided colon cancer. This observations led to the hypothesis of Watson *et al*. [35], that alcohol induced colorectal carcinogenesis might evolve through a molecular pathway distinct from Lynch-related CRC. On the other hand it is thought that alcohol can influence DNA methylation indirectly, via an antifolate effect, which might suggest that a high alcohol intake can influence risk of neoplasms in Lynch syndrome by methylation of a MMR gene.

Conclusions, implications and future research

The studies described in this thesis provide support for the hypothesis that modifiable risk factors are influencing risk of colorectal neoplasms among Lynch syndrome patients. The studies among sporadic colorectal adenoma patients did not confirm that associations for adenoma recurrence are similar to those for adenoma incidence. Although, the size of our study did not allow us to draw firm conclusions regarding advanced recurrence, findings indicated that diet may influence especially recurrence of advanced adenomas.

Clinical implications: What is the advice to those at high risk of colorectal cancer?

Lynch syndrome

Findings of the GEOLynch cohort indicate that current smokers and overweight men have higher risks of colorectal adenomas. Lynch syndrome patients have a 25-70% risk of colorectal cancer to age 70. The expression of the syndrome within affected-families varies: some patients develop CRC at a young age, others at an advanced age (e.g. >60 years).The associations with BMI and smoking seen in this thesis might explain part of this variability. Other parts of the variation may be explained by modifiable factors not studied within this thesis or by common polymorphic variants which have been evaluated in a number of studies [50-54]. These individuals with inherited high CRC risk who smoke and are overweight could be advised a more intensive screening programme than 'normal risk' Lynch syndrome patients. Furthermore, while regular colorectal screening is the only proven (secondary) prevention for colorectal cancer in Lynch syndrome patients at this moment [55,56], these patients should be advised to stay within the normal weight range and refrain from smoking. This advice, as well as the influence on risk, can be addressed by the clinical geneticist, for example when results of MMR mutation testing are discussed. Lynch syndrome patients also have medical specialists who are responsible for colorectal screening, often the gastroenterologists. They see their patients over the years, and could therefore advice the patient on this matter more regularly. In our current health care system there is not much room and time (read money) for primary prevention, and the main focus of the clinic logically is to treat rather than to prevent. In my opinion, also the general practitioner should have a more structural role in primary cancer prevention by advising these, and other, high risk patients.

Sporadic colorectal adenoma

The results on sporadic adenomas of this thesis (chapter 5 and 6) might not have a direct implication for the clinic, because no convincing effect of modifiable factors on recurrence of adenomas was observed. Furthermore, literature on modifiable factors and adenoma recurrences is limited for the exposures under study. Some studies [34] suggest an increased risk of adenoma recurrence for obese, or smoking individuals. Theoretically, risk factors for adenoma recurrence might be the same as those influencing first occurrence, but this was not confirmed in our studies. Associations might have been attenuated due to bias and lack of power. Larger studies should focus on recurrence of advanced adenomas.

In the coming years a national colorectal screening programme, using FOBT, will be launched in the Netherlands. This screening programme most probably will increase the prevalence of people in the general population who have had an adenoma. These

individuals are then identified as having a higher risk of colorectal cancer than the general population. Until the influence of modifiable factors on the recurrence of (advanced) adenomas is clear, it should be advised , as well as is done for the population at large, to follow lifestyle recommendations for cancer prevention as given by the WCRF [57,58].

Scientific implications and future research directions

Lynch syndrome

The research in this thesis show an association with BMI among men, but not among women with Lynch syndrome. Differences between both sexes in the strength of the BMI-colorectal-tumour associations are generally seen [54,58]. Difference could be due to a difference in fat distribution. Women have higher subcutaneous and lower intra-abdominal adipose tissue and probable lower intrahepatic cellular lipids than men within the same BMI-range [59] However, there is large variation in intra-abdominal fat at a given BMI or even waist circumference. A higher physical activity decreases intra-abdominal fat. This may result in less variety in abdominal fat or lower correlations between BMI and intra-abdominal fat, which could lead to less clear associations between BMI and colorectal tumour risk. While it is financially impossible to assess intra-abdominal fat among the whole GEOLynch study by MRI- or DEXA scan, valid measurement of physical activity and studying BMI stratified by physical activity levels might already provide some clarification on the association between BMI and colorectal tumours among women.

The question which is also relevant for Lynch syndrome patients is what the influence of modifiable risk factors is on the risk of colorectal cancer and other frequently occurring cancers in Lynch syndrome, such as endometrial cancer, in a regularly screened Lynch syndrome population. This question can be addressed with an extended follow-up of the current cohort, especially from those without a history of colorectal cancer. The influence of modifiable factors on risk of other cancers in Lynch syndrome is largely unknown. To study associations with endometrial cancer or with less frequently occurring tumours, a larger study population is needed. One way to achieve this is cooperating internationally, as there might not be enough patients with Lynch syndrome in the Netherlands.

Sporadic colorectal adenoma

In the POLIEP follow-up study no convincing associations between the modifiable risk factors studied and sporadic colorectal adenoma recurrences were observed. As discussed, a possible reason for the discrepant findings with the literature on incident adenomas could be an influence of index event bias. The selection of adenoma patients could have biased the association between risk factors and adenoma recurrence, due to shared risk factors for adenoma incidence and recurrence. Exploration of this potential bias and its influences on risk estimates are needed. Part of the bias might be explored by stratification of the risk factors, however a larger adenoma recurrence study, such as the pooling project of prospective adenoma studies [60] is needed to do so.

Furthermore, due to a limited number of advanced recurrences in the current POLIEP follow-up study, it was not possible to draw firm conclusions on the role of modifiable risk factors on advanced recurrences, which may be most relevant for ultimate colorectal cancer. Even a more relevant question to answer is whether these factors influence the risk of (advanced) adenoma recurrence in persons with an advanced

adenoma at baseline. These two research questions will also need a larger population and larger numbers of advanced recurrences to be answered with more certainty. To answer these questions, follow-up of this population should be optimal, which makes regular screening practices with removal and histological confirmation of detected polyps, not only from a clinical, but also from a scientific perspective highly valuable.

Dietary patterns

Within both populations we studied dietary patterns a priori, i.e. we constructed the patterns using principal component analysis (PCA), a data reduction technique based on the correlation matrix of foods included in the FFQ [61]. As discussed PCA tries to construct components which explain the largest possible variation in food intake. Another way to study dietary patterns is a posteriori, using current knowledge or existing dietary recommendations to classify subjects into several healthy or not so healthy patterns. These patterns can also include other lifestyle factors, such as physical activity level or smoking habits. Furthermore, recommendations can be studied to see whether these indeed lower risk of chronic disease.

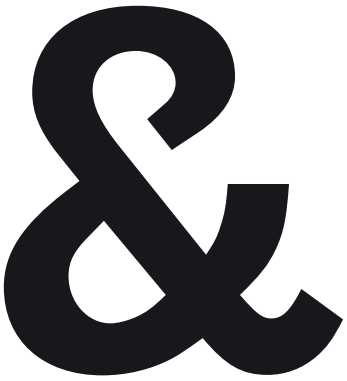
Summarizing, as far as future scientific research is concerned, we do need more studies, but especially more efficient, smarter use of existing, ongoing studies. Larger numbers, more research, researchers are like normal people, always wanting more, never satisfied.

References

1 Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. Epidemiology 15:615-625, 2004
2 Dahabreh IJ, Kent DM. Index event bias as an explanation for the paradoxes of recurrence risk research. JAMA 305:822-823, 2011
3 de Jong AE, Vasen HF. The frequency of a positive family history for colorectal cancer: a population-based study in the Netherlands. 64:367-370, 2006
4 Greenland S. Response and follow-up bias in cohort studies. Am J Epidemiol 106:184-187, 1977
5 Nagengast FM, Kaandorp CJE, namens Werkgroep Herziening Consensus Follow-up na poliepectomie. Herziene CBO-richtlijn 'Follow-up na poliepectomie'. Ned Tijdschr Geneeskd 145:2022-2025, 2001
6 CBS. Zelfgerapporteerde medische consumptie, gezondheid en leefstijl: Lengte en gewicht (20+) & Onder- en overgewicht (20+). Centraal Bureau voor de Statistiek, 2009
7 Rothman KJ, Greenland S: Modern epidemiology. Philadelphia [etc.], Wolters Kluwer/Lippincott Williams & Wilkins, 2008
8 Palmquist AEL, Upton R, Lee S, *et al*. Beliefs about cancer and diet among those considering genetic testing for colon cancer. J Nutr Educ Behav 43:150-156, 2011
9 Engstrom JL, Paterson SA, Doherty A, *et al*. Accuracy of self-reported height and weight in women: an integrative review of the literature. J Midwifery Womens Health 48:338-345, 2003
10 Gorber SC, Tremblay M, Moher D, *et al*. A comparison of direct vs. self-report measures for assessing height, weight and body mass index: a systematic review. Obes Rev 8:307-326, 2007
11 Gorber SC, Schofield-Hurwitz S, Hardt J, *et al*. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. Nicotine Tob Res 11:12-24, 2009
12 From Attebring M, Herlitz J, Berndt AK, *et al*. Are patients truthful about their smoking habits? A validation of self-report about smoking cessation with biochemical markers of smoking activity amongst patients with ischaemic heart disease. J Intern Med 249:145-151, 2001
13 Feunekes GIJ, van 't Veer P, van Staveren WA, *et al*. Alcohol intake assessment: the sober facts. Am J Epidemiol 150:105-112, 1999
14 Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, *et al*. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 26:S37-S48, 1997
15 Ocke MC, Bueno-de-Mesquita HB, Pols MA, *et al*. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 26:S49-S58, 1997
16 Willett W: Nutritional epidemiology. New York [etc.], Oxford University Press, 1998
17 Freedman LS, Schatzkin A, Midthune D, *et al*. Dealing with dietary measurement error in nutritional cohort studies. J Natl Cancer Inst 103:1086-1092, 2011
18 Morson BC. Evolution of cancer of the colon and rectum. Cancer 34:845-849, 1974
19 Winawer SJ, Fletcher RH, Miller L, *et al*. Colorectal cancer screening: Clinical guidelines and rationale. Gastroenterology 112:594-642, 1997
20 Vasen HFA, Nagengast FM, Meera Khan P. Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome). Lancet 345:1183-1184, 1995
21 de Jong AE, Morreau H, Van Puijenbroek M, *et al*. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 126:42-48, 2004
22 Jass JR, Stewart SM, Stewart J, *et al*. Hereditary non-polyposis colorectal cancer -- morphologies, genes and mutations. Mutat Res 310:125-133, 1994
23 Werkgroep erfelijke darmkanker: Erfelijke darmkanker: landelijke richtlijn versie 1.0, Vereniging Klinische Genetica Nederland, 2008
24 Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 20:488-495, 2009
25 VanderWeele TJ. On the relative nature of overadjustment and unnecessary adjustment. Epidemiology 20:496-499, 2009
26 Leufkens AM, van Duijnhoven FJB, Boshuizen HC, *et al*. Educational level and risk of colorectal cancer in EPIC with specific reference to tumor location. Int J Cancer Epub:DOI: 10.1002/ijc.26030, 2011
27 Aarts MJ, van der Aa MA, Coebergh JWW, *et al*. Reduction of socioeconomic inequality in cancer incidence in the South of the Netherlands during 1996-2008. Eur J Cancer 46:2633-2646, 2010
28 Win AK, Dowty JG, English DR, *et al*. Body mass index in early adulthood and colorectal cancer risk for carriers and non-carriers of germline mutations in DNA mismatch repair genes. Br J Cancer 105:162-169, 2011
29 Almendingen K, Hofstad B, Vatn MH. Does high body fatness increase the risk of presence and growth of colorectal adenomas followed up in situ for 3 years? Am J Gastroenterol 96:2238-2246, 2001
30 Davidow AL, Neugut AI, Jacobson JS, *et al*. Recurrent adenomatous polyps and body mass index. Cancer Epidemiol Biomarkers Prev 5:313-315, 1996
31 Jacobs ET, Martinez ME, Alberts DS, *et al*. Association between body size and colorectal adenoma recurrence. Clin Gastroenterol Hepatol 5:982-990, 2007

32 Sass DA, Schoen RE, Weissfeld JL, *et al.* Relationship of visceral adipose tissue to recurrence of adenomatous polyps. Am J Gastroenterol 99:687-693, 2004
33 Wallace K, Baron JA, Karagas MR, *et al.* The association of physical activity and body mass index with the risk of large bowel polyps. Cancer Epidemiol Biomarkers Prev 14:2082-2086, 2005
34 Jacobs ET, Ahnen DJ, Ashbeck EL, *et al.* Association between body mass index and colorectal neoplasia at follow-up colonoscopy: a pooling study. Am J Epidemiol 169:657-666, 2009
35 Watson P, Ashwathnarayan R, Lynch HT, *et al.* Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). Arch Intern Med 164:2429-2431, 2004
36 Pande M, Lynch PM, Hopper JL, *et al.* Smoking and colorectal cancer in Lynch syndrome: results from the Colon Cancer Family Registry and the University of Texas M.D. Anderson Cancer Center. Clin Cancer Res 16:1331-1339, 2010
37 Diergaarde B, Braam H, Vasen HF, *et al.* Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 5:736-742, 2007
38 Jacobson JS, Neugut AI, Murray T, *et al.* Cigarette smoking and other behavioral risk factors for recurrence of colorectal adenomatous polyps (New York City, NY, USA). Cancer Causes Control 5:215-220, 1994
39 Reid ME, Marshall JR, Roe D, *et al.* Smoking exposure as a risk factor for prevalent and recurrent colorectal adenomas. Cancer Epidemiol Biomarkers Prev 12:1006-1011, 2003
40 Paskett ED, Reeves K, Pineau B, *et al.* The association between cigarette smoking and colorectal polyp recurrence (United States). Cancer Causes Control 16:1021-1033, 2005
41 Baron JA, Sandler RS, Haile RW, *et al.* Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. J Natl Cancer Inst 90:57-62, 1998
42 Voskuil DW, Kampman E, Grubben MJAL, *et al.* Meat consumption and meat preparation in relation to colorectal adenomas among sporadic and HNPCC family patients in the Netherlands. Eur J Cancer 38:2300-2308, 2002
43 Cottet V, Bonithon-Kopp C, Kronborg O, *et al.* Dietary patterns and the risk of colorectal adenoma recurrence in a European intervention trial. Eur J Cancer Prev 14:21-29, 2005
44 Sansbury LB, Wanke KL, Albert PS, *et al.* The effect of strict adherence to a high-fiber, high-fruit and -vegetable, and low-fat eating pattern on adenoma recurrence. Am J Epidemiol 170:576-584, 2009
45 Schatzkin A, Lanza E, Corle D, *et al.* Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. N Engl J Med 342:1149-1155, 2000
46 Lanza E, Yu B, Murphy G, *et al.* The polyp prevention trial continued follow-up study: no effect of a low-fat, high-fiber, high-fruit, and -vegetable diet on adenoma recurrence eight years after randomization. Cancer Epidemiol Biomarkers Prev 16:1745-52, 2007
47 Herman JG, Graff JR, Myohanen S, *et al.* Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 93:9821-9826, 1996
48 Nagasaka T, Rhees J, Kloor M, *et al.* Somatic hypermethylation of *MSH2* is a frequent event in Lynch syndrome colorectal cancers. Cancer Res 70:3098-3108, 2010
49 Thygesen LC, Wu K, Grønbæk M, *et al.* Alcohol intake and colorectal cancer: a comparison of approaches for including repeated measures of alcohol consumption. Epidemiology 19:258-264, 2008
50 Wijnen JT, Brohet RM, van Eijk R, *et al.* Chromosome 8q23.3 and 11q23.1 variants modify colorectal cancer risk in Lynch syndrome. Gastroenterology 136:131-137, 2009
51 Krüger S, Engel C, Bier A, *et al.* The additive effect of p53 Arg72Pro and RNASEL Arg462Gln genotypes on age of disease onset in Lynch syndrome patients with pathogenic germline mutations in *MSH2* or *MLH1*. Cancer Lett 252:55-64, 2007
52 Campbell PT, Edwards L, McLaughlin JR, *et al.* Cytochrome P450 17A1 and catechol O-methyltransferase polymorphisms and age at Lynch syndrome colon cancer onset in Newfoundland. Clin Cancer Res 13:3783-3788, 2007
53 Zecevic M, Amos CI, Gu X, *et al.* *IGF1* gene polymorphism and risk for hereditary nonpolyposis colorectal cancer. J Natl Cancer Inst 98:139-143, 2006
54 Jones JS, Amos CI, Pande M, *et al.* DNMT3b polymorphism and hereditary nonpolyposis colorectal cancer age of onset. Cancer Epidemiol Biomarkers Prev 15:886-91, 2006
55 Järvinen HJ, Aarnio M, Mustonen H, *et al.* Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 118:829-834, 2000
56 Vasen HFA, Abdirahman M, Brohet R, *et al.* One to 2-year surveillance intervals reduce risk of colorectal cancer in families with Lynch syndrome. Gastroenterology 138:2300-2306, 2010
57 WCRF/AICR: Food, nutrition, physical activity, and the prevention of cancer: a global perspective. (ed 2nd). Washington DC, AICR, 2007
58 WCRF/AICR: Continuous update project interim report summary: food, nutrition and physical activity and the prevention of colorectal cancer, 2011
59 Thomas EL, Parkinson JR, Frost GS, et al. The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat. Obesity Epub:doi:10.1038/oby.2011.142, 2011
60 Martínez ME, Baron JA, Lieberman DA, et al. A pooled analysis of advanced colorectal neoplasia diagnoses after colonoscopic polypectomy. Gastroenterology 136:832-841, 2009
61 Slattery ML, Boucher KM, Caan BJ, *et al.* Eating patterns and risk of colon cancer. Am J Epidemiol 148:4-16, 1998

Samenvatting
Dankwoord
Curriculum Vitae
List of publications
Overview of completed training activities



Samenvatting

Beïnvloedbare risicofactoren en adenomateuze dikke darmpoliepen bij mensen met een verhoogd risico op dikke darmkanker

Uit resultaten van eerder uitgevoerd epidemiologisch onderzoek naar voeding en lichamelijke activiteit is gebleken dat verschillende beïnvloedbare factoren invloed hebben op het ontstaan van dikke darmkanker. Overgewicht ofwel een hoge body mass index (BMI: kg/m²), onvoldoende lichaamsbeweging, en een te hoge inname van rood vlees, bewerkt vlees, en/of alcohol verhoogt het risico op dikke darmkanker, terwijl voedingsmiddelen met een hoog vezelgehalte het risico mogelijk verlagen. Een westers voedingspatroon (hoog in rood vlees, & geraffineerde, zoete en vette producten) verhoogt het dikke darmkanker risico terwijl eetpatronen met een hogere inname van fruit, groente, vis en kip het risico mogelijk verlagen. Ook blijkt roken geassocieerd te zijn met een verhoogd risico op dikke darmkanker.

Adenomateuze dikke darmpoliepen zijn goedaardige gezwellen in de dikke darm. Zij worden over het algemeen gezien als voorlopers van dikke darmkanker, hoewel lang niet alle poliepen zullen ontaarden in kanker. Onderzoek heeft laten zien dat factoren die van invloed zijn op dikke darmkanker vaak invloed hebben op het ontstaan van adenomateuze dikke darmpoliepen. Sommige adenomateuze poliepen, de zogenaamde advanced poliepen, hebben specifieke kenmerken die de kans groter maken om uit te groeien tot een kwaadaardig gezwel. Als mensen adenomateuze poliepen in de dikke darm hebben gehad, met name wanneer dit advanced adenomateuze poliepen waren, is hun risico op nieuwe adenomateuze poliepen en daarmee mogelijke op dikke darmkanker verhoogt. Gemiddeld hebben mensen in Nederland 2,5% kans op het krijgen van dikke darmkanker tijdens hun eerste 70 levensjaren. Het risico op dikke darmkanker bij mensen na verwijdering van een advanced adenomateuze poliep lijkt ongeveer 2 keer zo groot als bij mensen die geen adenomateuze poliep in hun dikke darm hadden. Terwijl bij mensen die belast zijn met het erfelijke Lynch syndroom de kans op het krijgen van dikke darmkanker voor het 70e levensjaar tussen de 25 en 70% ligt.

De eerder genoemde onderzoeken naar voeding en lichamelijke activiteit en het ontstaan van dikke darmkanker hebben betrekking op mensen uit de algemene bevolking met een gemiddeld dikke darmkanker risico. In het huidige onderzoek wilden we nagaan of BMI, voedingspatronen, alcohol en roken ook geassocieerd zijn met het ontstaan van adenomateuze dikke darmpoliepen bij mensen met een relatief hoog risico op dikke darmkanker. Daarom hebben we gekeken naar verbanden tussen de eerder genoemde beïnvloedbare factoren en het ontstaan van adenomateuze dikke darmpoliepen in twee groepen met een ho(o)g(er) risico op dikke darmkanker, te weten 1) mensen met een erfelijke aanleg voor dikke darmkanker; het Lynch syndroom en 2) mensen zonder erfelijke aanleg die eerder een adenomateuze dikke darmpoliep hebben gehad.

Studiepopulaties

GEOLynch studie

Voor de onderzoeken beschreven in dit proefschrift hebben we twee prospectieve cohortonderzoeken opgezet, de GEOLynch studie en de POLIEP-vervolg studie. Voor de GEOLynch studie zijn tussen 2006 en 2008, 486 mannen en vrouwen in de leeftijd van 18 tot 80 jaar met het Lynch syndroom uit heel Nederland geworven. Al deze deelnemers hebben zowel een voedselvragenlijst als vragenlijsten over leefstijlfactoren en andere mogelijke risicofactoren ingevuld. Gegevens van de dikke darmonder-

zoeken en de mogelijk ontstane dikke darmtumoren zijn in de daarop volgende jaren tot juli 2009 verzameld via de Stichting Opsporing Erfelijke Tumoren (STOET), en via de Universitaire Medische Centra in Nijmegen en Groningen. Nadat de mensen gemiddeld 20 maanden in de studie waren opgenomen, werden bij 58 deelnemers adenomateuze dikke darmpoliepen ontdekt.

POLIEP-vervolg studie
Voor de POLIEP-vervolg studie zijn patiënten met een adenomateuze dikke darmpoliep in de leeftijd van 18 tot 75 jaar geïncludeerd. Deze deelnemers zijn tussen 1995 en 2002 geworven in tien verschillende ziekenhuizen in Nederland nadat zij een dikke darmonderzoek hadden ondergaan. Alle 565 patiënten beschreven in de hoofdstukken 5 en 6 van dit proefschrift hebben na de wervingsscopie ook minstens één vervolg scopie gehad. Medische gegevens over deze dikke darmonderzoeken zijn verzameld tot 2009. Ook in deze studie hebben de deelnemers vragenlijsten over voedings- en leefstijlgewoonten ingevuld. In de loop van het onderzoek hebben 165 personen adenomateuze dikke darmpoliepen teruggekregen, waarvan 37 deelnemers in die tijd een advanced adenomateuze poliep hebben ontwikkeld.

Resultaten
GEOLynch studie
In hoofdstuk 2 worden de resultaten beschreven voor BMI en adenomateuze dikke darmpoliepen bij mensen met het Lynch syndroom. In deze populatie met erfelijke belasting voor dikke darmkanker vonden we dat overgewicht, gedefinieerd als een BMI groter of gelijk aan 25 kg/m², geassocieerd was met een verhoogd risico op het krijgen van een eerste adenoom in de dikke darm bij mannen. Bij vrouwen zagen we dit verhoogde risico niet. Ook zagen we geen verhoging van het risico op adenomateuze dikke darmpoliepen met hogere BMI bij personen die al dikke darmtumoren hadden gehad. Uit een recent cohort onderzoek uit de Verenigde Staten (VS) in patienten met Lynch syndroom kwam naar voren dat een hoge BMI (>30 kg/m²) rond het 20e jaar ook het risico op dikke darmkanker verhoogd. De bevindingen van beide onderzoeken komen overeen met resultaten uit eerder uitgevoerde onderzoeken bij mensen met een gemiddeld dikke darmkankerrisico.
Hoofdstuk 3 beschrijft de resultaten voor rookgewoonten en alcoholgebruik bij mensen met Lynch syndroom. Rokers hadden een verhoogde kans op het ontstaan van adenomateuze poliepen in de dikke darm vergeleken met Lynch patiënten die nooit gerookt hebben. Bij voormalige rokers zagen we dit verhoogde risico ook, alleen was deze wel lager dan die van mensen die aangaven nog steeds te roken. Deze resultaten komen overeen met twee eerdere onderzoeken uit de VS bij mensen met het Lynch syndroom. In die onderzoeken werd een verhoogd risico op dikke darmkanker gezien bij rokers. Hoewel een hoge alcohol inname het risico op adenomateuze poliepen in de dikke darm ook lijkt te verhogen, zagen we geen overtuigend bewijs voor een relatie. Twee andere onderzoeken bij mensen met het erfelijke Lynch syndroom zagen ook geen overtuigend bewijs voor een verhoogd risico bij een hoge versus een lage alcohol consumptie.
In hoofdstuk 4 worden de resultaten gepresenteerd van de voedingspatronen in de Lynch syndroom populatie en de mogelijke verbanden met adenomateuze dikke darmpoliepen. Van de vier voedingspatronen die zijn geïdentificeerd binnen deze groep, zagen we dat het 'Snack' patroon geassocieerd was met een verhoging van het risico op adenomateuze poliepen in de dikke darm. Het 'Prudent', ofwel verstandige, voedingspatroon lijkt het risico iets te verlagen, terwijl het 'Vlees' en het 'Kosmopolitische', ofwel niet traditionele, eetpatroon het risico een beetje lijken te

verhogen, alleen zijn deze resultaten niet overtuigend genoeg om een conclusie te kunnen trekken. Er zijn geen andere onderzoeken gedaan naar verbanden tussen voedingspatronen en het risico op adenomateuze poliepen in Lynch syndroom. We kunnen daarom dit onderzoek alleen vergelijken met onderzoeken gedaan in personen met een gemiddeld risico op dikke darmkanker. In deze onderzoeken wordt vaak een verhoogd risico op het ontstaan van dikke darmtumoren gezien voor een Westers eetpatroon. Gedeeltelijk is dit terug te zien in de verhoging van het risico op adenomateuze poliepen bij een hogere inname van het 'Snack' patroon. Echter, voor het 'Vlees' patroon, dat ook als Westers kan worden beschouwd, wordt dit verband niet waargenomen.

POLIEP-vervolg studie
Hoofdstuk 5 beschrijft de resultaten voor BMI in de POLIEP-vervolg studie, waaraan patiënten met eerdere adenomateuze dikke darmpoliepen deelnamen. In dit hoofdstuk presenteren we ook het verband tussen veel voorkomende variaties in erfelijk materiaal van insuline-achtige groeifactoren (IGF), die het risico op terugkeer van adenomateuze poliepen in de dikke darm bij overgewicht extra kunnen beïnvloeden. We zagen geen verband tussen BMI en het terugkeren van adenomateuze dikke darmpoliepen, in tegenstelling tot een groot Amerikaans onderzoek waarin een hoger risico op het terugkeren van adenomen werd waargenomen bij personen met obesitas (BMI ≥30 kg/m²). Wel zagen we dat bepaalde varianten in het erfelijk materiaal die coderen voor IGF's het verband tussen BMI en adenomateuze poliepen mogelijk beïnvloeden. Echter, voordat we hier echte conclusies aan kunnen verbinden, is het nodig om deze resultaten in andere onderzoekspopulaties bevestigd te zien.
In hoofdstuk 6 zijn de resultaten tussen het verband van voedingspatronen met de terugkeer van adenomateuze poliepen in de dikke darm beschreven. In de POLIEP-vervolg studie werden drie voedingspatronen waargenomen, namelijk een 'Laagvlees' patroon, een 'Kosmopolitisch' patroon, en een 'Geraffineerde producten' patroon. Geen van deze voedingspatronen leken geassocieerd met het terugkeren van adenomateuze poliepen in de dikke darm. In dit hoofdstuk is ook gekeken of deze voedingspatronen invloed hadden op het ontstaan van advanced' adenomateuze poliepen. Het lijkt erop dat de voedingspatronen meer invloed hebben op deze advanced' poliepen dan op adenomateuze poliepen in het algemeen. Echter, het aantal advanced adenomen dat in de loop van de studie is ontstaan is klein. Hierdoor kunnen we geen harde conclusies verbinden aan deze bevindingen voordat er meer onderzoek is gedaan.

Ten slotte zijn de belangrijkste uitkomsten van dit promotieonderzoek samengevat in de algemene discussie (hoofdstuk 7). In dit zelfde hoofdstuk worden ook de sterke en minder sterke kanten van deze studies behandeld. Het beschreven onderzoek in de Lynch syndroom patiënten ondersteunt onze hypothese dat beïnvloedbare factoren van invloed kunnen zijn op het ontstaan van adenomateuze poliepen in de dikke darm in deze groep. Echter, we hebben geen bewijs gevonden in de POLIEP-vervolg studie dat dezelfde factoren het terugkeren van adenomateuze poliepen beïnvloeden bij mensen zonder erfelijke belasting die eerder al adenomateuze poliepen in hun dikke darm hebben gehad. Toekomstige, grotere studies met langere vervolg tijd, zullen zich vooral moeten richten op terugkeer van de zogenaamde advanced adenomen.

Dankwoord

Is het dan echt af? Ik geloof het wel. Mijn proefschrift is af. Zonder hulp was mij dit nooit gelukt. Graag wil ik iedereen bedanken die hier een bijdrage aan heeft geleverd.

Hartelijk dank

Ten eerste de deelnemers. Ik ben jullie heel dankbaar voor de medewerking aan de GEOLynch en POLIEP-vervolg studie, zonder jullie was er geen onderzoek en geen proefschrift. Nogmaals dank.

Ellen Kampman, mijn promotor, dagelijks begeleider en coach ineen. Heel erg bedankt voor je vertrouwen, je geduld en voor de kansen die jij mij hebt gegeven. Je hebt me een goede inkijk gegeven in de wetenschappelijke wereld, ik bewonder je ideeën, kennis en de vrijheid die ik van je heb gekregen om alles zelf uit te vinden. Ik waardeer je enorm als persoon en kijk met plezier terug op onze gesprekken over leven en wetenschap.

Hans Vasen, mijn 2e promotor, hartelijk dank voor het wegwijs maken in de wereld van de erfelijke dikke darmkanker, door jou voelde ik me thuis bij de InSIGHT congressen. Je plezier in de wetenschap is aanstekelijk.

Zeer veel dank ook voor mijn copromotor Fokko Nagengast. Veel deelnemers van de GEOLynch studie kwamen uit jouw ziekenhuis. Via jou was ook ik onderdeel van het Universitair Medisch Centrum Nijmegen, dank voor deze inkijk in de medische wereld.

Mary Velthuizen en Alice Donselaar van de Stichting Opsporing Erfelijke Tumoren wil ik heel hartelijk bedanken voor alle hulp bij de werving en dataverzameling van de GEOLynch studie. Door jullie en door Dieneke, Clasien, Inge, Marianne, Marjon, Mary G, Andrea, Wouter, Nandy, en Marry voelde ik me heel erg welkom!

Maria van Vugt, wat fijn dat jij mij hebt geholpen bij het opstarten van de POLIEP-vervolg studie in het Radboud. Gewoon doen zei je, er gaat altijd wel wat mis. Jouw hulp bij de GEOLynch en de POLIEP-vervolg studie was van onschatbare waarde.

Polly Newcomb, thank you so much for sharing your views during your stay in Wageningen, I really enjoyed it. I am honoured that you are part of my thesis committee.

De POLIEP-vervolg studie is uitgevoerd in 9 ziekenhuizen. Ik wil iedereen bedanken voor de prettige samenwerking bij de afdelingen Maag-, Darm- en Lever ziekten en de betrokken laboratoria van het Radboud Universitair Medisch Centrum, Ziekenhuis Gelderse Vallei, Ziekenhuis Rivierenland, Slingeland Ziekenhuis, St. Antonius Ziekenhuis, Meander Medische Centrum, Slotervaartziekenhuis, ziekenhuis Rijnstate en het Canisius-Wilhelmina Ziekenhuis.

Professor Kleibeuker, bedankt voor de prettige samenwerking bij de GEOLynch studie.

Leontien Witjes en Marlieke Visser hebben het overgrote deel van de werving van de POLIEP-vervolg studie op zich genomen. Zonder jullie was er geen vervolg geweest. Hartelijk dank voor al het werk en voor de leuke samenwerking.

Ursula Oldenhof bedankt voor het verzamelen en invoeren van de vele statusgegevens en vragenlijsten. Je nauwgezette aanpak heeft me erg geholpen.

Hartelijk dank Professor Han van Krieken, Iris Nagtegaal en Elisa Vink-Borger voor de samenwerking en kennis over tumorpathologie en immunohistochemie. Jan Hendriks, hartelijke bedankt voor je advies over de statististiek van de GEOLynch studie.

Brenda Diergaarde hartelijk dank voor het uitvoeren van de SNP analyses en voor de tijd die je hebt vrijgemaakt om mij kennis over SNP's bij te brengen.

Jan Harryvan bedankt voor al jouw labhulp, voor het uittesten van de speekselpotjes en het isoleren van DNA. Marga van der Steen, Pieter Versloot, en Betty van der Struijs dank voor jullie hulp omtrent bloedafname en labzaken. Saskia Meyboom en Karin Borgonjen hartelijk dank voor het berekenen van de voedingsgevens.

De tijdelijke maar broodnodige ondersteuning van Jacqueline Tol, Emma Steenbergen, Geline Ormel, en Evert Evers heb ik zeer kunnen waarderen. Bedankt voor de hulp bij het invoeren van de vragenlijsten en het aanschrijven van patiënten.
Van de afstudeervakstudenten die ik (mede) heb mogen begeleiden heb ik veel geleerd. Hopelijk was dat andersom ook zo. Cathelijne, Yvonne, José, Miriam, Lifang, Jozette en Marie, dank voor al het werk dat jullie hebben verzet binnen mijn studies.
POLIEP-studie AIO's, bedankt voor de werving van alle patiënten. Speciale dank voor Petra, Maureen en Mariken voor het overbrengen van alle ins and outs over deze studie. Bedankt kanker docs voor de discussies over mijn onderzoeken. Fränzel zonder je kritische blik en positieve motivatie zou er geen algemene discussie in mijn proefschrift staan, bedankt. Renate W, na mede-AIO en ganggenoot, ben je nu een kanker collega. Superfijn dat je altijd mijn manuscripten wilde lezen. Renate H bedankt voor de samenwerking, ik vind het prettig om de studies aan jou door te geven. Dieuwertje zonder jou was het printen van de leesversie nachtwerk geworden. Martinette, mijn SAS adviseur, het puzzelen had veel langer geduurd zonder jouw oplossingen.
Daarnaast zijn er veel collega's en oud-collega's van de afdeling Humane Voeding die indirect hebben bijgedragen aan het succes van het onderzoek en mijn werkplezier. Dank daarvoor. Alle collega's van de leerstoelgroep Voedingsepidemiologie wil ik bedanken voor de leuke methodologische discussies en voordrachten. Medebewoners van het Agro, met een bijzondere vermelding voor de 4e verdieping, jullie hebben mijn verblijf daar opgevrolijkt. Dank jullie wel voor alle small talk. Karen en Gabrielle, dank voor jullie secretariële ondersteuning, valt het uitdelen van dropjes daaronder? Hartstikke bedankt voor alle gezelligheid. Mijn mede AIO's wil ik graag bedanken voor het delen van praktische ervaringen over het opzetten van studies en analyses, maar met name voor de gezelligheid buiten alle werkzaamheden om. Speciale dank voor de PhD-tour groepen van 2005 en 2007. Beide reizen waren een hoogtepunt door jullie! Dear PhD tour committee, Simone, Mirre, Janette, Nicolien, Laeticia and Anand, it was a pleasure to organize the USA trip with you.
Een paar personen wil ik nog bij naam noemen, Sandra muchos gracias for throwing excellent cocktail parties and making evenings at the 4th flour lively. Mijn kamergenoten, Marja, Hendriek en Esther, zonder jullie is het stil. Dank voor de gezelligheid. Sanne en Pleunie, ambitiebeesten, mede door de ENLP zijn we i.p.v. goede collega's vrienden geworden. Dank voor alles. Gerda, jouw gastvrijheid en attentie zijn een voorbeeld, ik kon altijd komen logeren, onze trip naar Washington DC en NYC was super. Linda het is altijd gezellig met jou, en gedeelde smart is halve smart, en dat scheelt veel bij de afronding van een proefschrift. Elise mijn allereerste kamergenoot bij Humane Voeding, wat ben jij een kanjer. Ik ben heel blij dat je in Nederland bent en mijn paranimf kan zijn. Audrey Jung you are my partner in crime. Thanks for all your help with blood and tissue collection and all other issues surrounding our studies. I am really happy that you support me as my paranimf.
Lieve vrienden, familie en schoonfamilie, het afgelopen jaar, of waren het er twee, was ik steeds bijna klaar en heb ik jullie verwaarloosd. Het is af en ik zal me beteren. Bedankt voor jullie steun en interesse. Lieve papa en mama, wat ben ik blij dat jullie er zijn. De basis voor wie ik ben, komt bij jullie weg. Lieve grote broer Jelle en lieve Marijke. Wat fijn dat ik regelmatig bij jullie kon logeren, ik was nooit te veel. Jelle hartelijke dank voor alle tijd die je in het mooie ontwerp hebt gestopt. Mooier kon niet.

Lieve Xander, mijn proefschrift is echt af, nu ben ik er ook weer voor jou. Ik kan je niet genoeg bedanken voor alles wat jij voor mij hebt gedaan in de afgelopen jaren. Ik ben zo blij met jou.

Curriculum Vitae

Akke Botma was born on July 30th, 1979 in Leek, the Netherlands. After completing secondary school (Voorbereiding Wetenschappelijk Onderwijs) at 'Scholengemeenschap de Waezenburg', she obtained her Master's degree in Nutrition and Health at Wageningen University in June 2004. During her master program she wrote two master theses. Her first master thesis in International Nutrition focused on 'the availability of vitamin A-rich animal products for lactating women and children under five years of age in rural Bangladesh', for which she spent nearly 6 months in Bangladesh. Her second master thesis, for which she joined the Epidemiology group of Professor Floor van Leeuwen at the Netherlands Cancer Institute in Amsterdam, focused on 'long-term incidence of vascular disease following breast cancer treatment'. In September 2004, Akke was appointed as junior researcher and joined the WCRF Systematic Literature Review team of Wageningen University This team reviewed literature on the association between food, nutrition, physical activity and risk of gallbladder, liver and colorectal cancer. In June 2005, she was appointed as a PhD-fellow at Wageningen University in the Diet and Cancer group of Professor Ellen Kampman at the division of Human Nutrition. In her PhD-project she worked in close collaboration with Professor Hans Vasen, medical director of the Netherlands Foundation for the Detection of Hereditary Cancer. As a PhD-fellow, she was a member of the committee for Temporary Scientific Staff at the Division of Human Nutrition and chaired the organising committee of the biennial PhD Study tour to the north-eastern part of the USA in 2007. In 2011, she was selected for the European Nutrition Leadership Programme (ENLP). After finishing her PhD-thesis, Akke was appointed as researcher in the Diet and Cancer group of Professor Ellen Kampman.

List of publications

Publications in peer-reviewed journals

- Botma A, Nagengast FM, Braem MG, Hendriks JC, Kleibeuker JH, Vasen HF, Kampman E. Body mass index increases risk of colorectal adenomas in men with Lynch syndrome: the GEOLynch cohort study. J Clin Oncol 28:4346-4353, 2010
- Nieuwenhuis MH, De Vos Tot Nederveen Cappel W, Botma A, Nagengast FM, Kleibeuker JH, Mathus-Vliegen EM, Dekker E, Dees J, Wijnen J, Vasen HF. Desmoid tumors in a Dutch cohort of patients with familial adenomatous polyposis. Clin Gastroenterol Hepatol 6:215-219, 2008
- Hooning MJ, Botma A, Aleman BM, Baaijens MH, Bartelink H, Klijn JG, Taylor CW, van Leeuwen FE. Long-term risk of cardiovascular disease in 10-year survivors of breast cancer. J Natl Cancer Inst 99:365-375, 2007

Publications submitted to peer-reviewed journals

- Winkels RM[1], Botma A[1], van Duijnhoven FJB, Nagengast FM, Kleibeuker JH, Vasen HFA, Kampman E. Smoking Increases the risk for colorectal adenomas in persons with Lynch syndrome.

  [1] authors contributed equally to this work
- Botma A, Vasen HFA, van Duijnhoven FJB, Nagengast FM, Kampman E. Dietary patterns and colorectal adenomas in Lynch syndrome.
- Botma A, Diergaarde B, van Duijnhoven FJB, Nagengast FM, Depla ACTM, van Hees PAM, Witteman BJM, Vasen HFA, Kampman E. BMI, polymorphisms in insulin-like growth factor genes and colorectal adenoma recurrence.
- Botma A, Winkels RM, Witteman BJM, de Boer SY, van de Meeberg PC, Nagengast FM, Kampman E. Dietary patterns and colorectal adenoma recurrence.

Abstracts in scientific journals

- Winkels RM, Botma A, Nagengast FM, Vasen HFA, Kampman, E. Smoking and alcohol consumption and colorectal adenoma risk in Lynch syndrome: the GEOLynch cohort study. Fam Cancer 10:S8, 2011

Book contributions

- Contributor for the expert report 'Food, nutrition, physical activity and the prevention of cancer: a global perspective' of the World Cancer Research Fund / American Institute for Cancer Research, 2007

Overview of completed training activities

Discipline specific activities	Organizer & location	Year
Courses		
Cancer Epidemiology	NIHES, NKI-AVL, Amsterdam, NL	2005
Molecular Epidemiology - Biomarkers of exposures, susceptibility & disease	University of Leeds, Leeds, UK	2006
Basic Oncology - Introduction in fundamental & clinical oncology	NVVO, Ellecom, NL	2006
Master class 'Diet and Cancer'	Graduate school VLAG, NZO, Wageningen, NL	2007
Exposure assessment in nutrition research	Graduate school VLAG, Division of Human Nutrition, Wageningen, NL	2010
Conferences and meetings		
Annual Epidemiology conference with poster presentation 2005 with oral presentations 2007-2010	WEON, NL	2005, 2007-2010
Annual meeting NWO nutrition with oral presentations	NWO-Nutrition, Deurne, NL	2006-2008
STOET symposium with oral presentations	STOET, Utrecht, NL	2006, 2008
INSIGHT conference with oral presentation	INSIGHT-group, Yokohama, JP	2007
Food, nutrition, physical activity & the prevention of cancer, conference with poster presentation	AICR/WCRF, Washington, USA	2007
Wageningen nutritional sciences forum	Division of Human Nutrition, Wageningen, NL	2009
INSIGHT conference with oral presentation	INSIGHT-group, Dusseldorf, DE	2009
WCRF conference with poster presentation	WCRF, London, UK	2010
INSIGHT conference with oral presentation	INSIGHT-group, San Antonio, Texas, USA	2011
General courses		
PhD introduction week	Graduate school VLAG, Ermelo, NL	2006
Presentation skills	Language Centre, WUR, Wageningen, NL	2006
Negotiating	NWO, Den Haag, NL	2006
Talent day with courses 'Networking' & 'Creative thinking'	NWO, Utrecht, NL	2006
Project and time management	Wageningen Graduate Schools, Wageningen, NL	2007
Philosophy and ethics of food sciences and technology	Graduate school VLAG, Wageningen, NL	2008
Survival analysis	NIHES, Rotterdam, NL	2008
Master class 'Linear and Logistic regression'	Graduate school VLAG, Division of Human Nutrition, Wageningen, NL	2010
Optional courses and activities		
Master class 'Nutrigenomics'	Graduate school VLAG, Wageningen, NL	2005
Participating in PhD tour to UK and Ireland	Division of Human Nutrition	2005
Organising and participating in PhD tour	Division of Human Nutrition	2007
to North-eastern part of the USA Journal club	Division of Human Nutrition, Wageningen, NL	2005-2009
Research presentations	Division of Human Nutrition, Wageningen, NL	2005-2010
Methodology and epidemiology research meetings	Division of Human Nutrition, Wageningen, NL	2005-2011
17th European Nutrition Leadership Programme	ENLP. Luxembourg, LU	2011

Colophon

The research described in this thesis was financially supported by the Dutch Cancer Society (KWF, grant number UW 2005-3275) and was conducted at the Division of Human Nutrition of Wageningen University, the Netherlands. The analyses described in chapter 3 were additionally supported by the World Cancer Research Fund (WCRF). Financial support for the establishment of the cohort studies in this thesis was provided by the Dutch Cancer Society (KWF), the Netherlands Organization for Health Research and Development (ZONMW), and the Netherlands Foundation for Digestive Diseases (MLDS).

Financial support from Wageningen University and the Dutch Cancer Society for printing this thesis is gratefully acknowledged.

Cover design and layout
Jelle J. Botma

Font
Scala Sans Pro (Martin Majoor)

Printing
Printed by Grafisch Service Centrum Wageningen