COLORECTAL CANCER AND DIET IN SCOTLAND

By

Evropi Theodoratou

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF EDINBURGH

OCTOBER 2008

DECLARATION

Thesis: Colorectal cancer and diet in Scotland

I, Evropi Theodoratou hereby declare that I am the sole author of this thesis. I developed the hypotheses examined in this thesis and conducted all aspects of the research except when contribution of colleagues is acknowledged. This thesis has not been submitted for any other degree or professional qualification.

This thesis was based on the analysis of the Scottish Colorectal Cancer Study. The study was funded by the Cancer Research UK, the Medical Research Council and the Chief Scientist Office of the Scottish Executive and was headed by Professors Harry Campbell, Malcolm G Dunlop and Mary E Porteous. The recruitment process of study participants was co-ordinated by Dr Roseanne Cetnarskyj and conducted by trained research nurses. Finally, the nutrient data analysis of the Food Frequency Questionnaires was conducted by Dr Geraldine McNeill and her colleagues at the University of Aberdeen.

Signature:……. Date:…….

AKNOWLEDGMENTS

I would like to thank my principal supervisor **Professor Harry Campbell** for his guidance and encouragement, throughout my PhD.

I would also like to thank **Dr Niall Anderson**, my second supervisor, for his advice and suggestions mainly regarding the statistical issues of the thesis.

I wish to acknowledge **Professors Malcolm Dunlop and Mary Porteous**, who together with Professor Harry Campbell were the principal investigators of the case-control study that my thesis was based on.

I wish to acknowledge **Dr Roseanne Cetnarskyj** for coordinating the recruitment process of the study. In addition, I wish to acknowledge all the research nurses and the members of the administrative team for their help regarding data collection and management. I would like to specially thank **Mrs Gisela Johnstone** for her support throughout my PhD and during the writing of the thesis.

I wish to acknowledge **Dr Geraldine McNeill** and her colleagues at the University of Aberdeen for technical support regarding the Food Frequency Questionnaires and for providing advice on certain issues regarding nutrient data analysis.

I wish to acknowledge **Dr Susan Farrington** and **Dr Albert Tenesa,** for providing to me the genetic data, as well as advising me regarding specific aspects of the genetic analysis.

I wish to acknowledge the **State Scholarships Foundation** in Greece, for funding me and giving me the opportunity to conduct this thesis.

A special thank you to all **the SOCCS study participants**, for their kind and valuable participation.

Finally, I would like to thank **my fiancée Manos, my parents Thodoris and Katerina** and **my sister Emmanouela,** for their support, encouragement and patience throughout my PhD.

ABSTRACT

Introduction

Colorectal cancer is a cancer that forms in the tissues of the colon and/ or rectum and more than 95% of colorectal cancers are adenocarcinomas. It is the third most common cancer in incidence and mortality rates, accounting for 9% of all cancer cases and for 8% of all cancer related deaths (2002). The established risk factors of colorectal cancer include personal or family history of previous colorectal cancer or adenomatous polyps, chronic bowel inflammatory disease and presence of any of the hereditary syndromes. In addition, due to the fact that the majority of colorectal cancer cases (approximately 90%) occur after the age of 50, advanced age is also considered as a risk factor. Finally, evidence for significant associations between colorectal cancer and other risk factors, including diet, body weight, physical activity, smoking, alcohol intake, NSAIDs intake and HRT in post-menopausal women, is promising and increasing.

Aims and objectives

The main aims of this project were: 1) to investigate the associations between colorectal cancer and specific nutrients, including flavonoids, fatty acids, folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium (prior hypotheses 1-4) and 2) to conduct an overall as well as forward and backward stepwise regression analyses of demographic, lifestyle and dietary risk factors.

Methods

The analysis of this thesis was based on a population-based case-control study of colorectal cancer (Scottish Colorectal Cancer Study; SOCCS). In total 3,417 colorectal cancer cases and 3,396 controls were recruited in the study. Dietary and lifestyle data were collected by two questionnaires (Lifestyle & Cancer and Food Frequency Questionnaire) and were available for 2,061 cases and 2,776 controls. For the analysis of the first two hypotheses (flavonoids and fatty acids) a matched dataset of 1,489 case-control pairs was used and conditional logistic regression models were applied, whereas for the analysis of the last two hypotheses (folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium) an unmatched dataset including 2,061 cases and 2,776

controls was used and unconditional logistic regression models were applied. For the overall and stepwise regression analyses the unmatched dataset was used (2,061 cases and 2,776 controls). Forward and backward stepwise regression was applied on three different sets of variables and the stability of the resultant models was checked in 100 bootstrap samples.

Results

Regarding the first two hypotheses, statistically significant odds ratios (ORs) (matched on sex, age and health board are and adjusted for family history of cancer, BMI, physical activity, smoking, and intakes of total energy, fibre, alcohol and NSAIDs) for highest versus lowest intakes (quartiles) were observed for flavonols OR (95% CI), p-value for trend: 0.78 (0.60, 0.99), 0.08) and for the individual flavonoid compounds quercetin and catechin (OR (95% CI), p-value for trend: 0.77 (0.60, 0.99), 0.04; 0.75 (0.58-0.97), 0.02; respectively); for the ω3PUFAs fatty acids (OR (95% CI), p-value for trend: 0.75 (0.59, 0.97), 0.01) and for the individual fatty acids stearic acid, EPA and DHA (OR (95% CI), p-value for trend: 1.46 (1.11, 1.91), 0.01; 0.74 (0.58, 0.95), 0.02; 0.74 (0.58, 0.95), 0.02; respectively). Regarding the last two hypotheses, statistically significant odds ratios (ORs) (adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, and intakes of total energy, fibre, alcohol and NSAIDs) for highest versus lowest intakes (quartiles) were observed for vitamin B6, vitamin B12 and alcohol (OR (95% CI), p-value for trend: 0.86 (0.72, 1.03), 0.08; 0.80 (0.67, 0.97), 0.05; 0.83 (0.68, 1.00), 0.03); and for vitamin D (OR (95% CI), p-value for trend: 0.83 (0.69, 0.99), 0.03).

Regarding the second aim of the project, several risk factors were found to be significantly associated with colorectal cancer in the overall analysis including demographic and lifestyle factors (family history of cancer, NSAIDs intake, dietary energy intake, HRT intake and physical activity), food group variables (vegetables, eggs, sweets, fruit/ vegetable juice, oily fish, coffee, fruit, savoury foods and white fish) and nutrient variables (*t*MUFAs, ω3PUFAs, SFAs, *t*FAs, MUFAs, quercetin, catechin, phytoestrogen, cholesterol, fibre, protein, starch, magnesium, potassium, manganese, copper, iron, zinc, phosphorus, selenium, niacin, vitamin B6, carotenes, vitamin C,

vitamin A, potential niacin, biotin, folate, pantothenic acid, vitamin D, vitamin B1 and vitamin B12). In addition, the variables that were selected to be included in 100% of the models after applying forward and backward stepwise regression analyses were family history, NSAIDs, sweets and fruit/ vegetable juice. Finally according to the findings from the bootstrap analysis, the variables that were selected to be included in models for the majority of the bootstrap samples (more than 90%) were family history, NSAIDs, dietary energy, eggs, sweets, fruit/ vegetable juice and white fish.

Discussion

The particular dietary factors that were found to be inversely associated with colorectal cancer after applying several multivariable logistic regression models were: flavonols, quercetin, catechin, ω3PUFAs, EPA, DHA, vitamin B6, vitamin B12 and vitamin D. In addition, high intakes of stearic acid were found to be positively associated with colorectal cancer. In contrast, high intakes of dietary and total folate were associated with a decreased colorectal cancer risk in the energy-adjusted model, but this inverse association was attenuated after further adjustment for several confounding factors including fibre. Regarding alcohol intake, when it was divided into quartiles, high alcohol consumption was associated with a statistically significant and dose-dependent decreased colorectal cancer risk. However, when alcohol intake was divided in categories an increased colorectal cancer risk for intakes of higher than 60 g/day was observed. Intakes of ω3PUFAs, vitamin D and vitamin B12 were highly correlated due to having the same food source (oily fish) and therefore it is difficult to draw specific conclusions regarding which nutrient is truly associated with colorectal cancer and which not. Finally, it was observed that for calcium intakes to be inversely associated with colorectal cancer, a dosage of 1500mg/day or higher was necessary. The majority of these results are in accordance with results of previous epidemiological and laboratory studies; however their confirmation in further large-scale studies is required.

Results from the overall and stepwise regression analysis supported previous findings of an increased colorectal cancer risk due to a high or moderate family history risk. In addition, high intakes of dietary energy were found to be positively associated with increased colorectal cancer risk in the overall analysis and in addition dietary energy was

selected to be included in the majority of the stepwise regression models. On the other hand, regular intake of NSAIDs was found to be inversely associated with colorectal cancer risk in the overall analysis and in the majority of the stepwise regression models. Finally, the overall and stepwise regression analyses generated a few new hypotheses suggesting that low intakes of fruit/ vegetable juice, eggs, white fish and sweets (a combined variable of high-fat and high-sugar foods) and high intakes of coffee and magnesium were associated with a decreased colorectal cancer. These findings, though interesting and important for generation of new hypotheses, need further investigation (as prior hypotheses) in large-scale observational studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 COLORECTAL CANCER

1.1 Introduction

This chapter describes the epidemiology, natural history and progression of colorectal cancer. In addition, the prevalence, incidence and survival rates as evaluated in epidemiologic research are presented. Finally, the established genetic and non-genetic (environmental) risk factors are summarised.

1.2 Large intestine

The large intestine is the most distal part of the lower gastrointestinal tract and its main roles are: to absorb vitamins that are created by the colonic bacteria (over 700 different species), to absorb the remaining water from indigestible food matter, to maintain the fluid balance of the body and to compact and store faecal material until eliminated through the anus.

The main parts of the large intestine are the caecum, the appendix, the colon and the rectum. The caecum is the connection between the small and large intestines and its main role is to accept and store processed material of undigested food, water, vitamins and minerals and to move it towards the colon. The appendix is a small projection emerging from the caecum and it has no known function. The colon is the largest part of the large intestine and it has 4 sections (ascending, transverse, descending and sigmoid) that are located in the abdominal cavity. Within the colon, the processed material mixes with mucus and colonic bacteria to form faeces. In addition, the lining of the colon absorbs most of the water, some vitamins and minerals and the colonic bacteria chemically break down part of the fibre to produce nutrients for their own survival and to nourish the cells lining the colon. Through muscular movements of the colon, faeces are pushed along the colon and move into the rectum, which is the final part of the large intestine and where the faeces are stored before being excreted as a bowel motion.

Regarding the histology of the large intestine, the intestinal wall has four primary layers: 1) Serosa or adventitia, which is the outer layer responsible for keeping the digestive tract in the right position inside the body; 2) Muscularis externa, which is composed of a continuous inner layer of circular muscle and a discontinuous outer layer of longitudinal

muscle responsible for the motility of the lumen contents; 3) Submucosa, which is the connective tissue located between the layer of circular muscle and the mucosa; 4) Mucosa, which is the inner layer of the intestinal wall comprising a single layer of columnar epithelium (surface epithelium), connective tissue (lamina propria) and an outer muscle layer (lamina muscularis mucosa) and is characterised by the presence of numerous invaginations of the surface epithelium into the lamina propria glands, which are approximately 50 cells deep (crypts of Lieberkühn). These crypts are used mainly for water absorption. In addition, colon cells proliferate and differentiate (from stem cells) in the lower parts of the crypts and then migrate to the upper part of the crypts to renew the superficial epithelial cells (approximately every six days) (1). Several problems or disorders can arise in the large intestine including irritable bowel syndrome, inflammatory bowel disease (including Crohn's disease and ulcerative colitis), colorectal polyps and colorectal cancer.

1.3 Clinical characteristics of colorectal cancer

1.3.1 Definition of colorectal cancer

"Colorectal cancer is a cancer that forms either in the tissues of the colon, the longest part of the large intestine, or in the tissues of the rectum, the last part of the large intestine before the anus" (definition taken from National Cancer Institute; www.cancer.gov).

1.3.2 Types of colorectal cancer

The main types of colorectal cancer are: 1) adenocarcinomas, 2) squamous cell carcinomas, 3) carcinoid tumours, 4) sarcomas and 5) lymphomas. More than 95% of colorectal cancers are adenocarcinomas with the cancer starting in the gland cells in the lining of the intestinal wall. Colorectal adenocarcinomas can be of two types according to the microbiology of the cancer cells: mucinous (98-99% of adenocarcinomas; cancer cell in pools of mucus) or signet-ring tumours (1-2% of adenocarcinomas; mucus inside the cancer cells). This thesis will be examining the epidemiology of adenocarcinomas of the large intestine (colon and rectum).

Briefly the main characteristics of the other types are: squamous cell carcinomas, carcinoids, sarcomas and lymphomas. Squamous cell carcinomas are cancers that start from the skin-like cells that make up the bowel lining together with the gland cells. Carcinoid is an unusual type of slow growing tumour and is called a neuroendocrine tumour. These cancers grow in hormone producing tissues, usually in the digestive system and they are rare. Sarcomas are cancers of the supporting cells of the body (bone, muscle, etc.) and most of the colorectal sarcomas are leiomyosarcomas (started in the smooth muscle of the large intestine). Finally, lymphomas are cancers of the lymphatic system and only 0.01% of colorectal cancers are of lymphatic origin.

1.3.3 Classification of colorectal cancer

Colorectal cancer can be classified into three forms according to the way that is developed. In particular the three major forms are hereditary, familial and sporadic colorectal cancer. The proportion of each form may be different in different populations, but generally the majority of colorectal cancer cases in all populations are considered sporadic, whereas hereditary colorectal cancer is the least common form. Finally, 10-30% of colorectal cancer cases are considered to be linked to a familial risk (2).

1.3.3.1 Hereditary colorectal cancer syndromes

Colorectal cancer hereditary syndromes that result from inherited susceptibility due to rare high penetrance mutations may account for up to 5% of all cases. The most common hereditary syndrome is Hereditary Non-Polyposis Colorectal Cancer (HNPCC), also known as Lynch syndrome (2-5% of colorectal cancer cases). One of the characteristics of this syndrome is an unusually high occurrence of colorectal and specific extra-colonic cancers. In addition, the HNPCC syndrome has an earlier age of onset. Highly penetrant germline mutations in mismatch repair genes, *hMLH1* (located at chromosome 3p21–23) and *hMSH2* (located at chromosome 2p21) resulting in microsatellite instability in the tumour are responsible for the majority of the HNPCC cases. These genes are part of the DNA mismatch repair pathway and a HuGE review published in 2002 identified 45 polymorphisms in *hMLH1* and 55 polymorphisms in *hMSH2* (3). Regarding the population prevalence of *hMLH1*/*hMSH2* mutation carriers, it has been estimated to be 1 in 3,139 in a Scottish population aged 15–74 years (4). In

addition, according to available gene variant data there is no evidence suggesting any differences in frequency between populations, or between ethnic groups (3). It has been reported that the standardised incidence ratio for colorectal cancer for carriers of *hMLH1* or *hMSH2* mutations when compared with the general population is 68 (5) and the relative risk for colorectal cancer for first-degree relatives of mutation carriers compared with first degree relatives of non-carriers is 8.1 (6).

The second most common hereditary syndrome is a highly penetrant autosomal dominant cancer syndrome known as Familial Adenomatous Polyposis Coli (FAP; 1% of colorectal cancer cases) and it occurs due to germline mutations of the Adenomatous Polyposis Coli (*APC*) gene (tumour suppressor gene located at chromosome 5q21-22) (7). APC protein down-regulates the Wnt signalling pathway through its binding to β-catenin and axin and loss of the APC protein function due to *APC* mutations is associated with carcinogenesis (8). The main characteristic of FAP is the appearance of hundreds and in some cases of thousands of colorectal adenomas, which can develop into carcinomas if left untreated (9).

There are a number of rarer autosomal dominant disorders, including Juvenile Polyposis Syndrome, Cowden syndrome and Peutz-Jeghers syndrome. Juvenile Polyposis Syndrome appears usually under the age of 20 years old and it has been suggested that mutations in *SMAD4* (18q), *PTEN* (10q22-24) and *BMPR1A* genes are associated with this syndrome (10;11). Cowden syndrome, on the other hand is characterised by multiple hamartomas and it has been found to be associated with *PTEN* mutations (12). Finally, the Peutz-Jeghers syndrome has been suggested to be associated with mutations in the *LKB/STK11* gene (19p13.3) (13).

1.3.3.2 Familial colorectal cancer

An additional 20% of colorectal cancer cases are associated with a family history of colorectal cancer (with first degree relatives of a patient with colorectal cancer case having approximately a 2-4 times increased risk) and comprise the familial colorectal cancer cases. Low-penetrance *APC* mutations have been found to be associated with some types of familial colorectal cancer (14). In particular, the most common *APC* mutations that have been found to be associated with familial colorectal cancer include

I1307K (15) and E1317Q (16), whereas at least 12 additional variants of *APC* (8 of them being in exon 15) have been identified (17).

Another familial form of colorectal cancer which was first described in 2002, is MYH associated polyposis (MAP), (18). This form of colorectal cancer is due to bi-allelic mutations in *MUTYH* gene and its phenotype is clinically comparable to the FAP phenotype (18;19). However, MAP, which is recessively transmitted, generally results in a smaller number of adenomas and has a later age of onset (20). *MUTYH* (1p32.1-34.3) (21) is a base excision repair gene (21;22) and the two most common *MUTYH* variants accounting for >80% of disease causing alleles in whites are Y165C and G382D, whereas the E466X nonsense mutation has been identified in Indian families and the Y90X in Pakistani families (21). Finally, approximately 30 mutations 52 missense variants and three inframe insertions/ deletions have been identified (23).

1.3.3.3 Sporadic colorectal cancer

Most cases of colorectal cancers arise sporadically, namely with no background of a family history of the disease, and genetic and environmental factors are important (24). Somatic (occurring during an individual's lifetime) rather than germline (inherited) mutations in these genes play role in sporadic cancer, with somatic mutations of the *APC* gene to be found in as many as 80% of sporadic tumours (25).

1.3.4 Natural history of colorectal adenocarcinoma

1.3.4.1 Adenoma-carcinoma sequence

Colorectal adenocarcinomas start in the innermost layer and can grow through some or all of the other layers. The vast majority of them derive from adenomatous polyps, which are circumscribed aggregations of epithelial tissue characterised by uncontrolled cell division, following a sequence known as the adenoma-carcinoma sequence (1). Briefly, the first step in the development of tumours from normal epithelium is usually the onset of dysplasia. In particular, in the colonic crypt, the normal sequence of proliferation-differentiation of the colonic cells alters. Proliferated cells fail to differentiate taking up the whole crypt (dysplastic crypt). Single dysplastic crypts (unicryptal adenomas) are thought to be the first manifestations of tumour development (hyperproliferative epithelium). Adenomas (adenomatous polyps) can then gradually

grow in size and change from a tubular to a villous architecture. The cells show first mild then moderate and then severe dysplasia followed by malignant change resulting in local invasion with eventual metastasis to distant sites (24). However, most of the adenomatous polyps do not develop into malignant carcinomas, but they remain benign and asymptomatic (1). There is evidence suggesting that colorectal carcinomas can derive from other types of colorectal lesions besides the adenomatous polyps including serrated polyps and flat adenomas (1). Briefly, serrated polyps include several different types of lesions such as aberrant crypt foci, hyperplastic polyps, mixed polyps, serrated adenomas and sessile serrated adenomas. These lesions are normally small, smooth and sessile and occur mainly in the rectum and sigmoid colon. Recently, a serrated colorectal carcinogenesis pathway has been described, with some molecular differences with the conventional adenoma-carcinoma sequence (26). Regarding flat adenomas, they are superficial, non-polypoidal lesions and their malignant potential is considerably higher than the malignant potential of adenomatous or serrated polyps. In addition, it has been proposed that colorectal carcinomas deriving from flat adenomas also follow a different molecular pathway (27).

1.3.4.2 Molecular genetics of sporadic colorectal carcinogenesis

Like in many other tumour types, colorectal carcinogenesis derives from mutations in mainly oncogenes and tumour suppressor genes and in comparison to the inherited and familial colorectal cancer (germline mutations), sporadic colorectal cancer results from the accumulation of multiple somatic mutations. In addition sporadic colorectal cancer can have two different genomic profiles, which are known as: 1) chromosomal instability neoplasia (CIN) and 2) microsatellite instability neoplasia (MIN) (28).

The majority of sporadic colorectal cancers (85-90%) initiate due to mutation in the *APC* gene and are characterised by chromosomal instability. These tumours are generally associated with hyperploidy, allelic losses, frequent tumour suppressor gene mutations (*APC*, *p53*) and are mainly located in the left part of the colon. Mutations in the *APC* gene (loss of heterozygosity on chromosome 5q: *5qLOH*) occur early in the colorectal carcinogenesis and they are normally followed by mutations in the *k-ras* gene and later in the *p53* gene (*17pLOH*). In addition, mutations in three additional genes (*DCC,*

SMAD4, SMAD2) on chromosome 18q (*18qLOH*) have been found in advanced adenomas. The remaining 10-15% of sporadic colorectal tumours are characterised by microsatellite instability (MIN) and are mainly located in the proximal colon. They are euploid tumours without allelic losses, present infrequent suppressor gene mutations (*p53, APC*) and more frequent mutations in the *BRAF* and *PI3KCA* oncogenes and some other genes (*TGBβ-RII, BAX, TCF4, Caspase5, HIF1α*) (29).

1.3.5 Clinical grading and staging of colorectal cancer

Two systems can be applied to describe the extent of colorectal cancer in the body: the Dukes' and the American Joint Committee on Cancer (AJCC) systems. Modified Dukes' staging, which was originally published by Dukes CE (1932), is a pathological staging based on resection of the tumour and measures the depth of invasion through the mucosa and bowel wall. However, it does not take into account the level of nodal involvement or the grade of the tumour. There are four modified Dukes' stages (A-D): 1) Stage A, where the tumour penetrates into the mucosa of the bowel wall; 2) Stage B, where the tumour penetrates into (B1) and through (B2) the muscularis propria (the muscular layer) of the bowel wall; 3) Stage C, where the tumour penetrates into (C1) and through (C2) the muscularis propria of the bowel wall and there is pathologic evidence of colon or rectal cancer in the lymph nodes; 4) Stage D, where the tumour has spread beyond the borders of the lymph nodes (to organs such as the liver, lung or bone; Table 1).

The AJCC system is based on the TNM classification. In TNM classification, T stands for tumour and describes the extent of the tumour spread through the layers that form the bowel wall, N stands for nodes and indicates whether or not the cancer has spread to nearby lymph nodes and, if so, how many lymph nodes are affected and M stands for metastasis and indicates whether or not the cancer has spread to distant organs. Each of these three elements is categorised separately and classified with a number. There are five stages for tumour describing its extent through the bowel wall (Tis, T1-T4): 1) Tis, where tumour involves only the mucosa; 2) T1, where tumour invades submucosa; 3) T2, where tumour invades muscularis propria; 4) T3, where tumour invades through the muscularis propria into the subserosa, or into the pericolic or perirectal tissues; 5) T4, where tumour directly invades other organs or structures, and/or perforates. There are

three stages for node describing the cancer spread to nearby lymph nodes (N0-N2): 1) N0, where there is no spread in regional lymph node; 2) N1, where there is spread in one to three regional lymph nodes; 3) N2, where there is spread in four or more regional lymph nodes. Finally, there are two stages for metastasis describing the cancer spread to distant organs (M0-M1): 1) M0, where there is no distant metastasis; 2) M1, where distant metastasis is present. In case of incomplete information regarding the tumour invasion, nodes affected and presence or not of metastasis, the stage code becomes Tx, Nx or Mx, respectively (Table 2).

When the three TNM numbers are combined (stage grouping), the AJCC stage is formed (0, I-IV): 1) Stage 0 for Tis, N0 and M0; 2) Stage I for T1, N0 and M0 or T2, N0 and M0; 3) Stage IIA for T3, N0 and M0; 4) Stage IIB for T4, N0 and M0; 5) Stage IIIA for T1, N1 and M0 or T2, N1 and M0; 6) Stage IIIB for T3, N1 and M0 or T4, N1 and M0; 7) Stage IIIC for any T, N2 and M0; 8) Stage IV for any T, any N and M1 (Table 3); (information taken from the American cancer society; http://www.cancer.org/).

Table 1 Summary of Duke's staging system*

Stage	Description
A	tumour penetrates into the mucosa of the bowel wall
B1	tumour penetrates into the muscularis propria (the muscular layer) of the bowel wall
B2	tumour penetrates into and through the muscularis propria (the muscular layer) of the bowel wall
C1	tumour penetrates into the muscularis propria of the bowel wall pathologic evidence of colon or rectal cancer in the lymph nodes
C2	tumour penetrates into and through the muscularis propria of the bowel wall pathologic evidence of colon or rectal cancer in the lymph nodes
D	tumour has spread beyond the confines of the lymph nodes (to organs such as the liver, lung or bone)

* Information taken from http://www.cancer.org/

Table 2 Summary of TNM classification*

Tumour (T)		Lymph nodes (N)		Distant metastasis (M)	
Tis	tumour involves only the mucosa	N0	there is no metastasis in regional lymph node	M0	there is no distant metastasis
T1	tumour invades submucosa	N1	there is metastasis in 1 to 3 regional lymph nodes	M1	there is distant metastasis
T2	tumour invades muscularis propria	N2	there is metastasis in 4 or more regional lymph nodes	Mx	incomplete information regarding distant metastasis
T3	tumour invades through the muscularis propria into the subserosa, or into the pericolic or perirectal tissues	Nx	incomplete information regarding number of affected lymph nodes		
T4	tumour directly invades other organs or structures, and/or perforates				
Tx	incomplete information regarding tumour invasion				

Table 3 Summary of AJCC staging system*

Stage	TNM stage equivalent	Description
0	Tis, N0, M0	carcinoma in situ or intramucosal carcinoma
I	T1, N0, M0 or T2, N0, M0	Cancer has begun to spread, but is still in the inner lining
IIA	T3, N0, M0	Cancer has spread to other organs near the colon or rectum, but it has not reached lymph nodes
IIB	T4, N0, M0	
IIIA	T1, N1, M0 or T2, N1, M0	Cancer has spread to lymph nodes, but has not been carried to distant organs of the body
IIIB	T3, N1, M0 or T4, N1, M0	
IIIC	any T, N2, M0	
IV	any T, any N, M1	Distant organs metastasis (i.e. lungs and liver)

* Information taken from http://www.cancer.org/

1.4 Epidemiology of colorectal cancer

1.4.1 Prevalence of colorectal cancer

According to the International Agency for Research on Cancer (IARC) 5-year world prevalence of colorectal cancer in 2002 was approximately 0.05%. For more developed countries (including all countries of Europe, all countries of North America, Japan, Australia and New Zealand) 5-year prevalence was higher than for less developed countries (including all countries of Africa, Latin America, the Caribbean, Asia - excluding Japan, Micronesia, Polynesia and Melanesia) (0.17% and 0.016% respectively) (IARC). In particular, 5-year prevalence of colorectal cancer for Europe and the UK in 2005 were 0.12% and 0.15% respectively (IARC). In addition, according to the Scottish Cancer Registry, 5-year prevalence of colorectal cancer in 2005 in Scotland was 0.14% (0.15% for men and 0.12% for women).

1.4.2 Incidence of colorectal cancer

1.4.2.1 Geographical trends

Colorectal cancer is the third most common cancer in Scotland for both males and females (12.9% of all cancers, 2005), with 3,412 individuals (1,854 men and 1,558 women) affected in 2005 (Scottish Cancer Registry). The crude incidence rates were 75.5/100,000 for men and 59.0/100,000 for women. Age-standardised (European standard population) incidence rates (EASR) by sex are presented separately for each Scottish Health Board (Figure 1) as well as for North, South East and West of Scotland (Figure 2). The EASR incidence for Scotland in 2005 was 61.3/100,000 for men and 38.1/100,000 for women. Age-standardised (World standard population) incidence rate (WASR) for Scotland in 2005 was 40.2/100,000 for men and 25.2/100,000 for women. The highest EASR incidence rates were observed in the West of Scotland for men (62.4/100,000) and in the North of Scotland for women (39.5/100,000) (Scottish Cancer Registry).

Incidence rates for the UK and separately for England, Wales, Scotland and N. Ireland were obtained from Cancer Research UK (2004). In 2004, 36,109 British individuals were affected from colorectal cancer (19,657 men and 16,452 women) and the crude incidence rate was 67.2/100,000 for men and 53.9/100,000 for women. EASR incidence rates for the UK, England, Wales, Scotland and N. Ireland are presented in Figure 3.

The EASR incidence rate for the UK was 55.3/100,000 for men and 35.5/100,000 for women. The highest EASR incidence rate was observed in Scotland for men (65/100,000) and in N. Ireland for women (40.5/100,000). Colorectal cancer incidence rates for 2005 were available for Scotland (Scottish Cancer Registry), Wales (Welsh Cancer Intelligence & Surveillance Unit) and for N. Ireland (N. Ireland Cancer research). However, at the point that this thesis was written, 2005 data were not available for England. EASR incidence rates for 2005 were 61.3/100,000 (men) and 38.1/100,000 (women) for Scotland, 58.8/100,000 (men) and 34.4/100,000 (women) for Wales and 64.2/100,000 (men) and 35.4/100,000 (women) for N. Ireland.

Incidence rates of colorectal cancer in countries of the European Union (EU) according to the 2006 estimates (Cancer Research UK) varied by a factor of 3 for men and a factor of 2 for women, with the lowest EASR incidence rates to be observed in Greece (31/100,000 for men and 21.3/100,000 for women) and the highest EASR incidence rates to be observed in Hungary (106/100,000 for men and 50.6/100,000 for women). EASR estimates for the EU are 59/100,000 for men and 35.6/100,000 for women and together with the EASR 2006 estimates for each country member of the EU are presented in Figure 4.

According to the IARC, approximately 1,023,152 new cases of colorectal cancer were diagnosed in 2002 (9% of all new cancer cases) making colorectal cancer the third most common cancer worldwide. 65% of the new cases of colorectal cancer in 2002 were recorded in the more developed regions. Large variations in incidence rates were observed with the lowest WASR incidence rate to be observed in Africa (WASR incidence rate in middle Africa: 2.3/100,000 for men and 3.3/100,000 for women) and the highest to be observed in Australia, N. America and Europe (highest WASR incidence rate in Australia/ N. Zealand: 48.2/100,000 for men and 36.9/100,000 for women). WASR incidence rates are presented in a bar chart in Figure 5 and in a world map in Figure 6 separately for men and women.

1.4.2.2 International temporal trends

In Scotland male colorectal cancer incidence rates rose slowly each year between 1982 and 1995 (1982 EASR incidence rate: 51.2/100,000; 1995 EASR incidence rate: 62.0/100,000). In 1996 there was an almost 6% increase in colorectal cancer incidence

reaching a 69.7/100,000 EASR incidence rate (highest EASR incidence rate from 1982 to 2005). Since 1997, there has been an almost constant gradual decrease of EASR incidence rates (2005 EASR incidence rate: 61.3/100,000). The lowest male colorectal cancer EASR incidence rate was observed in 1982 (51.2/100,000) (Figure 7). Over the same period female colorectal cancer incidence rates were generally constant, with slight fluctuations. The highest EASR incidence rate was observed also in 1996 (45.6/100,000) and the lowest EASR incidence rate was observed in 2005 (38.1/100,000) (Figure 7). According to Cancer Research UK, male colorectal cancer incidence rates in Great Britain rose slowly by an average of 1% each year between 1982 and 1999. Since 1999 and until 2004 there has been a slight decrease. The highest EASR incidence rate was observed in 1999 (58.2/100,000) and the lowest in 1982 (48.8/100,000) (Figure 8). Over the same period the female colorectal cancer incidence rates have changed very little. The highest EASR incidence rate was observed in 1992 (38.16/100,000) and the lowest EASR incidence rate was observed in 2003 (34.9/100,000) (Figure 8).

There is no clear trend in global age standardised incidence rates of colorectal cancer. In countries of relatively low-income economy, which have recently made a transition to a higher-income economy (e.g. eastern and southern European countries, Japan, Singapore), a rapid increase in incidence rates has been observed (30). However in countries with traditionally high colorectal cancer incidence rates a slight decrease has been observed in the last few years (e.g. Canada, USA and New Zealand/Australia) (30). Trends in age standardised incidence rates for male and female colorectal cancer are presented in Figure 9 and Figure 10 for selected countries.

Despite the decrease of the age-standardised incidence rates particularly in countries of high-income economy, the absolute number of colorectal cancer cases continues to increase, mainly because of the increasing age of the population. For Scotland, in particular, in 1982 2,726 men and women were diagnosed with colorectal cancer, whereas in 2005 3,412 people were diagnosed with, a 25.2% increase. In addition, a report published in 2006 from the European Network of Cancer Registries (ENCR), estimated that between 2004 and 2006 there was a 10.3% increase in absolute number of all cancers in Europe and concluded that absolute numbers of cancer will continue to

increase even if age-specific incidence rates remain constant or decrease, mainly due to the ageing European population (31).

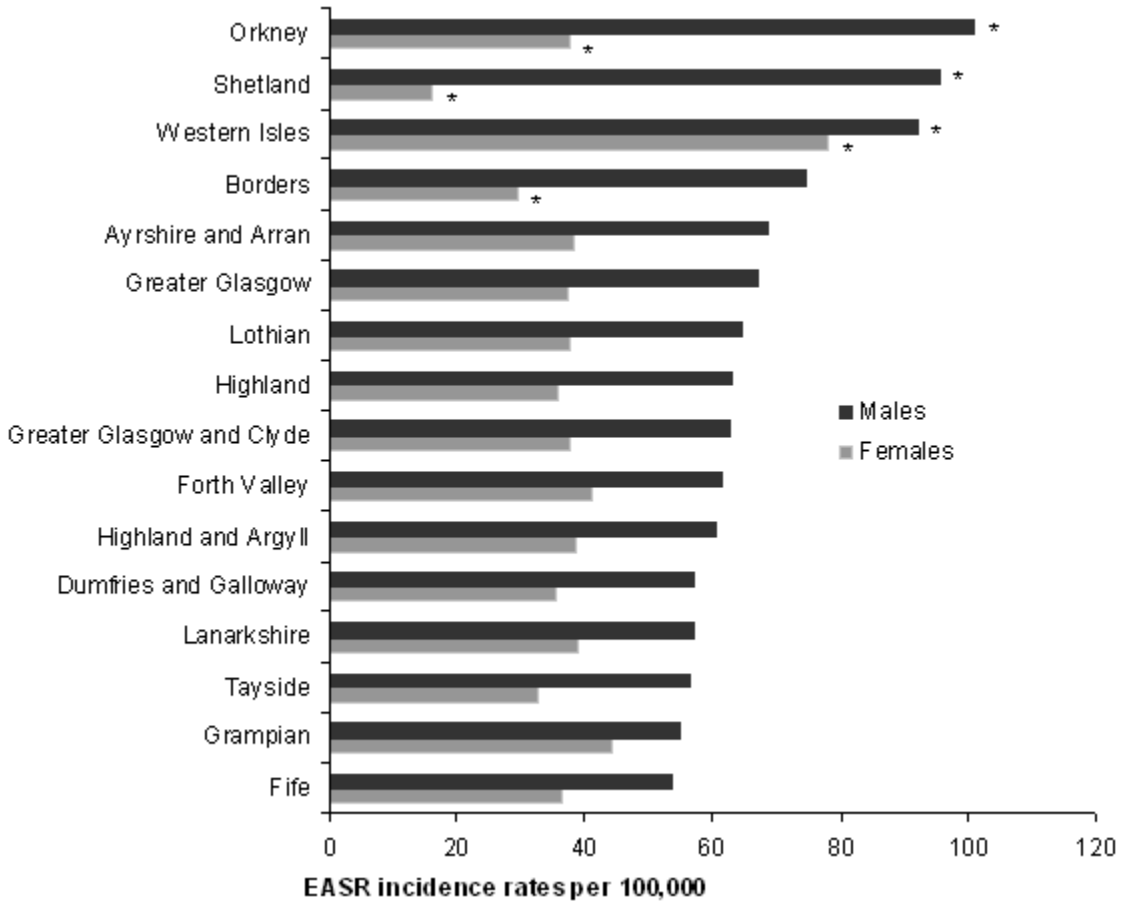


Figure 1 Age standardised (European standard population) incidence rates of colorectal cancer (per 100,000) in Scottish Health Boards by sex. Incidence rates marked with a star (*) were based on low numbers (≤50); (2005, Cancer Registry Scotland, ISD).

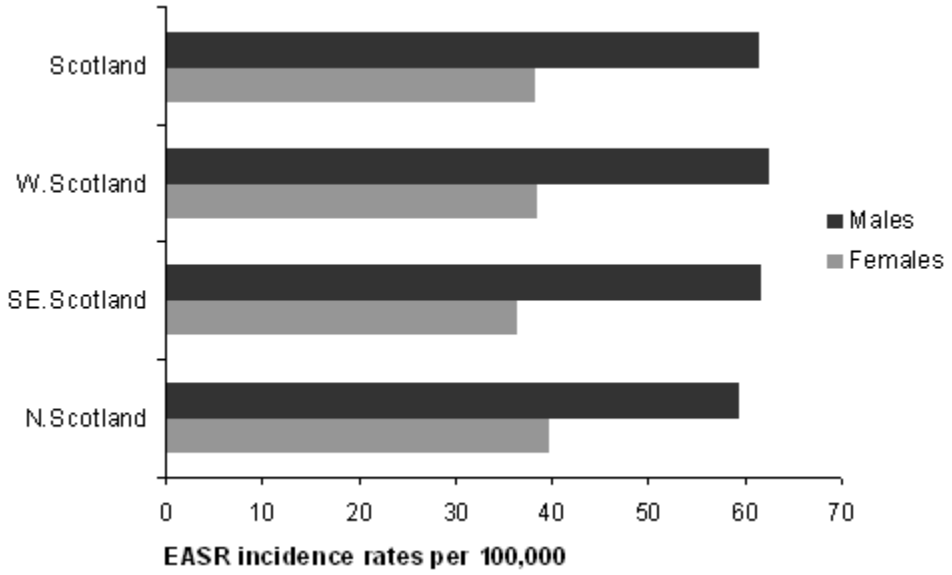


Figure 2 Age standardised (European standard population) incidence rates of colorectal cancer (per 100,000) in Scotland by sex (2005; Cancer Registry Scotland, ISD)

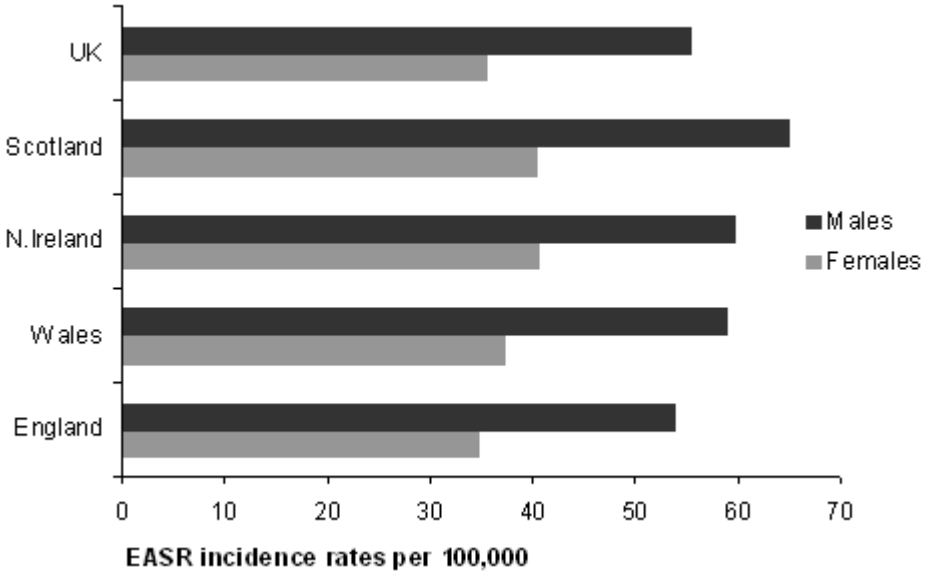


Figure 3 Age standardised (European standard population) incidence rates of colorectal cancer (per 100,000) in the UK by sex (2004; Cancer Research UK)

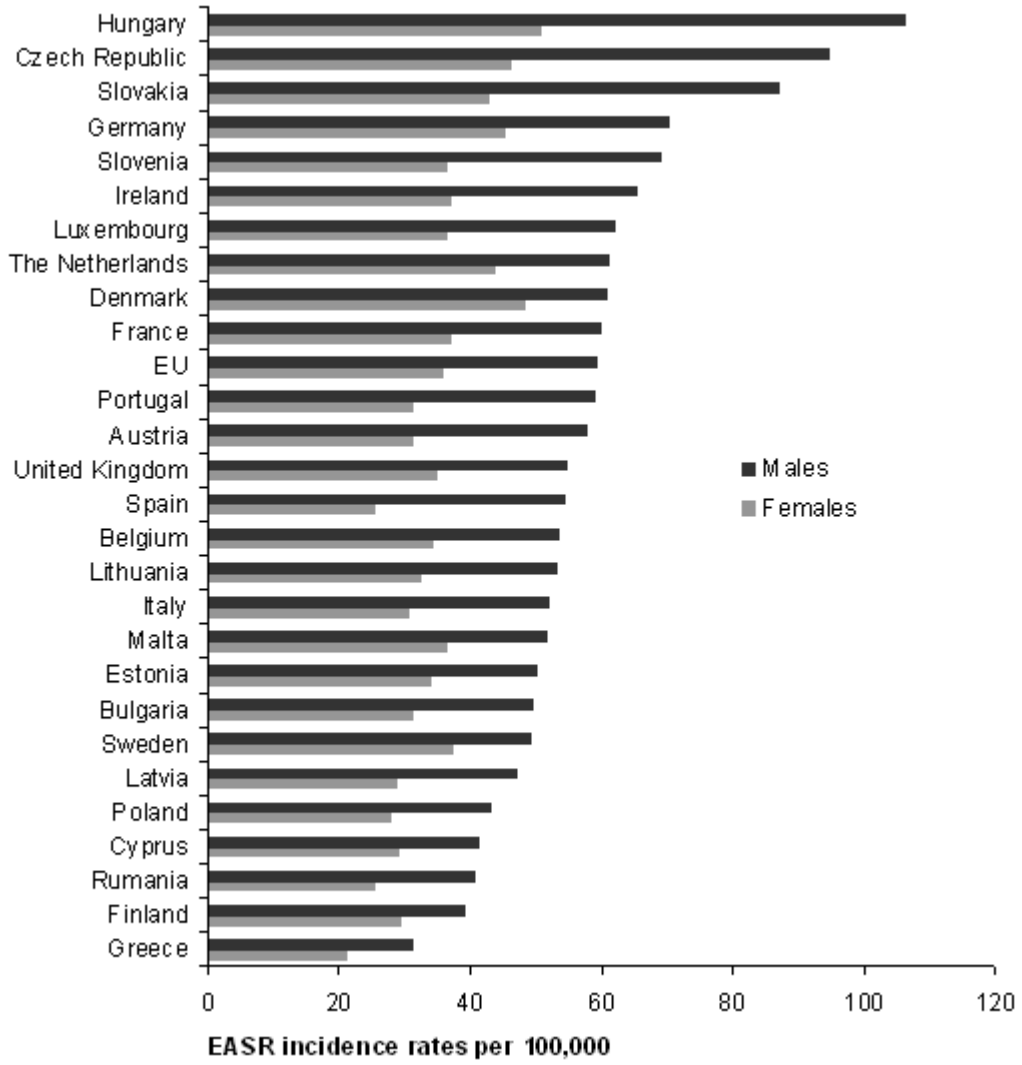


Figure 4 Age standardised (European standard population) incidence rates of colorectal cancer (per 100,000) in Europe by sex (2006 estimates; Cancer Research UK)

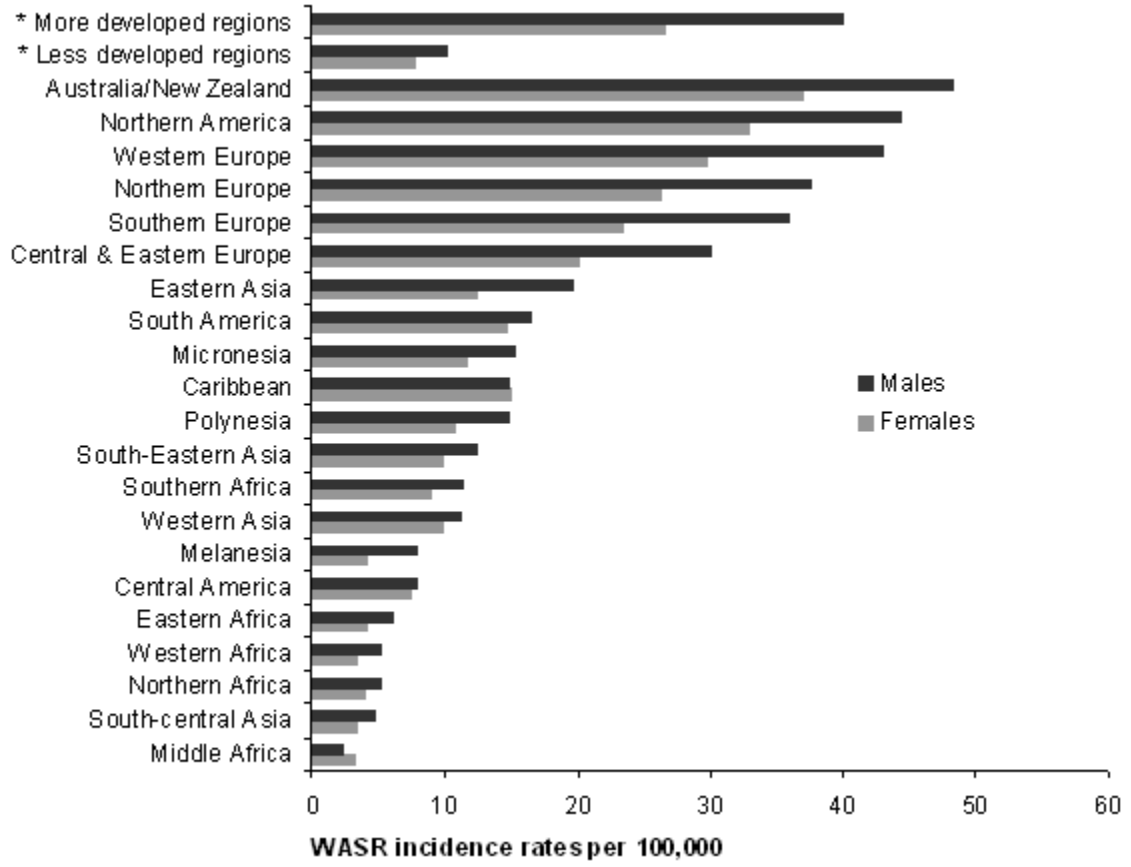
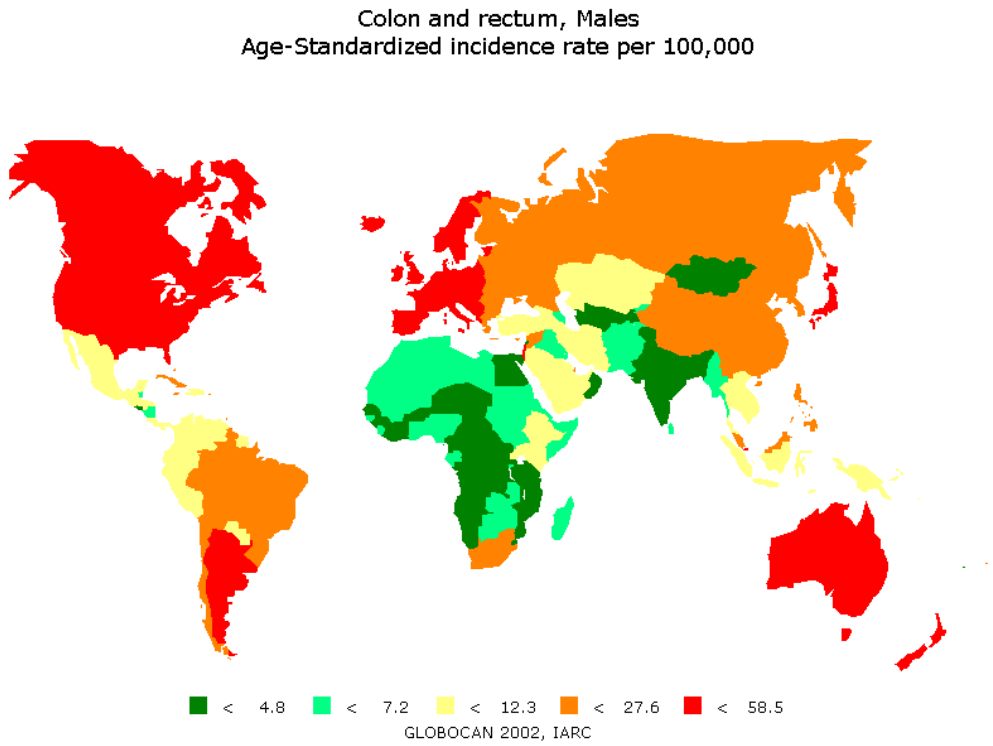


Figure 5 Age standardised (World standard population) incidence rates of colorectal cancer (per 100,000) worldwide by sex (2002 estimates; International Agency for research in cancer); (*More developed regions include: all countries of Europe, Japan, Australia, New Zealand and all countries of North America; Less developed regions include all countries of: Africa, Latin America, the Caribbean, Asia -excluding Japan, Micronesia, Polynesia and Melanesia)



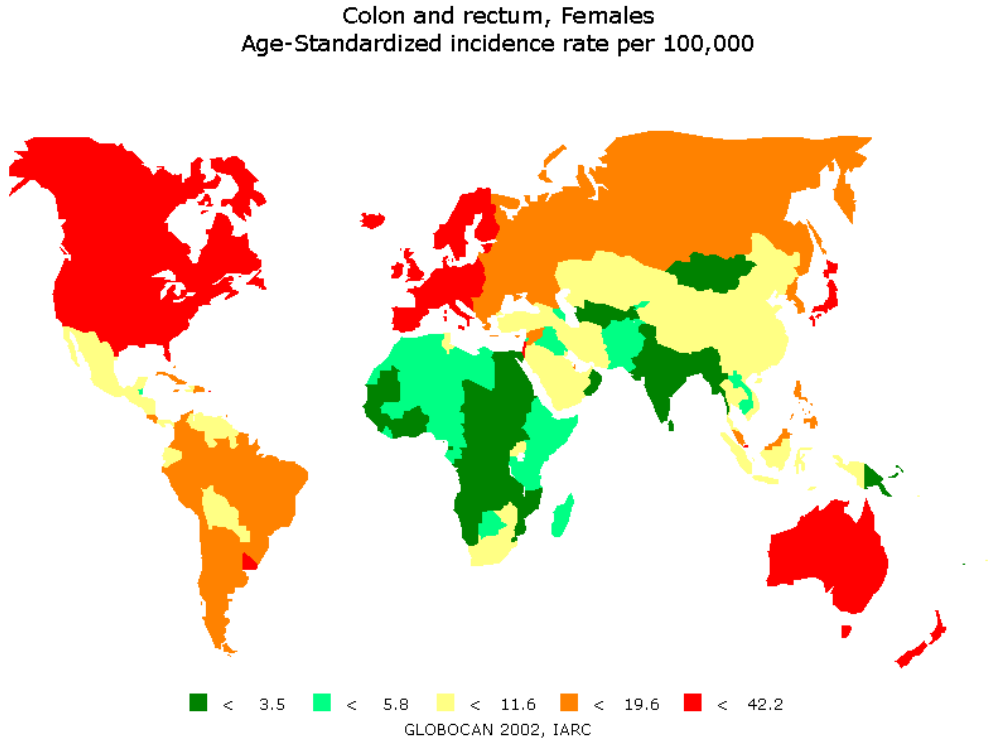


Figure 6 Maps of age standardised incidence rates of colorectal cancer (World Standard population) separately for men and women; Source: International Agency for research on cancer (2002 estimates)

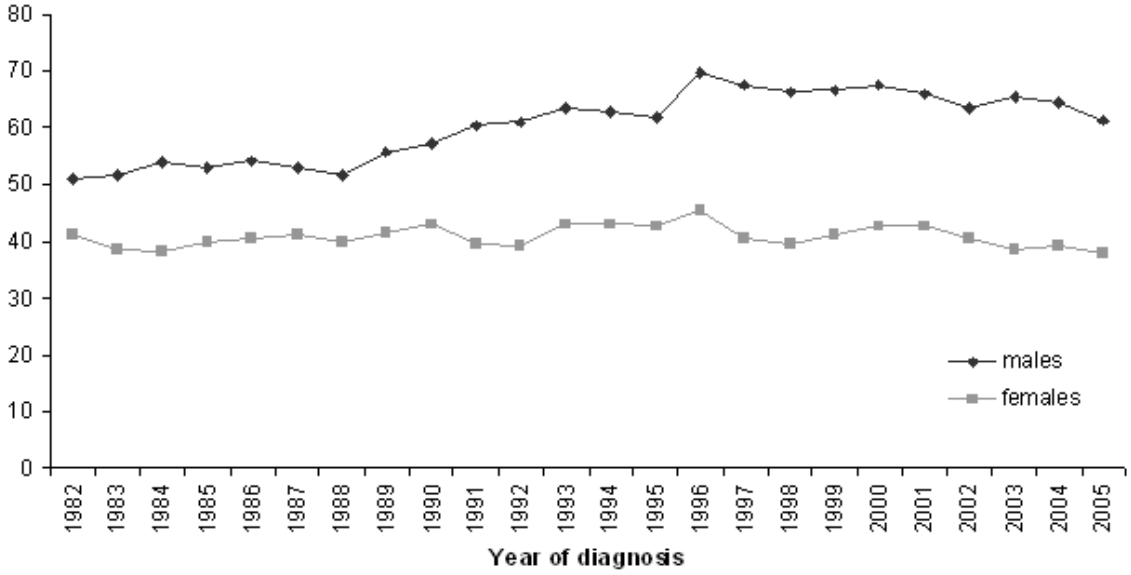


Figure 7 Age standardised (European standard population) incidence rates of colorectal cancer (per 100,000) in Scotland by sex from 1982 to 2005 (Cancer Registry Scotland, ISD)

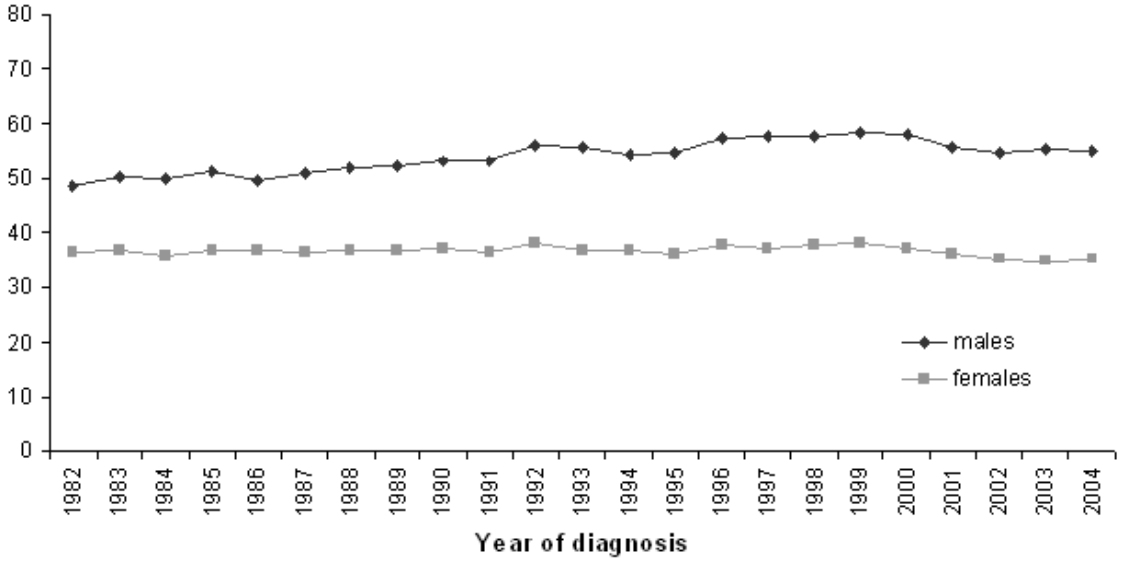


Figure 8 Age standardised (European standard population) incidence rates of colorectal cancer (per 100,000) in Great Britain by sex from 1982 to 2005 (Cancer Research UK)

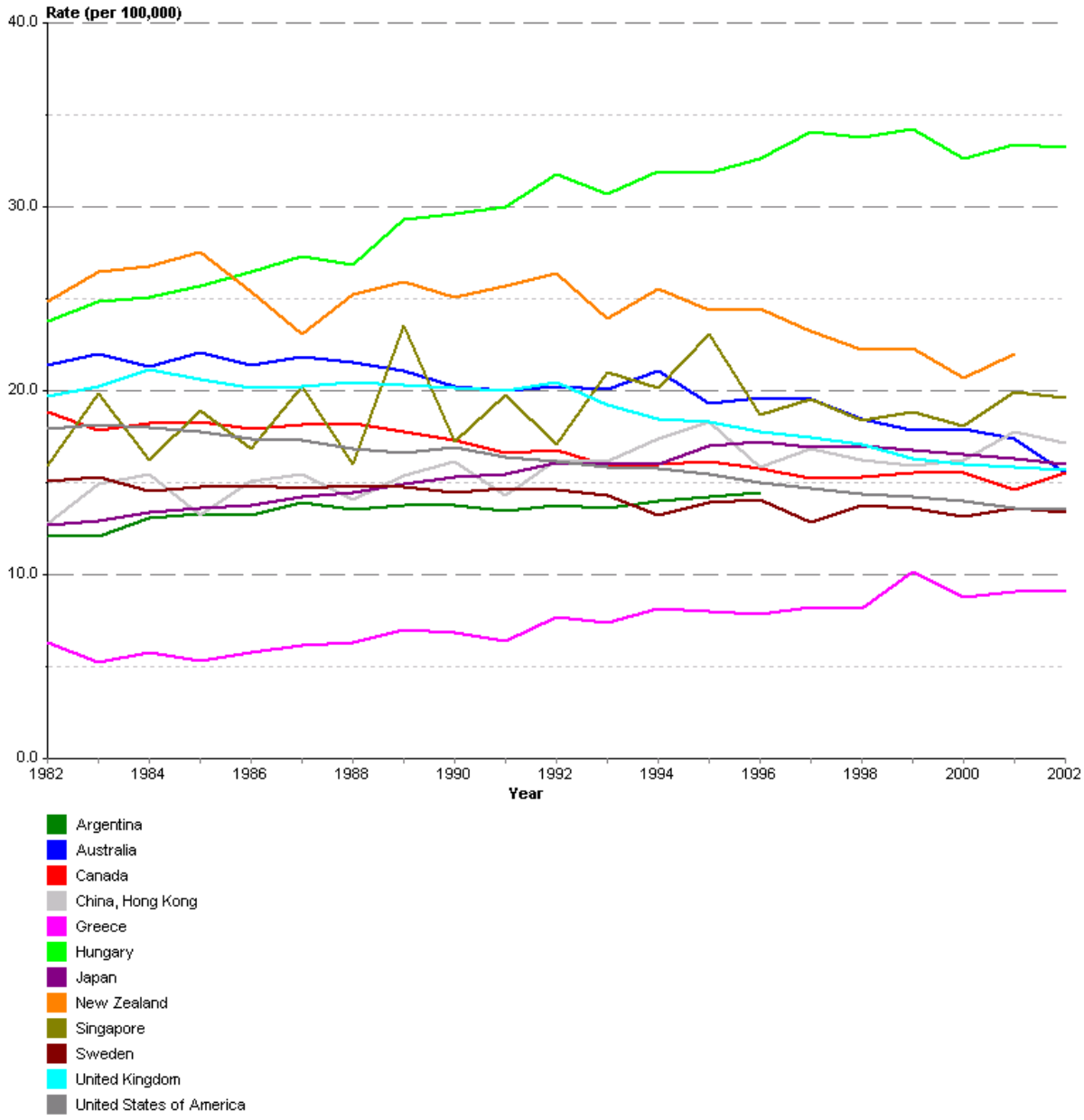


Figure 9 Age standardised (World standard population) incidence rates of male colorectal cancer (per 100,000) in selected countries from 1982 to 2002 (International Agency for research on cancer)

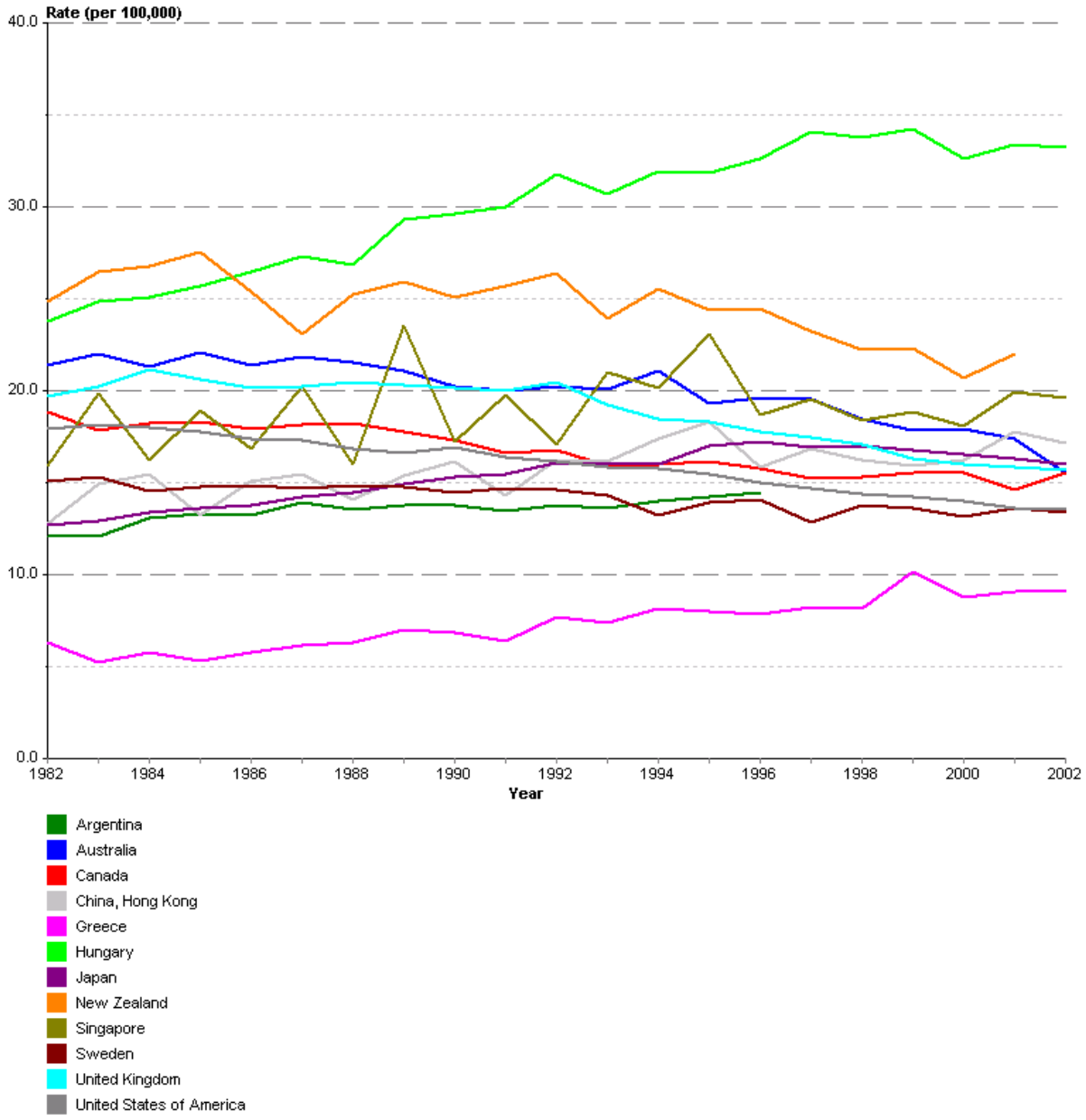


Figure 10 Age standardised (World standard population) incidence rates of female colorectal cancer (per 100,000) in selected countries from 1982 to 2002 (International Agency for research on cancer)

1.4.3 Mortality rates of colorectal cancer

1.4.3.1 Geographical trends

Colorectal cancer was the second most common cause of death from cancer in Scotland for males and the third for females (10.3% of all deaths from cancer for both sexes, 2005), with 1,550 individuals (835 men and 715 women) having died in 2006 (Scottish Cancer Registry). Crude mortality rate was 33.8/100,000 for men and 27.0/100,000 for women. EASR mortality rates by sex are presented separately for each Scottish Health Board (Figure 11) as well as for North, South East and West of Scotland (Figure 12). The EASR and WASR mortality rates for Scotland in 2006 was 27.0/100,000 and 17.2/100,000 for men and 15.8/100,000 and 10.0/100,000 for women. The highest EASR mortality rates were observed in the West of Scotland for men (28.3/100,000) and in the North of Scotland for women (17.3/100,000) (Scottish Cancer Registry).

Mortality rates for the UK and separately for England, Wales, Scotland and N. Ireland were obtained from Cancer Research UK (2005). In 2005, 16,092 British individuals died from colorectal cancer (8,637 men and 7,455 women) and the crude mortality rate was 29.4/100,000 for men and 24.3/100,000 for women. EASR mortality rates for the UK, England, Wales, Scotland and N. Ireland are presented in Figure 13. The EASR mortality rate for the UK was 23.3/100,000 for men and 14.3/100,000 for women and geographic distribution was similar with a relatively small variation. The highest EASR mortality rates for both men and women were observed in N. Ireland (16.1/100,000 and 11.7/100,000 respectively). Colorectal cancer mortality rates for 2006 were available for Scotland (Scottish Cancer Registry) and for N. Ireland (N. Ireland Cancer research). However, at the point that this thesis was written 2006 mortality data were not available for England and Wales. EASR mortality rates for 2006 were 27.0/100,000 (men) and 15.8/100,000 (women) for Scotland and 24.6/100,000 (men) and 13.7/100,000 (women) for N. Ireland.

Mortality rates of colorectal cancer in countries of the EU according to the 2002 data from the IARC varied by a factor of 3 for both men and women, with the lowest WASR mortality rates to be observed in Greece (9.7/100,000 for men and 8.0/100,000 for

women) and the highest WASR mortality rates to be observed in Hungary (35.6/100,000 for men and 21.2/100,000 for women). WASR mortality rates for 2002 estimates of each country member of the EU are presented in Figure 14.

According to the IARC, approximately 528,978 individuals died from colorectal cancer in 2002 (8% of all cancer related deaths) and 60% of colorectal cancer deaths were recorded in the more developed regions. Large variations in mortality rates were observed with the lowest WASR mortality rate to be observed in Africa (WASR mortality rate in middle Africa: 2.2/100,000 for men and 3.0/100,000 for women) and the highest to be observed in Europe, Australia, N. America (highest WASR mortality rate in Central and Eastern Europe: 19.7/100,000 for men and 12.9/100,000 for women). WASR incidence rates are presented in a bar chart in Figure 15 and in a world map in Figure 16 separately for men and women.

1.4.3.2 International temporal trends

In Scotland male colorectal cancer mortality rates were unstable from 1983 to 1997, with moderate fluctuations. Since 1997, a steady decline in EASR male mortality rates has been observed (18% difference between 1997 and 2006). The lowest EASR mortality rate in male colorectal cancer was observed in 2006 (27.0/100,000) and the highest was observed in 1993 (34.4/100,000) (Figure 17). Over the same period (1983-2006), there was a constant decline in female colorectal cancer mortality rates with slight fluctuations (38% difference between 1983 and 2006). The highest EASR mortality rate was observed also in 1983 (25.3/100,000) and the lowest were observed in 2004 and 2005 (15.7/100,000) (Figure 17). Regarding the absolute number of deaths, 1,714 men and women died from colorectal cancer in Scotland in 1983, whereas there was a 9.6% decrease in 2006, with 1,550 colorectal cancer deaths.

Male colorectal cancer mortality rates in the UK were generally constant from 1982 to 1992 with two peaks in 1984 (EASR mortality rate: 33.0/100,000) and in 1992 (EASR mortality rate: 31.9/100,000). Since 1992 there has been a constant decline in mortality rates with an almost 27% difference between the years 1992 and 2005. The highest EASR mortality rate was observed in 1984 (33.0/100,000) and the lowest in 2005 (23.3/100,000) (Figure 18). Over the same period (1982-2005), there was a constant

decline in female colorectal cancer mortality rates (36% difference between 1982 and 2005). The highest EASR mortality rate was observed in 1995 (23.4/100,000) and the lowest EASR mortality rate was observed in 2005 (14.3/100,000) (Figure 18).

Generally global mortality rates of colorectal cancer for both men and women have been either constant or slightly increasing over time. However there are some exceptions with greater increase in colorectal cancer mortality rates especially in countries that have recently adopted a more western type of lifestyle (e.g. Japan and countries of the Eastern and Southern Europe). In contrast decreases in mortality rates have been observed over time for some countries (e.g. the UK, Sweden).

According to the ENCR report, in contrast to what was observed in Scotland, a 1.8% increase in absolute number of deaths from colorectal cancer for men and women was reported from 2004 to 2006 (31). Changes in absolute numbers of colorectal cancer deaths for selected countries are presented in Figure 19 and Figure 20.

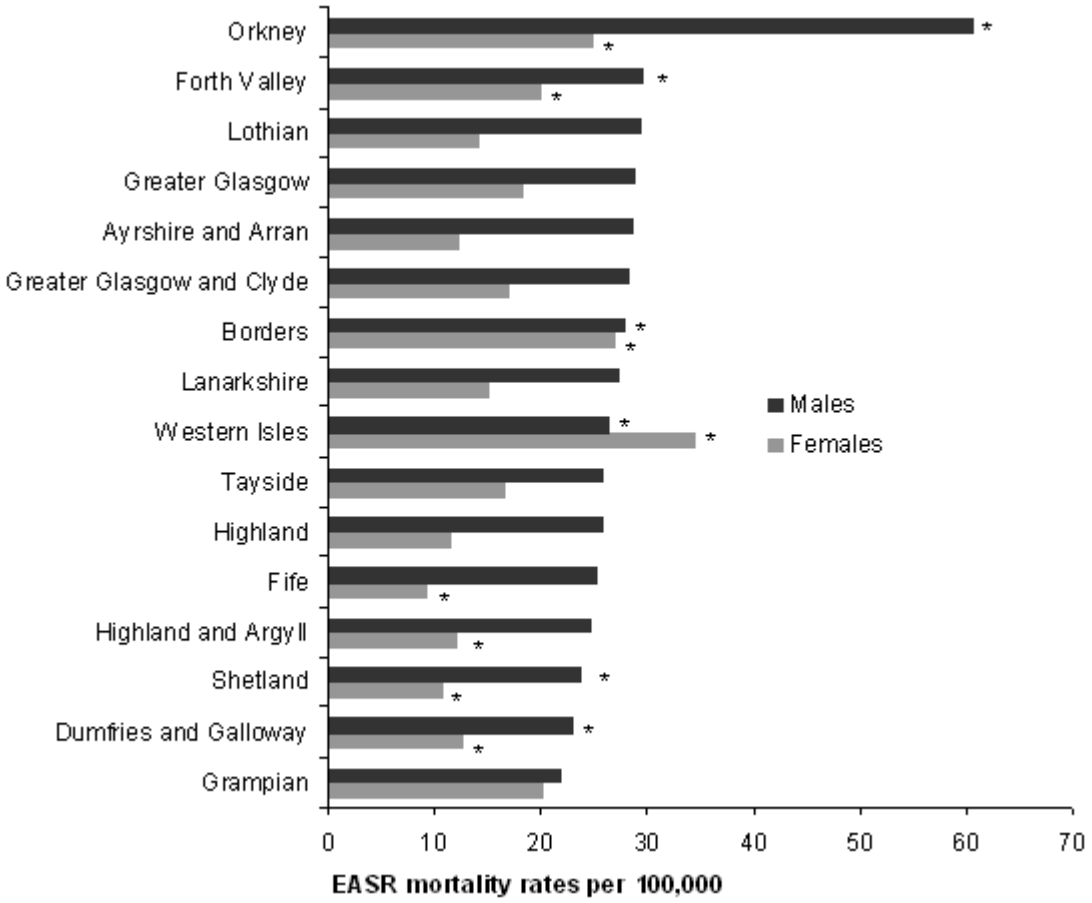


Figure 11 Age standardised (European standard population) mortality rates of colorectal cancer (per 100,000) in Scottish Health Boards by sex. Mortality rates marked with a star (*) were based on low numbers (≤50); (2006, Cancer Registry Scotland, ISD)

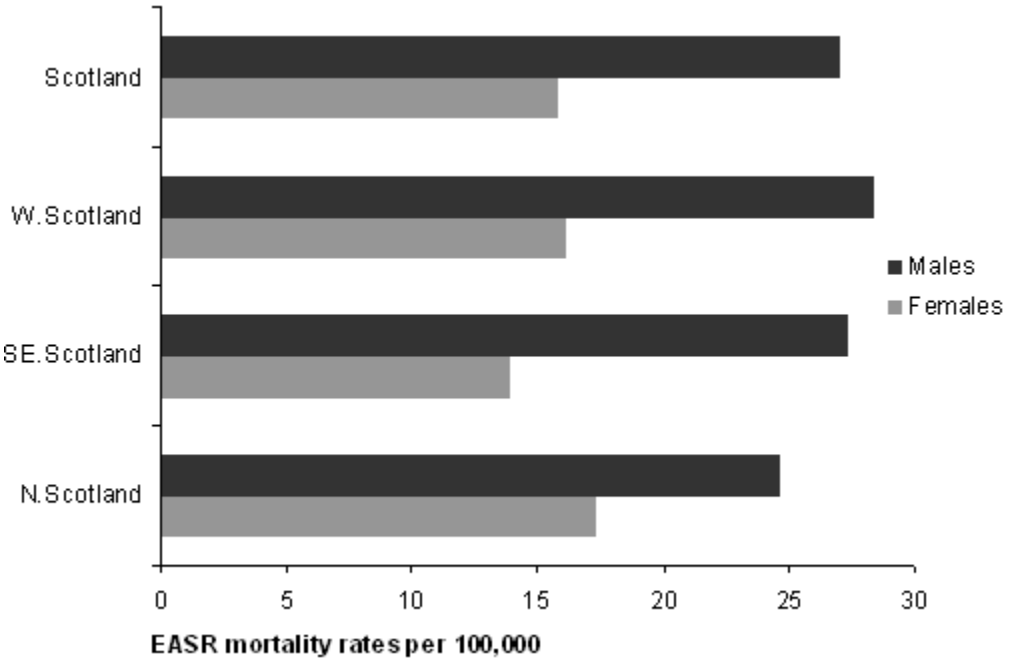


Figure 12 Age standardised (European standard population) mortality rates of colorectal cancer (per 100,000) in Scotland by sex (2006; Cancer Registry Scotland, ISD)

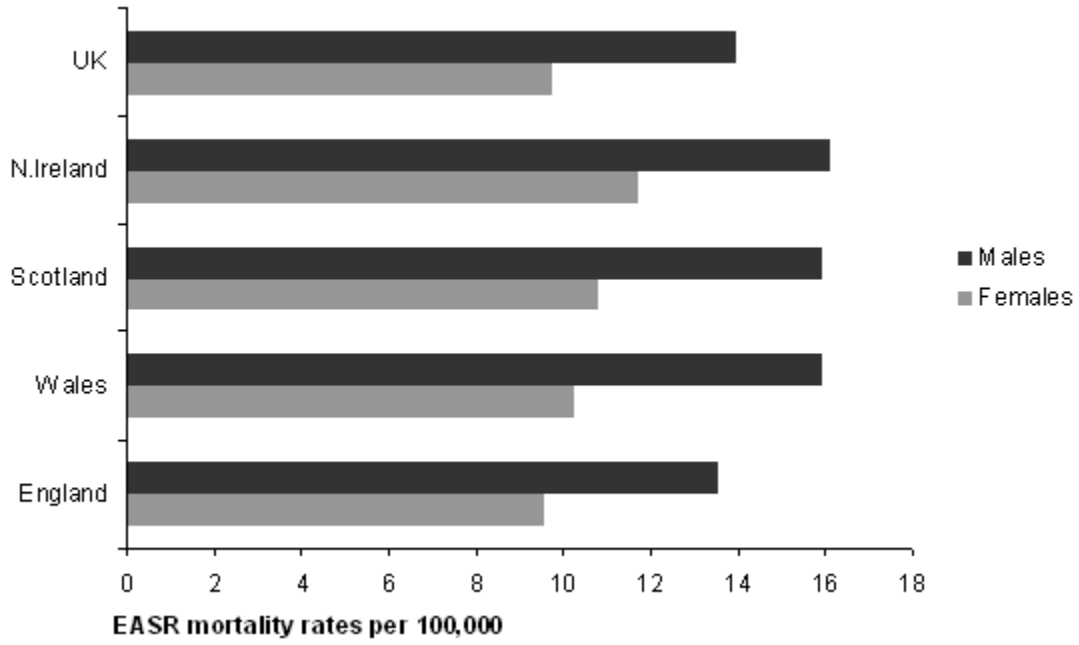


Figure 13 Age standardised (European standard population) mortality rates of colorectal cancer (per 100,000) in the UK by sex (2005; Cancer Research UK)

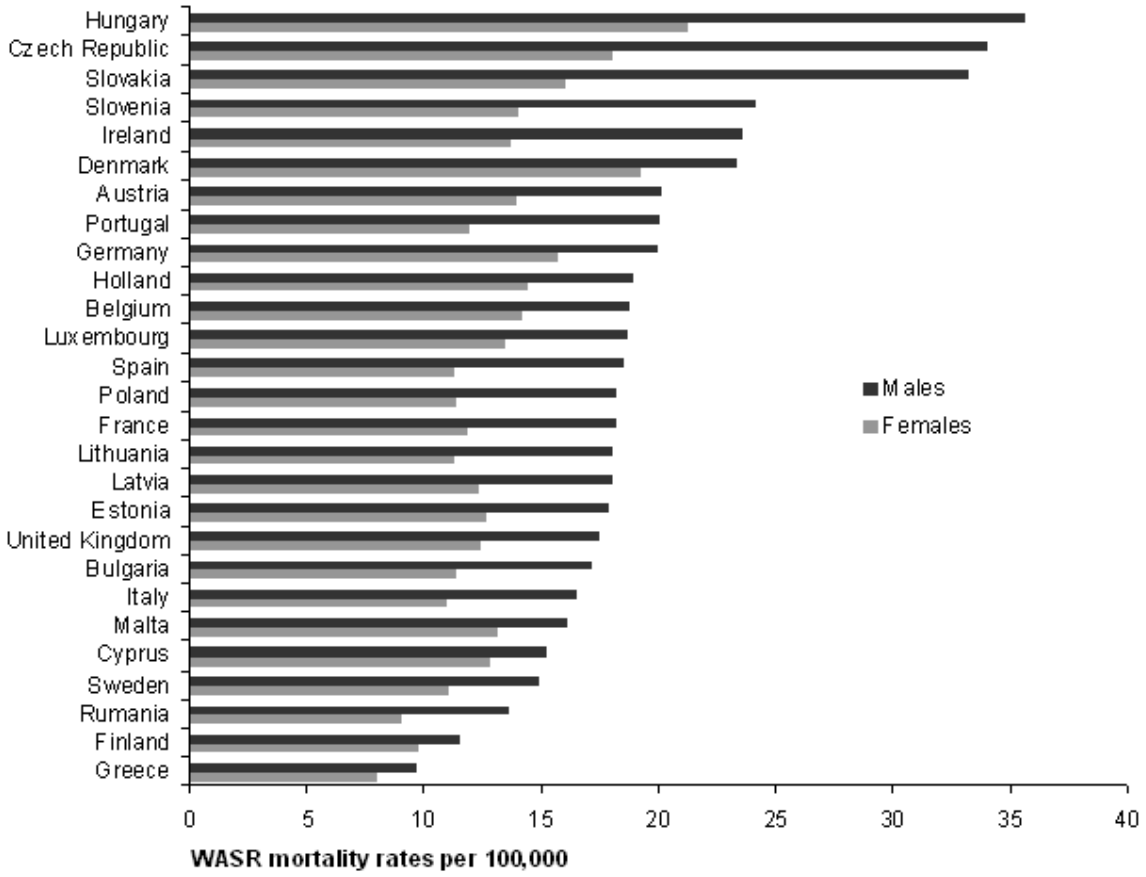


Figure 14 Age standardised (World standard population) mortality rates of colorectal cancer (per 100,000) in Europe by sex (2002 estimates; International Agency for research on cancer)

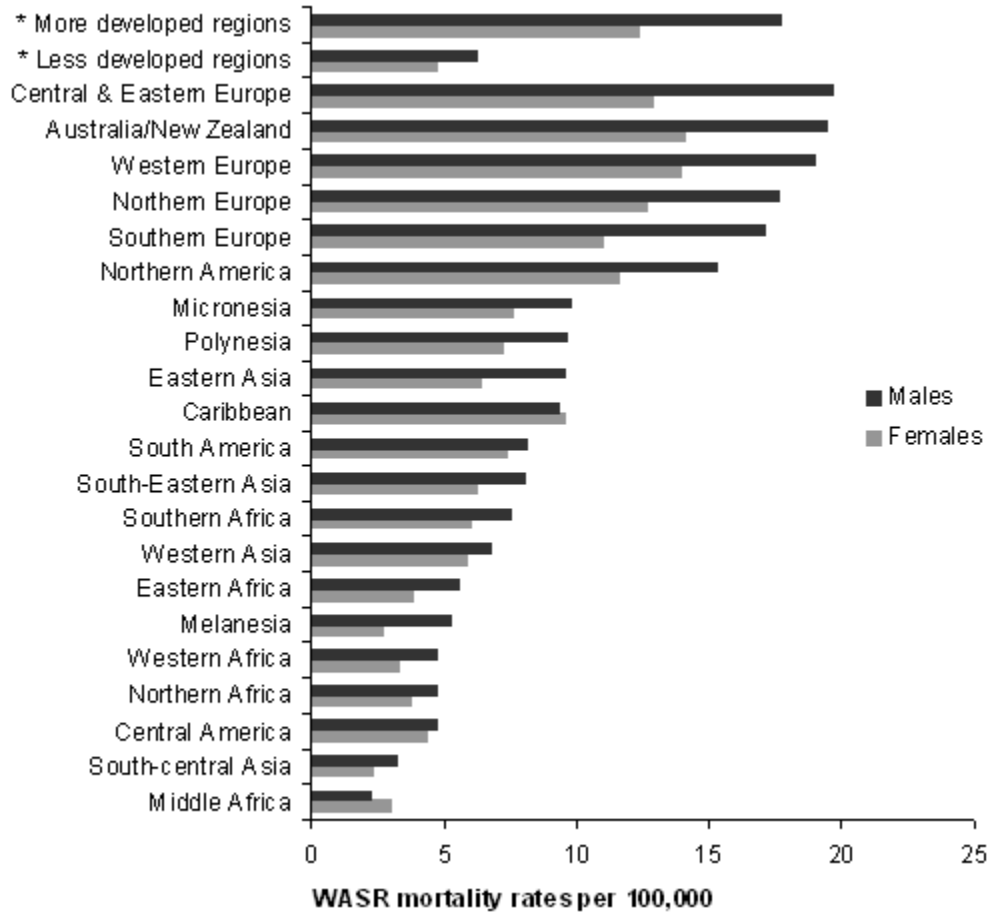
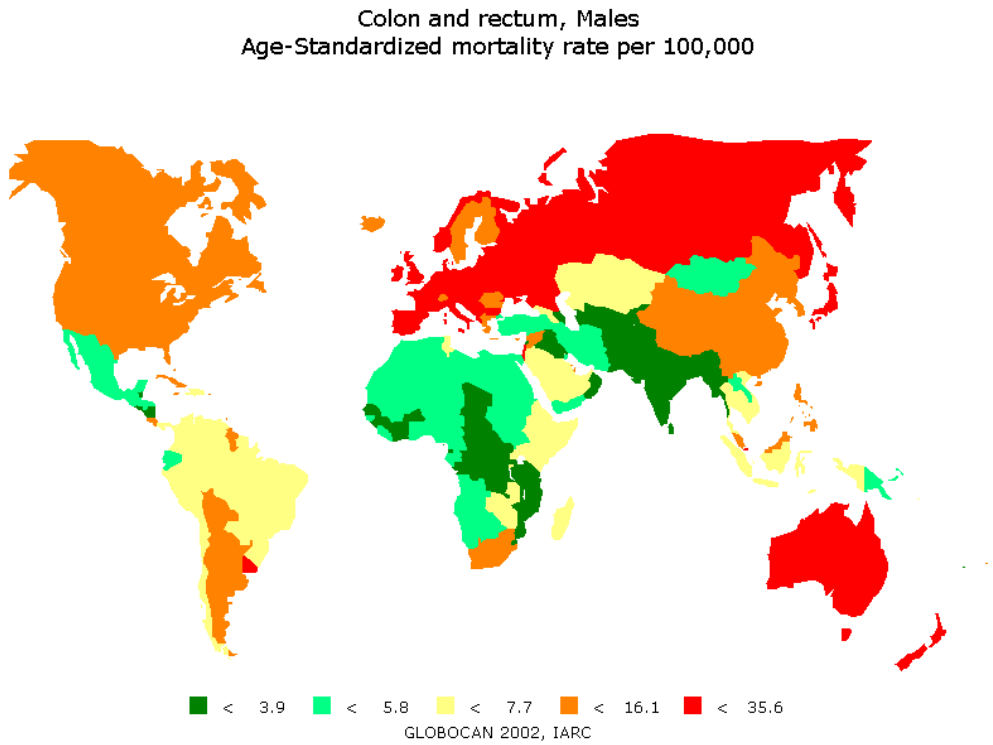


Figure 15 Age standardised (World standard population) mortality rates of colorectal cancer (per 100,000) worldwide by sex (2002 estimates; International Agency for research on cancer); (*More developed regions include: all countries of Europe, Japan, Australia, New Zealand and all countries of North America; Less developed regions include all countries of: Africa, Latin America, the Caribbean, Asia -excluding Japan, Micronesia, Polynesia and Melanesia)



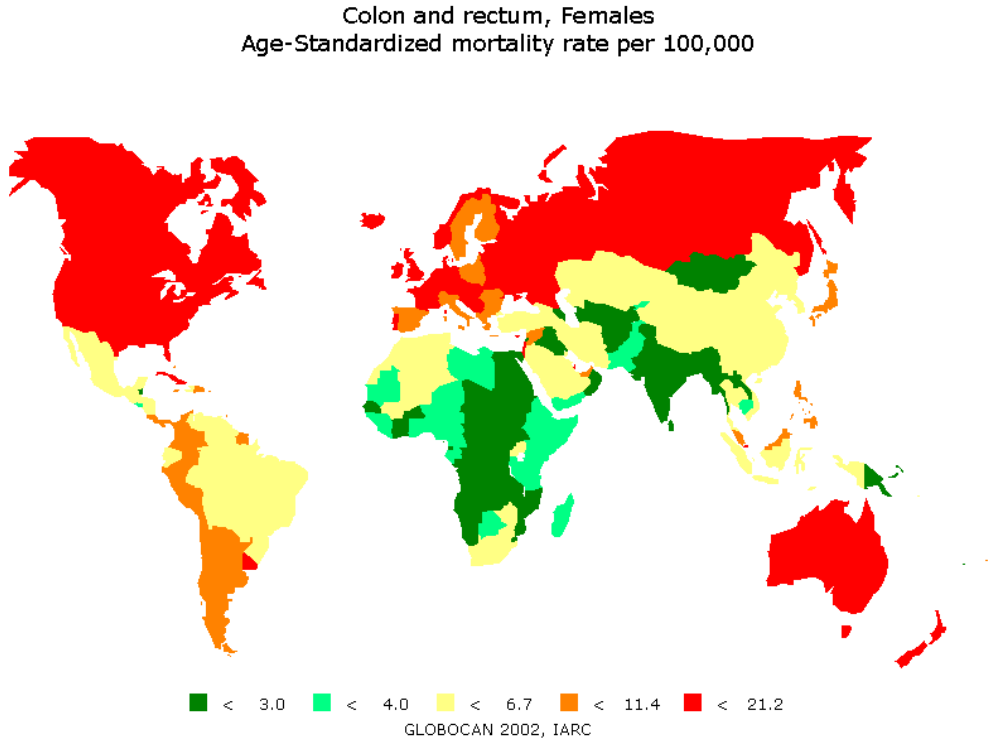


Figure 16 Maps of age standardised mortality rates of colorectal cancer (World Standard population) separately for men and women; Source: International Agency for research on cancer (2002 estimates)

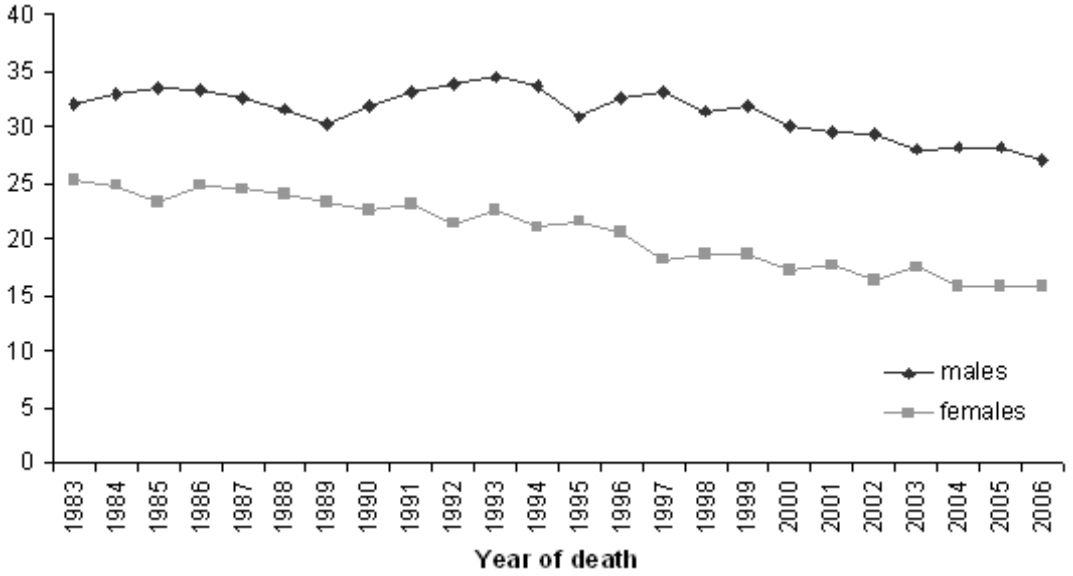


Figure 17 Age standardised (European standard population) mortality rates of colorectal cancer (per 100,000) in Scotland by sex from 1983 to 2006 (Cancer Registry Scotland, ISD)

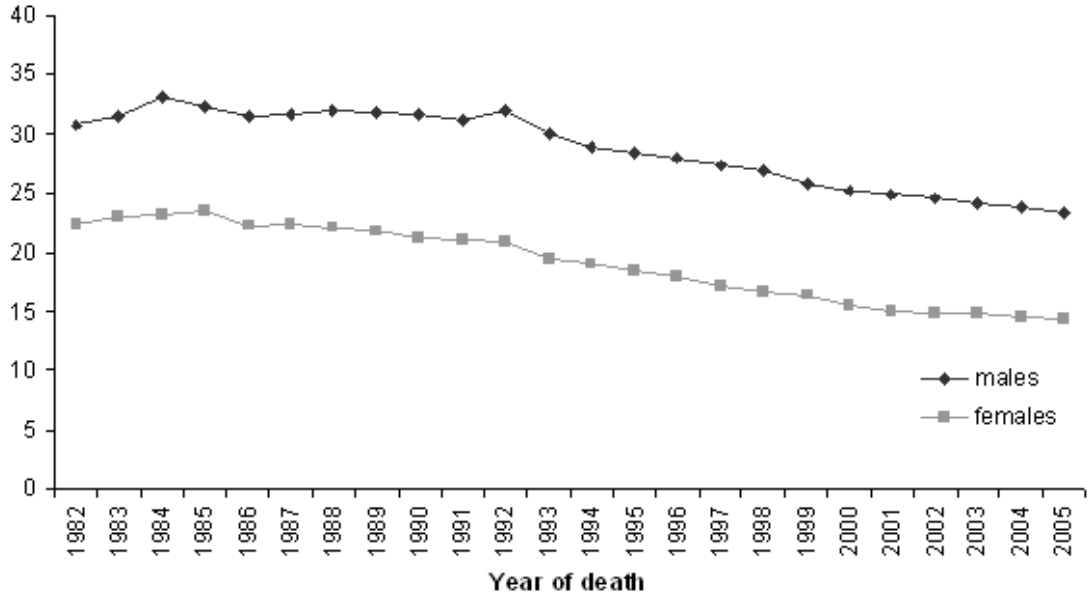


Figure 18 Age standardised (European standard population) mortality rates of colorectal cancer (per 100,000) in the UK by sex from 1982 to 2005 (Cancer Research UK)

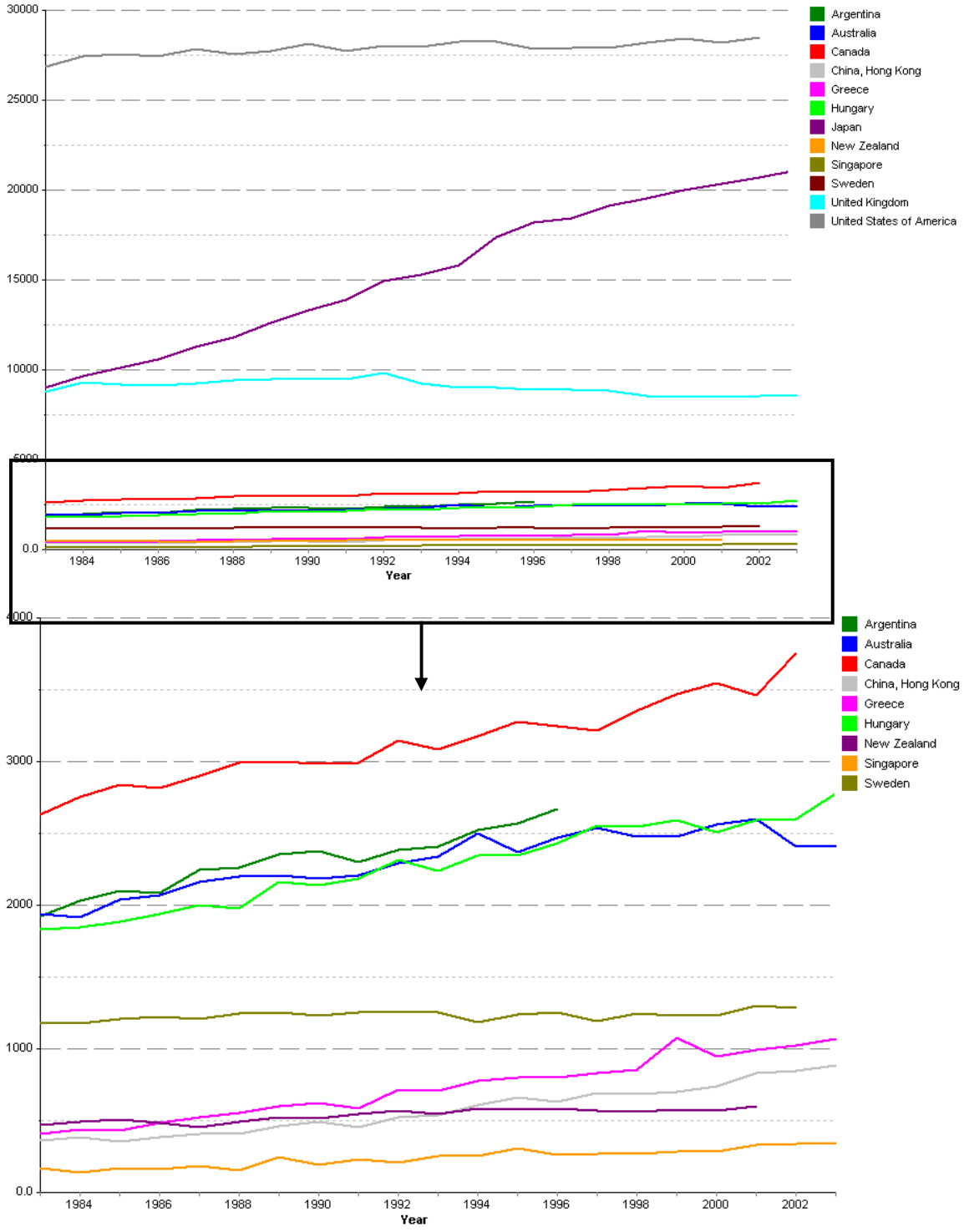


Figure 19 Number of deaths from colorectal cancer for men in selected countries from 1983 to 2003 (International Agency for research in cancer)

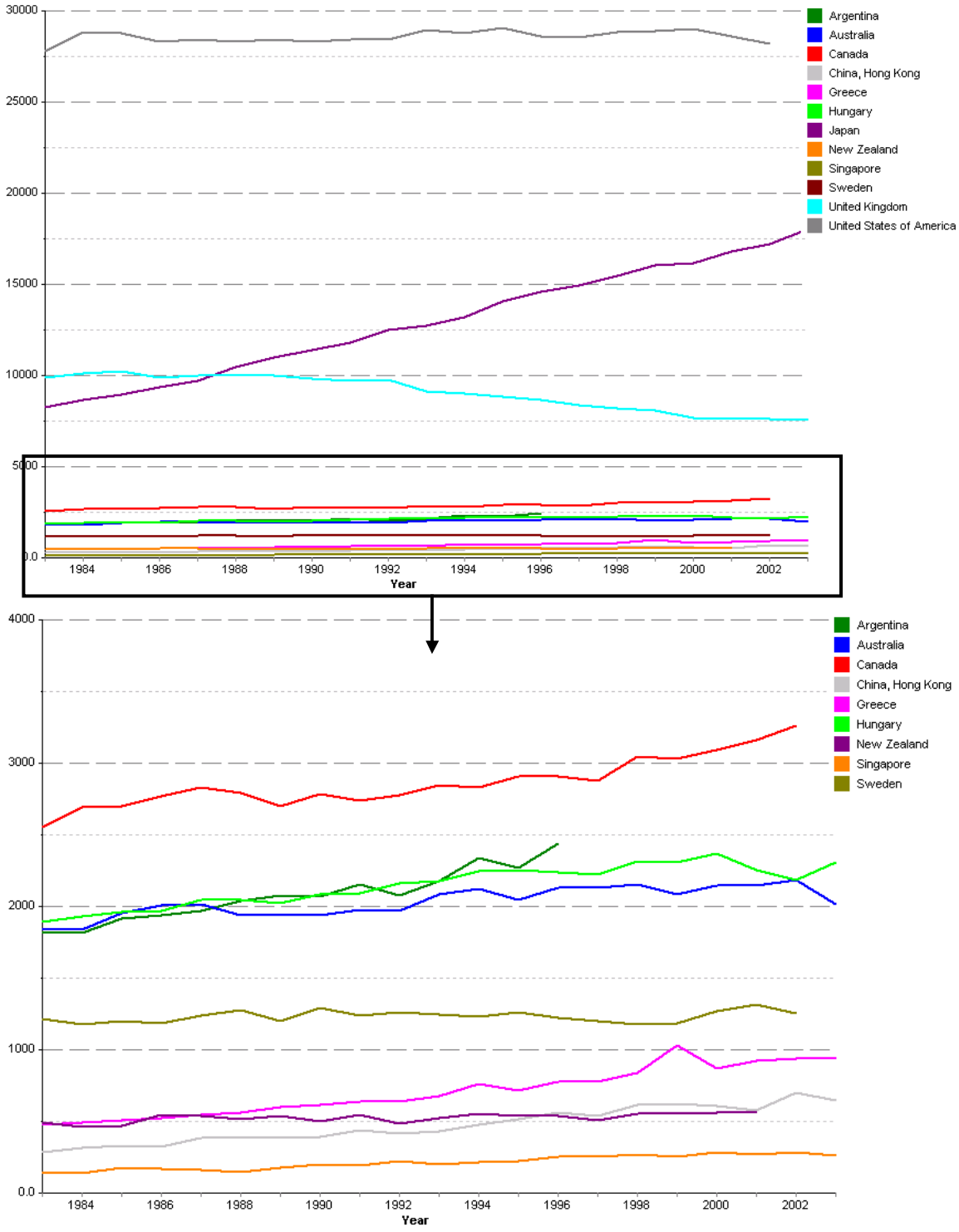


Figure 20 Number of deaths from colorectal cancer for women in selected countries from 1983 to 2003 (International Agency for research in cancer)

1.4.4 Survival rates: Geographical and temporal trends

Survival rates for colorectal cancer have been significantly improved the last 25 years both for Scotland and the UK, a pattern that has been observed for many cancers. In particular, 1-year and 5-year relative survival rates in Scotland were 75.8% and 54.9% for men and 74.1% and 55.1% for women (time period 2000-2004; Scottish Cancer Registry, ISD). One year relative survival rates in Scotland have increased by 30% for men and by 26% for women and 5-year relative survival rates have been increased by 50% for men and 51% for women (relative increases, time period 1980-2004; Figure 21). In addition median survival after diagnosis has been increased from 1.9 years in 1980-1984 to 4.1 years in 1995-1999. Survival rates and survival rate changes were similar for England, Wales and the N. Ireland. In particular, 1- and 5-year relative survival rates in 2000-2001 were 74% and 52% for men and 73% and 53% for women (Cancer Research UK, N. Ireland Cancer Registry; Figure 22). Survival has been considered to depend highly on stage at diagnosis, with more advanced cancers having poorer prognosis. In particular, approximate 5-year survival rates for the UK have been estimated to be 83% for Dukes' stage A, 64% for stage B, 38% for stage C and 3% for stage D (Cancer Research UK).

According to the EUROCARE study, the mean age-adjusted 5-year survival rate for colorectal cancer in Europe was 53.8% in the time period 1995-1999, a rate which is significantly higher than the survival rates that were observed in previous time periods. In particular the relative difference in 5-year survival rates in Europe between the time periods 1990-1994 and 1995-1999 was 8.5% (32). The European 5-year survival rates were higher than those observed in Great Britain in the same time-period (32). The variation of colorectal cancer survival with geography was similar to other common cancers (including lung, breast, and prostate). In particular, the highest 5-year survival rates were observed in Nordic countries (except Denmark) and central Europe, intermediate in southern Europe, low in the UK and Ireland, and the lowest in Eastern Europe (32). It has been suggested that these differences within Europe are mainly due to the stage at diagnosis as well as due to less effective treatments (Cancer Research UK, (33;34)). However, the between-countries and inter-regional differences in colorectal

cancer survival rates that were observed in 1995-1999 have been narrowed significantly compared to previous years (33;35;36).

Colorectal cancer survival rates in other parts of the world show a similar pattern of increase. In particular, 5-year relative survival rates in USA in 1996-2004 were 65.4% for men and 65.2% for women, showing an 8% and a 6% relative increase when compared to survival rates in 1993-1995 (Surveillance Epidemiology and End Results, National Cancer Institute). In Australia, 5-year survival rates in 1998-2004 were 61.3% for men and 62.4% for women, showing an 8% and 9% relative increase when compared to survival rates in 1992-1997 (Australia's Health 2008, Australian Institute of Health and Welfare). Finally, 5-year survival rate for colorectal cancer in Japan in 1993-1996 was 64.6% for both sexes (National Cancer Centre, Japan).

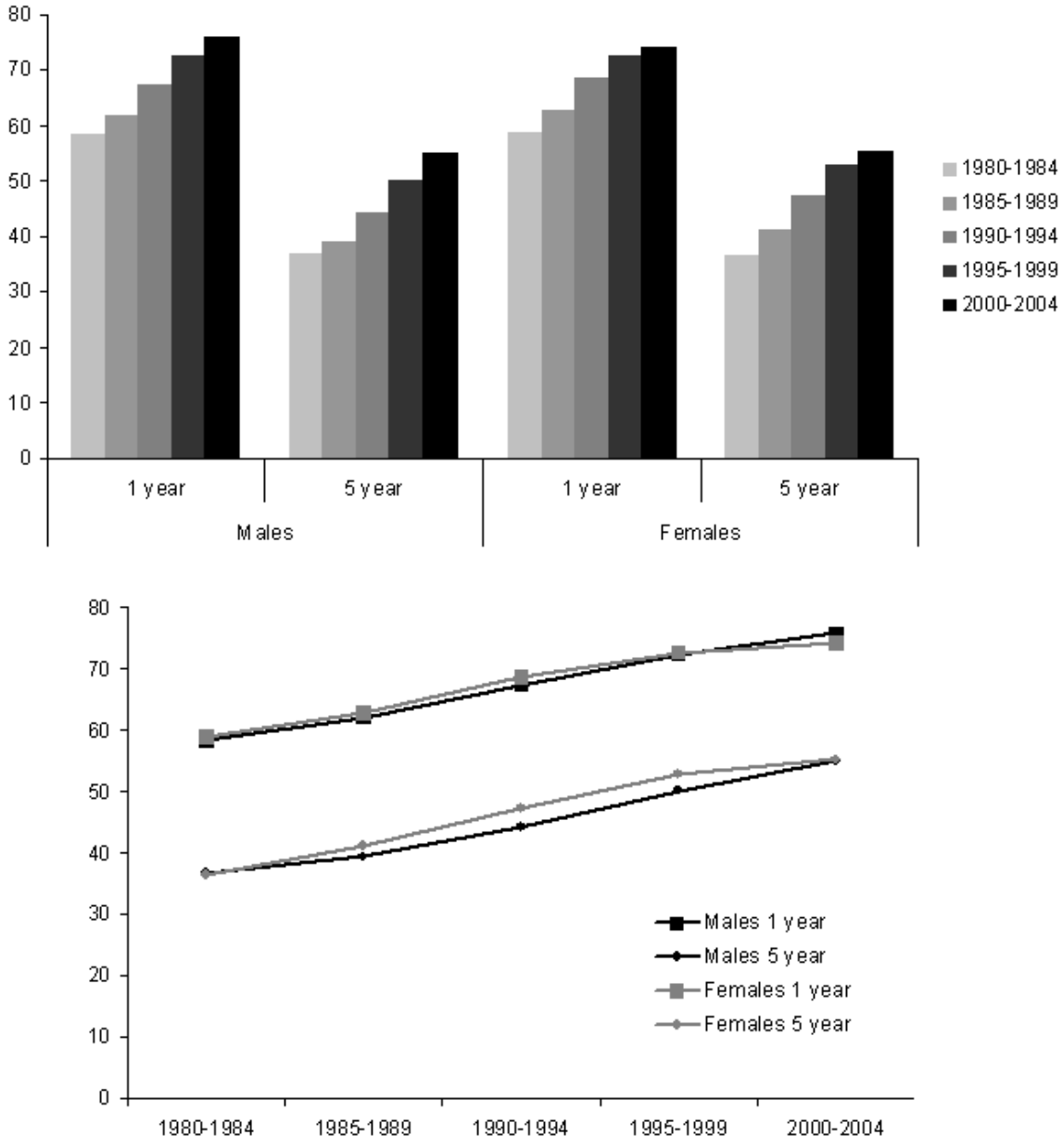


Figure 21 Age standardised one-year and five-year relative survival rates (European Cancer Patient Population - EUROCARE-4) for patients diagnosed in Scotland, 1980-2004 (Cancer Registry Scotland, ISD). (Note: 5-year survival rates for time period 2000-2004 are based on estimates).

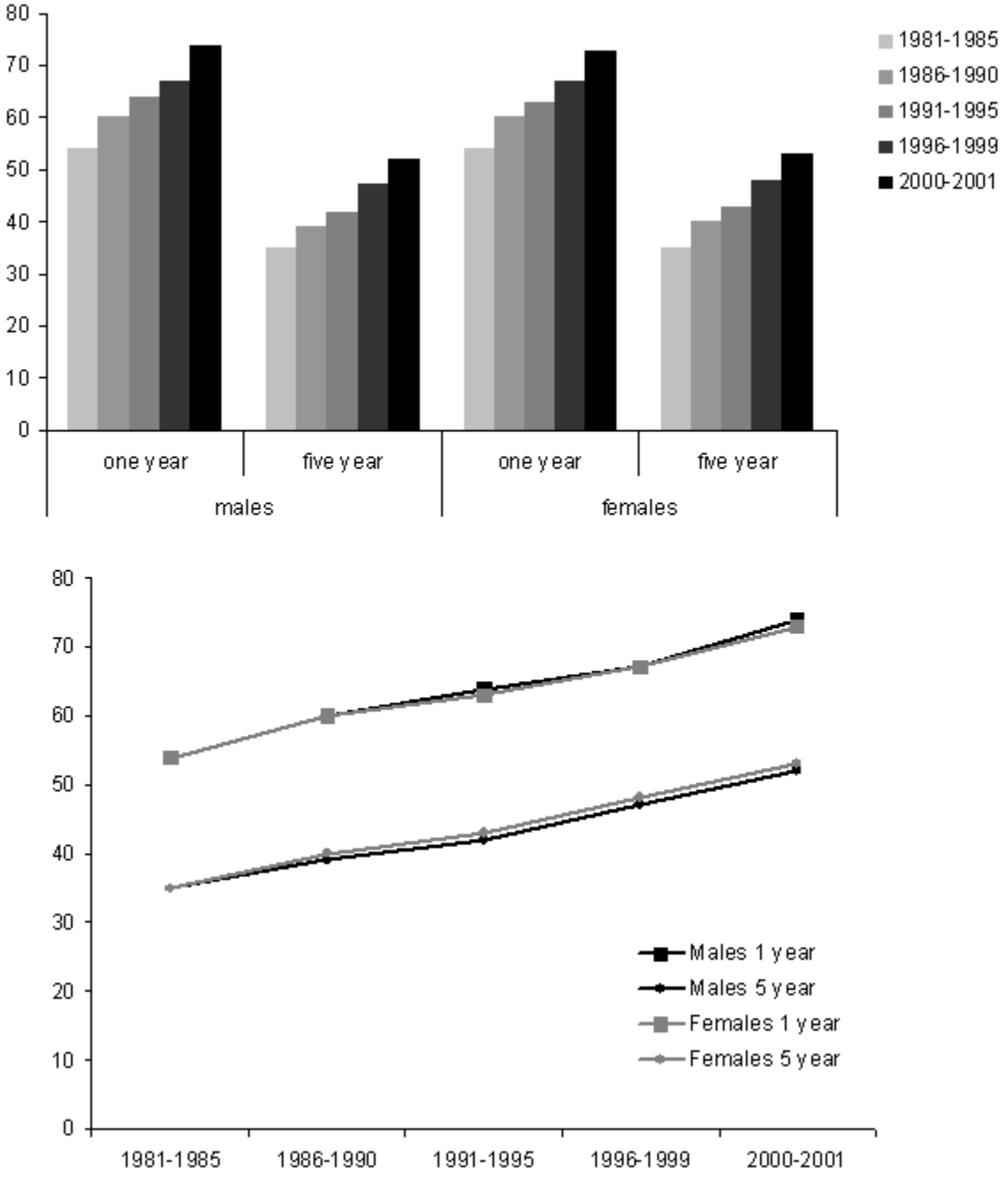


Figure 22 Age standardised one-year and five-year relative survival rates for patients diagnosed in England, Wales and N. Ireland, 1981-2001 (Cancer Research UK). (Note: 1- and 5-year survival rates for time period 2000-2001 are based on estimates).

1.4.5 Colorectal cancer projections for Scotland

In 2004 the updated cancer incidence projections for Scotland (2001-2020) were released from Scottish government. It is estimated that over 168,000 adult individuals will be diagnosed with cancer during 2016-2020 (approximately 33,700 new cases per year), which represents a 28% increase in the number of cancer cases (comparing number of cases in 2001 with number of cases in 2020). An increase in the number of cases is predicted for several types of cancer (including colorectal) with notable exceptions being stomach, lung and cervical cancers, which are predicted to decline. Most of the estimated increase is predicted to be due to the growing number of elderly people in the Scottish population, but for some types of cancer risk is thought to increase independently of the the high number of elderly people (The Scottish Government, Statistics).

For colorectal cancer, during 2016-2020 24,643 cases are predicted to be diagnosed (42.4% more than the number of colorectal cancer cases diagnosed in 1996-2000). This number comprises 12,472 individuals younger than 75 years old (50.6%) and 12,171 individuals older than 75 years old (49.4%) (The Scottish Government, Statistics). Incidence projections of colorectal cancer for the years 2001-2005, 2006-2010, 2011-2015 and 2016-2020 are presented in Figure 23 for the whole population and separately for individuals younger and older than 75 years old.

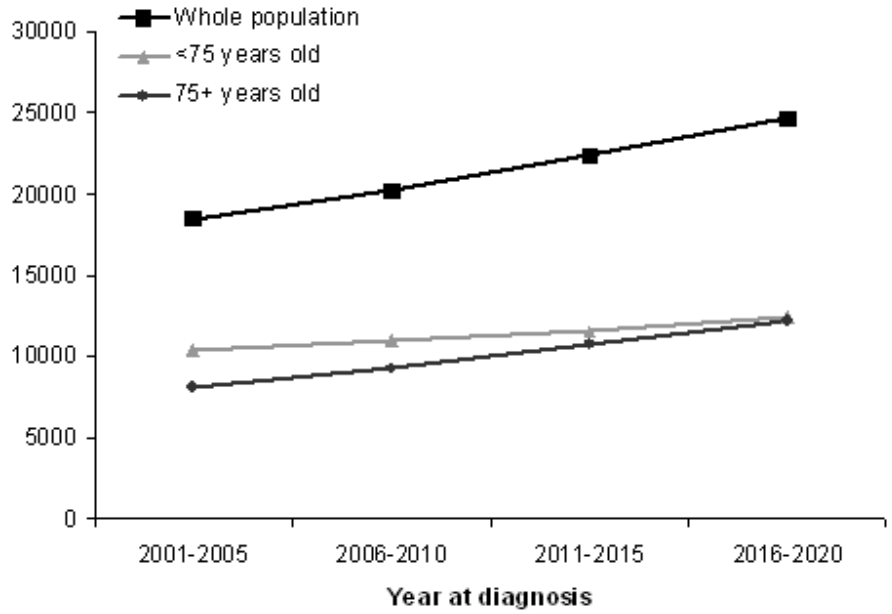


Figure 23 Colorectal cancer incidence projections for Scotland (2001-2020) for the whole population and after age stratification (<75, 75+ years old). (The Scottish Government Statistics)

1.5 Main risk factors

1.5.1 Introduction

Many factors have been found to be positively or inversely associated with colorectal cancer. Age, personal history of previous colorectal cancer or adenomatous polyps, family history of colorectal cancer, chronic bowel inflammatory disease, and presence of either HNPCC or FAP are considered as established risk factors of colorectal cancer. According to the American Cancer Society, individuals that: 1) have a personal history of colorectal cancer, 2) have a personal history of adenomatous polyps, or 3) have a family history of colorectal cancer, are at increased risk of developing colorectal cancer. Individuals that: 1) have a history of inflammatory bowel disease (including ulcerative colitis and Crohn's disease) of significant duration or 2) have one of the two hereditary syndromes (HNPCC or FAP), are at high risk of developing colorectal cancer. For individuals at increased and high colorectal cancer risk screening and surveillance techniques should be provided to decrease incidence and mortality rates (37). Finally, it has been suggested that colorectal cancer risk rises significantly from the age of 50 and therefore in many countries screening programmes for those older than 50 years old have been recommended. In Scotland in particular, individuals aged from 50 to 74 years old are invited every two years for bowel screening.

Evidence for other risk factors, including diet, body weight, physical activity, smoking, alcohol intake, non steroidal anti-inflammatory drugs (NSAIDs) intake and hormone replacement therapy (HRT) in post-menopausal women will be described in this chapter.

1.5.2 Age

Colorectal cancer risk increases with age and it is more likely to occur in individuals older than 50 years old (National Cancer Institute). In Scotland in 2005, 95% of colorectal cancer cases were older than 50 years old (95.3% for men and 94.5% for women) and the distribution of patients and incidence rates according to age separately for men and women are presented in Figure 24 (Cancer Registry Scotland, 2005). The distribution of colorectal cancer cases according to age in the UK (Cancer Research UK, 2004) and selected countries of the world (IARC, 2002) is similar to the Scottish distribution (Figure 25, Figure 26, Figure 27).

In addition, age affects survival rates with older patients having poorer prognosis. This might be due to various reasons. For example they may seek medical advice at a later

stage of the disease or due to advanced age they may not be able to receive the appropriate treatment or they may have poorer surgical prognosis (33). Age specific colorectal cancer 1-year and 5-year survival rates for Scotland, England and Wales are presented in Figure 28 and Figure 29.

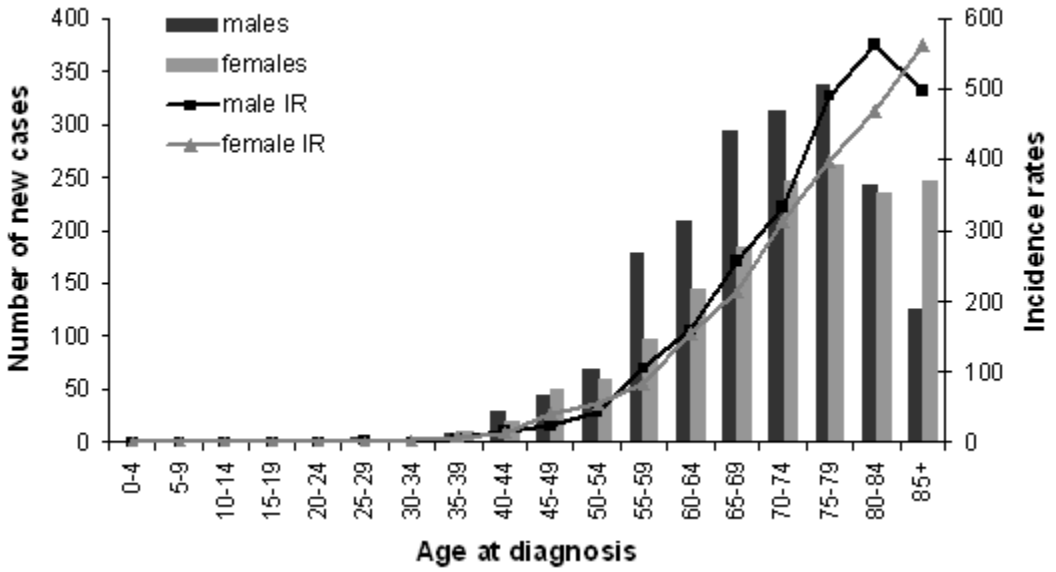


Figure 24 Numbers of new cases and age-specific incidence rates by sex for colorectal cancer in Scotland (2005, Cancer Registry Scotland, ISD)

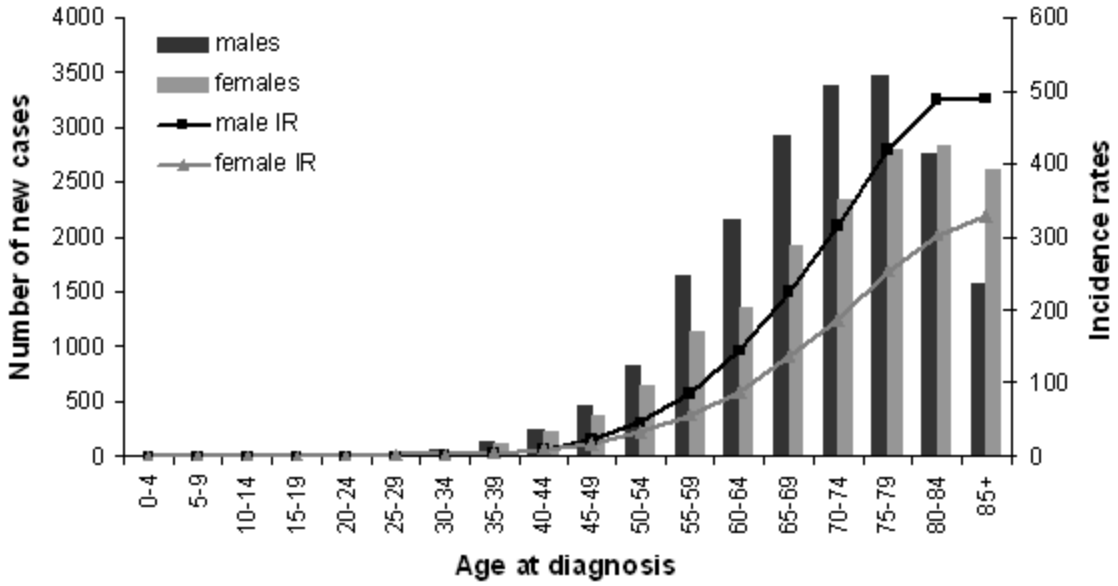


Figure 25 Numbers of new cases and age-specific incidence rates by sex for colorectal cancer in the UK (2004, Cancer Research UK)

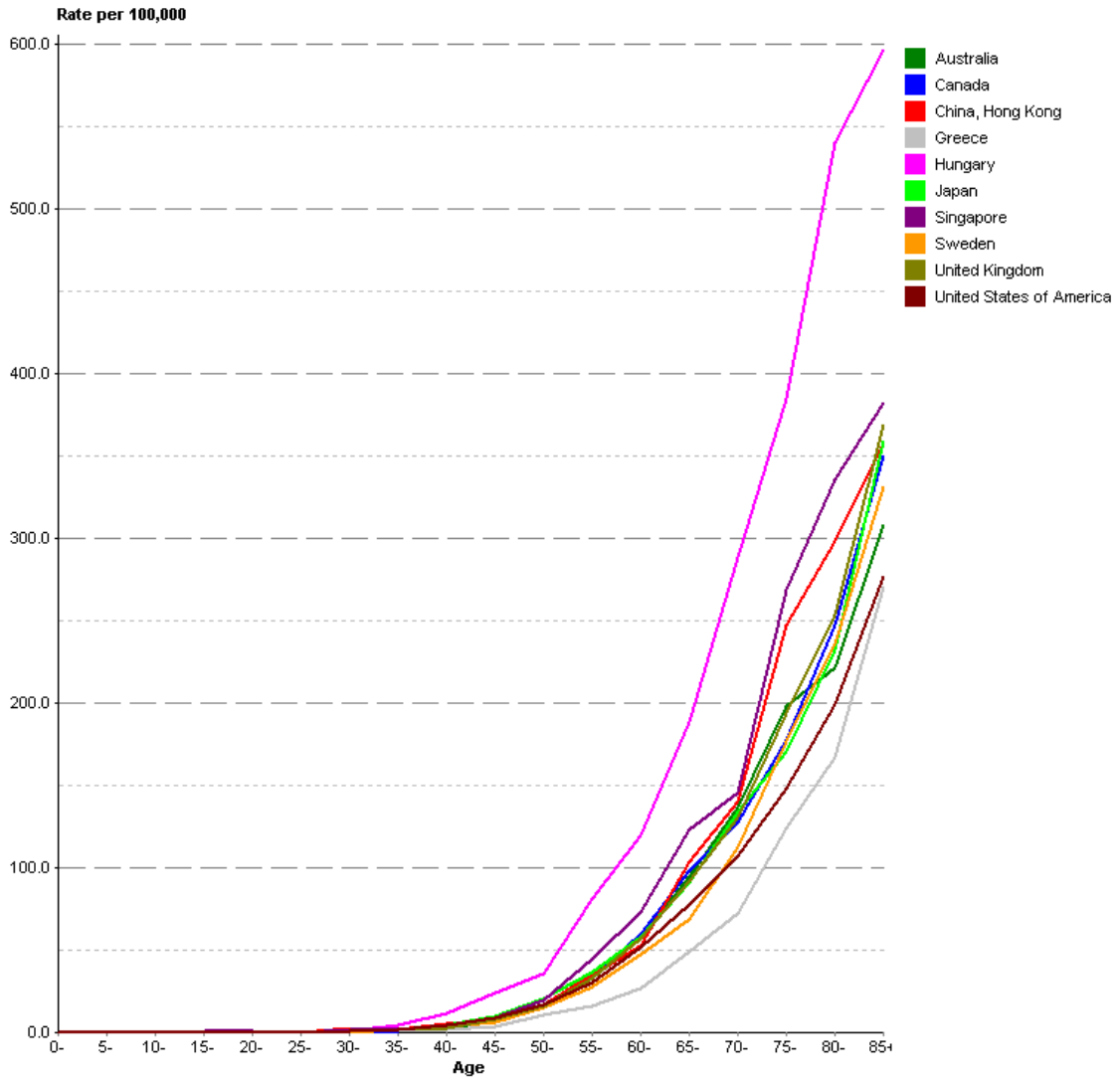


Figure 26 Numbers of new cases and age-specific incidence rates for colorectal cancer in men in selected countries (2002, International Agency for research in cancer)

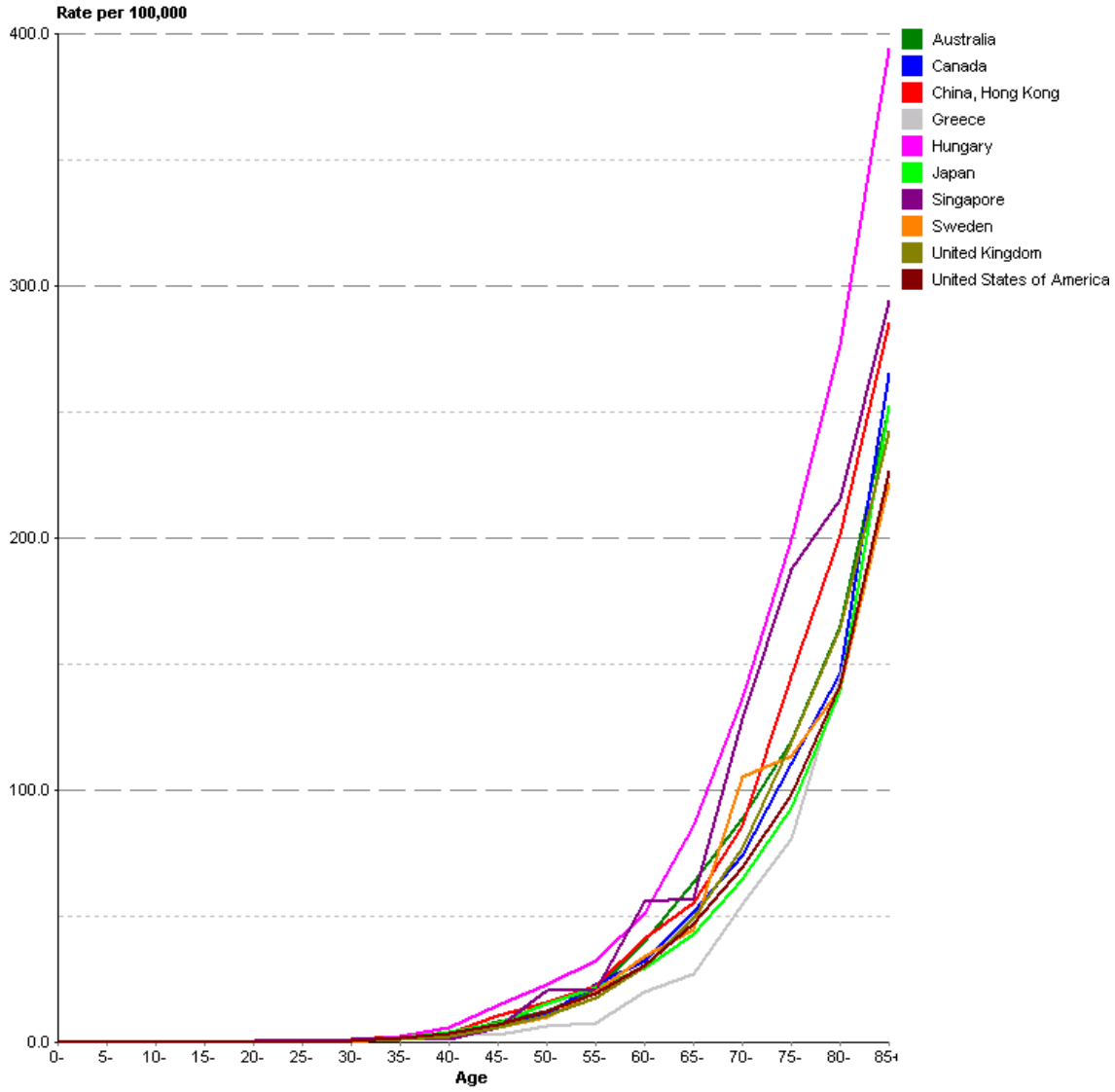


Figure 27 Numbers of new cases and age-specific incidence rates for colorectal cancer in women in selected countries (2002, International Agency for research in cancer)

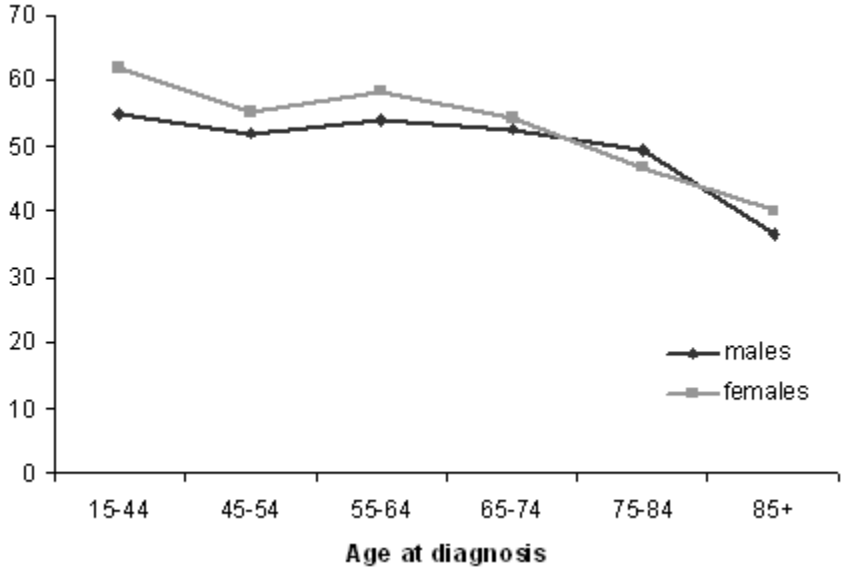


Figure 28 Age specific 5-year relative survival (%) in Scotland for 1995-1999 (Cancer Registry Scotland, ISD)

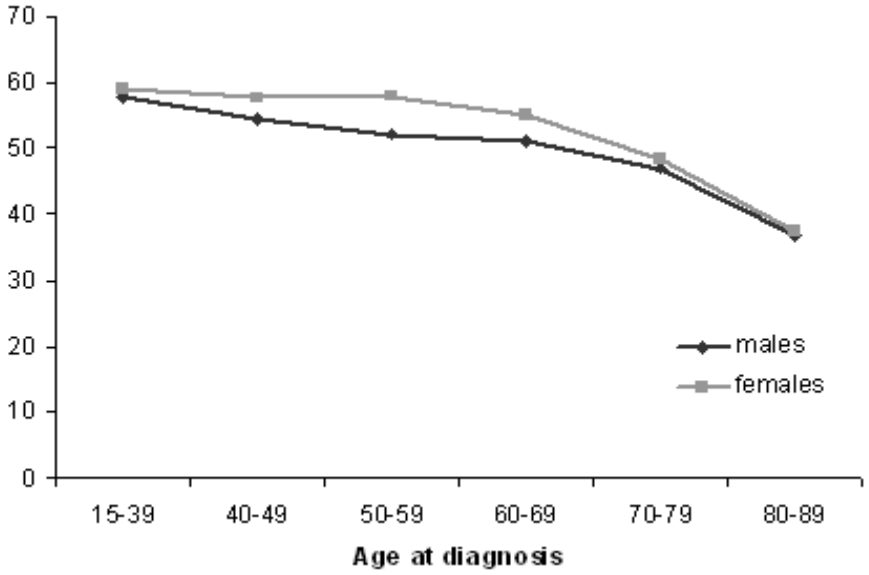


Figure 29 Age specific 5-year relative survival (%) in England and Wales for 1996-1999 (Cancer Research, UK)

1.5.3 Previous colorectal cancer or adenomatous polyps

Patients with previous colorectal cancer are at risk of developing recurrent or metachronous cancers and therefore long-term colonoscopic surveillance is necessary (38). However, the frequency and epidemiological characteristics of metachronous (a new primary cancer in a person with a history of cancer) colorectal cancers is still unknown. According to the findings of a recent population-based study in France, the cumulative risk of metachronous cancers was 2% among 5-year survivors and 7% among 20-year survivors (39). In addition, a two to three fold increased incidence risk of colorectal cancer was observed in four other population-based studies (Connecticut, Utah, Sweden and Finland) (40-43).

Adenomatous polyps are neoplastic benign epithelial tumours and most adenocarcinomas of the colon and rectum arise from pre-existing adenomatous polyps via the adenoma–carcinoma sequence (44). Numerous studies have demonstrated that these patients have a higher risk of recurrent adenomas and/ or of developing colorectal cancer than the general population (45). Both the risk of adenomas recurrence and colorectal cancer is associated with the size and number of the initially detected adenomas (46). Approximately between 15 to 60% of polypectomy patients develop a recurrence and the risk of colorectal cancer for these patients has been estimated to be at least twice the risk of the general population (46). Regarding the other types of adenomas, serrated adenomas are considered as lesions of non-neoplastic characteristics and with no or low malignant potential. (26). In contrast, the colorectal cancer risk of flat adenomas is considerably higher than for adenomatous or serrated polyps (27).

1.5.4 Family history of colorectal cancer

According to the Scottish Executive cancer guidelines (http://www.sehd.scot.nhs.uk/), the criteria for high family history risk of colorectal cancer are: 1) at least three family members affected by colorectal cancer or at least two with colorectal cancer and one with endometrial cancer in at least two generations; one affected relative must be ≤50 years old at diagnosis and one of the relatives must be a first degree relative of the other two; or 2) presence of the HNPCC syndrome; or 3) untested first degree relatives of

known gene carriers. The criteria for moderate risk are: 1) one first degree relative affected by colorectal cancer when aged <45 years old; or 2) two affected first degree relatives with one aged <55 years old; or 3) three affected relatives with colorectal or endometrial cancer, who are first degree relatives of each other and one a first degree relative of the consultant. Individuals that do not fulfil all the above criteria are classified as low family history risk (Scottish Executive cancer guidelines). According to a meta-analysis, which was published in 2006 and included 59 studies (published from 1958 to 2004), the pooled colorectal cancer relative risk estimate when at least one first degree relative was affected was 2.24 (95% CI 2.06, 2.43) and it rose to 3.97 (95% CI 2.60, 6.06) when there were at least two affected relatives. In addition, the absolute risk by age of 70 for a 50-year old individual was 3.4% (95% CI 2.8 to 4.0) with at least one affected relative or 6.9% (95% CI 4.5 to 10.4) with two or more, which is considerable higher than the 1.8% population lifetime risk for a 50-year old (47).

1.5.5 Inflammatory bowel disease

Inflammatory bowel disease is a group of idiopathic (of unknown cause) inflammatory conditions of the large intestine and it comprises ulcerative colitis and Crohn's disease. Ulcerative colitis mainly affects the large intestine and it mainly occurs with inflammation of the mucosa. In contrast, Crohn's disease can develop in any part of the gastrointestinal tract but most commonly affects the distal part of the small intestine and parts of the large intestine. In addition, inflammation in Crohn's disease extends much deeper into the layers of the intestinal wall than in ulcerative colitis (48).

According to a meta-analysis of 116 studies, the overall prevalence of colorectal cancer in patients with ulcerative colitis is 3.7%. In addition, an estimation of the cumulative colorectal cancer risk according to the duration of ulcerative colitis was calculated to be 2% at 10 years, 8% at 20 years, and 18% at 30 years (49). The evidence for the link between Crohn's disease and colorectal cancer is less clear than for ulcerative colitis. According to a meta-analysis conducted in 2007, patients with Crohn's disease were found to have a 2.4-fold increase in risk of colorectal cancer, which was however associated with significant heterogeneity. After cancer site stratification, the risk of

colon cancer was found to increase by a factor of 2.59 (no significant heterogeneity) but rectal cancer risk was not significantly associated with Crohn's disease (50).

When considering geographic variations the risk of colorectal cancer was found to be significantly higher in North America and the United Kingdom compared with Scandinavian and other countries for both patients with ulcerative colitis and Crohn's disease (49;50). Compared with sporadic colorectal cancer, colorectal cancer arising in patients with inflammatory bowel disease affects individuals at a younger age, progresses to invasive adenocarcinoma from flat and non-polypoid dysplasia more frequently and exhibits a mucinous and signet ring cell histology in a higher proportion of cases (48).

1.5.6 Diet

According to the second report of Food, Nutrition, Physical Activity and the Prevention of Cancer of the American Institute for Cancer Research / World Cancer Research Fund (AICR/WCRF), which was released in November of 2007 diet has a very important role in the prevention and causation of colorectal cancer (30). It has also been thought that the role of diet in colorectal carcinogenesis is particularly important when a poor diet is combined with a generally unhealthy lifestyle, consisting of excess calorie intake and weight gain, physical inactivity and high consumption of alcohol (51). The roles of several foods and nutrients in colorectal carcinogenesis have been investigated in many observational studies; however the evidence regarding the effect of particular dietary factors is still generally inconsistent. The foods and nutrients, on which there is most published data, are red and processed meat, dietary fibre, fruit and vegetables, folate, vitamin D and calcium.

In this chapter, findings regarding red and processed meat, dietary fibre and fruit and vegetables will be summarised. Evidence regarding specific nutrients, which associations with colorectal cancer were investigated in this thesis will be presented in chapter 3. Thus a detailed literature search will be presented for the following nutrients: a) flavonoids, b) fatty acids, c) folate, vitamin B2, vitamin B6 and vitamin B12 and d) vitamin D and calcium (see chapter 3 on page 66).

1.5.6.1 Red and processed meat

Evidence regarding the positive association between colorectal cancer and intake of red and processed meat is quite consistent. In the second report of AICR/WCRF, a systematic review and meta-analysis of observational analytical studies of risks associated with intake of red meat and processed meat showed a positive association with colorectal cancer (30). Regarding red meat, 16 cohort and 71 case-control studies were included with nearly all of them showing a positive association with colorectal cancer. A meta-analysis of the cohort data showed that every 50g/day increase of red meat intake was associated with a 15% increase in colorectal cancer risk. Fourteen cohort and 44 case-control studies investigating the association with processed meat were included in this report and meta-analysis of the cohort studies showed a positive association with colorectal cancer risk (30). Another recent meta-analysis of prospective studies of meat and colorectal cancer reported a significantly elevated summary relative risks for both red meat (RR (95% CI): 1.28 (1.15, 1.42)) and processed meat (1.20 (1.11, 1.31)) in the highest versus lowest category of intake (52). Finally, results from a recent large prospective study (NIH-AARP Diet and Health Study, USA), which included over 5,000 colorectal cancer cases reported a statistically significant positive association between colorectal cancer risk and intakes of both red (HR (95% CI): 1.24 (1.12, 1.36)) and processed meat (1.20 (1.09, 1.32)) (53).

1.5.6.2 Dietary fibre

The first observation that high fibre intake may decrease colorectal cancer risk was published in 1969 (54). Since then many studies (case-control studies, cohort studies and meta-analyses) have been published, but the relationship of dietary fibre intake with the development of colorectal cancer is still not completely understood. In the second AICR/WCRF report, 16 cohort and 91 case-control studies were investigated and meta-analysis of the cohort studies showed a 10% decreased risk per 10g/day of fibre intake (30). However, a pooled analysis of 8,100 colorectal cancer cases, followed up for 6–20 years, showed a statistically non-significant decreased risk for the groups that consumed the most dietary fibre (55). Recent results from the Multiethnic Cohort study in Hawaii

and Los Angeles (2,110 cases) showed that fibre was inversely associated with colorectal cancer for both men and women (age and ethnicity adjusted model: RR (95% CI): men: 0.49 (0.41, 0.60); women: 0.75 (0.61, 0.92)). However after further adjustment (family history of colorectal cancer, history of colorectal polyp, pack-years of cigarette smoking, BMI, hours of vigorous activity, aspirin use, multivitamin use, HRT, alcohol, red meat, folate and vitamin D) this inverse association remained statistically significant only in men (RR (95% CI): 0.62 (0.48, 0.79)) (56). In the Pooling Project of Prospective Studies of Diet and Cancer (8,081 colorectal cancer cases; 13 cohort studies) a statistically significant inverse association was found in an age-adjusted model (RR (95% CI): 0.84 (0.77–0.92)), but the association was diluted after further adjustment (RR (95% CI): 0.94 (0.86, 1.03); adjusted for: age, body mass index, height, education, family history of colorectal cancer, use of postmenopausal hormone therapy, oral contraceptive use, use of NSAIDs, multivitamin use, smoking, and intake of dietary energy, dietary folate, red meat, total milk and alcohol) (55). Further analyses of the Pooling Project though showed a statistically significant increased risk for colorectal cancer among participants with a very low intake of fibre (dietary fibre intake of <10 g/day versus intake of ≥30 g/day, RR (95% CI): 1.18 (1.05–1.31)). Finally, results from the European Prospective Investigation into Cancer and Nutrition (EPIC; 1,721 cases, nine European countries) showed a statistically significant lower risk for colorectal cancer associated with high-fibre intake (model adjusted for age, sex, energy from fat and non-fat sources, height, weight, folate, physical activity, alcohol, smoking, educational level, and intake of red and processed meat; RR (95% CI): 0.79 (0.63–0.99)) (57).

1.5.6.3 Fruit and vegetables

According to the results of the first AICR/WCRF published in 1997, evidence that vegetables protect against colorectal cancer was judged as convincing (58). However, analysis of more recent cohort and case-control studies challenged this hypothesis of reduced risk and the conclusion of the second AICR/WCRF report (2007) suggested that there is limited evidence of a protective colorectal cancer effect of both fruit and vegetables (30). In particular, 17 cohort and 71 case-control studies investigating the

effect of non-starchy vegetables were included in the second AICR/WCRF report (2007) and meta-analysis of the cohort data produced no evidence of an inverse association. Similarly, analysis of 20 cohort and 57 case-control studies, which have investigated the effect of fruit intakes showed no clear evidence of an overall association (30). However, a comparison of the groups of the highest vegetable intakes against those with the lowest suggested a possible inverse association. In addition, fruit intake in women was inversely associated with colorectal cancer risk (30). A review of nine case-control studies conducted from the International Agency for Research on Cancer in 2003 (59) summarised that colorectal cancer risk was lowered by 13% (odds ratio (OR) (95% CI): 0.87, (0.78, 0.97)) and 37% (OR (95% CI): 0.63 (0.56, 0.70)) when the highest versus the lowest category of respectively fruit and vegetable intakes were compared. However, a review of 11 prospective cohort studies published in the same report (59) found that fruit and vegetable intakes were not related to risk of colorectal cancer. Finally, the Women's Health Initiative Randomised Controlled (WHI) Dietary Modification Trial concluded that daily intake of at least five servings of fruits and vegetables along with a low-fat diet over an approximately 8 year period, did not lower risk of colorectal cancer in postmenopausal women (60).

1.5.7 Dietary energy intake

Specific biological functions of the body need the intake of energy to be performed. These include body's functions and processes at rest (basal metabolic rate - BMR), digestion and assimilation of food and physical activities (30). Energy requirements of the individuals depend on their sex, age, size and physical exercise levels (30). Positive energy balance, which leads to weight gain, occurs when an individual consumes more energy than the energy that is expended by his or her biological functions. On the other hand, negative energy balance, which leads to weight loss, occurs when an individual consumes less energy than the energy that is expended by his or her biological functions (30). A number of observational studies have investigated the effect of high dietary energy intake on colorectal cancer (61). In particular, findings from case-controls studies suggest that there is a positive and dose-dependent association between dietary energy intake and colorectal cancer risk, whereas findings from prospective studies, do not

support strong inverse associations, suggesting that the case-control findings might be biased (62-69). A recent case-control investigated the joint effect of energy intake, body mass index (BMI) and physical activity and suggested that dietary energy intakes are inversely associated with colorectal cancer only among the individuals of low physical activity, a finding that might explain the inconsistent results (70).

1.5.8 Obesity

Results from observational studies have concluded that obesity is an important risk factor in several cancers, including colorectal cancer (71). In the second AICR/WCRF (2007), analysis of 68 cohort and of 86 case-control studies that investigated the effect of body fatness measured by BMI (kg/m^2), showed a strong positive association (meta-analysis of cohort data: 15% increase in risk per 5 kg/m^2 increase in BMI). In addition, analysis of 13 cohort and six case-control studies investigating abdominal fatness measured by either waist circumference or waist to hip ratio also showed a strong positive association (30). Therefore, the panel of the second AICR/WCRF report (2007) concluded that the evidence that obesity (both body and abdominal) is causally linked with colorectal cancer is convincing (30). In addition, results from the EPIC study, published in 2006 showed that the highest quintile of waist circumference was associated with a RR for colon cancer of 1.4 (95% CI 1.0, 1.9) in men and 1.5 (95% CI 1.1, 2.0) in women (72). One recent meta-analysis, which was published in 2007 and included 30 prospective studies (1966-2007) concluded that overall, a 5 kg/m^2 increase in BMI was related to an increased risk of colon cancer in both men (RR (95% CI): 1.30 (1.25, 1.35)) and women (RR (95% CI): 1.12 (1.07, 1.18)). BMI was positively associated with rectal cancer in men (RR (95% CI): 1.12 (1.09, 1.16)) but not in women (RR (95% CI): 1.03 (0.99, 1.08)). Regarding abdominal fatness, colon cancer risk increased with increasing waist circumference (per 10 cm increase) in both men and women (RR (95% CI): 1.33 (1.19, 1.49); 1.16 (1.09, 1.23), respectively) (73). Results from a second meta-analysis also published in 2007 (31 studies: 23 cohort, 8 case-control) indicated that the RR of colorectal cancer for the obese (BMI$\geq$ 30 kg/m^2) versus the normal weight individuals (<25 kg/m^2) was 1.19 (1.11, 1.29) and the RR comparing those with the highest, to the lowest, level of central obesity was 1.45 (1.31, 1.61) (74).

Finally, a third meta-analysis published in 2008, reported a strong association between BMI and colon cancer (per 5 kg/m^2 increase in BMI: RR (95% CI), p-value: 1.24 (1.20, 1.28), <0.0001) and a weak association between BMI and rectal cancer in men (per 5 kg/m^2 increase in BMI: RR (95% CI), p-value: 1.09 (1.06, 1.12), <0.0001). In addition, it reported a weak association between colon cancer and a 5 kg/m^2 increase in BMI in women (RR (95% CI): 1.09 (1.05, 1.13), <0.0001) (75).

1.5.9 Physical activity

Results from both cohort and case-control studies have consistently indicated that increased physical activity is inversely associated with male colon cancer risk, reporting risk reductions of about 40% with high versus low levels of physical activity (76). In addition, results of observational studies regarding the relationship between physical activity and female colon cancer have reported less strong but similar associations (76). However, results for the association between physical activity and rectal cancer are much less consistent, with only a small proportion of the published studies showing a statistically significant inverse association (77). The second AICR/WCRF report (2007), after reviewing evidence from 11 cohort studies of total physical activity, 12 cohort studies of occupational physical activity and 24 cohort studies of recreational physical activity, concluded that there is enough evidence that high levels, greater frequency and greater intensity of physical activity lowers colon cancer risk, but there is not enough evidence regarding rectal cancer risk (30). In addition, a meta-analyses of 19 cohort studies published in 2005 reported that increased physical activity was linked to a statistically significant reduction in the risk of male colon cancer (RR (95% CI): occupational physical activity 0.79 (0.72, 0.87); recreational physical activity 0.78 (0.68, 0.91). For women though, only recreational physical activity was protective against colon cancer (RR (95% CI): 0.71 (0.57-0.88). In addition, no protection against rectal cancer was observed in either sex (78). Finally, results from the EPIC study including 1,094 colon and 599 rectal cases, showed an inverse association between physical activity and colon cancer (RR (95% CI): 078 (0.59, 1.03)), but no association with rectal cancer (77).

1.5.10 Alcohol

In a recent monograph by WHO International Agency for Research on Cancer (IARC) it was stated that colorectal cancer is causally related to alcohol consumption (79), a conclusion that is in accordance with the conclusion from the second AICR/WCRF report (2007). In particular, in the second AICR/WCRF (2007) report meta-analysis from 24 cohort studies investigating consumption of alcoholic drinks and from 13 cohort studies investigating ethanol intakes showed that intake of more than 30g per day of ethanol is causally linked with male colorectal cancer and probably linked with female colorectal cancer (30). Results from the EPIC study suggested that both lifetime and baseline alcohol intake were significantly associated with colorectal cancer incidence for alcohol intakes of 30-59.9 g/day compared to 0.1-4.9 g/day (23% and 26% increase in risk, respectively) (80). In addition, a recent meta-analysis, which included 16 prospective cohort studies of colorectal cancer reported that high alcohol intake was significantly associated with increased risk of both colon and rectal cancer (highest versus lowest category of alcohol intake RR (95% CI): 1.50 (1.25, 1.79); 1.63 (1.35, 1.97); respectively) (81). Finally, another recent pooled meta-analysis of eight cohort studies found an increased risk of colorectal cancer with alcohol consumption but this positive association was again limited to consumption of more than 30 g/day (RR (95% CI): 0 g/day vs. 30-45 g/day 1.16 (0.99, 1.36); 0 g/day vs. ≥45 g/day 1.41 (1.16 to 1.72)) (82).

Regarding the associations between colorectal cancer and specific types of alcohol (i.e. wine, beer, spirits), findings from observational studies are mainly inconsistent (83). The main concept is that different alcoholic beverages contain many other different substances apart from alcohol, which might have different effects on colorectal cancer. One example is the hypothesis that beer might increase rectal cancer risk due to its high content in volatile nitrosamines (83). However, results from various large studies, including results from a meta-analysis published in 1990 (84), from the Pooling Project (82), from the EPIC study (80) and from the Netherlands Cohort Study (83), did not provide strong evidence for a different colorectal cancer risk (overall or site specific) according to the type of alcohol.

1.5.11 Smoking

Cigarette smoking has been consistently linked with risk of colorectal adenomatous polyps. A recent meta-analysis combining findings from 42 case-control and nested case-control studies (15,354 cases and 100,011 controls) reported pooled colorectal adenoma ORs of 2.14 (1.86, 2.46) for current vs. never smokers, of 1.47 (1.29, 1.67) for former vs. never smokers and of 1.82 (1.65, 2.00) for ever versus never smokers (85). In the same meta-analysis the authors found that smoking was also more strongly associated with high risk adenomas than with low risk adenomas and therefore they concluded that smoking is an important risk factor for both the formation and aggressiveness of adenomatous polyps (85). In addition, a systematic review conducted in 2001, after reviewing 22 studies on the association between colorectal adenomas and smoking, reported that long-term heavy smokers have a 2 to 3 fold increased risk to develop colorectal adenomas (86). However, evidence of a causal link between smoking and colorectal cancer is still debatable, and it was not considered as an established risk factor for colorectal cancer by the IARC (85). Early studies (before the 1970s) reported no associations whereas more recent studies reported positive associations between cigarette smoking and colorectal cancer (87). A possible explanation of this difference is that early studies may not have considered a sufficiently long time lag between smoking exposure and time of risk (86). However, inconsistencies in the relationship between smoking and colorectal cancer risk have been reported also in more recent studies, with some studies reporting statistically significant associations only with rectal cancer (87-89), other studies reporting statistically significant associations only among men (90;91) and some other studies reporting generally no significant associations (92;93).

1.5.12 Non steroidal anti-inflammatory drugs and aspirin

The protective short-term effect of NSAIDs and/ or aspirin on colorectal adenomas in patients with a history of colorectal adenomas or colorectal cancer has been demonstrated in three recent randomised clinical trials (94-96). In addition, results from three other randomised control studies showed a 40% reduction in colorectal adenomas recurrence with the use of either celecoxib or rofecoxib, which are also cyclo-

oxygenase-2 enzyme (COX-2) inhibitors (97-99). However, the effect of NSAIDs or aspirin on colorectal cancer risk is still not well established, possibly due to the long time that colorectal cancer needs to develop (100). Two large randomised trials, the Physicians' Health Study (101) and the Women's Health Study (102), failed to show a protective benefit of low-dose aspirin on risk of colorectal cancer in men and women. However, this failure to detect a protective effect of aspirin might be due to either low doses or insufficient duration of the treatment and results from a recent secondary analysis (103) of data pooled from two other randomised trials (104;105) support this argument (pooled HR (95% CI), p-value: 0.74 (0.56, 0.97) 0.02 overall; 0.63 (0.47, 0.85, 0.002 for 5 years or more). In addition, results from the Health Professional Follow-up Study after 18 years of follow up, reported that regular, long-term aspirin use reduces risk of colorectal cancer among men, but the benefit of aspirin requires at least 6 years of continuous and consistent use (100). Finally, both a systematic review of randomised, controlled trials, case-control and cohort studies (106) and a meta-analysis of observational studies, including data from 19 case-control and 11 cohort studies (103) reported that regular use of aspirin or NSAIDs was consistently associated with a reduced risk of colorectal cancer, especially in high doses and after use for more than 10 years.

1.5.13 Hormone replacement therapy

Post menopausal HRT has been found to be inversely associated with colorectal cancer in several observational studies (summarised in (107-109)). A meta-analysis of 18 observational studies, published in 1999 reported a 34% reduction in colorectal cancer risk for current versus no HRT (RR (95% CI): 0.66 (0.59, 0.74)) and a 20% reduction in risk for ever versus never users of HRT (RR (95% CI): 0.80 (0.74, 0.86)) (107). A systematic review and meta-analysis of 15 randomised clinical trials was published in 2005 from the Cochrane Collaboration and investigated the effects of long term HRT for peri-menopausal and post-menopausal women on several chronic diseases including colorectal cancer (110). Colorectal cancer outcome was measured in four of these trials (111-114), however only the WHI trial data were included in the meta-analysis due to the very small size of the remaining three clinical trials. Therefore, according to the

findings of this study, for women taking oestrogen combined with progesterone HRT there was no statistically significant difference in the incidence of colorectal cancer when compared to women taking placebo after one to four years' follow-up. However, women taking combined continuous HRT for five or more years had a statistically significant lower incidence of colon cancer (RR (95% CI): 0.62 (0.43 to 0.89)) (114). Furthermore cancers, which were diagnosed in women who were taking combined HRT, had greater lymph node involvement and were of a more advanced stage (114). However, the statistically significant lower colorectal cancer risk observed in the combined continuous HRT group during the intervention phase of the WHI trial did not persist three years after stopping the intervention (HR (95% CI): 1.08 (0.66-1.77)) (115).

1.6 Summary

Colorectal cancer is a cancer that forms either in the tissues of the colon or the rectum, and more than 95% of colorectal cancers are adenocarcinomas, deriving from colorectal adenomatous polyps. Approximately 25% of colorectal cancer cases are due to an inherited predisposition (5-10% hereditary syndromes, 15-20% familial colorectal cancer) with the remaining 75% having no obvious genetic predisposition (sporadic colorectal cancer). Sporadic colorectal cancer might therefore occur due to low-penetrance genetic mutations, due to effects of environmental risk factors or due to specific gene-environment interactions.

Colorectal cancer is the third most common cancer in global incidence and mortality rates accounting for 9% of all cancer cases and for 8% of all cancer related deaths (2002). However, large geographical variations in incidence rates are observed with the lowest rates to be recorded in Africa and the highest in N. America, Europe and Australia. Temporal trends in incidence rates of colorectal cancer differ between countries, with countries that have recently made a transition to a higher-income economy (e.g. eastern and southern European countries, Japan, Singapore) to show a rapid increase and countries with traditionally higher colorectal cancer incidence rates to

show a slight decrease in the last few years. Survival rates of colorectal cancer though have been significantly improved in most countries the last 25 years.

The established risk factors of colorectal cancer include personal history of previous colorectal cancer or adenomatous polyps, family history of colorectal cancer, chronic bowel inflammatory disease and presence of any of the hereditary syndromes. In addition, due to the fact that the majority of colorectal cancer cases (approximately 90%) occur after the age of 50, advanced age is also considered as a risk factor and in many countries colorectal cancer screening is recommended for those older than 50 years old. Finally, evidence for significant associations between colorectal cancer and other risk factors, including diet, body weight, physical activity, smoking, alcohol intake, NSAIDs intake and HRT in post-menopausal women, is promising and increasing.

2 AIMS AND OBJECTIVES

2.1 Introduction

In chapter 1, the fact that colorectal cancer is a common cancer accounting for 9% of all cancer cases and for 8% of all cancer related deaths was highlighted. At least 75% of colorectal cancer cases occur without a specific genetic background (sporadic colorectal cancer) and some established non-genetic risk factors (including age, personal history of previous colorectal cancer or adenomatous polyps, chronic bowel inflammatory disease, specific dietary aspects, body weight, physical activity, smoking, alcohol intake, NSAIDs intake and HRT) are thought to affect colorectal carcinogenesis. In this chapter the main aims and objectives of the thesis will be presented. In particular, this thesis had two aims, for the investigation of which a population-based case-control study of colorectal cancer was used (described in detail in chapter four, on page 141).

2.2 Aims

2.2.1 Aim 1: To investigate the association between specific nutrients and colorectal cancer

The first aim of this thesis was to determine whether particular nutrients are associated with colorectal cancer in a hypothesis-driven type of analysis. The dietary risk factors that were selected for this part of the analysis (part 1) were of two types. The first type (hypotheses 1 and 2) included relatively novel dietary risk factors, whose associations with colorectal cancer were not widely investigated in observational studies. In particular, this group included the following risk factors: 1) the flavonoid subgroups: flavonols, flavones, flavan3ols, procyanidins, flavanones and the individual flavonoid compounds: quercetin, catechin, epicatechin, naringenin and hesperetin (hypothesis 1); and 2) total fatty acids (FAs), the fatty acid subgroups saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), poly-unsaturated fatty acids (PUFAs), omega-3 PUFAs (ω3PUFAs), omega-6 PUFAs (ω6PUFAs), trans fatty acids (*t*FAs) and trans mono-unsaturated fatty acids (*t*MUFAs) and the individual fatty acid compounds

palmitic acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid, arachidonic acid, α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) (hypothesis 2). The second type of the dietary risk factors (hypotheses 3 and 4) that were included in the hypothesis-driven analysis part (part 1) consisted of dietary risk factors that were more widely studied in other observational studies, but their role in colorectal carcinogenesis is still not well established. In addition, for hypotheses 3 and 4, associations between colorectal cancer and particular genetic factors closely linked to the dietary factors were investigated. In particular the risk factors included in hypothesis 3 were the dietary risk factors folate, vitamin B2, vitamin B6, vitamin B12 and alcohol, which are involved in the one-carbon metabolic pathway (folate metabolic pathway) and the four following single nucleotide polymorphisms (SNPs) of three genes also involved in the one-carbon metabolic pathway: rs1801133 (*MTHFR* C677T), rs1801131 (*MTHFR* A1298C), rs1805087 (*MTR* A2756G) and rs1801394 (*MTRR* A66G) (genetic risk factors). Finally, the risk factors that were included in hypothesis 4 were vitamin D and calcium (dietary risk factors) and the four following SNPs of the vitamin D receptor (*VDR*) gene: rs10735810 (*Fok*I), rs1544410 (*Bsm*I), rs11568820 and rs7975232 (*Apa*I).

2.2.2 Aim 2: To conduct an overall analysis of the study and to identify the risk factors that better explain colorectal cancer risk in this population by applying forward and backward stepwise regression

The second aim of this thesis was to investigate the relationship between all the lifestyle and dietary risk factors that were collected from the Scottish Colorectal Cancer Study and colorectal cancer (overall analysis). In addition, stepwise regression models were applied in order to identify the risk factors that explained better colorectal cancer risk. The main goal of this part of the thesis (part 2) was to generate new hypotheses for future studies and not to draw any specific conclusions about the associations of these risk factors with colorectal cancer.

2.3 Objectives

The main objectives of this thesis are described below separately for aims 1 and 2.

2.3.1 Objectives of aim 1 (Hypotheses 1-4)

2.3.1.1 Hypotheses 1 and 2

1) To summarise the dietary intake of the novel dietary risk factors (flavonoid and fatty acid subgroups and individual compounds) for all subjects and after case/ control status stratification (mean, standard deviation, median, interquartile range of the dietary intakes; calculation of the t-test and Wilcoxon rank test).
2) To investigate the univariable associations between the novel dietary risk factors (same as above) and colorectal cancer using a crude conditional logistic regression model.
3) To investigate the multivariable associations between the novel dietary risk factors (same as above) and colorectal cancer using four conditional logistic regression models adjusted for different potential confounding factors.
4) To investigate the multivariable associations between the novel dietary risk factors (same as above) and colorectal cancer using a conditional logistic regression model adjusted for potential confounding factors, after sex, age and cancer site stratification.

2.3.1.2 Hypotheses 3 and 4

1) To summarise the dietary intake of the additional dietary risk factors (folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium) for all subjects and after case/ control status stratification (mean, standard deviation, median, interquartile range of the dietary intakes; calculation of the t-test and Wilcoxon rank test).
2) To investigate the univariable associations between the additional dietary risk factors (same as above) and colorectal cancer using a crude unconditional logistic regression model.
3) To investigate the multivariable associations between the additional dietary risk factors (same as above) and colorectal cancer using three unconditional logistic regression models adjusted for different potential confounding factors.

4) To investigate the multivariable associations between the additional dietary risk factors (same as above) and colorectal cancer using an unconditional logistic regression model adjusted for potential confounding factors, after sex, age and cancer site stratification.
5) To investigate the univariable and multivariable associations between the genetic factors and colorectal cancer using a crude and a simply adjusted unconditional logistic regression model.
6) To investigate the multivariable associations between the additional dietary risk factors (same as above) and colorectal cancer using an unconditional logistic regression model adjusted for potential confounding factors, after stratification according to the genetic factors and to investigate the interaction relationships between the genetic factors and the dietary risk factors.

2.3.2 Objectives of aim 2

1) To summarise all the explanatory variables that were to be included in the second part of the analysis by presenting percentages of the categorical variables and mean (with standard deviations) and median intakes (with interquartile ranges) of the continuous variables (for the whole sample and after case/ control status stratification).
2) To examine the correlation relationships (calculating Spearman rank correlation coefficient) between each individual continuous explanatory variable.
3) To investigate the univariable associations between each explanatory variable (quartiles for continuous variables; categories for categorical variables) and colorectal cancer using a crude unconditional logistic regression model. (Note: food and nutrient variables were adjusted for dietary energy intake by using the residual or the standard method of energy adjustment.)
4) To apply forward and backward stepwise regression to three different sets of explanatory variables (quartile form of continuous variables): a) Set 1: demographic factors, lifestyle variables and food variables; b) Set 2: demographic factors, lifestyle variables and nutrients; c) Set 3: demographic factors, lifestyle variables, food variables and nutrients.

5) To reapply, forward and backward stepwise regression on all three sets of variables (quartile form of continuous variables) separately for males and females.
6) To examine the stability of the built models by selecting 100 bootstrap samples and then for each bootstrap sample, applying forward and backward stepwise regression to the three different sets of the variables in the whole sample (bootstrap method).

3 LITERATURE REVIEW OF EXAMINED DIETARY RISK FACTORS

3.1 Introduction

In chapter 1, epidemiological evidence for the most clearly established dietary factors including red and processed meat, dietary fibre and fruit and vegetables was presented. In this chapter the dietary risk factors that were examined in the first part of this thesis comprising the prior hypotheses (aim 1; see chapter 2, on page 61) are described and evidence from observational studies is presented. These factors include: flavonoids, fatty acids, nutrients involved in the one-carbon metabolic pathway (folate, vitamin B2, vitamin B6, and vitamin B12), vitamin D and calcium.

Literature searches for each dietary factor examined in this thesis (as part of the prior hypothesis) were carried out in the PUBMED (MEDLINE) database limited to humans, English language and from years 1990 to 2008. The exact words used for each literature search as well as the results of each search are presented in Appendix I.

The first step of relevant references involved looking at the title of the study in order to identify whether the publication was applicable for inclusion. If necessary information for inclusion or exclusion were not available in study's title, the abstract of the study was examined (second step). Studies that appeared relevant on first and second step were entered into a Reference Manager database. The selected studies were then examined at a whole-article level review to see if they met the inclusion/exclusion criteria (third step). Those studies that did not meet the inclusion criteria were excluded. The inclusion criteria were studies, which were: 1) Observational (prospective or retrospective); 2) Having as primary or secondary endpoint colon and/or rectal adenocarcinoma; 3) Investigating the associations with a) the dietary nutrient intake (using a validated assessment of diet) or b) serum/ plasma concentration of a valid metabolite (biomarker) of the nutrient under examination; 4) Providing RRs or ORs and 95% confidence interval (95% CI) or information allowing us to calculate them. Additional studies were identified through published reviews, systematic literature

reviews and meta-analyses and/ or citations from the included studies. Summary tables are presented in the end of each section.

3.2 Flavonoids

3.2.1 Introduction

One type of plant secondary metabolite is a group of biologically active polyphenolic compounds widely distributed in a variety of plants. These compounds are of two types: flavonoids (consist of a C_{15} skeleton based on 1,3-diphenylpropane) and isoflavones (consist of a C_{15} skeleton based on 1,2-diphenylpropan). More than 10,000 plant flavonoids have been described, and they have been classified into at least ten chemical subgroups according to their structural patterns and their diverse bioactivities (116). However, laboratory and epidemiologic studies have focused on isoflavones and six flavonoid subgroups: flavonols, flavones, flavan3ols, anthocyanidins, pro- or anthocyanidins and flavanones.

The main dietary sources of these flavonoids differ widely among subgroups (117-120). Flavonols (main representatives: quercetin, kaempferol, myricetin) are mainly present in leafy vegetables, apples, onions and berries and these are the most abundant flavonoids in foods. Flavones (main representatives: apigenin, luteolin) and procyanidins are in low quantities in some vegetables and wine respectively. Flavan3ols are found in green tea, black tea, grapes, apples, chocolate and red wine. Flavanones, such as naringenin and hesperetin known also as citrus flavonoids are found in citrus fruits and their juices (121). The last subgroup, isoflavones, can be found in soya beans and together with lignans, whose precursors are present in a wide variety of plant foods, form the subgroup of phytoestrogens (122).

Flavonoids have many biological activities including antioxidant effects, inhibiting inflammation, antimutagenic and antiproliferative properties and involvement in the cell cycle regulation and apoptosis (117). In addition, results from laboratory studies show that flavonoids affect both molecular and cellular mechanisms that are involved in carcinogenesis (119). For colorectal cancer, in particular, in vitro colon cell lines and in vivo animal studies have reported anticarcinogenic properties associated with

flavonoids, including free radical scavenging, modifying or inactivating enzymes that activate or detoxify carcinogens, inhibiting the induction of transcription factors such as activator protein-1 (AP-1) activity and inducing apoptosis (123;124).

3.2.2 Evidence from observational studies

A few observational studies, have reported associations between flavonoid intake and incidence of different types of cancer (breast, lung, stomach, prostate, urothelial, bladder and colorectal) (125-130), but the most consistent findings have been observed for a reduced lung cancer risk (131). Regarding colorectal cancer, 13 observational studies (nine cohort and four case-control studies) that have examined the association between flavonoid and isoflavone intakes and colorectal cancer have been identified and 12 of them are presented in Table 4 (cohort studies) and Table 5 (case-control studies) (118;125;128;129;131-139). Four of the nine cohort studies were small with less than 200 cases and thus had very limited power to detect moderate or weak associations (118;128;129;134). In addition, the three larger cohort studies did not investigate all 6 subgroups of flavonoids (125;132;133;139) and only one, the Iowa Women's Health study reported statistically significant associations (125). This explanatory study examined associations between flavan3ols and many types of cancer and was restricted to postmenopausal women. The authors reported an inverse association with rectal cancer but did not correct statistical significance levels to account for the many tests performed and concluded that the role of flavonoid intake in colorectal cancer should be studied further (125). In a more recent analysis of the Iowa Women's Health study, the association between total flavonoids and the main subgroups (flavonols, flavones, flavan3ols, anthocyanidins, procyanidins, flavanones and isoflavones) and incidence of several types of cancer (including colorectal) was examined (131). However, no statistically significant associations between colorectal cancer and total flavonoid or any of the main subgroups was observed and the main finding of this study was a further support of an inverse association between flavonoids and lung cancer (not enough data to be presented in Table 4) (131).

All four case-control studies reported statistically significant inverse associations between flavonoid subgroups or compounds and colorectal cancer. In the Italian case-

control study the effect of the main six flavonoid subgroups was examined and the authors have reported a statistically significant inverse association for flavonols, flavones, anthocyanidins and isoflavones (135). The Canadian and Chinese case-control studies examined the associations between colorectal cancer and specific flavonoids and reported significant findings for phytoestrogens (and separately for lignans and isoflavones) and for specific flavan3ols, respectively (137;138).

Table 4 Colorectal cancer risk and flavonoid intake; Results from published cohort studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Flavonoid	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p†
Mursu J, 2008 (134)	Finland; Kuopio Ischaemic Heart Disease Risk Factor Study; 2590 FM	4-day food recording; quartiles	flavonols	highest vs. lowest quartile (mg/d)	colorectal	55	age, examination years, BMI, smoking, PA, alcohol, fat, SF and energy adjusted intake of fibre, vitamin C and E	1.53 (0.72, 3.23)	0.59
			flavones					0.71 (0.30, 1.65)	0.56
			flavan3ols					1.37 (0.65, 2.89)	0.82
			anthocyanidins					0.59 (0.24, 1.41)	0.97
			flavanones					0.90 (0.37, 2.20)	0.52
			total flavonoids					1.16 (0.58, 2.34)	0.83
Oba S, 2007 (139)	Japan; Prospective Takayama study; 30221 FM	FFQ; tertiles	isoflavones	M: 59.58 vs. 22.45 mg/d	colon	111	energy, age, height, alcohol, smoking, BMI, PA, coffee, use of HRT (women)	1.47 (0.90, 2.40)	0.12
				F: 59.58 vs. 22.45 mg/d		102		0.73 (0.44, 1.18)	0.20
Lin J, 2006 (133)	USA; Nurses' Health Study, Health Professionals Follow-up Study; 10741FM	FFQ; quintiles	total flavonoids	M >30.5 vs. <10.7 mg/d	colorectal	380	age, BMI, FH, history of CR polyps, prior sigmoidoscopy screening, PA, pack-years of smoking, red meat, alcohol, energy, calcium, folate, fibre, aspirin, multivitamin	1.28 (0.89, 1.83)	0.21
				F>31.1 vs. <0.96 mg/d		498		1.13 (0.83, 1.52)	0.42
			quercetin			380		1.16 (0.80, 1.68)	0.40
						498		1.01 (0.75, 1.35)	0.40
			kaempferol			380		1.09 (0.78, 1.52)	0.29
						498		1.14 (0.85, 1.52)	0.55
			myricetin			380		1.33 (0.93, 1.89)	0.43
						498		0.89 (0.67, 1.18)	0.96
Arts IC, 2002 (125)	USA; Iowa Women's Health Study; 34651F	FFQ; quintiles	catechins	>75.1 vs. <3.6 mg/d	colon	635	age, energy, BMI, waist-to-hip ratio, PA, pack-years of smoking, smoking, number of years since quit smoking, alcohol, fruit	1.10 (0.85, 1.44)	0.63
				>24.7 vs. <3.6 mg/d	rectal	132		0.55 (0.32, 0.95)	0.002
			catechin + epicatechin	>24.3 vs. <3.2 mg/d	colon	635		1.04 (0.71, 1.29)	0.90
				>15.7 vs. <3.2 mg/d	rectal	132		0.92 (0.50, 1.71)	0.75

			gallates	>50.8 vs. <0.4 mg/d	colon	635	and vegetable	0.95 (0.72, 1.25)	0.44
				>8.9 vs. <0.4 mg/d	rectal	132		0.39 (0.22, 0.71)	0.02
Knekt P, 2002 (118)	Finland; Finnish Mobile Clinic Health Examination Survey; 10054 FM	diet history; quartiles	quercetin	M >3.9 vs. <1.5 mg/d F >4.7 vs. <1.8 mg/d	colorectal	90	sex, age, geographic area, occupation, smoking, BMI	0.62 (0.33, 1.17)	0.22
			kaempferol	M >0.8 vs. <0.1 mg/d F >0.9 vs. <0.1 mg/d				1.13 (0.60, 2.12)	0.96
			myricetin	M >0.1 vs. <0.06 mg/d F >0.2 vs. <0.03 mg/d				1.31 (0.71, 2.43)	0.39
			hesperetin	M >15.4 vs. 0 mg/d F >26.8 vs. <3.2 mg/d				0.97 (0.50, 1.90)	0.84
			naringenin	M >4.7 vs. <4.7 mg/d F >7.7 vs. <0.9 mg/d				0.93 (0.48, 1.82)	0.99
			total	M >26.9 vs. <4.3 mg/d F >39.5 vs. <8.5 mg/d				0.84 (0.43, 1.64)	0.95
Hirvonen T, 2001 (128)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27110 M	diet history; quartiles	flavonols + flavones	16.3 vs. 4.2 mg/d	colorectal	133	age; supplement group	1.70 (1.00, 2.70)	0.10
Goldbohm RA, 1998 (132)	Netherlands; Netherlands Cohort Study; 3726FM		flavonols + luteolin	43.5 vs. 12.7 mg/d	colorectal	603		0.97 (0.71, 1.32)	0.92

Knekt P, 1997 (129)	Finland; Finnish Mobile Clinic Health Examination Survey; 9959FM	diet history interview; quartiles	flavonols flavones	M >4.8 vs. <2.1 mg/d F >5.5 vs. <2.4 mg/d	colorectal	72	sex, age, geographic area, occupation, BMI, smoking, energy, vitamin C, vitamin E, beta carotene, fibre, SFAs, MUFAs, PUFAs, cholesterol	0.74 (0.32, 1.68)

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SFAs: saturated fatty acids; MUFAs: mono-unsaturated fatty acids; PUFAs: poly-unsaturated fatty acids

[†] P-value for trend

Table 5 Colorectal cancer risk and flavonoid intake; Results from published case-control studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Flavonoid	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p[†]
Theodoratou E, 2007[‡] (136)	Scotland; SOCCS study; 2912 FM	FFQ; quartiles	flavonols flavones flavan3ols procyanidins flavanones phytoestrogens	>36.8 vs. <16.0 mg/d >1.9 vs. <0.5 mg/d >162.1 vs. 42.6 mg/d >45.2 vs. <16.7 mg/d >40.6 vs. <7.4 mg/d >857.6 vs. <402.7 µg/d	colorectal	1456	matched on age, sex, residence area; adjusted for FH; BMI; PA; smoking; and intakes of energy (residual), fibre, alcohol and NSAIDs	0.73 (0.59, 0.91) 1.11 (0.88, 1.40) 0.81 (0.65, 1.01) 0.81 (0.65, 1.00) 1.13 (0.89, 1.43) 0.93 (0.74, 1.15)	0.02 0.60 0.08 0.08 0.37 0.67
Yuan MJ, 2006 (137)	China; nested case-control of the Shanghai Cohort; 968 FM	urine metabolite measurements; 4 categories	EGC EC	>7.82 vs. 0 µmol/g Cr >2.00 vs. 0 µmol/g Cr	colorectal	162	matched on age, date of blood collection and neighbourhood of residence; adjusted for: smoking (cigarettes/day, and number of years), alcohol, number of alcoholic beverages	0.76 (0.47, 1.24) 0.91 (0.55, 1.51)	0.28 0.59
Cotterchio M, 2006 (138)	Canada, Ontario Familial Colorectal Cancer Registry; 2985 FM	FFQ; tertiles	lignans isoflavones phytoestrogens	>0.26 vs. <0.16 mg/d >1.10 vs. <0.29 mg/d >1.34 vs. <0.53 mg/d	colorectal	1095	lignans adjusted for: age, sex, dietary fibre, and energy; isoflavones and phytoestrogens adjusted for age, sex, and energy	0.73 (0.56, 0.94) 0.71 (0.58, 0.86) 0.71 (0.59, 0.86)	0.01 <0.01 <0.01
Rossi M, 2006 (135)	Italy; 6107 FM	FFQ; quintiles	flavonols flavones flavan3ols anthocyanidins	>28.5 vs. <13.2 mg/d >0.7 vs. <0.3 mg/d >88.5 vs. <20.8 mg/d >31.7 vs. <5.3 mg/d	colorectal	1953	age, sex, study centre, FH, education, alcohol, BMI, occupational PA, energy (residual)	0.64 (0.54-0.77) 0.78 (0.65-0.93) 0.98 (0.82-1.18) 0.67 (0.54-0.82)	<0.001 0.004 0.74 <0.001

	flavanones	>67.0 vs. <12.5 mg/d	0.96 (0.81-1.15)	0.43
	isoflavones	>33.9 vs. <14.4 µg/d	0.76 (0.63-0.91)	0.001
	total flavonoids	>191.1 vs. <75.3 mg/d	0.97 (0.81-1.16)	0.50

* Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

† P-value for trend

‡ Results that are part of the current thesis and will be presented in detail in the following chapters

3.3 Fatty acids

3.3.1 Introduction

All fats consist of fatty acids (organic (carboxylic) acids), which are classified as either saturated or unsaturated, depending on their chemical structure. SFAs have no double bonds between the carbon atoms of the fatty acid chain. The most abundant SFAs are butyric acid, which is the main product of fibre fermentation in the large bowel, and palmitic and stearic acids, which are mainly found in meat products. SFAs are also found in butter, lard, coconut oil, cream and cheese. Unsaturated fatty acids have one (MUFAs) or more (PUFAs) double bonds in the fatty acid chain. The most common MUFAs are palmitoleic acid and oleic acid and they are mainly present in nuts, avocados and olive oil. PUFAs are further divided in ω3PUFAs and ω6PUFAs fatty acids. The difference is that ω3PUFAs have a double bond, three carbons away from the methyl carbon, whereas ω6PUFAs have it six carbons away from the methyl carbon. Omega-3 PUFA subgroup consists of α-linolenic acid mainly found in seeds (rapeseed, soybeans, walnuts and flaxseed) oils, and EPA and DHA acids mainly found in oily fish (herring, salmon, mackerel and halibut) and sea food. Omega-6 PUFAs consist of linoleic, γ-linolenic and arachidonic acids and their main sources are sunflower and safflower oil. Unlike other fatty acids, linoleic and α-linolenic acids (essential fatty acids) cannot be synthesised by the body and therefore intake through the diet is required (140). Trans fatty acids are a type of either MUFAs or PUFAs and occur naturally in small quantities in meat and dairy products from ruminates. However, most *t*FAs consumed today, are industrially produced through partial hydrogenation of plant oils and animal fats (141).

Animal and cell-line studies have suggested that the effect of fats depends not only on their quantity but also on composition of fatty acids, which might explain the differences in the observed associations (142). Several hypothesised mechanisms regarding the role of specific fatty acids on the development of colon cancer have been described. One example is the anticarcinogenic properties of butyric acid, which is mainly produced in the large bowel as result of fibre fermentation (142). Furthermore it has been shown that MUFAs and *t*FAs promote human colon growth through increase in fatty acid oxidation and disturbance of membrane enzymes (142). In addition, there is increasing interest on

the possible protective effects of ω3PUFAs in contrast to the increased risk of ω6PUFAs. The different effects of these two series are related to their enzymatic competition for their metabolic conversion in eicosanoids, which effect many physiological processes, including apoptosis, cell proliferation and immune cell function (143;144).

3.3.2 Evidence from observational studies

Consumption of fats and oils varies throughout the world, with intake in more developed regions of the world (Europe, North America, Australia and New Zealand) being higher (approximately 30-40 % of total energy intake) than in less developed countries (Africa, Asia and Latin America; approximately 20-30% of total energy intake) (30). Since the first study that suggested that dietary fats might affect colorectal carcinogenesis (145) many studies have investigated the colorectal cancer effect of fat according to its amount (total fat), its type (saturated, mono-unsaturated and poly-unsaturated fat) and its origin (animal, vegetable, fish derived fat) (142). Neither case-control nor cohort studies have found that high total fat intakes increase risk of colorectal cancer (64). In addition a meta-analysis of case-control studies (conducted from 1976 to 1988) concluded that there were no energy-independent associations between the three major fat subclasses (SFAs, MUFAs or PUFAs) and colorectal cancer (142). Regarding specific fatty acid subgroups (SFAs, MUFAs, PUFAs, ω3PUFAs, ω6PUFAs and *t*FAs), few observational studies have studied their associations with colorectal cancer. Regarding the fat origin, results from ecological studies indicate that diets particularly high in animal fat to be generally associated with increased risk in colorectal cancer, in contrast to diets high in vegetable or fish derived fat (146). In addition, according to the AICR/WCRF report (2007), the evidence that high intake of animal fat is causally linked to colorectal cancer is fairly consistent but limited and a summary relative risk of three prospective studies was 1.16 (95% CI 0.92, 1.38) per 20g/day (30). In addition, according to the findings of the same report the evidence that high fish intake is inversely associated with colorectal cancer is limited (summary RR of seven prospective studies (95% CI): 0.96 (0.92, 1.00)) (30).

We identified nine cohort (Table 6) and six case-control studies (Table 7) that studied the association between colorectal cancer and saturated fat (62;63;65;66;141;147-156)

and two cohort (Table 6) and nine case-control studies (Table 7) that studied the association between colorectal cancer and SFAs (157-167). Only one case-control reported a statistically significant positive association between SFAs and colorectal cancer (160). Regarding MUFAs, we identified seven cohort (Table 8) and four case-control studies (Table 9) that examined the association between mono-unsaturated fat and colorectal cancer (62;63;66;141;147-150;152;153;155) and three cohort (Table 8) and nine case-control studies (Table 9) that examined the association between MUFAs and colorectal cancer (157-168). One cohort study reported a statistically significant inverse association between MUFAs and colon cancer (168) and one case-control study a significant inverse association between MUFAs and colorectal cancer (66). Regarding PUFAs, we identified four cohort (Table 10) and four case-control studies (Table 11) that examined the association between poly-unsaturated fat and colorectal cancer (62;66;147;148;150;152;153;155) and two cohort (Table 10) and seven case-control studies (Table 11) that examined the association between PUFAs and colorectal cancer (157;160-167). Three case-control studies reported significant inverse associations between PUFAs or poly-unsaturated fat and colorectal cancer (66;160;165).

A few studies have investigated the associations between colorectal cancer and ω3PUFAs or ω6PUFAs, separately. We identified six cohort (Table 12) and 10 case-control studies (Table 13) that tested association between risk of colorectal cancer and ω3PUFAs or the individual ω3PUFAs EPA, DHA and/or α-linolenic acid (141;147;150;158-164;166;169-173). In total, one cohort and four case-control studies reported a statistically significant inverse association between ω3PUFAs and colorectal cancer (158;161;164;170;172). Regarding ω6PUFAs, we identified three cohort (Table 14) and 10 case-control studies (Table 15) that investigated their associations (or the associations with linoleic acid) with colorectal cancer (141;150;158-164;166;169;173;174), with only one case-control study reporting a significant inverse association (160). Finally, we identified three cohort (Table 16) and three case-control (Table 17) studies that examined the association between *t*FAs and colorectal cancer (141;161;162;164;175;176), with one case-control study reporting significant positive association for female colorectal cancer cases (175).

Table 6 Colorectal cancer risk and saturated fat or saturated fatty acids; Results from published cohort studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p†
Weijenberg MP, 2007 (152)	The Netherlands; Netherlands Cohort Study; 120852 FM	FFQ; quartiles	SF	M: 45.8 vs. 28.9 g/d F: 36.6 vs. 23.9 g/d	colon	434	age, sex, BMI, smoking, energy, FH of CRC	0.94 (0.69, 1.27)	0.54
Brink M, 2004 (148)	The Netherlands; Netherlands Cohort Study; 3346 FM	FFQ; quartiles	SF	M: 45.8 vs. 28.9 g/d F: 36.6 vs. 23.9 g/d	rectum	160	age, sex, BMI, smoking, energy intake, FH of CRC	0.73 (0.46, 1.17)	0.37
Lin J, 2004 (141)	USA; Women's Health Study; 37547 F	FFQ; quintiles	SF	13 vs. 7 % energy	colorectal	202	age, random treatment assignment, BMI, FH of CRC, history of colorectal polyps, PA, cigarette smoking, alcohol consumption, postmenopausal HRT, and energy	0.92 (0.61, 1.41)	0.44
Flood A, 2003 (156)	USA; Breast Cancer Detection Demonstration Project; 45496 F	FFQ; quintiles	SF	15.7 vs. 7.1 g/d	colorectal	487	energy	1.02 (0.77, 1.34)	0.74
Jarvinen R, 2001 (157)	Finland; Finnish Mobile Clinic Health Examination Survey;9959 FM	diet history; quartiles	SFAs	M: >86.6 vs. 53.5 g/d F: >60.1 vs. <35.6 g/d	colorectal	109	age, sex, BMI, occupation, smoking, geographical area, energy, vegetables, fruits and cereals	1.47 (0.56, 3.83)	
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol,	diet history; quartiles	SFAs	65.1 vs. 33.8 g/d	colorectal	185	age, supplement group, smoking years, BMI,	0.9 (0.6, 1.4)	0.27

	Beta-Carotene Cancer Prevention Study; 27111M						alcohol, education, PA at work, calcium		
Kato I, 1997 (65)	USA; New York's University Health Study; 14727 F	FFQ; quartiles	SF	high vs. low quartile	colorectal	100	total calorie intake, age, place at enrolment, highest level of education	1.05 (0.59, 1.88)	0.51
Gaard M, 1996 (63)	Norway; 50535FM	FFQ	SF		colon	143	energy	no association	
Bostick RM, 1994 (147)	USA; Iowa's Women's Health; 32215 F	FFQ; quintiles	SF	>31.7 vs. <16.0 g/d	colon	212	age, energy, height, parity, vitamin E, vitamin E x age term, vitamin A supplement intake, residual energy adjustment	1.21 (0.78, 1.89)	0.98
Giovannucci E, 1994 (149)	USA; Health Professionals Follow-up Study; 47949 M	FFQ; quintiles	SF	33.0 vs. 17.4 g/d	colon	205	age, energy (residual)	0.88 (0.56, 1.37)	0.79
Goldbohm RA, 1994 (155)	The Netherlands; Netherlands Cohort Study; 3500 FM	FFQ; quintiles	SF	M: 47 vs. 28 g/d F: 27 vs. 23 g/d	colon	215	age, energy, dietary fibre	1.07 (0.69, 1.66)	0.91

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; SF: saturated fat; SFAs: saturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 7 Colorectal cancer risk and saturated fat or saturated fatty acids; Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Theodoratou E, 2007‡ (164)	Scotland; SOCCS; 2910FM	FFQ; quartiles	SFAs	≥43.64 vs. <31.72 g/d	colorectal	1458	matched on age, sex, are of residence, adjusted for FH, total energy intake (residual method), fibre intake, alcohol intake, use of NSAIDs, smoking, BMI, PA	1.27 (1.00, 1.61)	0.08
Kimura Y, 2007 (158)	Japan; Fukuoka Colorectal Cancer Study; 1575FM	FFQ; quintiles	SFAs	22.10 vs. 11.39 g/d	colorectal	782	energy (residual), age, sex, residential area, BMI 10 years before, parental CRC, smoking, alcohol use, type of job, leisure-time PA, dietary calcium and fibre intake	1.04 (0.71, 1.51)	0.52
Kuriki K, 2006 (160)	Japan; 295 FM	Erythrocyte measurements using gas-liquid chromatography; tertiles	SFAs	>52.80 vs. <50.89 mol%	colorectal	74	BMI, habitual exercise, drinking and smoking status, green-yellow vegetable intake, FH	8.20 (2.86, 23.52)	<0.0001
Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	SFAs	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC<	0.83 (0.57, 1.20) 0.86 (0.56, 1.33)	0.35 0.57

							BMI, exercise, alcohol, smoking, multivitamin use		
Kojima M, 2005 (159)	Japan; Japan Collaborative Cohort Group; 650 FM	serum; quartiles	SFAs	M: ≥36.1 vs. <31.9 weight % of total serum lipids F: ≥35.4 vs. <31.4 weight % of total serum lipids	colorectal	83 86	matched on age and participating institution; adjusted for FH of CRC, BMI, education, smoking, alcohol, green leafy vegetable intake, PA	1.71 (0.66, 4.47) 0.59 (0.23, 1.52)	0.36 0.51
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	SFAs	M: >50.8 vs. <8.9 g/d F: >44.0 vs. <7.7 g/d	colorectal	171	age, sex, energy, BMI, PA	1.4 (0.6, 3.2)	0.50
Nkondjock A, 2003 (161)	Canada; 1070 FM	FFQ; quartiles	SFAs	>123.3 vs. 66.14 g/d	colorectal	402	energy (residual), age, marital status, history of colorectal cancer in first-degree relatives, BMI one year prior to diagnosis, and PA	0.97 (0.68, 1.38)	0.53
Levi F, 2002 (66)	Switzerland; 836 FM	FFQ; tertiles	SF	>312 vs. <205 g/d	colorectal	286	age, sex, education, PA and residual energy	1.4 (0.9, 2.2)	>0.05
Franceschi S, 1998 (62)	Italy; 6107 FM	FFQ; per 100 kcal of total energy/day	SF	mean: 230 kcal/day	colorectal	1953	age, sex, study centre, education, PA and alcohol intake	1.12 (0.98, 1.28)	
Slattery ML, 1997 (163)	USA; 4403 FM	CARDIA diet history; quintiles	SFAs	M >69.3 vs. <42.0 g/MJ F >68.0 vs. 39.2 g/MJ	colon	1095 888	age at diagnosis or selection, energy intake, dietary fibre, cholesterol, calcium, BMI, physical activity, FH of CRC, NSAIDs	0.88 (0.64, 1.22) 0.96 (0.67, 1.37)	

Le Marchand L, 1997 (150)	USA (Hawaii); 2384 FM	FFQ; quartiles	SF	M >27 vs. <18 g/d F >20 vs. <14 g/d	colorectal	698 494	age, FH of CRC, alcoholic drinks/week, pack-years, lifetime recreational PA, BMI five years ago, and caloric, dietary fibre and calcium intakes; residual Calorie-adjustment	1.2 (0.8, 1.8) 1.5 (0.9, 2.4)	0.4 0.09
Ghadirian P, 1997 (153)	Canada; 1070 FM	FFQ; quartiles	SF	high vs. low quartile	colon	402	sex, age, marital status, history of colon carcinoma in first-degree relatives, energy	0.71 (0.49, 1.03)	0.09
De Stefani E, 1997 (154)	Uruguay; 846 FM	quartiles	SF	>35.3 vs. ≤25.8 mg/d	colorectal	282	age, sex, residence, urban/rural status, energy, calcium, vitamin D, folate	1.52 (0.84, 2.77)	0.18
Trichopoulou A, 1992 (151)	Greece; 200 FM	FFQ	SF	continuous	colorectal	100	age, gender, energy	1.28 (0.71, 2.30)	>0.05
Zaridze D, 1992 (165)	Russia; 434 FM	FFQ; quartiles	SFAs	M: >80.8 vs. <48.3 g/d F: >74.8 vs. <44.5 g/d	colorectal	217	energy, education	1.56 (0.59, 4.18)	0.40

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; SF: saturated fat; SFAs: saturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

Table 8 Colorectal cancer risk and mono-unsaturated fat or mono-unsaturated fatty acids; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Weijenberg MP, 2007 (152)	The Netherlands; Netherlands Cohort Study; 120852 FM	FFQ; quartiles	MUF	M: 42.5 vs. 28.2 g/d F: 33.1 vs. 22.4 g/d	colon	434	age, sex, BMI, smoking, energy intake and FH of CRC	0.99 (0.73, 1.34)	0.79
Brink M, 2004 (148)	The Netherlands; Netherlands Cohort Study; 3346 FM	FFQ; quartiles	MUF	M: 42.5 vs. 28.2 g/d F: 33.0 vs. 22.4 g/d	rectum	160	age, sex, BMI, smoking, energy intake and FH of CRC	0.87 (0.56, 1.37)	0.80
Lin J, 2004 (141)	USA; Women's Health Study; 37547 F	FFQ; quintiles	MUF	15 vs. 8 % energy	colorectal	202	age, random treatment assignment, BMI, FH of CRC, history of colorectal polyps, PA, smoking, alcohol consumption, HRT, energy	1.09 (0.68, 1.73)	0.72
Jarvinen R, 2001 (157)	Finland; Finnish Mobile Clinic Health Examination Survey; 9959 FM	diet history; quartiles	MUFAs	M: >49.2 vs. 30.5 g/d F: >34.0 vs. <20.8	colorectal	109	age, sex, BMI, occupation, smoking, geographical area, energy intake, vegetables, fruits and cereals	2.37 (0.86, 6.51)	
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111M	modified diet history; quartiles	MUFAs	40.7 vs. 28.4 g/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	1.2 (0.8, 1.8)	0.44
Gaard M, 1996 (63)	Norway; 50535 FM	FFQ	MUF		colon	143	energy	no association	

Chyou PH, 1996 (168)	Japanese-American (USA); 7945 M	24 hour diet history;quartiles	MUFAs	≥41 vs. <22 g/d	colon rectum	330 123	age	0.73 (0.53, 1.00) 1.47 (0.88, 2.47)	0.02 0.47
Bostick RM, 1994 (147)	USA; Iowa's Women's Health; 32215 F	FFQ; quintiles	MUF	>33.1 vs. <16.6 g/d	colon	212	age, energy (residual), height, parity, vitamin E, vitamin E x age term, vitamin A supplement	0.85 (0.54, 1.35)	0.70
Giovannucci E, 1994 (149)	USA; Health Professionals Follow-up Study; 47949 M	FFQ; quintiles	MUF	34.2 vs. 19.1 g/d	colon	205	age, energy (residual)	1.07 (0.68, 1.69)	0.68
Goldbohm RA, 1994 (155)	The Netherlands; Netherlands Cohort Study; 3500 FM	FFQ; quintiles	MUF	M: 43 vs. 27 g/d F: 27 vs. 23 g/d	colon	215	age, energy, dietary fibre	1.00 (0.63, 1.57)	0.88

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; MUF: mono-unsaturated fat; MUFAs: mono-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 9 Colorectal cancer risk and mono-unsaturated fat or mono-unsaturated fatty acids; Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Theodoratou E, 2007‡ (164)	Scotland; SOCCS; 2910FM	FFQ; quartiles	MUFAs	≥36.16 vs. <28.72 g/d	colorectal	1458	matched on age, sex, are of residence; adjusted for FH, total energy intake (residual), fibre intake, alcohol intake, use of NSAIDs, smoking, BMI, PA	1.33 (1.05, 1.68)	0.06
Kimura Y, 2007 (158)	Japan; Fukuoka Colorectal Cancer Study; 1575FM	FFQ; quintiles	MUFAs	28.06 vs. 15.29 g/d	colorectal	782	Energy (residual), age, sex, residential area, BMI 10 years before, parental CRC, smoking, alcohol use, type of job, leisure-time PA, dietary calcium and fibre intake	0.88 (0.62, 1.25)	0.44
Kuriki K, 2006 (160)	Japan; 295FM	Erythrocyte measurements using gas-liquid chromatography; tertiles	MUFAs	>18.85 vs. <17.56 mol%	colorectal	74	BMI, habitual exercise, drinking and smoking status, green-yellow vegetable intake, and FH colorectal cancer	1.93 (0.88, 4.23)	0.15
Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	MUFAs	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin	0.92 (0.62, 1.36) 0.76 (0.51, 1.14)	0.80 0.15
Kojima M, 2005 (159)	Japan; nested case-	serum; quartiles	MUFAs	M: ≥24.7 vs. <20.8 weight % of total	colorectal	83	matched on age and participating institution;	2.05 (0.86, 4.89)	0.06

	control of Japan Collaborative Cohort Group; 650 FM			serum lipids F: ≥24.1 vs. <20.4 weight % of total serum lipids		86	adjusted for FH of CRC, BMI, education, smoking, alcohol, green leafy vegetable intake, PA	0.83 (0.36, 1.92)	0.51
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	MUFAs	M: >44.5 vs. <10.0 g/d F: >35.6 vs. <8.1 g/d	colorectal	171	age, sex, energy, BMI, PA	1.0 (0.4, 2.4)	0.95
Nkondjock A, 2003 (161)	Canada; 1070 FM	FFQ; quartiles	MUFAs	>50.01 vs. 25.99 g/d	colorectal	402	energy (residual), age, marital status, history of colorectal cancer in first-degree relatives, BMI one year prior to diagnosis, and PA	0.99 (0.69, 1.41)	0.96
Levi F, 2002 (66)	Switzerland; 836 FM	FFQ; tertiles	MUF	>335 vs. <249 g/d	colorectal	286	age, sex, education, PA and residual energy	0.6 (0.4, 0.9)	<0.05
Franceschi S, 1998 (62)	Italy; 6107 FM	FFQ; per 100 kcal of total energy/day	MUF	mean: 264 kcal/day	colorectal	1953	age, sex, study centre, education, PA and alcohol intake	1.00 (0.91, 1.10)	
Slattery ML, 1997 (163)	USA; 4403 FM	CARDIA diet history; quintiles	MUFAs	M >66 vs. <44 g/MJ F >63 vs. <39 g/MJ	colon	1095 888	age at diagnosis or selection, energy intake, dietary fibre, cholesterol, calcium, BMI, PA, FH of CRC, NSAIDs	0.89 (0.65, 1.21) 0.94 (0.66, 1.34)	
Le Marchand L, 1997 (150)	USA (Hawaii); 2384 FM	FFQ; quartiles	MUF	M >33 vs. <24 g/d F >20 vs. <14 g/d	colorectal	698 494	age, FH of CRC, alcoholic drinks/week, pack-years, lifetime recreational PA, BMI five years ago, and caloric, dietary fibre and calcium intakes; residual Calorie-adjustment	1.4 (0.9, 2.1) 1.4 (0.9, 2.2)	0.06 0.1

Ghadirian P, 1997 (153)	Canada; 1070 FM	FFQ; quartiles	MUF	high vs. low quartile	colon	402	sex, age, marital status, history of colon carcinoma in first-degree relatives, energy	0.89 (0.61, 1.30)	0.63
Zaridze D, 1992 (165)	Russia; 434 FM	FFQ; quartiles	MUFAs	M: >72.4 vs. <45.9 g/d F: >69.4 vs. <42.8 g/d	colorectal	217	energy, education	0.54 (0.20, 1.51)	0.23

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; MUF: mono-unsaturated fat; MUFAs: mono-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

Table 10 Colorectal cancer risk and poly-unsaturated fat or poly-unsaturated fatty acids; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Weijenberg MP, 2007 (152)	The Netherlands; Netherlands Cohort Study; 120852 FM	FFQ; quartiles	PUF	M: 29.3 vs. 11.6 g/d F: 22.5 vs. 8.8 g/d	colon	434	age, sex, BMI, smoking, energy intake and FH of CRC	1.21 (0.89, 1.63)	0.38
Brink M, 2004 (148)	The Netherlands; Netherlands Cohort Study; 3346 FM	FFQ; quartiles	PUF	M: 29.1 vs. 11.6 g/d F: 22.5 vs. 8.8 g/d	rectum	160	age, sex, BMI, smoking, energy intake and FH of CRC	0.83 (0.53, 1.29)	0.54
Jarvinen R, 2001 (157)	Finland; Finnish Mobile Clinic Health Examination Survey; 9959 FM	diet history; quartiles	PUFAs	M: >10.3 vs. 5.9 g/d F: >7.5 vs. <4.1	colorectal	109	age, sex, BMI, occupation, smoking, geographical area, energy intake and consumption of vegetables, fruits and cereals	1.13 (0.56, 2.26)	
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111M	modified diet history; quartiles	PUFAs	19.4 vs. 6.5 g/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	1.4 (0.9, 2.1)	0.18
Bostick RM, 1994 (147)	USA; Iowa's Women's Health; 32,215 F	FFQ; quintiles	PUF	>16.2 vs. <8.0 g/d	colon	212	age, energy (residual), height, parity, vitamin E, vitamin E x age term, vitamin A supplement	0.74 (0.49, 1.12)	0.53
Goldbohm RA, 1994 (155)	The Netherlands; Netherlands Cohort Study; 3500 FM	FFQ; quintiles	PUF	M: 31 vs. 11 g/d F: 24 vs. 8 g/d	colon	215	age, energy, dietary fibre	1.38 (0.88, 2.16)	0.19

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; PUF: poly-unsaturated fat; PUFAs: poly-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 11 Colorectal cancer risk and poly-unsaturated fat or poly-unsaturated fatty acids; Results from published case-control studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p[†]
Theodoratou E, 2007[‡] (164)	Scotland; SOCCS; 2910 FM	FFQ; quartiles	PUFAs	≥16.75 vs. <12.01 g/d	colorectal	1458	matched on age, sex, are of residence; adjusted for FH, total energy intake (residual method), fibre intake, alcohol intake, use of NSAIDs, smoking, BMI, PA	0.97 (0.77, 1.23)	0.54
Kuriki K, 2006 (160)	Japan; 295 FM	Erythrocyte measurements using gas-liquid chromatography; tertiles	PUFAs	>31.09 vs. <28.21 mol%	colorectal	74	BMI, habitual exercise, drinking and smoking status, green-yellow vegetable intake, FH	0.15 (0.05, 0.46)	<0.005
Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	PUFAs	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin use	0.90 (0.61, 1.31) 0.76 (0.51, 1.12)	0.88 0.47
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	PUFAs	M: >18.8 vs. <2.6 g/d F: >14.4 vs. <2.1 g/d	colorectal	171	age, sex, energy, BMI, PA	1.4 (0.7, 2.9)	0.28
Nkondjock A, 2003 (161)	Canada; 1070 FM	FFQ; quartiles	PUFAs	>21.55 vs. 11.15 g/d	colorectal	402	energy (residual), age, marital status, FH of CRC, BMI one year prior to diagnosis, and PA	1.04 (0.74, 1.48)	0.69

Levi F, 2002 (66)	Switzerland; 836 FM	FFQ; tertiles	PUF	>194 vs. <138 g/d	colorectal	286	age, sex, education, PA and residual energy	0.6 (0.4, 0.9)	<0.05
Franceschi S, 1998 (62)	Italy; 6107 FM	FFQ; per 100 kcal of energy	PUF	mean: 96 kcal/day	colorectal	1953	age, sex, study centre, education, PA and alcohol	0.89 (0.76, 1.02)	
Slattery ML, 1997 (163)	USA; 4403 FM	CARDIA diet history; quintiles	PUFAs	M >34.7 vs. <21.3 g/MJ F >8.26 vs. 4.94 g/MJ	colon	1095 888	age at diagnosis or selection, energy intake, dietary fibre, cholesterol, calcium, BMI, PA, FH of CRC, NSAIDs	1.07 (0.82, 1.41) 1.05 (0.77, 1.43)	
Le Marchand L, 1997 (150)	USA (Hawaii); 2384 FM	FFQ; quartiles	PUF	M >28 vs. <19 g/d F >22 vs. <15 g/d	colorectal	698 494	age, FH of CRC, alcoholic drinks/week, pack-years, lifetime recreational PA, BMI five years ago, and caloric, dietary fibre and calcium intakes; residual Calorie-adjustment	0.7 (0.5, 1.1) 0.9 (0.6, 1.5)	0.2 0.7
Ghadirian P, 1997 (153)	Canada; 1070 FM	FFQ; quartiles	PUF	high vs. low quartile	colon	402	sex, age, marital status, history of colon carcinoma in first-degree relatives, energy	0.96 (0.65, 1.42)	0.51
Zaridze D, 1992 (165)	Russia; 434 FM	FFQ; quartiles	PUFAs	M: >30.4 vs. <15.1 g/d F: >31.2 vs. <17.0 g/d	colorectal	217	energy, education	0.29 (0.13, 0.64)	0.004

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; PUF: poly-unsaturated fat; PUFAs: poly-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

Table 12 Colorectal cancer risk and omega-3 poly-unsaturated fatty acids; Results from published cohort studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p†
Hall MN, 2008 (170)	USA; Physicians' Health Study; 22071 M	FFQ; quartiles	ω3PUFAs (from fish)	high vs. low quartile	colorectal	500	age, smoking, BMI, multivitamin use, history of diabetes, random assignment to aspirin or placebo, vigorous exercise, alcohol intake, red meat intake	0.76 (0.59, 0.98)	0.02
Lin J, 2004 (141)	USA; Women's Health Study; 37547 F	FFQ; quintiles	ω3PUFAs	0.21 vs. 0.03 %energy	colorectal	202	age, random treatment assignment, BMI, FH of CRC, history of colorectal polyps, PA, cigarette smoking, alcohol consumption, postmenopausal HRT, and total energy intake	1.11 (0.73, 1.69)	0.43
Kobayashi M, 2004 (171)	Japan; Japan Public Health Centre-based Study; 95.376 FM	FFQ; quartiles	EPA	F 0.31 vs. 0.06 g/d	colon	156	age, area, FH of CRC,	1.04 (0.60, 1.80)	0.87
					rectum	68	BMI, PA, smoking,	0.57 (0.29, 1.15)	0.27
				M 0.39 vs. 0.07 g/d	colon	300	alcohol, use of vitamin	1.06 (0.71, 1.58)	0.74
					rectum	154	supplements, energy,	1.37 (0.81, 2.32)	0.28
			DHA	F 0.50 vs. 0.11 g/d	colon	156	cereal, vegetable and	1.08 (0.63, 1.87)	0.59
					rectum	68	meat intake	0.66 (0.33, 1.33)	0.47
				M 0.64 vs. 0.14 g/d	colon	300		0.98 (0.66, 1.46)	0.93
					rectum	154		1.17 (0.70, 1.96)	0.61
Terry P, 2001 (173)	Sweden; Swedish Mammography Screening Cohort 61463 F	FFQ; quartiles	ALA	0.70 vs. 0.45 g/d	colorectal	460	age, BMI, education level,	0.99 (0.75, 1.32)	0.99
			EPA	0.09 vs. 0.03 g/d			energy intake, intakes of	0.96 (0.72, 1.28)	0.91
			DHA	0.18 vs. 0.08 g/d			red meat and alcohol,	0.90 (0.60, 1.20)	0.49
							dietary fibre, calcium, vitamin C, folic acid,		

							vitamin D, SFAs, MUFAs, PUFAs		
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111M	modified dietary history; quartiles	ω3PUFAs (from fish)	0.7 vs. 0.2 g/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	1.2 (0.8, 1.9)	0.84
Bostick RM, 1994 (147)	USA; Iowa's Women's Health; 32215 F	FFQ; quintiles	ω3PUFAs	>0.18 vs. <0.03 g/d	colon	212	age, energy (residual), height, parity, vitamin E, vitamin E x age term, vitamin A supplement	0.70 (0.45, 1.09)	0.26

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; ω3PUFAs: omega 3 poly-unsaturated fatty acids; SFAs: saturated fatty acids; MUFAs: mono-unsaturated fatty acids; PUFAs: poly-unsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ALA: α-linolenic acid; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 13 Colorectal cancer risk and omega-3 poly-unsaturated fatty acids; Results from published case-control and nested case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Hall MN, 2007 (169)	USA; Physicians' Health Study; 460M	blood measurements; quartiles	ω3PUFAs	>6.06 vs. <4.43 %TF	colorectal	178	BMI, multivitamin use, history of diabetes, random assignment to aspirin or placebo, vigorous exercise, alcohol intake, red meat	0.60 (0.32, 1.11)	0.10
Theodoratou E, 2007‡ (164)	Scotland; SOCCS; 2910FM	FFQ; quartiles	ω3PUFAs	≥2.82 vs. <1.85 g/d	colorectal	1458	matched on age, sex, are of residence; adjusted for FH, total energy intake (residual), fibre intake, alcohol intake, use of NSAIDs, smoking, BMI, PA	0.63 (0.50, 0.80)	<0.0005
Kimura Y, 2007 (158)	Japan; Fukuoka Colorectal Cancer Study; 1575FM	FFQ; quintiles	ω3PUFAs	3.94 vs. 1.99 g/d	colorectal	782	Energy (residual), age, sex, residential area, BMI 10 years before, parental CRC, smoking, alcohol use, type of job, leisure-time PA, dietary calcium and fibre intake	0.74 (0.52, 1.06)	0.05
Kuriki K, 2006 (160)	Japan; 295FM	Erythrocyte measurements using gas-liquid chromatography; tertiles	ω3PUFAs	>9.75 vs. <7.98 mol%	colorectal	74	BMI, habitual exercise, drinking and smoking status, green-yellow vegetable intake, and FH colorectal cancer	0.41 (0.15, 1.09)	0.16

Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	ω3PUFAs	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin use	0.89 (0.61, 1.30) 0.85 (0.57, 1.27)	0.72 0.37
Kojima M, 2005 (159)	Japan; Japan Collaborative Cohort Group; 650 FM	serum; quartiles	ω3PUFAs	M ≥12.0 vs. <7.7 weight % of total serum lipids F ≥11.0 vs. <7.8 weight % of total serum lipids	colorectal	83 86	matched on age and participating institution; adjusted for FH of CRC, BMI, education, smoking, alcohol, green leafy vegetable intake, PA	0.24 (0.08, 0.76) 0.85 (0.38, 1.91)	0.08 0.96
Tavani A, 2004 (172)	Italy/ Switzerland; 7045 FM	FFQ; quintiles	ω3PUFAs	>1.46 vs. <0.55 g/w	colorectal	2280	age, sex, study centre, education, BMI, energy, alcohol, smoking, PA	0.7 (0.6, 0.9)	<0.0001
Nkondjock A, 2003 (161)	Canada; 1070 FM	FFQ; quartiles	ω3PUFAs	>2.92 vs. <1.46 g/d	colorectal	402	energy (residual), age, marital status, history of colorectal cancer in first-degree relatives, BMI one year prior to diagnosis, and PA	0.73 (0.51, 1.05)	0.02
Slattery ML, 1997 (163)	USA; 4403 FM	CARDIA diet history; quintiles	ω3PUFAs	M >3.36 vs. <2.14 g/MJ F >0.84 vs. <0.22 g/MJ	colon	1095 888	age at diagnosis or selection, energy intake, dietary fibre, cholesterol, calcium, BMI, physical activity, FH of CRC, NSAIDs	1.00 (0.76, 1.31) 0.89 (0.66, 1.22)	

Le Marchand L, 1997 (150)	USA (Hawaii); 2384 FM	FFQ; quartiles	ω3PUFAs	M >2.6 vs. <1.7 g/d F >2.1 vs. <1.3 g/d	colorectal	698 494	age, FH of CRC, alcoholic drinks/week, pack-years, lifetime recreational PA, BMI five years ago, and caloric, dietary fibre and calcium intakes; energy (residual)	0.8 (0.5, 1.1) 1.4 (0.9, 2.2)	0.1 0.4

* Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; ω3PUFAs: omega 3 poly-unsaturated fatty acids; SFAs: saturated fatty acids; MUFAs: mono-unsaturated fatty acids; PUFAs: poly-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

† P-value for trend

‡ Results that are part of the current thesis and will be presented in detail in the following chapters

Table 14 Colorectal cancer risk and omega-6 poly-unsaturated fatty acids; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Lin J, 2004 (141)	USA; Women's Health Study; 37547 F	FFQ; quintiles	ω6PUFAs	7.6 vs. 3.8 % energy	colorectal	202	age, random treatment assignment, BMI, FH of CRC, history of colorectal polyps, PA, cigarette smoking, alcohol consumption, postmenopausal HRT, and total energy intake	1.60 (0.98, 2.60)	0.16
Terry P, 2001 (173)	Sweden; 61463 F	FFQ; quartiles	linoleic acid	7.4 vs. 3.7 g/d	colorectal	460	age, BMI, education level, energy intake, intakes of red meat and alcohol, dietary fibre, calcium, vitamin C, folic acid, vitamin D, SFAs, MUFAs, PUFAs	1.06 (0.78, 1.45)	0.53
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111M	modified dietary history; quartiles	linoleic acid	16.4 vs. 4.5 g/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	1.3 (0.8, 2.0)	0.20

[*] Abbreviations: F: females; FFQ: food frequency questionnaire; ω6PUFAs: omega 6 poly-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 15 Colorectal cancer risk and omega-6 poly-unsaturated fatty acids; Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Hall MN, 2007 (169)	USA; Physicians' Health Study; 460 M	blood measurements; quartiles	ω6PUFAs	>40.1 vs. <36.1 %TF	colorectal	178	BMI, multivitamin use, history of diabetes, random assignment to aspirin or placebo, vigorous exercise, alcohol intake, red meat intake.	0.64 (0.35, 1.17)	0.16
Theodoratou E, 2007‡ (164)	Scotland; SOCCS; 2910 FM	FFQ; quartiles	ω6PUFAs	≥13.12 vs. <8.90 g/d	colorectal	1458	matched on age, sex, are of residence; adjusted for FH, total energy intake (residual method), fibre intake, alcohol intake, use of NSAIDs, smoking, BMI, PA	1.03 (0.81, 1.30)	0.86
Kimura Y, 2007 (158)	Japan; Fukuoka Colorectal Cancer Study; 1575 FM	FFQ; quintiles	ω6PUFAs	15.23 vs. 7.98 g/d	colorectal	782	Energy (residual), age, sex, residential area, BMI 10 years before, parental CRC, smoking, alcohol use, type of job, leisure-time PA, dietary calcium and fibre intake	0.77 (0.54, 1.10)	0.17
Kuriki K, 2006 (160)	Japan; 295 FM	Erythrocyte measurements using gas-liquid chromatography; tertiles	ω6PUFAs	>21.51 vs. <19.74 mol%	colorectal	74	BMI, habitual exercise, drinking and smoking status, green-yellow vegetable intake, and FH colorectal cancer	0.24 (0.10, 0.59)	<0.005

Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	ω6PUFAs	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin	0.84 (0.57, 1.24) 0.97 (0.65, 1.45)	0.77 0.78
Kojima M, 2005 (159)	Japan; Japan Collaborative Cohort Group; 650 FM	serum; quartiles	ω6PUFAs	M ≥36.1 vs. <28.9 weight % of total serum lipids F ≥37.5 vs. <31.9 weight % of total serum lipids	colorectal	83 86	matched on age and participating institution; adjusted for FH of CRC, BMI, education, smoking, alcohol, green leafy vegetable intake, PA	0.69 (0.30, 1.61) 1.15 (0.48, 2.75)	0.36 0.32
Koh WP, 2004 (174)	China (Singapore); Singapore Chinese Health Study; 1487 FM	FFQ; quartiles	ω6PUFAs	high vs. low quartile	colorectal	310	age, year of recruitment, gender, dialect, education, BMI, smoking, alcohol, FH of CRC	1.04 (0.63, 1.70)	
Nkondjock, 2003 (161)	Canada; 1070 FM	FFQ; quartiles	ω6PUFAs	>18.06 vs. 19.05 g/d	colorectal	402	energy (residual), age, marital status, history of colorectal cancer in first-degree relatives, BMI one year prior to diagnosis, and PA	1.07 (0.76, 1.54)	0.27
Slattery ML, 1997 (163)	USA; 4403 FM	CARDIA diet history; quintiles	linoleic acid	M: >30.9 vs. <18.4 g/MJ F: >7.31 vs. <4.24	colon	1095 888	age at diagnosis or selection, energy intake, dietary fibre, cholesterol, calcium, BMI, physical activity, FH of CRC, NSAIDs	1.12 (0.85, 1.47) 1.07 (0.79, 1.46)	

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; ω6PUFAs: omega 6 poly-unsaturated fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

Table 16 Colorectal cancer risk and *trans* fatty acids; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Limburg PJ, 2008 (176)	USA; Iowa Women's Health Study; 35216 F	FFQ; quartiles	*t*FAs	>3.28 vs. ≤1.96 g/d	colorectal	1229	age, total energy intake (residual), body mass index, physical activity level, oestrogen use, self-reported diabetes mellitus, smoking status, and intake of total fat, red meat, fruits and vegetables, calcium, folate, vitamin E and alcohol	1.06 (0.88, 1.28)	0.40
Lin J, 2004 (141)	USA; Women's Health Study; 37547 F	FFQ; quintiles	*t*FAs	1.9 vs. 0.6% energy	colorectal	202	age, random treatment assignment, BMI, FH of CRC, history of colorectal polyps, PA, cigarette smoking, alcohol consumption, postmenopausal HRT, and total energy intake	1.30 (0.89, 2.05)	0.18
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111 M	modified diet history; quartiles	*t*FAs	5.7 vs. 1.8 g/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	1.1 (0.7, 1.6)	0.49

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; *t*FAs: trans fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 17 Colorectal cancer risk and *trans* fatty acids; Results from published case-control studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Fatty acid subgroup	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p[†]
Theodoratou E, 2007[‡] (164)	Scotland; SOCCS; 2910FM	FFQ; quartiles	*t*FAs	≥4.24 vs. <2.88 g/d	colorectal	1458	matched on age, sex, are of residence; adjusted for FH, total energy intake (residual method), fibre intake, alcohol intake, use of NSAIDs, smoking, BMI, PA	1.28 (1.01, 1.62)	0.07
Nkondjock, 2003 (161)	Canada; 1070 FM	FFQ; quartiles	*t*FAs	>1.60 vs. 0.32 g/d	colorectal	402	energy (residual), age, marital status, history of colorectal cancer in first-degree relatives, BMI one year prior to diagnosis, and PA	0.83 (0.58, 1.19)	0.31
Slattery ML, 2001 (175)	USA; 4403 FM	CARDIA diet history; quintiles	*t*FAs	M: >3.34 vs. ≤1.69 g/1000kcal	colon	1149	age, BMI, PA, energy, fibre and calcium intake, oestrogen status (women)	1.2 (0.9, 1.7)	0.34
				F: >2.99 vs. ≤1.69 g/1000kcal		894		1.5 (1.1, 2.0)	0.04

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; *t*FAs: trans fatty acids; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

3.4 Folate, vitamin B2, vitamin B6, vitamin B12

3.4.1 Introduction

Folate, vitamin B2 (riboflavin), vitamin B6 and vitamin B12 are water soluble vitamins that occur naturally in food. In addition, their synthetic forms can be taken as supplements. Their main dietary sources of folate are: broccoli, brussels sprouts, asparagus, peas, chickpeas, brown rice and fortified breakfast cereals; of vitamin B2 are: milk, eggs, fortified breakfast cereals, rice and mushrooms; of vitamin B6 are: poultry, fish, meat, legumes, nuts, potatoes, whole grains and fortified breakfast cereals; and of vitamin B12 are: meat, salmon, cod, milk, cheese, eggs, yeast extract, and fortified breakfast cereals (Food Standards Agency). The recommended daily intake for adults for folate is 0.2mg (0.4mg for pregnant women), for vitamin B2 1.3mg for men and 1.1mg for women, for vitamin B6 1.4mg for men and 1.2mg for women and for vitamin B12 0.0015mg (Food Standards Agency).

The metabolic pathway of folate, also known as one-carbon metabolism is very important for DNA synthesis, repair and methylation and vitamins B2, B6 and B12 act as co-enzymes in different steps of the pathway (Figure 30). Briefly, folate or folic acid is converted initially to 5,10-methylene thetrahydrofolate (5,10-MTHF; co-enzyme: vitamin B6), which is the compound needed for the nucleotide synthesis and DNA methylation. 5,10-MTHF is then converted to 5-MTHF by the enzyme MTHF reductase (MTHFR) and the co-enzyme vitamin B2. In the next step, 5,10-MTHF gives homocysteine and methionine and the enzyme that catalyses the latter reaction is methionine synthase (MTR; co-enzyme: vitamin B12) which is activated by the MTR reductase (MTRR) (177;178). In the final step homocysteine, through the action of the enzyme cystathionine β-synthase (CBS) and the co-enzyme vitamin B6, is catabolised to glutathione, a detoxification enzyme that inactivates many carcinogenic compounds and protect cells from oxidative stress and DNA damage (177-179) (Figure 30).

The role of folate in preventing neural tube defects (NTDs) is well established and in 1998 mandatory folic acid fortification was introduced in the USA and Canada in order to reduce the number of children born with that defect (180). Indeed, the rate of NTDs

during the full fortification period (2000-2002) was decreased by 46% in Canada when compared to the pre-fortification period (1993-1997) (181). In 2007, the Standing Advisory Committee on Nutrition of the United Kingdom decided that mandatory folic acid fortification (by adding folic acid to either bread or flour) should be introduced also in the UK (182). However, a recent temporal study reported a statistically significant increase in colorectal cancer absolute rates both in the USA and Canada for the period that followed the full folic-acid fortification (180). And since it has been hypothesised that folate and in particular folic acid might have some enhancing effects on cancer, including colorectal cancer (183), folic acid fortification in the UK has been postponed until the release of the results of two clinical trials investigating the relationship between folic acid and several types of cancer.

3.4.2 Evidence from observational studies

According to the WCRF/AICR second report (2007), there is limited evidence that folate is inversely associated with colorectal cancer, and due to the inconsistency of the results of the different studies, residual confounding from other nutrients (e.g. fibre) cannot be ruled out (30). In addition, a meta-analysis, which was published in 2005 and included seven cohort and nine case-control studies, reported that there is some evidence of a protective effect of dietary folate (and not supplementary folic acid) on colorectal cancer, but due to significant heterogeneity (particularly among case-control studies) this effect might be confounded by other dietary nutrients (e.g. fibre) (184). We identified 14 cohort (Table 18) and 24 case-control studies (Table 19) that investigated the association between folate/folic acid and colorectal cancer (166;167;185-220). Four cohort and five case-control studies reported a statistically significant inverse association between dietary folate (185;190;192;193;199;201;206;218;220) and colorectal cancer, two case-control studies reported a statistically significant inverse association between serum folate levels and colorectal cancer (198;209) and one case-control study reported a statistically significant inverse association between supplementary folic acid and colon cancer (219).

A smaller number of observational studies have investigated the associations between colorectal cancer and vitamin B2, vitamin B6 and vitamin B12. We identified: one

cohort (Table 20) and six case-control studies (Table 21) investigating the association between vitamin B2 and colorectal cancer (185;203;206;207;212;221;222), with only one case-control study reporting a statistically significant inverse association (221); four cohort (Table 22) and 11 case-control (Table 23) investigating the association between vitamin B6 and colorectal cancer (167;185;195;196;200;203;206;207;212;213;218;221; 223-225), with three cohort studies and five case-control studies reporting a statistically significant inverse association between colorectal cancer and dietary vitamin B6 (196;200;213;218;221;223-225) and one cohort study reporting a statistically significant positive association between rectal cancer and total vitamin B6 (195); and two cohort (Table 24) and nine case-control (Table 25) investigating the association between vitamin B12 and colorectal cancer (167;185;195;196;200;203;206;207;209;212;226), with one cohort study reporting a significant positive association between dietary vitamin B12 and colorectal cancer (196).

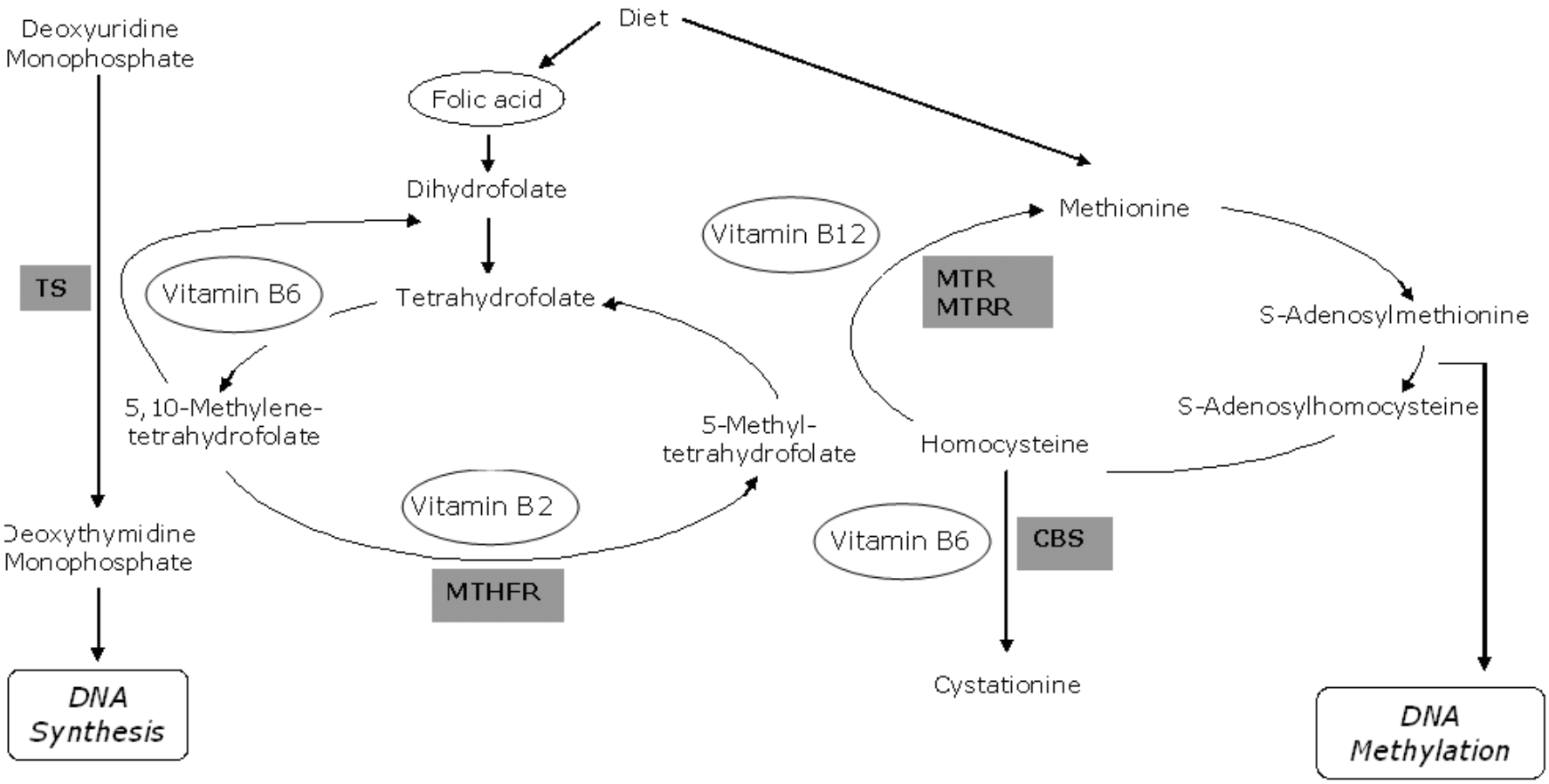


Figure 30 Simplified diagram of the one-carbon (folate) metabolic pathway. Adapted from Sharp & Little, AJE, 2007

Table 18 Colorectal cancer risk and folate; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Ishihara J, 2007 (196)	Japan; Japan Public Health Centre-based Prospective Study; 81184 FM	FFQ; quartiles	folate; Diet	M: 530 vs. 214 µg/d F: 564 vs. 238 µg/d	colorectal	335 191	age, alcohol, smoking, BMI, supplement use, PA, calcium, vitamin D, meat intake, study area	1.20 (0.85, 1.71) 1.33 (0.85, 2.09)	0.46 0.14
de Vogel S, 2006 (189)	The Netherlands; Netherlands Cohort Study; 4728 FM	FFQ; tertiles	folate; Diet	M: 279.9 vs. 162.7 µg/d F: 248.0 vs. 142.5 µg/d	colon	213 186	age, FH, BMI, iron, fibre, energy, riboflavin, vitamin B6, vitamin C, and methionine	0.96 (0.61, 1.54) 0.82 (0.45, 1.49)	0.84 0.53
Rossi E, 2006 (210)	Australia; 1969 & 1978 Busselton Health survey; 1035 FM	serum and red cell; quartiles	folate	Serum: <2.99 µg/l vs. ≥6.00 Red cell: <199.9 vs. ≥350.0 µg/l (high quartile is the reference category)	colorectal	41	age, sex, smoking, alcohol, BMI	2.15 (0.73, 6.31) 2.00 (0.82, 4.83)	
Zhang SM, 2006 (218)	USA Women's Health Study; 37916 F	FFQ; quintiles	folate; Diet + Supplements	≥614 vs. <259 µg/d (total) ≥385 vs. <244 µg/d (diet) ≥385 vs. <244 µg/d (diet, excluding supplement users)	colorectal	220 220 139	age, randomised treatment assignment, BMI, FH of CRC, history of colon polyps, PA, smoking status, red meat, alcohol, energy, menopausal status, HRT, aspirin	1.16 (0.76, 1.79) 0.67 (0.43, 1.03) 0.46 (0.26, 0.81)	0.46 0.21 0.02
Brink M, 2005 (187)	The Netherlands, Netherlands Cohort Study; 3656 FM	FFQ; per 100 µg/d increase	folate; Diet	M: per 100 µg/d increase F: per 100 µg/d increase	colon rectal colon rectal	231 99 199 51	age, BMI, smoking, alcohol, fresh meat, energy, FH of CRC, vitamin C, iron, fibre	0.87 (0.66, 1.14) 0.58 (0.36, 0.93) 0.98 (0.62, 1.56) 1.85 (1.13, 3.02)	

Larsson SC, 2005 (202)	Sweden; Swedish Mammography Cohort; 61433 F	FFQ; quintiles	folate; Diet	≥212 vs. <150 µg/d	colorectal	805	age, BMI, education, energy, intake of red meat, SF, calcium, vitamin B6, beta-carotene, cereal fibre	0.80 (0.60, 1.06)	0.11
Wei EK, 2004 (217)	USA; Nurses' Health Study, Health Professionals Follow-Up Study; 87733 F, 46632 M	FFQ; quartiles	folate; Diet	>400 vs. ≤200 µg/d	colon rectal	1139 339	age, FH, BMI, PA, beef, pork or lamb as a main dish, processed meat, alcohol, calcium, height, pack-years smoking before age 30, history of endoscopy, sex	0.82 (0.68, 0.99) 1.18 (0.80, 1.74)	0.06 0.83
Konings EJM, 2002 (199)	The Netherlands; The Netherlands Cohort Study; 120852 FM	FFQ; quintiles	folate; Diet	M: >266 vs. <168 µg/d F: >243 vs. <150 µg/d	colon rectal colon rectal	400 259 360 152	age, alcohol intake, energy intake, FH of CRC, iron intake, vitamin C intake, and dietary fibre intake	0.73 (0.46, 1.17) 0.66 (0.35, 1.21) 0.68 (0.39, 1.20) 1.26 (0.58, 2.76)	0.03 0.03 0.18 0.55
Flood A, 2002 (191)	USA; Breast Cancer Detection Demonstration Project; 45264 F	FFQ; quintiles	folate; Diet + Supplements	Diet: >272 vs. <142 µg/d Total: >633 vs. <188 µg/d	colorectal	490	energy, methionine, and alcohol, and for total fat (for the analysis of the total fat)	0.86 (0.65, 1.13) 1.01 (0.75, 1.35)	0.14 0.67
Terry P, 2002 (215)	Canada; Canadian National Breast Screening Study; 5629 F	FFQ; quintiles	folate; Diet	>367 vs. ≤233 µg/d	colorectal	295	age, smoking, BMI, hours of vigorous PA, education, and intakes of total fat and energy	0.6 (0.4, 1.1)	0.25
Harnack L, 2002 (195)	USA; Iowa Women's Health Study; 32215 F	FFQ; quintiles for colon, tertiles for rectal cancer	folate; Diet + Supplements	>634.03 vs. <231.12 µg/d >463.37 vs. <281.85 µg/d	colon rectal	598 123	age, pack-years of cigarettes, BMI, oestrogen use, and intakes of calcium, vitamin E and energy	1.12 (0.77, 1.63) 0.89 (0.52, 1.51)	0.67 0.44

Su JL, 2001 (214)	USA; NHANES I Epidemiologic Follow-up Study; 14407 FM	24-hour recall interview; quartiles	folate; Diet	>249.0 vs. <103.3 µg/d	colon	219	baseline age, race, gender, education level, dietary intakes of calories, fat, vitamin B6, vitamin B12, alcohol	0.57 (0.34, 0.97)	0.18
Giovannucci E, 1998 (193)	USA; Nurses' Health Study; 88756 F	FFQ; quartiles	folate; Diet + Supplements	>400 vs. ≤200 µg/d	colon	442	energy, smoking, FH of CRC; PA, BMI, aspirin use; and intakes of red meat, alcohol, and fibre	0.69 (0.52, 0.93)	0.01
Sellers TA, 1998 (220)	USA; Iowa Women's Health Study; 35216 F	FFQ; tertiles	folate; Diet	No FH of CRC >413.49 vs. ≤255.38 µg/d	colon	180	age, energy, history of rectal cancer polyps	0.7 (0.5, 1.0)	0.05
				FH of CRC >413.49 vs. ≤255.38 µg/d		62		0.9 (0.5, 1.7)	0.8

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

Table 19 Colorectal cancer risk and folate; Results from published case-control studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p[†]
Sharp L, 2008 (212)	Scotland; 672 FM	FFQ; quartiles	folate; Diet + Supplements	≥348.6 vs. ≤263.9 µg/d	colorectal	264	sex, age, total energy, PA, FH of CRC, NSAIDs, sex × NSAID	1.37 (0.79, 2.36)	0.40
Otani T, 2008 (208)	Japan; nested case-controls of Public Health Centre-based prospective study; 1125 FM	plasma measurement; quartiles	folate	M: ≥8.6 vs. <5.6 ng/m F: ≥10.6 vs. <6.6 ng/ml	colorectal	163 160	matched pairs with adjustment for pack-years of smoking, alcohol, BMI, PA, vitamin supplement use, and FH of CRC	0.86 (0.45, 1.60) 1.00 (0.56, 1.90)	0.88 0.63
Coogan PF, 2007 (188)	USA; 2394 FM	FFQ; quartiles	folate; Diet	≥370.6 vs. ≤216.5 µg/d	colorectal	1229	age, sex, NSAIDs, screening colonoscopy, doctor visits 2 years before index date, alcohol, education, calcium supplement use, vitamin E use, SF, cholesterol, fibre, methionine, energy, folate containing supplement use	0.7 (0.4, 1.1)	0.1
Murtaugh MA, 2007 (206)	USA; 1730 FM	Dietary history (CARDIA); tertiles	folate; Diet + Supplements	Diet: >475 vs. ≤323 µg/d Total: >743 vs. ≤441 µg/d	rectal	751	age, sex, BMI, PA, energy, fibre, calcium, ibuprofen use, and smoking	0.66 (0.48, 0.92) 0.82 (0.64, 1.05)	0.01 0.09
Van Guelpen B, 2006 (216)	Sweden; nested case-control of Northern Sweden Health and Disease Cohort; 663 FM	plasma measurement; quintiles	folate	M: >11.3 vs. <5.1 µmol/l F: >13.0 vs. <5.7 µmol/l	colorectal	226	BMI, current smoking, recreational and occupational PA, and alcohol intake	1.34 (0.72, 2.50)	0.33

Kune G, 2006 (200)	Australia; 1442 FM	FFQ; quintiles	folate; Diet	>419 vs. <246 μg/d	colorectal	715	age, sex, alcohol, BMI, energy intake, FH of CRC, oral contraceptive pill use, cigarette pack-years, aspirin use	1.24 (0.81, 1.89)	
Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	folate; Diet	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin use	0.75 (0.51, 1.11) 0.81 (0.53, 1.23)	0.32 0.20
Jiang Q, 2005 (197)	China; 469 FM	FFQ; quartiles	folate; Diet	≥172.08 vs. <115.64 μg/d	colon rectal	53 73	sex, age, methionine, smoking status, drinking status, and zinc	0.91 (0.69, 1.19) 1.39 (0.56, 3.50)	0.41 0.41
Otani T, 2005 (207)	Japan; 331 FM	FFQ; tertiles	folate; Diet	≥485 vs. <343 μg/d	colorectal	107	Matched on sex, age, residence area; adjusted for smoking, alcohol consumption, BMI, dietary fibre intake	1.3 (0.49, 3.4)	0.62
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	folate; Diet	M: >350.3 vs. <79.8 μg/d F: >300.7 vs. <116.8 μg/d	colorectal	171	age, sex, energy, BMI, PA	1.1 (0.6, 2.0)	0.96
Satia-Abouta J, 2003 (211)	USA; North Carolina Colon Cancer Study; 1609 FM	FFQ; quartiles	folate; Diet + Supplements	Whites : 741 vs. 196 μg/d African/Americans: 642 vs. 147 μg/d	colon	337 276	energy, other potential confounders examined include age, sex, education, BMI, smoking, PA, FH of CRC, NSAIDs, supplement use, fat, dietary fibre, calcium, folate, fruits, vegetables	0.8 (0.5, 1.2) 0.9 (0.5, 1.6)	0.11 0.70
Pufulete M, 2003 (209)	UK; 104 FM	FFQ; serum	folate; score based	high vs. low tertile	colorectal	28	sex, age, BMI, smoking, and alcohol intake	0.09 (0.01, 0.57)	0.01

		measurements; erythrocyte measurements; tertiles	on dietary intakes and serum and erythrocyte measurements						
La Vecchia C, 2002 (201)	Italy; 6107 FM	FFQ; quintiles	folate; Diet	>330.8 vs. <197.6 µg/d	colorectal	1953	energy, sex, age, study centre, education, PA and FH	0.72 (0.60, 0.86)	0.01
Le Marchand L, 2002 (203)	USA; 1454 FM	FFQ; quintiles	folate; Diet + Supplements	Diet : >406 vs. ≤252 µg/d Total: >2430 vs. ≤297 µg/d	colorectal	727	Matched on sex, age, ethnicity; adjusted for energy (residual method), pack-years of cigarette smoking, lifetime recreational PA, lifetime aspirin use, BMI, years of schooling, intakes of non-starch polysaccharides from vegetables, calcium from foods and supplements	0.9 (0.6, 1.3) 0.8 (0.6, 1.1)	0.43 0.23
Levi F, 2000 (204)	Switzerland; 714 FM	FFQ ; tertiles	folate; Diet	1144.9 vs. 431.2 µg/d	colorectal	223	age, sex, years of education, smoking, alcohol, BMI, PA, total energy and fibre intake	1.54 (0.8, 3.1)	>0.05
Kato I, 1999 (198)	USA ; nested case-control of New York University Women's Health Study cohort; 628 F	FFQ and serum measurements; quartiles	folate; Diet + Supplements	FFQ: ≥626 vs. ≤224 µg/d Serum: ≥31.04 vs. ≤12.23 nmol/l	colorectal	105	FH of CRC, beer intake, prior occult blood testing and number of hours spent in sport activities in their early 30	0.88 (0.46, 1.69) 0.52 (0.27, 0.97)	0.67 0.04

Slattery ML, 1997 (213)	USA; 4403 FM	Diet history (CARDIA); quartiles	folate; intakes only from plant foods	M: ≥210 vs. ≤120 µg/1000 kcal	colon	1,099	age, BMI, lifetime vigorous leisure time PA, use of	1.2 (0.8, 1.6)	0.70
				F: ≥230 vs. ≤130 µg/1000kcal		849	aspirin/ NSAIDs, presence or absence of a first degree relative with CRC, total energy intake, calcium	0.9 (0.6, 1.3)	0.38
White E, 1997 (219)	USA; 871 FM	Supplements questionnaire; 3 categories	folic acid; Supplements	≥400 vs. 0 µg/d	colon	444	age, sex	0.51 (0.34, 0.77)	<0.001
Glynn SA, 1996 (194)	Finland; Alpha-Tocopherol Beta-Carotene Study; 385 M	FFQ and serum; quartiles	folate; Diet and Supplements	FFQ: 388 vs. 268 µg/d	colon	86	total energy intake, and energy-adjusted intakes of vitamin A and starch (residuals)	0.51 (0.20, 1.31)	0.15
					rectal	50		2.12 (0.43, 2.54)	0.26
				Serum:>5.2 vs. ≤2.9 ng/ml	colon	86		0.96 (0.40, 2.30)	0.83
					rectal	50		2.94 (0.84, 10.33)	0.10
Boutron-Ruault MC, 1996 (186)	France; 480 FM	diet history; quintiles	folate: Diet	M: >360 vs. <110 µg/d F: >320 vs. <185 µg/d	colorectal	171	-	1.00 (0.5, 2.00)	>0.05
Ferraroni M, 1994 (190)	Italy; 3350 FM	FFQ; quintiles	folate; Diet	>261.49 vs. <162.63 µg/d	colorectal	1326	age, sex, education, FH of CRC, BMI, energy	0.52 (0.40, 0.68)	<0.05
Meyer F, 1993 (205)	USA; 838 FM	FFQ; quartiles	folate; Diet	high vs. low quartile	colon	424	age, interviewer, dietary energy, alcohol, fibre	M: 2.08 F: 0.73	
Benito E, 1991 (185)	Spain; 784 FM	FFQ; quartiles	folate; Diet	>227 vs. <146 µg/d	colorectal	286	energy, age, sex, weight	0.61	<0.05
Freudenheim JL, 1991 (192)	USA, 1600 FM	FFQ; quartiles (tertiles for female rectal)	folate; Diet	M: >380 vs. <240 µg/d	colon	205	energy	1.03 (0.56, 1.89)	>0.05
				>385 vs. <250 µg/d	rectal	227		0.31 (0.16, 0.59)	<0.001
				F: >340 vs. <210 µg/d	colon	223		0.69 (0.36, 1.30)	>0.05
				>310 vs. <220 µg/d	rectal	145		0.50 (0.24, 1.03)	>0.05

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

Table 20 Colorectal cancer risk and vitamin B2 (riboflavin); Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Shin A, 2006 (222)	China; Shanghai Women's Health Study; 73314 F	FFQ; quintiles	vitamin B2; Diet	>1.12 vs. ≤0.61mg/d	colorectal	283	age, menopausal status, education, cigarette smoking, alcohol consumption, exercise, FH of CRC, vitamin supplements use and calorie intake	1.4 (0.9, 2.4)	0.36

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 21 Colorectal cancer risk and vitamin B2 (riboflavin); Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Sharp L, 2008 (212)	Scotland; 672 FM	FFQ; quartiles	vitamin B2; Diet + Supplements	≥2.49 vs. ≤1.87 mg/d	colorectal	264	sex, age, total energy, PA, FH of CRC, NSAIDs, sex × NSAID	1.44 (0.83, 2.47)	0.17
Murtaugh MA, 2007 (206)	USA; 1730 FM	diet history (CARDIA); tertiles	vitamin B2; Diet + Supplements	Diet: >2.68 vs. ≤1.84 mg/d Total: >4.00 vs. ≤2.49 mg/d	rectal	751	age, sex, BMI, PA, energy, fibre, calcium, ibuprofen use, and smoking (pack-years)	1.27 (0.91, 1.77) 0.94 (0.73, 1.22)	0.19 0.65
Otani T, 2005 (207)	Japan; 331 FM	FFQ; tertiles	vitamin B2; Diet	≥1.85 vs. <1.49 mg/d	colorectal	107	Matched on sex, age, residence area; adjusted for smoking, alcohol consumption, BMI, dietary fibre intake	1.1 (0.52, 2.5)	0.64
Le Marchand L, 2002 (203)	USA; 1454 FM	FFQ; quintiles	vitamin B2; Diet + Supplements	Total: >13.31 vs. ≤1.52 mg/d	colorectal	727	Matched on sex, age, ethnicity; adjusted for energy (residual method), pack-years of cigarette smoking, lifetime recreational PA, lifetime aspirin use, BMI, years of schooling, intakes of non-starch polysaccharides from vegetables, calcium from foods and supplements	1.4 (1.0, 1.9)	0.13
La Vecchia C, 1997 (221)	Italy; 6107 FM	FFQ; quartiles	vitamin B2; Diet	≥2.23 vs. ≤1.29 mg/d	colorectal	1953	age, centre, sex, education, PA, energy, fibre	0.72 (0.6, 0.9)	<0.01

Benito E, 1991 (185)	Spain; 784 FM	FFQ; quartiles	vitamin B2; Diet	>1.87 vs. <1.17 µg/d	colorectal	286	energy, age, sex, weight	1.41	>0.05

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 22 Colorectal cancer risk and vitamin B6; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Ishihara J, 2007 (196)	Japan; Japan Public Health Centre-based Prospective Study; 81184 FM	FFQ; quartiles	vitamin B6; Diet	M: 1. 91 vs. 1.09 mg/d F: 1.80 vs. 1.02 mg/d	colorectal	335 191	age, alcohol, smoking, BMI, supplement use, PA, calcium, vitamin D, meat intake, study area	0.69 (0.48, 0.98) 1.10 (0.67, 1.83)	0.03 0.99
Zhang SM, 2006 (218)	USA Women's Health Study; 37,916 F	FFQ; quintiles	vitamin B6; Diet + Supplements	≥4.00 vs. <1.78 mg/d (total) ≥2.40 vs. <1.69 mg/d (diet) ≥2.40 vs. <1.69 mg/d (diet, excluding supplement users)	colorectal	220 220 139	age, randomised treatment assignment, BMI, FH of CRC, history of colon polyps, PA, smoking, red meat, alcohol, energy, menopausal status, HRT, aspirin	1.14 (0.77, 1.69) 0.84 (0.56, 1.27) 0.69 (0.41, 1.15)	0.07 0.18 0.05
Larsson SC, 2005 (223)	Sweden; Swedish Mammography Cohort; 61433 F	FFQ; quintiles	vitamin B6; Diet	≥2.05 vs. <1.53 mg/d	colorectal	805	age, BMI, education, energy, intake of red meat, SF, calcium, folate, beta-carotene, cereal fibre	0.66 (0.50, 0.86)	0.002
Harnack L, 2002 (195)	USA; Iowa Women's Health Study; 32215 F	FFQ; quintiles for colon, tertiles for rectal cancer	vitamin B6; Diet + Supplements	>4.35 vs. <1.59 mg/d >3.27 vs. <1.93 mg/d	colon rectal	598 123	age, pack-years of cigarettes, BMI, oestrogen use, and intakes of calcium, vitamin E and energy	0.95 (0.67, 1.36) 1.97 (1.08, 3.62)	0.88 0.03

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

Table 23 Colorectal cancer risk and vitamin B6; Results from published case-control studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p[†]
Sharp L, 2008 (212)	Scotland; 672 FM	FFQ; quartiles	vitamin B6; Diet + Supplements	≥3.04 vs. ≤2.29 mg/d	colorectal	264	sex, age, total energy, PA, FH of CRC, NSAIDs, sex × NSAID	1.07 (0.63, 1.81)	0.86
Theodoratou E, 2008[‡] (224)	Scotland; SOCCS study; 4750 FM	FFQ; quartiles	vitamin B6; Diet + Supplements	Diet: ≥3.26 vs. ≤2.55 mg/d Total: ≥3.39 vs. ≤2.58 mg/d	colorectal	2028	energy (residual), age, sex, folate, fibre, alcohol, smoking, BMI, PA NSAIDs, FH of CRC	0.77 (0.61, 0.98) 0.86 (0.69, 1.07)	0.03 0.12
Murtaugh MA, 2007 (206)	USA; 1730 FM	diet history (CARDIA); tertiles	vitamin B6; Diet + Supplements	Diet: >2.6 vs. ≤1.79 mg/d Total: >4.08 vs. ≤2.44 mg/d	rectal	751	age, sex, BMI, PA, energy, fibre, calcium, ibuprofen use, and smoking (pack-years)	0.92 (0.72, 1.17) 0.92 (0.72, 1.17)	0.59 0.46
Kune G, 2006 (200)	Australia; 1442 FM	FFQ; quintiles	vitamin B6; Diet	>3.4 vs. <1.7 mg/d	colorectal	715	age, sex, alcohol, BMI, energy intake, FH of CRC, oral contraceptive pill use, cigarette pack-years, aspirin use	0.52 (0.34, 0.80)	
Wei EK, 2005 (225)	USA; nested case-control of Nurses' Health Study; 544 F	FFQ; quartiles	vitamin B6; diet + Supplements	8.6 vs. 1.6 mg/d	colorectal	194	Matched on year of birth, month and year of blood collection, fasting status; adjusted for BMI, PA,	0.60 (0.34, 1.06)	0.03
			plasma PLP concentration; quartiles	131.2 vs. 23.9 pmol/ml		188	smoking, menopausal status, post menopausal HRT, duration of regular aspirin use, FH of CRC, intake of alcohol and red meat, plasma vitamin D, history of endoscopy	0.56 (0.31, 1.01)	0.07

Otani T, 2005 (207)	Japan; 331 FM	FFQ; tertiles	vitamin B6 diet	≥1.74 vs. <1.46 mg/d	colorectal	107	Matched on sex, age, residence area; adjusted for smoking, alcohol consumption, BMI, dietary fibre intake	0.88 (0.41, 1.9)	0.77
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	vitamin B6; Diet	M: >2.2 vs. <0.6 mg/d F: >1.7 vs. <0.7 mg/d	colorectal	171	age, sex, energy, BMI, PA	1.9 (0.9, 4.0)	0.13
Le Marchand L, 2002 (203)	USA; 1454 FM	FFQ; quintiles	vitamin B6; Diet	>2.46 vs. ≤1.69 mg/d	colorectal	727	Matched on sex, age, ethnicity; adjusted for energy (residual), smoking, lifetime recreational PA, lifetime aspirin use, BMI, years of schooling, intakes of non-starch polysaccharides from vegetables, calcium from foods and supplements	1.0 (0.7, 1.4)	0.74
La Vecchia C, 1997 (221)	Italy; 6,107 FM	FFQ; quartiles	vitamin B6; Diet	≥2.78 vs. ≤2.04 mg/d	colorectal	1953	age, centre, sex, education, PA, energy, fibre	0.53 (0.4, 0.7)	<0.001
Slattery ML, 1997 (213)	USA; 4403 FM	Diet history (CARDIA); quartiles	vitamin B6; intakes only from plant foods	M: ≥1.18 vs. ≤0.75 mg/1000 kcal F: ≥1.28 vs. ≤0.82 mg/1000kcal	colon	1,099 849	age, BMI, lifetime vigorous leisure time PA, use of aspirin/ NSAIDs, presence or absence of a first degree relative with CRC, total energy intake, calcium	0.7 (0.6, 1.0) 0.6 (0.5, 0.8)	<0.01 <0.01

Benito E, 1991 (185)	Spain; 784	FFQ; quartiles	vitamin B6; Diet	>2.20 vs. <1.40 mg/d	colorectal	286	energy, age, sex, weight	0.85	>0.05

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

Table 24 Colorectal cancer risk and vitamin B12; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Ishihara J, 2007 (196)	Japan; Japan Public Health Centre-based Prospective Study; 81184 FM	FFQ; quartiles	vitamin B12; Diet	M: 13.7 vs. 4.2 µg/d F: 12.8 vs. 4.0 µg/d	colorectal	335 191	age, alcohol, smoking, BMI, supplement use, PA, calcium, vitamin D, meat intake, study area	1.50 (0.96, 2.35) 1.70 (0.96, 3.01)	0.05 0.07
Harnack L, 2002 (195)	USA; Iowa Women's Health Study; 32215 F	FFQ; quintiles for colon, tertiles for rectal cancer	vitamin B6; Diet + Supplements	>18.36 vs. <5.12 µg/d >14.67 vs. <7.17 µg/d	colon rectal	598 123	age, pack-years of cigarettes, BMI, oestrogen use, and intakes of calcium, vitamin E and energy	0.94 (0.69, 1.27) 1.29 (0.78, 2.14)	0.86 0.35

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

Table 25 Colorectal cancer risk and vitamin B12; Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Dahlin AM, 2008 (226)	Sweden; Northern Sweden Health and Disease Study; 678 FM	plasma measurements; quintiles	plasma vitamin B12	M: ≥351.2 vs. <220.2 pmol/L F: ≥391.9 vs. <232.1 pmol/L	colorectal	226	BMI, current smoking, recreational and occupational PA, alcohol, and plasma folate and total homocysteine	0.82 (0.46, 1.45)	0.71
Sharp L, 2008 (212)	Scotland; 672 FM	FFQ; quartiles	vitamin B12; Diet + Supplements	≥7.98 vs. ≤5.25 µg/d	colorectal	264	sex, age, total energy, PA, FH of CRC, NSAIDs, sex × NSAID	0.95 (0.56, 1.62)	0.73
Murtaugh MA, 2007 (206)	USA; 1730 FM	diet history (CARDIA); tertiles	vitamin B12; Diet + Supplements	Diet: >6.57 vs. ≤3.92 µg/d Total: >11.2 vs. ≤6.09 µg/	rectal	751	age, sex, BMI, PA, energy, fibre, calcium, ibuprofen use, and smoking (pack-years)	1.13 (0.86, 1.51) 0.91 (0.71, 1.15)	0.37 0.37
Kune G, 2006 (200)	Australia; 1442 FM	FFQ; quintiles	vitamin B12; Diet	>11.1 vs. <4.1 µg/d	colorectal	715	age, sex, alcohol, BMI, energy intake, FH of CRC, oral contraceptive pill use, cigarette pack-years, aspirin use	0.49 (0.34, 0.71)	
Otani T, 2005 (207)	Japan; 331 FM	FFQ; tertiles	vitamin B12; Diet	≥11.2 vs. <7.3 µg/d	colorectal	107	Matched on sex, age, residence area; adjusted for smoking, alcohol, BMI, dietary fibre intake	1.1 (0.55, 2.2)	0.77
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	vitamin B12; Diet	M: >13.5 vs. <2.0 µg/d F: >9.87 vs. <2.0 µg/d	colorectal	171	age, sex, energy, BMI, PA	1.4 (0.8, 2.5)	0.21
Pufulete M, 2003 (209)	UK; 104 FM	serum measurements; tertiles	vitamin B12	high vs. low tertile µg/l	colorectal	28	sex, age, BMI, smoking, and alcohol intake	0.25 (0.04, 1.72)	0.22

Le Marchand L, 2002 (203)	USA; 1454 FM	FFQ; quintiles	vitamin B12; Diet	>4.99 vs. ≤2.89 µg/d	colorectal	727	Matched on sex, age, ethnicity; adjusted for energy (residual), smoking, lifetime recreational PA, lifetime aspirin use, BMI, years of schooling, non-starch polysaccharides from vegetables, calcium from foods and supplements	1.1 (0.8, 1.6)	0.69
Benito E, 1991 (185)	Spain; 784	FFQ; quartiles	vitamin B12; Diet	>22.09 vs. <3.93 µg/d	colorectal	286	energy, age, sex, weight	0.61	>0.05

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs

[†] P-value for trend

3.5 Vitamin D and calcium

3.5.1 Introduction

Vitamin D can be ingested or synthesized in the skin from inactive precursors through the action of UV sunlight. Its active form, $1\alpha,25(OH)_2D_3$ is produced after two hydroxylation steps in the liver and kidneys (227). Foods that are good sources of vitamin D include oily fish and eggs, as well as fortified margarine, breakfast cereals and powdered milk. The recommended dietary intake of vitamin D is 10µg per day (Food Standards Agency). Calcium is mainly found in dairy products including milk and cheese. Other calcium sources include green leafy vegetables, soya products with added calcium (such as soya beans, tofu, soya drinks), nuts, bread and anything made with fortified flour. The recommended daily intake of calcium is currently 700 mg in the UK (Food Standards Agency). It has been suggested that prevalence of vitamin D deficiency (<75 nmol/l of 25(OH)D) in Scotland is high not only among elderly housebound individuals, but also among the middle aged ones, with persons that live in Scotland having a double risk of having less than 40 nmol/l of 25(OH)D than those who live in England or Wales (228). One of the main reasons is the high latitude of Scotland, with skin being unable to make vitamin D effectively during the winter months. Therefore, routine vitamin D and calcium supplementation especially for the ones that are housebound (>65 years old) is recommended (229).

Vitamin D regulates the blood concentration and absorption of calcium (227). Several biological mechanisms regarding the way that vitamin D and calcium might affect colorectal carcinogenesis have been described in laboratory studies. Briefly, some of the mechanisms of vitamin D and calcium include binding of long-chain fatty acids and bile acids in the small intestine or on the colonic lumen and therefore protecting the colonic mucosa from their mutagenic actions (230;231). They may also affect colorectal cancer risk via binding to the VDR influencing cell proliferation, differentiation, apoptosis and angiogenesis (231;232) or affecting insulin resistance (233).

3.5.2 Evidence from observational studies and randomised clinical trials

According to the findings of the second WCRF/AICR report (2007), the evidence that vitamin D status is associated with a decreased colorectal cancer risk is limited (30). In addition, a randomised clinical trial investigating the effects of daily calcium and vitamin D supplementation for seven years showed no effect on colorectal cancer incidence among postmenopausal women (234).

We identified 13 cohort (162;193;220;231;235-243) and 13 case-control studies (166;167;185;190;204;221;244-250) that examined the associations between dietary or total vitamin D intake and colorectal cancer and their results are inconclusive (Table 26, Table 27). Briefly, five cohort and four case-control studies reported a significant inverse association between total or dietary calcium intake and colorectal cancer or colon cancer (190;220;221;231;237;240;241;246;250). Results from serum/ plasma studies (234;251-255) are more consistent, indicating an inverse association with colorectal cancer (Table 28). In addition, a pooled meta-analysis of five studies examining the association between serum 25(OH)D and colorectal cancer risk, reported a significant and dose dependent ($p<0.0001$) association with the OR and 95% CI of the highest versus the lowest quintile being 0.46 (0.32, 0.64) (256).

Regarding calcium, a pooled meta-analysis of 10 cohort studies reported a statistically significant reduced risk of colorectal cancer for the highest versus the lowest calcium intake (RR (95% CI): 0.86 (0.78, 0.95)) (257). In addition a meta-analysis of 10 cohort studies conducted in the second WCRF/AICR report (2007), reported a RR for colorectal cancer of 0.98 (95% CI: 0.95, 1.00) per 200mg increase of calcium intake (30).

We identified 21 cohort (63;65;162;217;220;222;230;231;235-243;258-261) and 24 case-control studies (126;153;166;167;185;190;204;205;211;219;221;244-249;262-267) investigating the association between calcium and colorectal cancer risk (Table 29, Table 30). Briefly, ten cohort and ten case-control studies reported a statistically significant inverse association between total or dietary calcium and colorectal cancer risk (65;153;162;166;211;219-221;230;231;235;241-243;245-247;258;262;267).

Table 26 Colorectal cancer risk and vitamin D; Results from published cohort studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p†
Park S-Y, 2007 (231)	USA; Multiethnic cohort study; 191011 FM	FFQ; quintiles	Vitamin D; Diet + Supplements	Total:	colorectal		ethnicity, time since		
				M: ≥276 vs. ≤39 IU/1000kcal/d		1138	cohort entry, age, pack-	0.72 (0.51, 1.00)	0.03
				F: ≥276 vs. ≤39 IU/1000kcal/d		972	years of cigarette	0.89 (0.63, 1.27)	0.80
				Diet:			smoking, FH of CRC,		
				M: ≥96 vs. ≤31 IU/1000kcal/d		1138	PA, history of intestinal	0.91 (0.73, 1.13)	0.27
				F: ≥96 vs. ≤31 IU/1000kcal/d		972	polyps, NSAIDs, BMI,	0.78 (0.63, 0.96)	0.12
				Supplements:			energy, dietary fibre,		
				M: >400 vs. 0 IU/d		1138	HRT (women),	0.65 (0.49, 0.84)	0.001
				F: >400 vs. 0 IU/d		972	multivitamins	0.97 (0.75.1.26)	0.81
				Diet no supplement users					
				M: ≥96 vs. ≤31 IU/1000kcal/d		1138		0.87 (0.66, 1.13)	0.29
				F: ≥96 vs. ≤31 IU/1000kcal/d		972		0.69 (0.52, 0.93)	0.03
Kesse E, 2005 (238)	France; E3N-EPIC; 73034 F	FFQ; quartiles	vitamin D; Diet	>3.23 vs. <1.72 µg/d	colorectal	172	educational level, current smoking status, FH of CRC BMI, PA, energy, alcohol	0.89 (0.58, 1.36)	0.37
Lin J, 2005 (239)	USA; Women's Health Study; 39976 F	FFQ; quintiles	vitamin D; Diet + Supplements	≥545 vs. <161 IU/d (total)	colorectal	223	age, randomised	1.34 (0.84, 2.13)	0.08
				≥333 vs. <125 IU/d (diet)			treatment assignment,	0.96 (0.60, 1.55)	0.99
				>0-400 vs. 0 µg/d (Supplements)			BMI, FH of CRC,	1.36 (0.95, 1.95)	0.10
							history of colon polyps, PA, smoking status, red meat, alcohol, total energy, SF, multivitamin use, menopausal status, HRT		
McCullough ML, 2003 (241)	USA; Cancer Prevention	FFQ; quintiles	vitamin D; Diet + Multivitamins	>525 vs. <110 IU/d (total)	colorectal	683	age, smoking, BMI,	0.80 (0.62, 1.02)	0.02
				>240 vs. <90 IU/d (diet)			education, PA, FH of	0.92 (0.71, 1.18)	0.19
							CRC, energy, %SF,		

	Study II Nutrition; 127749 FM			M: >240 vs. <90 IU/d (diet) (excluding multivitamin users) F: >240 vs. <90 IU/d (diet) (excluding multivitamin users)		259 140	fruit, vegetables, multivitamins, HRT (women)	0.74 (0.50, 1.11) 1.13 (0.63, 2.04)	0.07 0.79
Terry P, 2002 (242)	Sweden; Swedish Mammography Screening Cohort 61463 F	FFQ; quartiles	vitamin D ; Diet	≥3.8 vs. <2.6 µg/d	colorectal	572	energy, age, BMI, education level, red meat, alcohol, energy adjusted SF, folic acid, vitamin C, calcium	1.05 (0.83, 1.33)	0.73
Jarvinen R, 2001 (236)	Finland; Finnish Mobile Clinic Health Examination Survey; 9959 FM	FFQ; quartiles	vitamin D; Diet	M: ≥4.89 vs. <2.58 µg/d F: ≥3.42 vs. <1.82 µg/d	colorectal	72	age, sex, BMI, occupation, smoking, geographical area, energy	1.74 (0.82, 3.68)	0.13
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111 M	FFQ; quartiles	vitamin D; Diet	8.62 vs. 2.58 µg/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	1.00 (0.70, 1.50)	0.77
Zheng W, 1998 (243)	USA; Iowa Women's Health Study; 34702 F	FFQ; tertiles	vitamin D; Diet + Supplements	>475.5 vs. <224.1 IU/d	rectal	144	age, smoking status, pack-years of smoking, HRT, energy	0.76 (0.50, 1.16)	0.20
Giovannucci E, 1998 (193)	USA; Nurses' Health Study; 88756 F	FFQ; quartiles	vitamin D; Diet + Supplements	high vs. low quartile	colon	442	energy, smoking, FH of CRC; PA, BMI, aspirin use; and intakes of red meat, alcohol, fibre, folate	0.86 (0.60, 1.28)	>0.20

Sellers TA, 1998 (220)	USA; Iowa Women's Health Study; 35216 F	FFQ; tertiles	vitamin D; Diet + Supplements	No FH of CRC >478.2 vs. ≤226.3 IU/d (total) high vs. low tertile (diet) >400 vs. 0 IU/d (supplement) FH of CRC >478.2 vs. ≤226.3 IU/d (total) high vs. low tertile (diet) >400 vs. 0 IU/d (supplement)	colon	180 62 	age, energy, history of rectal cancer polyps	0.6 (0.4, 0.9) 0.7 (0.5, 1.0) 0.8 (0.5, 1.3) 0.9 (0.5, 1.7) 0.8 (0.4, 1.7) 1.1 (0.5, 2.4)	0.02 0.06 0.3 0.7 0.6 0.9
Martinez EM, 1996 (240)	USA; Nurses Health Study; 89448 F	FFQ; quintiles	vitamin D; Diet + Supplements	>477 vs. <92 IU/d (total) >477 vs. <92 IU/d (total) (women with unchanged milk intake) >245 vs. <76 IU/d (diet) >245 vs. <76 IU/d (diet) (women with unchanged milk intake)	colorectal	501 346 501 346	age, BMI, PA, FH of CRC, aspirin, cigarette smoking, red-meat intake, and alcohol	0.88 (0.66, 1.16) 0.67 (0.47, 0.95) 0.84 (0.63, 1.13) 0.77 (0.54, 1.09)	0.23 0.02 0.16 0.11
Kearney J, 1996 (237)	USA; Health Professionals; 47935 M	FFQ; quintiles	vitamin D; Diet + Supplements	≥613 vs. <161 IU/d (total) ≥358 vs. <134 IU/d (dietary) ≥448 vs. <4.0 IU/d (supplements)	colon	203	age, total calories, FH of CRC, previous polyps screening, past history of smoking, alcohol, aspirin, PA, BMI, red meat, SF, dietary fibre	0.66 (0.42, 1.05) 0.88 (0.54, 1.42) 0.48 (0.22, 1.02)	0.02 0.55 0.11
Bostick RM, 1993 (235)	USA; Iowa Women's Health Study; 32216 F	FFQ; quintiles	vitamin D; Diet + Supplements	>618 vs. <159 IU/d (total) >373 vs. <127 IU/d (diet) >400 vs. 0 IU/d (Supplements)	colon	212	age, energy, height, parity, low fat meat intake, vitamin E, a vitamin E x age interaction term	0.73 (0.45, 1.18) 0.98 (0.61, 1.58) 0.67 (0.40, 1.13)	0.42 0.98 0.13

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

Table 27 Colorectal cancer risk and vitamin D; Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Theodoratou E, 2008‡ (250)	Scotland; SOCCS study; 4750 FM	FFQ; quintiles	vitamin D; Diet + Supplements	Diet: ≥6.00 vs. ≤2.51 µg/d Total: ≥8.31 vs. ≤2.76 µg/d	colorectal	2070	energy (residual method), energy (included as a covariate), age, sex, deprivation score, fibre, FH of CRC, BMI, smoking, NSAIDs, PA	0.77 (0.63, 0.94) 0.80 (0.65, 0.98)	0.01 0.01
Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	vitamin D; Diet	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin use	1.04 (0.72, 1.51) 0.97 (0.66, 1.44)	0.92 0.91
Slattery ML, 2004 (249)	USA; 2143 FM	Diet history (CARDIA); tertiles	vitamin D; Diet	M: >10.2 vs. <4.2 µg/d F: >8.3 vs. <3.1 µg/d	rectal	556 390	age, PA, energy, fibre, BMI, NSAIDs	1.08 (0.73, 1.60) 0.52 (0.32, 0.85)	
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	vitamin D; Diet	M: >5.3 vs. <0.6 µg/d F: >4.3 vs. <0.7 µg/d	colorectal	171	age, sex, energy, BMI, PA	1.1 (0.6, 2.0)	0.66
Levi F, 2000 (204)	Switzerland; 7140 FM	FFQ diet; tertiles	vitamin D; Diet	2.6 vs. 1.2 µg/d	colorectal	223	age, sex, years of education, smoking, alcohol drinking, BMI, PA, energy, fibre	1.46 (0.90, 2.30)	>0.05
Kampman E, 2000 (245)	USA; 4403 FM	Dietary history (CARDIA); quintiles	vitamin D; Diet	M: >11.2 vs. <3.6 µg/d F: >8.6 vs. <2.6 µg/d	colon	1086 880	age, BMI, FH, aspirin and./ or NSAIDs, energy, long-term vigorous activity, fibre, calcium	1.40 (1.00, 2.20) 1.10 (0.70, 1.70)	

Marcus PM, 1998 (246)	USA; 1190 FM	FFQ; quintiles	vitamin D; Diet + Supplements	≥557 vs. <148 IU/d (total)	colon	348	age, energy, fibre	0.70 (0.40, 1.10)	0.05
				≥336 vs. <122 IU/d (diet)				0.80 (0.50, 1.30)	0.45
				≥400 vs. 0 IU/d (Supplement)				0.80 (0.60, 1.10)	0.12
				≥557 vs. <148 IU/d (total)	rectal	164		0.80 (0.50, 1.50)	0.42
				≥336 vs. <122 IU/d (diet)				0.90 (0.40, 1.60)	0.99
				≥400 vs. 0 IU/d (Supplement)				0.90 (0.60, 1.40)	0.16
La Vecchia C, 1997 (221)	Italy; 4154 FM	FFQ; quintiles	vitamin D; Diet	≥4.28 vs. <2.02 µg/d	colorectal	1953	age, area of residence, sex, education, PA, energy, fibre	0.77 (0.60, 0.90)	<0.01
Pritchard RS, 1996 (248)	Sweden; 1081 FM	FFQ; quartiles	vitamin D; Diet	≥7 vs. ≤2.8 µg/d	colon	352	age, sex, energy, protein	0.60 (0.40, 1.00)	0.08
					rectal	217		0.50 (0.30, 0.90)	0.08
Boutron MC, 1996 (244)	France; 480 FM	Diet history; quintiles	vitamin D; Diet	M: >5.7 vs. <2.5 µg/d F: >4.7 vs. <2.1 µg/d	colorectal	171	age, sex and caloric intake	0.80 (0.40, 1.60)	0.77
Ferraroni M, 1994 (190)	Italy; 3350 FM	FFQ; quintiles	vitamin D; Diet	>1.97 vs. <0.79 µg/d	colorectal	1326	age, sex, education, FH of CRC, BMI, energy	0.74 (0.58, 0.95)	<0.05
Peters RK, 1992 (247)	USA; 1492 FM	FFQ; per 108 IU increase/day	vitamin D; Diet	per 108 IU increase/day	colon	746	fat, protein, carbohydrates, alcohol, calcium, FH, weight, PA, pregnancies (females)	1.08 (0.97, 1.20)	
Benito E, 1991 (185)	Spain; 784 FM	FFQ; quartiles	vitamin D; Diet	>1.66 vs. <0.32 µg/d	colorectal	286	energy, age, sex, weight	0.74	>0.05

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

Table 28 Colorectal cancer risk and serum/ plasma vitamin D metabolites; Results from published nested case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Metabolite	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Wu K, 2007 (255)	USA; Health Professionals Follow-up Study; 535 M	plasma measurements; quintiles	25(OH)D	39.4 vs. 18.4 ng/ml	colorectal	179	FH, aspirin use, PA, folate, calcium, retinol, pack-years of smoking, alcohol, meat intake (total red and processed meat)	0.83 (0.45, 1.52)	0.24
Otani T, 2007 (253)	Japan; Japan Public Health Centre-based Prospective Study; 1125 FM	plasma measurements; quartiles	25(OH)D	M: >32.1 vs. <22.9 ng/ml	colorectal	163	matched on sex, age, study area, date of blood draw, fasting time; adjusted for pack-years of smoking, alcohol, BMI, PA, vitamin supplement use, FH of CRC	0.73 (0.35, 1.5)	0.39
				F: >27.0 vs. 18.7 ng/ml	colorectal	160		1.1 (0.50, 2.3)	0.74
Wactawski-Wende J, 2006 (234)	USA; Women's Health Initiative; 612 F	serum measurements; quartiles	25(OH)D	≥23 vs. <12 ng/mL	colorectal	306	matched on age, centre, race or ethnic group, date of blood sampling	0.4 (0.2, 0.8)	0.01
Feskanich D, 2004 (252)	USA; Nurses' Health Study; 579 F	plasma measurements; quintiles	25(OH)D	39.9 vs. 16.2 ng/mL	colorectal	193	matched on year of birth, month of blood draw; adjusted for BMI, PA, pack-years of smoking, menopausal status, HRT, aspirin, FH of CRC, calcium, folate, methionine, retinol, red meat, alcohol	0.53 (0.27, 1.04)	0.02
			$1,25(OH)_2D$	43.0 vs. 21.7 pg/ml				1.77 (0.93, 3.36)	0.51

Tangrea J, 1997 (254)	Finland; Alpha-Tocepherol Beta-Carotene Prevention Study; 438 M	serum measurements; quartiles	25(OH)D $1,25(OH)_2D$	>19.3 vs. ≤9.8 ng/l >43.1 vs. ≤31.7 ng/l	colorectal	146	matched on age, date of baseline blood draw, study clinic	0.6 (0.3, 1.1) 0.9 (0.5, 1.7)	0.13 0.76
Braun MM, 1995 (251)	USA; 171 FM	serum measurements; quintiles	25(OH)D $1,25(OH)_2D$	>30.1 vs. <17.2 ng/mL >41.3 vs. <26.6 pg/ml	colon	57	matched on age, race, sex, date of blood draw	0.40 (0.1, 1.4) 1.1 (0.4, 3.2)	0.57 0.88

* Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

† P-value for trend

Table 29 Colorectal cancer risk and calcium; Results from published cohort studies (1990-2008)[*]

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	RR (95% CI)	p[†]
Park S-Y, 2007 (231)	USA; Multiethnic cohort study; 191011 FM	FFQ; quintiles	calcium; Diet + Supplements	Total: M: ≥611 vs. ≤288 mg/1000kcal/d F: ≥611 vs. ≤288 mg/1000kcal/d Diet: M: ≥466 vs. ≤260 mg/1000kcal/d F: ≥466 vs. ≤260 mg/1000kcal/d Supplements M: ≥200 vs. 0 mg/d F: ≥200 vs. 0 mg/d Diet excluding supplement users: M: ≥466 vs. ≤260 mg/1000kcal/d F≥466 vs. ≤260 mg/1000kcal/d	colorectal	1138 972 1138 972 1138 972 1138 972	ethnicity, time since cohort entry, age, pack-years of cigarette smoking, FH of CRC, PA, history of intestinal polyps, NSAIDs, BMI, energy, dietary fibre, HRT (women), multivitamins	 0.70 (0.52, 0.93) 0.64 (0.50, 0.83) 0.76 (0.59, 0.96) 0.91 (0.72, 1.17) 0.74 (0.60, 0.90) 0.82 (0.69, 0.98) 0.73 (0.54, 1.00) 0.70 (0.50, 0.97)	 0.006 0.003 0.02 0.61 0.003 0.02 0.06 0.02
Shin A, 2006 (222)	China; Shanghai Women's Health Study; 73314 F	FFQ; quintiles	calcium; Diet	>610.8 vs. ≤291.9 mg/d	colorectal	283	age, menopausal status, education, cigarette smoking, alcohol consumption, exercise, FH of CRC, vitamin supplements use and calorie intake	0.9 (0.6, 1.4)	0.48
Larsson SC, 2006 (230)	Sweden; Cohort of Swedish Men; 45306 M	FFQ; quartiles	calcium; Diet + Supplements	≥1445 vs. <956 mg/d	colorectal	449	age, education, FH of CRC, BMI, exercise, history of diabetes, smoking, aspirin, multivitamin use, energy, SF, total vitamin D, alcohol, fruit, vegetables, red meat	0.68 (0.51, 0.91)	0.01

Kesse E, 2005 (238)	France; E3N-EPIC; 73034 F	FFQ; quartiles	calcium; Diet	total Ca: >1201.8 vs. <766.2 mg/d dairy Ca: >736.0 vs. <359.2 mg/d	colorectal	172	educational level, current smoking status, FH of CRC BMI, PA, energy, alcohol	0.72 (0.47, 1.10) 0.86 (0.56, 1.32)	0.08 0.25
Lin J, 2005 (239)	USA; Women's Health Study; 39976 F	FFQ; quintiles	calcium; Diet + Supplements	≥1357 vs. <614 mg/d (total) ≥1083 vs. <480 mg/d (diet) ≥500 vs. 0 ug/d (Supplements)	colorectal	223	age, randomised treatment assignment, BMI, FH of CRC, history of colon polyps, PA, smoking status, red meat, alcohol, total energy, SF, multivitamin use, menopausal status, HRT	1.20 (0.79, 1.85) 0.90 (0.53, 1.54) 1.30 (0.90, 1.87)	0.21 0.81 0.13
Flood A, 2005 (258)	USA; Breast Cancer Detection Demonstration Project; 45354 F	FFQ; quintiles	calcium; Diet + Supplements	>1270 vs. <472 mg/d (total) >830 vs. <412 mg/d (diet) >800 vs. 0 ug/d (Supplements)	colorectal	482	energy, age	0.74 (0.55, 0.99) 0.74 (0.56, 0.98) 0.76 (0.56, 0.98)	0.02 0.05 0.09
Wei EK, 2004 (217)	USA; Nurses' Health Study, Health Professionals Follow-Up Study; 87733 F, 46632 M	FFQ; quartiles	calcium; Diet	>1100 vs. <600 µg/d	colon rectal	1139 339	age, FH, BMI, PA, beef, pork or lamb as a main dish, processed meat, alcohol, calcium, height, pack-years smoking before age 30, history of endoscopy, sex	0.88 (0.73, 1.07) 0.92 (0.65, 1.30)	0.17 0.66
McCullough ML, 2003 (241)	USA; Cancer Prevention Study II	FFQ quintiles	calcium; Diet + Supplements	>988 vs. <504 mg/d (diet) >500 vs. 0 mg/d (Supplements) >1255 vs. <561 mg/d (total)	colorectal	683	age, smoking, BMI, education, PA, FH of CRC, energy, %SF, fruit, vegetables,	0.92 (0.72, 1.17) 0.69 (0.49, 0.96) 0.87 (0.67, 1.12)	0.28 0.03 0.02

	Nutrition; 127749 FM						multivitamins, HRT (women)		
Wu K, 2002 (261)	USA; Nurses' Health Study, Health Professionals Follow-Up Study; 87998F 47344 M	FFQ; number of categories: 7 for total Ca; 6 for dietary Ca; 6 for dairy Ca; 6 for non-dairy Ca	calcium; Diet + Supplements	M: >1250 vs. ≤500 mg/d (total) F: >1250 vs. ≤500 mg/d (total) M: >1000 vs. ≤500 mg/d (diet) F: >1000 vs. ≤500 mg/d (diet) M: >800 vs. ≤200 mg/d (dairy) F: >800 vs. ≤200 mg/d (dairy) M: >350 vs. ≤250 mg/d (non-dairy) F: >350 vs. ≤250 mg/d (non-dairy)	colon	399 626	age, FH, BMI, PA, pack-years of smoking before age of 30, aspirin, red meat, alcohol; for women: HRT, menopausal status	0.64 (0.43, 0.95) 0.94 (0.66, 1.33) 0.67 (0.46, 0.96) 0.97 (0.68, 1.38) 0.78 (0.53, 1.16) 0.78 (0.50, 1.21) 1.02 (0.73, 1.43) 1.03 (0.70, 1.54)	0.17 0.35 0.24 0.21 0.33 0.26 0.37 0.43
Terry P, 2002 (242)	Sweden; Swedish Mammography Screening Cohort 61463 F	FFQ; quartiles	calcium ; Diet	914 vs. 486 mg/d	colorectal	572	energy, age, BMI, education level, red meat, alcohol, energy adjusted SF, folic acid, vitamin C, calcium	0.72 (0.56, 0.93)	0.02
Jarvinen R, 2001 (236)	Finland; Finnish Mobile Clinic Health Examination Survey; 9959 FM	FFQ; quartiles	calcium; Diet	M: ≥1953.3 vs. <1178.2 mg/d F: ≥1416.7 vs. <862.5 mg/d	colorectal	72	age, sex, BMI, occupation, smoking, geographical area, energy	1.43 (0.61, 3.39)	0.97
Pietinen P, 1999 (162)	Finland; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; 27111 M	FFQ; quartiles	calcium; Diet	1789 vs. 856 mg/d	colorectal	185	age, supplement group, smoking years, BMI, alcohol, education, PA at work, calcium	0.6 (0.4, 0.9)	0.04

Zheng W, 1998 (243)	USA; Iowa Women's Health Study; 34702 F	FFQ; tertiles	calcium; Diet + Supplements	>1278.7 vs. <800.8 mg/d	rectal	144	age, smoking status, pack-years of smoking, HRT, energy	0.59 (0.37, 0.94)	0.02
Sellers TA, 1998 (220)	USA; Iowa Women's Health Study; 35216 F	FFQ; tertiles	calcium; Diet + Supplements	No FH of CRC >1296.6 vs. ≤820.7 mg/d (total) >964.7 vs. ≤615 mg/d (diet) >500 vs. 0 IU/d (supplement) FH of CRC >1296.6 vs. ≤820.7 mg/d (total) >964.7 vs. ≤615 mg/d (diet) >500 vs. 0 IU/d (supplement)	colon	180 62 	age, energy, history of rectal cancer polyps	 0.5 (0.3, 0.7) 0.7 (0.4, 1.0) 0.6 (0.4, 0.9) 1.2 (0.6, 2.2) 0.8 (0.4, 1.7) 1.0 (0.5, 2.1)	 0.001 0.06 0.02 0.7 0.6 1.0
Kato I, 1997 (65)	USA; New York's University Health Study; 14727 F	FFQ; quartiles	calcium	high vs. low quartiles (total) high vs. low quartiles (from fish/shellfish) high vs. low quartiles (from dairy)	colorectal	100	total calorie intake, age, place at enrolment, highest level of education	0.71 (0.39, 1.28) 0.41 (0.22, 0.74) 0.65 (0.38, 1.11)	0.18 0.001 0.04
Gaard M, 1996 (63)	Norway; 50535 FM	FFQ	calcium		colon	143	energy	no association	
Martinez EM, 1996 (240)	USA; Nurses Health Study; 89448 F	FFQ; quintiles	calcium; Diet	>957 vs. <475 mg/d (diet) >957 vs. <475 mg/d (diet) (women with unchanged milk intake)	colorectal	501 346	age, BMI, PA, FH of CRC, aspirin, cigarette smoking, red-meat intake, and alcohol	0.80 (0.60, 1.07) 0.74 (0.53, 1.05)	0.25 0.12
Kearney J, 1996 (237)	USA; Health Professionals; 47935 M	FFQ; quintiles	calcium; Diet + Supplements	≥1213 vs. <631 mg/d (total) ≥1051 vs. <605 mg/d (dietary) ≥620 vs. <137 mg/d (dairy) ≥864 vs. <119 mg/d (non-dairy)	colon	203	age, total calories, FH of CRC, previous polyps screening, past history of smoking, alcohol, aspirin, PA, BMI, red meat, SF, dietary fibre	0.75 (0.48, 1.15) 0.81 (0.52, 1.28) 0.68 (0.42, 1.09) 0.86 (0.50, 1.48)	0.22 0.62 0.28 0.30
Kampman E, 1994	The Netherlands;	FFQ; quintiles	calcium; Diet	1288 vs. 596 mg/d (diet) 417 vs. 238 mg/d (non-dairy)	colorectal	478	age, gender, FH of CRC, energy, energy-adjusted	0.92 (0.64, 1.34) 1.77 (1.08, 2.90)	0.89 0.01

(259)	Netherlands Cohort; 3346 FM			634 vs. 64 mg/d (fermented dairy) 540 vs. 45 mg/d (unfermented dairy)			intake of fat and dietary fibre, BMI, history of gallbladder surgery	1.14 (0.77, 1.68) 0.71 (0.48, 1.05)	0.32 0.11
Bostick RM, 1993 (235)	USA; Iowa Women's Health Study; 32216 F	FFQ; quintiles	calcium; Diet + Supplements	>1547 vs. <629 mg/d (total) >1186 vs. <496 IU/d (diet) >500 vs. 0 mg/d (Supplements)	colon	212	age, energy, height, parity, low fat meat intake, vitamin E, a vitamin E x age interaction term	0.52 (0.33, 0.82) 0.73 (0.48, 1.13) 0.57 (0.37, 0.88)	0.01 0.28 0.03
Stemmermann GN, 1990 (260)	Hawaii (Japanese); 7572 M	24 hr diet recall; tertiles	calcium; Diet	low vs. high (total) low vs. high (dairy) low vs. high (non-dairy)	colon	189	age	1.3 (0.9, 1.8) 1.2 (0.9, 1.8) 1.1 (0.8, 1.6)	0.16 0.27 0.55

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

Table 30 Colorectal cancer risk and calcium; Results from published case-control studies (1990-2008)*

Study	Country; Study; Sample	Assessment	Nutrient	Comparison (high vs. low)	Outcome	Cases	Adjustments	OR (95% CI)	p†
Theodoratou E, 2008‡ (250)	Scotland; SOCCS study; 4750 FM	FFQ; quintiles	calcium; Diet + Supplements	Diet: ≥1.32 vs. ≤0.89 g/d Total: ≥1.34 vs. ≤0.89 g/d	colorectal	2070	energy (residual method), energy (included as a covariate), age, sex, deprivation score, fibre, FH of CRC, BMI, smoking, NSAIDs, PA	0.96 (0.78, 1.17) 0.89 (0.72, 1.09)	0.86 0.62
Wakai K, 2006 (166)	Japan; 2535 FM	FFQ; quartiles	calcium; Diet	high vs. low quartile	colon rectal	265 242	energy, sex, age, year and season of first visit to the hospital, reason for visit, FH of CRC< BMI, exercise, alcohol, smoking, multivitamin use	0.67 (0.46, 1.00) 0.97 (0.63, 1.50)	0.04 0.99
Slattery ML, 2004 (249)	USA; 2143 FM	Diet history (CARDIA); tertiles	calcium; Diet	M: >1543 vs. <743 mg/d F: >1275 vs. <628 mg/d	rectal	556 390	age, PA, energy, fibre, BMI, NSAIDs	1.02 (0.66, 1.56) 0.39 (0.24, 0.64)	
Senesse P, 2004 (167)	France; 480 FM	diet history; quartiles	calcium; Diet	M: >1241.3 vs. <321.1 mg/d F: >1168.6 vs. <365.6 mg/d	colorectal	171	age, sex, energy, BMI, PA	1.4 (0.8, 2.6)	0.38
Satia-Abouta J, 2003 (211)	USA; North Carolina Colon Cancer Study; 1609 FM	FFQ; quartiles	calcium; Diet + Supplements	Whites : 1691 vs. 456 mg/d African/Americans: 1143 vs. 304 mg/d	colon 276	337	energy, other potential confounders examined include age, sex, education, BMI, smoking, PA, FH of CRC, NSAIDs, supplement use, fat, dietary fibre, calcium, folate, fruits, vegetables	0.4 (0.3, 0.6) 0.6 (0.3, 1.1)	<0.0001 0.08

Ma J, 2001 (265)	USA; Health Professionals Study; 511 M	FFQ; tertiles	calcium; dairy foods	≥340 vs. ≤132 mg/d (dairy) ≥291 vs. ≤42 mg/d (milk)	colorectal	193	age, smoking, BMI, alcohol, multivitamin use, aspirin, exercise, molar ratio of IGF-I to IGFBP-3	0.62 (0.38, 1.02) 0.66 (0.40, 1.09)	0.09 0.06
Levi F, 2000 (204)	Switzerland; 7140 FM	FFQ diet; tertiles	calcium; Diet	1144.9 vs. 431.2 mg/d	colorectal	223	age, sex, years of education, smoking, alcohol drinking, BMI, PA, energy, fibre	0.96 (0.5, 1.7)	>0.05
Kampman E, 2000 (245)	USA; 4403 FM	Dietary history (CARDIA); quintiles	calcium; Diet	M: >1701 vs. <681 mg/d F: >1330 vs. <546 mg/d	colon	1086 880	age, BMI, FH, aspirin and./ or NSAIDs, energy, long-term vigorous activity, fibre	0.7 (0.5, 0.9) 0.6 (0.4, 0.9)	
Marcus PM, 1998 (246)	USA; 1190 F	FFQ; quintiles	calcium; Diet + Supplements	≥1396 vs. <532 mg/d (total) ≥1121 vs. <466 mg/d (diet) ≥800 vs. 0 mg/d (Supplement) ≥1396 vs. <532 mg/d (total) ≥1121 vs. <466 mg/d (diet) ≥800 vs. 0 mg/d (Supplement)	colon rectal 	348 164 	age, energy, fibre, SF, animal fat,	0.6 (0.4, 1.0) 0.8 (0.5, 1.4) 1.0 (0.7, 1.6) 0.6 (0.3, 1.1) 0.7 (0.3, 1.3) 0.8 (0.5, 1.6)	0.03 0.13 0.68 0.07 0.53 0.34
La Vecchia C, 1997 (221)	Italy; 4154 FM	FFQ; quintiles	calcium; Diet	≥1495 vs. <799 ug/d	colorectal	1953	age, area of residence, sex, education, PA, energy, fibre	0.72 (0.6, 0.9)	<0.01
White E, 1997 (219)	USA; 871 FM	Questionnaire for Supplements; 4 categories	calcium; Supplements	>100 vs. 0 mg/d	colon	444	age, sex	0.78 (0.52, 1.18)	0.03
Ghadirian P, 1997 (153)	Canada; 1070 FM	FFQ; quartiles	calcium; Diet	high vs. low quartile	colon	402	sex, age, marital status, history of colon carcinoma in first-degree relatives, energy	0.69 (0.47, 1.00)	0.04
De Stefani E, 1997 (126)	Uruguay; 846 FM	quartiles	calcium; Diet	>951.9 vs. ≤554.3 mg/d	colorectal	282	age, sex, residence, urban/rural status,	0.41 (0.24, 0.69)	0.001

							energy, protein, fat, folate		
Pritchard RS, 1996 (248)	Sweden; 1081 FM	FFQ; quartiles	calcium; Diet	≥1057 vs. ≤640 mg/d	colon rectal	352 217	age, sex, energy, protein	1.2 (0.7, 2.1) 1.0 (0.5, 1.9)	0.62 0.73
Boutron MC, 1996 (244)	France; 480 FM	Diet history; quintiles	calcium; Diet	high vs. low quintile (diet) high vs. low quintile (non-dairy) high vs. low quintile (dairy)	colorectal	171	age, sex and caloric intake	1.7 (0.8, 2.3) 1.6 (0.8, 3.0) 1.8 (0.9, 3.4)	0.33 0.11 0.17
Ferraroni M, 1994 (190)	Italy; 3350 FM	FFQ; quintiles	calcium; Diet	>1029.7 vs. <468.1 mg/d	colorectal	1326	age, sex, education, FH of CRC, BMI, energy	0.84 (0.65, 1.08)	>0.05
Slattery ML, 1994 (267)	USA; 715 FM	FFQ; quartiles	calcium; Diet	M: >1401.7 vs. ≤641.2 mg/d F: >1141.0 vs. ≤592.5 mg/d	colon	324	age, BMI, energy, fibre	0.3 (0.1, 0.8) 0.6 (0.2, 1.3)	
Kampman E, 1994 (264)	The Netherlands; 491 FM	diet history	calcium; Diet	Diet >1480 vs. ≤1010 mg/d From fermented dairy >683 vs. ≤333 mg/d From unfermented dairy >530 vs. ≤170 mg/d From non-dairy >402 vs. ≤296 mg/d	colon	232	age, gender, urbanization level, energy, FH of CRC, cholecystectomy and energy-adjusted intake of total fat, dietary fibre, vitamin C and alcohol	1.81 (1.05, 3.12) 1.26 (0.74, 2.16) 1.10 (0.63, 1.91) 0.69 (0.36, 1.32)	0.02 0.32 0.94 0.34
Meyer F, 1993 (205)	USA; 838 FM	FFQ; quartiles	calcium; Diet	high vs. low quartile	colon	424	age, interviewer, dietary energy, alcohol, fibre	M: 1.37 F: 0.35	
Peters RK, 1992 (247)	USA; 1492 FM	FFQ; continuous	calcium; Diet	continuous	colon	746	FH, weight, PA, pregnancies (females)	0.85 (0.78, 0.93)	<0.001
Arbman G, 1992 (262)	Sweden; 82 FM	diet history; 2 categories	calcium; Diet	above vs. below the median intake	colorectal	41	energy	0.33 (0.10, 0.94)	<0.05
Benito E, 1991 (185)	Spain; 784 FM	FFQ; quartiles	calcium; Diet	>1034 vs. <545 mg/d	colorectal	286	energy, age, sex, weight	1.48	>0.05
Freudenheim JL, 1990 (263)	USA; 844 FM	FFQ; quartiles (men), tertiles (women)	calcium; Diet	M: high vs. low quartile F: high vs. low quartile	rectal	277 145		1.51 (0.94, 2.44) 1.63 (0.91, 2.91)	>0.05 >0.05

Negri E, 1990 (266)	Italy; 1942 FM	FFQ; quintiles	calcium; Diet	>1046 vs. <480 mg/d	colon rectal	558 352	age, sex, education, area of residence, and consumption of selected indicator foods	1.1 (0.8, 1.6) 1.2 (0.8, 1.9)	>0.05 >0.05

[*] Abbreviations: F: females; M: males; FFQ: food frequency questionnaire; BMI: body mass index; FH: family history; CRC: colorectal cancer; PA: physical activity; HRT: hormone replacement therapy; NSAIDs: Non-steroidal anti-inflammatory drugs; SF: saturated fat

[†] P-value for trend

[‡] Results that are part of the current thesis and will be presented in detail in the following chapters

4 METHODS

4.1 Introduction

In this chapter the overall methodology of the thesis is described. In the first part, the population-based case-control study that the analysis was based on is presented. In the second part of the chapter the specific elements of data collection and process (prior to analysis) are described. Finally, in part three of the chapter the overall statistical methodology is outlined.

4.2 Scottish Colorectal Cancer Study

4.2.1 Study design

This thesis was based on a population-based case-control study of colorectal cancer (Scottish Colorectal Cancer Study; SOCCS) in relation to genetic susceptibility, lifestyle and dietary risk factors. The recruitment for this study commenced in February 1999 and ended in December 2006. It was funded by the Cancer Research UK (CR-UK), the Medical Research Council (MRC) and the Chief Scientist Office of the Scottish Executive (CSO) and was headed by Professors Malcolm G Dunlop, Harry Campbell and Mary E Porteous. The main aims of the study were to identify genetic factors that influence colorectal carcinogenesis but also to investigate what are the effects of diet and general lifestyle on colorectal cancer.

4.2.2 Ethical approval and consultant consent

Ethical approval for the SOCCS study was obtained from the MultiCentre Research Ethics committee for Scotland (MREC; approval number MREC/ 01/0/0), 18 Local Research Ethics committees, 18 Caldicott guardians and 16 NHS Trust management committees (Appendix II). The principles and procedures detailed in the MRC document "Human tissue and biological samples for use in research", November 1999 were followed and the model consent form proposed by MRC was used. Individual informed consent was received on the basis that the DNA sample and other data about the individual could be stored by the research team at Edinburgh University for uses in future research and may be shared with other medical research groups (with appropriate

ethical approvals first being obtained where necessary). This consent includes interactions with researchers working for commercial companies (Appendix II).

Consultant surgeons in all Scottish hospitals were asked permission for their eligible patients to receive information on the SOCCS studies. More than 100 surgeons allowed and only two surgeons refused to allow their patients to be informed.

4.2.3 Case recruitment

4.2.3.1 Eligibility for the study

All cases between 16 and 79 years old with colorectal adenocarcinoma diagnosed after February of 1999 and permanently resident in Scotland were eligible to take part in the study. In each case the diagnosis was confirmed histologically and with reference to the pathological report.

Cases that 1) were not normally resident in Scotland, 2) were recurrent colorectal cancer cases or 3) had a) squamous cell carcinoma of the anus, b) melanoma of the rectum or c) carcinoid tumours of the colon, were not eligible to be included in the study. In addition cases that could not give informed consent, because they 1) were too ill, 2) had mental health problems, 3) had learning difficulties or 4) had dementia, were excluded from the study.

4.2.3.2 Recruitment

Eight research nurses were trained by the principal investigators, the project co-ordinator and the research nurse co-ordinator (3-day training session) and appointed to eight geographical areas. Study awareness in 41 NHS and private funded Scottish hospitals was ensured by several presentations to the medical and nursing staff delivered by the research nurse co-ordinator. Following this presentation a recruitment strategy to ascertain eligible patients and to provide them with the study's information pack was developed. For eligible cases that did not wish to participate (non-participants) or did not respond within two months of initial approach (non-responders) a non-participant form was completed, recording sex, age of diagnosis, consultant, health board (where treated), reason of non-participation (if given) and type of surgery (if applicable; curative or palliative).

The initial recruitment plan for the study was that each research nurse would visit and recruit patients in the last few days of their hospital stay. However, because of a new discharge policy, which was implemented soon after recruitment commenced, eligible cases were recruited in their homes after having been discharged from the hospital.

4.2.3.3 Recruitment protocol

According to the recruitment protocol, each patient received an information pack containing a patient invitation letter, a patient information sheet, a sample consent form, a patient detail sheet and a prepaid envelope, 24 hours prior to the recruitment visit (Appendix II). The main steps of the patient recruitment were:

- Discussion with the patient about the main aims and elements of the study;
- Check that all necessary details on the patient details sheet were completed and legible;
- Permission to take a family history and drawing of it on sheet provided;
- Assessment of family history risk (moderate and high risk patients were offered genetic testing or were referred to a cancer genetic clinic);
- Recording of any previous cancer(s) (including year and hospital of diagnosis);
- Completion of study's consent form;
- If patient wished for his/ her blood sample to be taken, also the DNA storage consent form was completed;
- Completion of next of kin sheet (in case the patient did not wish his/ her blood sample to be taken);
- Completion of the treatment questionnaire;
- Patient was asked to complete the lifestyle (Appendix III) and food frequency questionnaires (Appendix IV) to the best of his/her ability and return in pre-paid envelope (this step was included for cases recruited after 01 September 2001).

4.2.4 Control recruitment

4.2.4.1 Selection procedure

Controls eligible for the study were randomly identified through the Community Health Index (CHI), which is a NHS population-based register. CHI is a national register of all

individuals who are registered with a general practitioner (GP) in Scotland. The completeness of the CHI has been estimated to be greater than 95% and it thus represents an excellent sampling frame for the selection of population-based controls (268). The controls were drawn following a matching protocol applied to the CHI and they were recruited through clinics set up in over 40 locations across Scotland. Access to the CHI for research purposes has recently been restricted following implementation of the Data Protection Act 1998 on 1 March 2000. However, the study received MREC (Scotland) approval to collaborate with the guardians of the CHI to use it as a sampling frame but without knowing the identity of individuals until they agreed to participate.

Controls were selected at random according to the study's instructions, by the Practitioner Services Division (PSD) of the Information and Statistics Division (ISD) of the NHS in Scotland (Step 1) and invitations passed on to these individuals via their GPs (Step 2). In particular, the GPs' information pack sent by post contained information and forms 1) for the GPs (covering letter, reply form, explanatory letter) and 2) for the controls (information sheet, reply form (Appendix II), lifestyle and food frequency questionnaires (Appendix III, IV). In case of no reply from the GP (within a 4 week period), a second information pack was sent repeating Step 2. If the GP refused to inform the eligible control, the type of response (NO) was recorded in the control recruitment recording form, the next eligible control for a particular case was approached and steps 1 and 2 were repeated (Step 3). If the GP agreed to inform the eligible control, the type of response (YES) was recorded in the control recruitment recording form and the study office waited for 3 weeks for the control to reply via ISD (Step 3). In case of no reply (within a 3 week period) a reminder letter was sent directly to the control (Step 4). If the control refused to take part, the type of response (NO) was recorded in the control recruitment recording form, the next eligible control for a particular case was approached and steps 1 and 2 were repeated (Step 5). If the control agreed to participate, the type of response was recorded (YES) in the control recruitment recording form and the details were passed to the research nurse responsible for the recruitment visit (Step 6) (Figure 31).

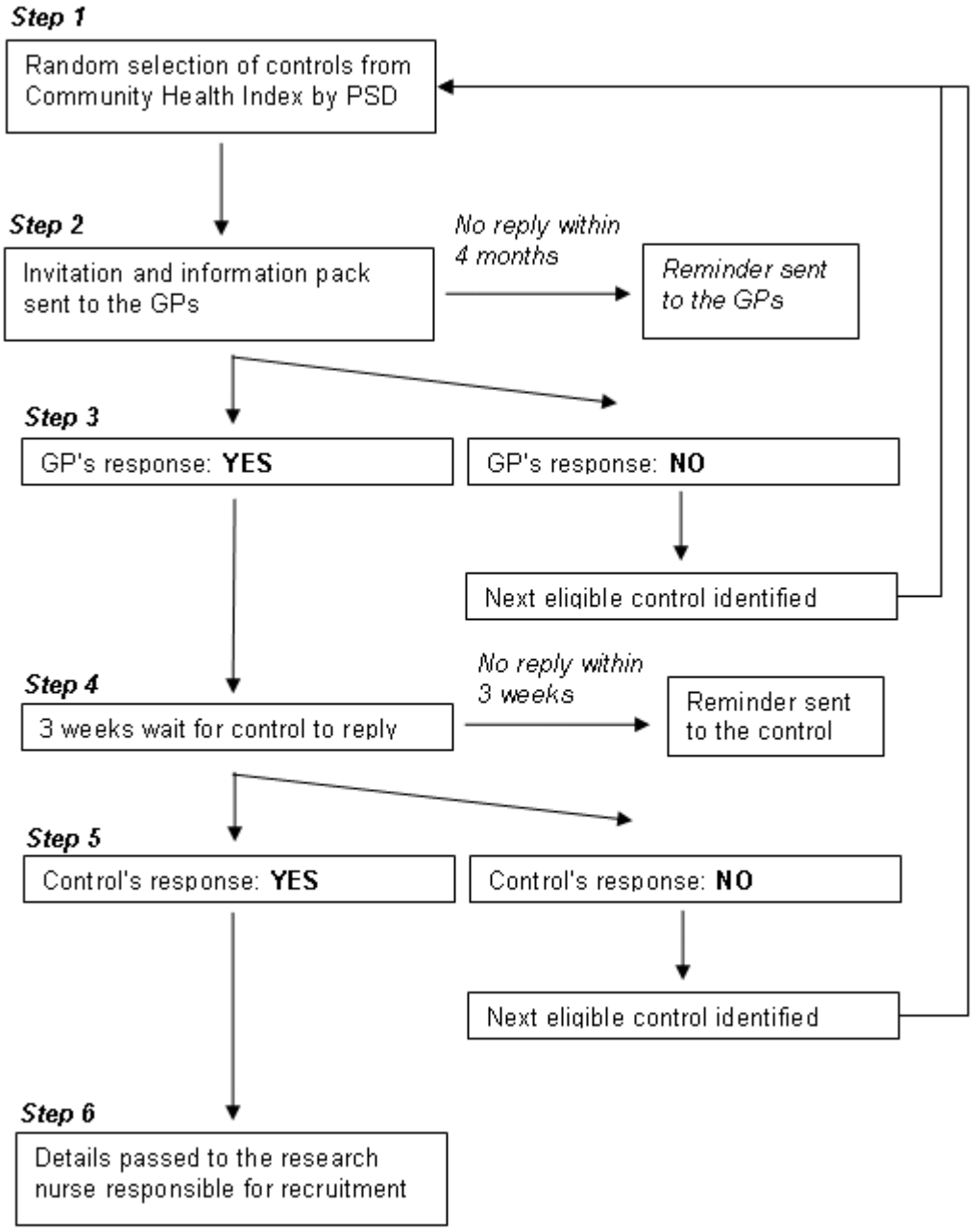


Figure 31 Selection procedure of controls

4.2.4.2 Recruitment protocol

According to the recruitment protocol, each control received the lifestyle and food frequency questionnaires prior to the recruitment meeting and controls were responsible to bring them to the recruitment meeting. The main steps of the control recruitment were:

- Discussion with the control about the main aims and elements of the study;
- Check that all necessary details were completed and legible on the control details sheet;
- Permission to take a family history and drawing of it on sheet provided;
- Assessment of family history risk (moderate and high risk patients were offered genetic testing or were referred to a cancer genetic clinic);
- Recording of any previous cancer(s) (including year and hospital of diagnosis);
- Completion of study's consent form;
- If control wished for his/ her blood sample to be taken, also the DNA storage consent form was completed.
- Completion of next of kin sheet (in case the control did not wish his/ her blood sample to be taken);
- Control was asked whether he/ she had brought along the lifestyle (Appendix III) and food frequency questionnaires (Appendix IV). If:
    - Yes; Questionnaires were quickly checked through and any necessary help was given.
    - No (forgot to bring along); A prepaid reply envelope was provided.
    - No (did not receive questionnaires); Blank questionnaires and a prepaid reply envelope were provided.
    - No (refused to complete); this answer was recorded in the controls detail form.

4.2.5 Subject data processing and management

4.2.5.1 Assignment of ID numbers

Subjects were assigned to unique identification (ID) numbers (Study ID). The Study ID consisted of four ID parts: a) "Case" ID (4 digits); b) "Status" ID (1 digit); c) "Relevant number" ID (2 digits); d) "Nurse number" ID (2 digits). For cases, "Case" ID numbers were assigned consecutively according to the order in which they were identified from the hospital files. Control subjects were assigned a "Case" ID identical to the "Case" ID of the case subjects that they were matched to. "Status" ID was used to separate cases from control subjects by having the value 0 for cases and the value 2 or 4 for the controls. "Relevant number" ID was used when multiple controls were recruited for one case. Therefore, it always had the value 00 for cases and from 00 up to 06 for the controls. Finally each research nurse had each own code number ("Nurse number" ID). For storing, elaborating and analysis of the collected data this ID system was used in order to protect the subjects' identity.

4.2.5.2 Main database

Recruitment and subject details were entered in an Access database (Main database). Details for non-participants and participants were held in separate tables. The main information contained in the non-participant and participant tables are listed separately for cases and controls in Table 31 and Table 32.

Table 31 List of details included in the Main database: Cases

Title	Data
Participants	
Study ID	Unique ID number
CHI number	National unique identification number assigned from the Community Health Index
Recruitment Date	Date of recruitment
Name	3 columns for case's title, forename and surname
Address	6 columns for case's address: Address line 1, address line 2, post code, post code area, health board area, post code area for calculation of deprivation score
Deprivation score	Deprivation score
Date of birth	Case's date of birth
Sex	Sex
MRC consent	Study participation consent (Yes/ No)
C note consent	Case medical records consent (Yes/ No)
GP details	7 columns for case's GP details: Name, surgery, address (2 columns), town, postcode, telephone number
Hospital	Name of hospital that the case was treated and recruited
Blood sample details	9 columns for blood sample receipt
Questionnaire	Whether lifestyle and food frequency questionnaires were given and returned
Risk	CRC family history risk (low, moderate, high, unclear, not applicable, missing)
Withdrawn	3 columns: Case withdrawn (yes/no), date of withdrawn, reason of withdrawn
Non-participants	
ID number	Unique ID number
Sex	Sex
Age	Age (at time of approach for recruitment)
Under 55	Whether the case was under 55 years old
Hospital	Name of the hospital the case was treated
Consultant	2 columns for the consultant and the associated consultant that treated the case
Date of invite	Date that the case was invited to take part
Health board	Health board of residence

Reason	Reason of no participation
Surgery	Whether the case had colorectal cancer surgery or not (yes/no)
Curative/ Palliative	Whether the surgery was curative, palliative or not recorded
Additional Info	Additional information regarding the reason of no participation

Table 32 List of details included in the Main database: Controls

Title	Data
Participants	
Study ID	Unique ID number
CHI number	National unique identification number assigned from the Community Health Index
PSD* Date	Date that control's details received from PSD
Name	3 columns for control's title, forename and surname
Address	6 columns for control's address: Address line 1, address line 2, post code, post code area, health board area, post code area for calculation of deprivation score
Deprivation score	Deprivation score
Date of birth	Control's date of birth
Sex	Sex
GP details	7 columns for control's GP: Name, surgery, address (2 columns), town, postcode, telephone number
Appointment date	Date of appointment of control with research nurse
MRC consent	Study participation consent (Yes/ No)
Blood sample details	9 columns for blood sample receipt
Questionnaire	Whether lifestyle and food frequency exposure questionnaires were given and returned
Risk	CRC family history risk (low, moderate, high, unclear, not applicable, missing)
Withdrawn	3 columns: Control withdrawn (yes/no), date of withdrawn, reason of withdrawn
Non-participants	
ID number	Unique ID number
Case ID	ID of the case that controls was approached for
Sex	Sex
PSD* Centre	PSD centre
For each control	The following variables were completed for all the approached controls

approached:	
PSD*	PSD sector
Control	Start recruiting control (yes/no)
Control accept	Control accepted to be recruited to the study (yes/no)
Post code area	Post code area for calculation of deprivation score
For a subset of the approached controls:	The following variables were completed for a subset of the controls approached for recruitment. (Up to 15 controls for each case could have been approached)
PSD*	PSD sector
Control	Start recruiting control (yes/no)
GP write 1	Write to the GP for the first time regarding control (yes/no)
GP re-write	Write to the GP for a second time regarding control (yes/no)
GP reply	GP replied regarding control (yes/no)
GP accept	GP accepted to send study information package to control (yes/no)
Control re-write	Write for a second time to control (yes/no)
Control reply	Reply from the control (yes/no)
Control accept	Control accepted to be recruited to the study (yes/no)
Post code area	Post code area for calculation of deprivation score
Deprivation score	Deprivation score
Reason	Reason why the GP or control has not agreed to participate (if applicable)
Other notes	Additional information regarding control recruitment status

* PSD: Practitioner Services Division

4.2.6 Subject participation analysis

In total, 6,678 eligible cases were identified, of which 3,471 cases agreed to participate (overall participation rate: 52.0%). Of the 3,471 recruited cases 54 withdrew from the study and **3,417** cases were finally included in the study (98.4% of the recruited cases). The main reasons of cases withdrawn were: 1) 43 cases did not fulfil the inclusion criteria, 2) five cases withdrew their consent, 3) five cases were duplicates and 4) one case was never recruited.

Regarding controls, 10,593 population-based controls were identified, of which 4,134 agreed to participate (overall participation rate: 39.0%). Of the 4,134 recruited controls, 737 withdrew from the study and **3,396** controls were finally included in the study (82.2% of the recruited controls). The main reasons of controls withdrawn were: 1) 364 controls withdrew their consent, 2) 185 controls were never recruited, 3) 105 controls could not be contacted or moved house, 4) 40 controls did not give blood or their DNA yield was insufficient, 5) 10 controls did not attend the appointments, 6) 10 controls developed colorectal cancer, 7) 23 controls for other reasons.

Distribution of cases was examined across sex, age, and health board area of residence for participants, non-participants and withdrawn subjects (Table 33). Among the non-participants, reason of no response was also examined (Table 34). Distribution of controls was examined across sex, age, health board area of residence, and deprivation score for participants, non-participants and withdrawn subjects (Table 35). For deprivation score information see chapter 4.4.1.

Table 33 Distribution of cases across sex, age, and health board area of residence for participants, non-participants and withdrawn subjects

Cases	Participants* (P) (n=3417)	Non-participants† (NP) (n=3207)	Withdrawn cases (W) (n=54)	p-value P vs. NP	p-value P vs. W
Sex					
Men	1958 (57.3%)	1858 (57.9%)	31 (57.4%)		
Women	1459 (42.7%)	1342 (41.8%)	23 (42.6%)		
Not recorded	0 (0.0%)	7 (0.2%)	0 (0.0%)	0.02	0.99
Age					
Mean (SD)	59.9 (11.6)	67.0 (9.8)‡	60.6 (12.4)§	$<5x10^{-5}$	0.67
Health board area					
Argyll & Clyde	249 (7.3%)	199 (6.2%)	2 (3.7%)		
Ayrshire & Arran	228(6.7%)	239 (7.5%)	3 (5.6%)		
Borders	97 (2.8%)	81 (2.5%)	1 (1.8%)		
Dumfries & Galloway	102 (3.0%)	106 (3.3%)	0 (0.0%)		
Fife	220 (6.4%)	180 (5.6%)	5 (9.3%)		
Forth Valley	187 (5.5%)	154 (4.8%)	4 (7.4%)		
Grampian	497 (14.5%)	282 (8.8%)	13 (24.1%)		
Greater Glasgow	520 (15.2%)	746 (23.3%)	7 (13.0%)		
Highland	165 (4.8%)	116 (3.6%)	3 (5.6%)		
Lanarkshire	315 (9.2%)	350 (10.9%)	2 (3.7%)		
Lothian	533 (15.6%)	447 (13.9%)	7 (13.0%)		
Orkney	11 (0.3%)	4 (0.1%)	0 (0.0%)		
Shetland	16 (0.5%)	9 (0.3%)	0 (0.0%)		
Tayside	263 (7.7%)	281 (8.8%)	2 (3.7%)		
Western Isles	12 (0.3%)	8 (0.3%)	1 (1.8%)		
Not recorded	2 (0.1%)**	5 (0.2%)	4 (7.4%)	<0.0005	<0.0005

* Agreed to participate

† Did not agree to participate

‡ Missing data for 56 non-participants

§ Missing data for 3 withdrawn participants

** Move to England

Table 34 Reason of no response for non-participants

Type of "no" response	Cases (non-participants: n=3207)
Unable to take part*	1276 (39.8%)
Did not want to take part	1877 (58.5%)
Not recorded	54 (1.7%)

* Reasons for being unable to take part: deceased (n=377), exact reason not recorded (n=289), patient too ill to participate (n=276), advanced disease (n=52), unaware of diagnosis (n=33), dementia (n=29), learning difficulties (n=28), not appropriate (n=26), limited understanding (n=18), consultant not agreed for patient to be approached (n=18), patient confused (n=18), mental health problems (n=17), not approached (n=8), unable to give informed consent (n=7), communication problems (n=7), Alzheimer's disease/ Parkinson's disease/ Schizophrenia (n=7), unconfirmed diagnosis (n=6), patient too anxious (n=6), memory problems (n=5), patient did not speak English (n=5), patient depressed (n=3), patient did not live in Scotland (n=3), other reason (n=38).

Table 35 Distribution of controls across sex, age, health board area of residence and Carstairs deprivation index for participants, non-participants and withdrawn subjects

Controls	Participants* (P) (n=3396)	Non-participants† (NP) (n=7291)	Withdrawn controls (W) (n=737)	p-value P vs. NP	p-value P vs. W
Sex‡					
Men	1908 (56.2%)	4194 (57.52%)	410 (55.63%)		
Women	1488 (43.8%)	3088 (42.35%)	327 (44.37%)		
Not recorded	0 (0.0%)	9 (0.12%)	0 (%)	0.05§	0.78
Age‡					
Mean (SD)	61.2 (10.9)**	63.26 (11.43)††	63.23 (11.30)‡‡	$<5x10^{-5}$	$<5x10^{-5}$
Health board area‡					
Argyll & Clyde	224 (6.6%)	615 (8.4%)	57 (7.7%)		
Ayrshire & Arran	233 (6.9%)	616 (8.4%)	37 (5.0%)		
Borders	111 (3.3%)	177 (2.4%)	23 (3.1%)		
Dumfries & Galloway	132 (3.9%)	245 (3.4%)	28 (3.8%)		
Fife	236 (6.9%)	354 (4.8%)	52 (7.1%)		
Forth Valley	187 (5.5%)	373 (5.1%)	59 (8.0%)		
Grampian	540 (15.9%)	780 (10.7%)	111 (15.1%)		
Greater Glasgow	416 (12.2%)	1496 (20.1%)	92 (12.5%)		
Highland	195 (5.7%)	257 (3.5%)	34 (4.6%)		
Lanarkshire	255 (7.5%)	829 (11.4%)	59 (8.0%)		
Lothian	568 (16.7%)	956 (13.1%)	84 (11.4%)		
Orkney	14 (0.4%)	17 (0.2%)	4 (0.5%)		
Shetland	13 (0.4%)	21 (0.3%)	8 (1.1%)		
Tayside	264 (7.8%)	537 (7.4%)	79 (10.7%)		
Western Isles	8 (0.2%)	36 (0.5%)	10 (10.7%)		
Not recorded	0 (0.0%)	9 (0.1%)	0 (0.0%)	<0.0005§§	<0.0005
Carstairs deprivation index					
1	318 (9.4%)	270 (3.7%)	52 (7.1%)		
2	686 (20.2%)	675 (9.3%)	128 (17.4%)		
3	923 (27.2%)	1086 (14.9%)	186 (25.2%)		

4	794 (23.4%)	1310 (18.0%)	183 (24.8%)		
5	365 (10.7%)	714 (9.8%)	99 (13.4%)		
6	218 (6.4%)	547 (7.5%)	61 (8.3%)		
7	92 (2.7%)	341 (4.7%)	28 (3.8%)		
Not recorded	0 (0.0%)	2348 (32.2%)	0 (0.0%)	<0.0005***	0.01

* Agreed to participate

† Did not agree to participate

‡ Sex, age and Health Board information for non-participants population controls was obtained from the cases the non-participant population controls were matched to.

§ The chi-square test p-value was 0.17, when we compared men and women distributions (participants versus non-participants) ignoring the 9 subjects, whose sex was not recorded.

** For 17 participants, age was calculated based on the date that the PSD report was returned to the study office and for 4 participants age could not be calculated.

†† Age is missing for 9 non-participants population controls.

‡‡ For 467 withdrawn population controls, age was calculated based on the date that the PSD report was returned to the study office and for 4 withdrawn population controls age could not be calculated.

§§ The chi-square test p-value was <0.0005, when we compared Health Board distributions (participants versus non-participants) ignoring the 9 subjects, whose health board information was not recorded.

*** The chi-square p-value was <0.0005 when we compared Carstairs Deprivation Index distributions (participants versus non-participants) ignoring the 2348 subjects, whose post code sector information was either not recorded or inadequate.

4.2.7 Biological materials

Materials collected from case and control subjects comprised blood, from which DNA, peripheral blood lymphocytes and plasma were prepared and stored in a custom made facility. In addition, tumour material and matched tumour/ normal material were collected from cases under 55 years old. Tumour material was also collected from older cases (>55 years old), but the rate of success was lower than for those >55 years old.

4.2.7.1 DNA preparation, storage and quality assurance

Blood samples were transferred to the academic campus at the Western General Hospital within 72 hours of sampling. Three aliquots of 10ml of blood were collected from each subject in two sodium Ethylene Diamine Tetraacetic Acid (EDTA) tubes and one Acid Citrate Dextrose (ACD) tube. Samples were received centrally, logged and bar-coded in the Wellcome Trust Millennial Clinical Research Facility (WT-CRF). DNA extraction was carried out using Nucleon kits. Samples were bar coded for sample tracking and management. Standard operating procedures appropriate for CPA accreditation were followed by the laboratory. One EDTA sample was directly extracted to DNA, the other was stored frozen as a white blood cell pallet in case of extraction failure. Median DNA yield on samples was 327µg (maximum yield 1197µg). The minimum yield was 50 µg of DNA since patients were asked to give a further blood sample in event of lower yields. Quality control procedures included spectrophotometric readings of every sample (either A260/280 or PicoGreen™), agarose gel electrophoresis of uncut and restriction enzyme cut DNA from 2% of samples and a control PCR on 1% of samples. Stock DNA concentration is currently stored at a target concentration of 1 mg/ml.

4.2.7.2 Peripheral Blood Lymphocytes

Peripheral blood lymphocytes processing and cryopreservation was carried out in the Cytogenetics Service of the South East Scotland Clinical Genetic Service, which is aligned with the WT-CRF. 10ml of blood in ACD anticoagulant tubes were bar coded. Peripheral blood lymphocytes were separated from whole blood by centrifugation over Ficoll-Hypaque. After centrifugation, mononuclear cells were isolated from the interface of the buffer, washed in media and preserved in FCS/DMSO. After controlled cooling,

the cells were stored in liquid nitrogen in two aliquots, if sufficient blood in good condition was received. If fewer lymphocytes were obtained they were stored in one aliquot. The mean cell count of project samples was 4.4 x 10^6 cells (maximum cell count has been 80 x 10^6 cells).

4.2.7.3 Plasma

Plasma was prepared by gentle centrifugation of sodium EDTA tubes prior to DNA extraction. 1500 µl of plasma was stored for each case and control for future proteomic studies. Plasma samples were all bar-coded and stored at -80ºC.

4.2.7.4 Tumour material

Formalin-fixed, paraffin-embedded tumour and normal material from all colorectal cancer patients aged <55yrs at diagnosis was collected. In addition, matched tumour/ normal material was stored for cases aged <55yrs.

4.2.8 Phenotype data collected

4.2.8.1 Tumour related parameters, clinical data and treatment details

Tumour related parameters, clinical data and treatment details were extracted from medical records by medical students or research nurses trained by the research nurse co-ordinator. In particular, information on tumour site, histological type, degree of differentiation, presence of synchronous or metachronous polyps, co-existent inflammatory bowel disease, Charlson co-morbidity index and types of symptoms before diagnosis were gained from pathology reports and medical records. In addition details on surgery procedure, adjuvant chemotherapy, radiotherapy and/ or palliative treatment were extracted from medical records.

To extract tumour stage details, a Specialist Registrar looked at all the pathology reports. However pathology reports of some subjects lacked information regarding metastasis status. For those cases, the Specialist Registrar looked at their Computerised Tomography (CT) scans (Health boards of: Lothian, Fife) and/ or wrote to the cases consultant (hospital) doctors and/ or to the cases GP doctors to get the necessary

information. Two systems were used to describe the extent of colorectal cancer in the patient bodies: the Dukes' and AJCC systems.

4.2.8.2 Personal, demographic and family history data

Ethnicity and ancestry data were recorded for all study participants. Demographic data were derived directly from participants and NHS clinical notes. Data were also collected from central NHS data and held by the Information and Statistics Division of the NHS in Scotland.

In addition, if participants agreed, a three-generation family history was constructed by a trained research nurse at recruitment time. Each family history then was assessed according to the risk levels published in the Scottish Executive cancer guidelines for colorectal cancer.

4.2.9 Self-administered lifestyle and food frequency questionnaires

Two standard questionnaires (the Lifestyle and Cancer Questionnaire - LCQ and the Scottish Collaborative Group Food Frequency Questionnaire - SCG-FFQ) were administered gathering data on use of aspirin/ NSAIDs, reproductive history/ hormonal factors, occupation, inflammatory bowel disease and on lifestyle characteristics such as diet, physical activity, tobacco smoking and alcohol intake. These questionnaires consisted of validated instruments used in other studies (i.e. the physical activity section was based on a modified version of the standard EPIC questionnaire, the women's reproduction section was based on the Million Women Study questionnaire and for measuring dietary intakes the SCG-FFQ was used, which was validated in Scottish populations). The reference (exposure) period for both questionnaires was one year prior recruitment for controls or one year prior to diagnosis for cases.

4.2.9.1 Lifestyle and Cancer Questionnaire

The LCQ was used in order to gather information about the general lifestyle of the subjects and the questions were referred in a time period of one year before diagnosis (cases) or recruitment (controls). It consisted of 69 questions grouped in 8 categories (Medical history, Lifestyle, Physical activity, Height and weight, Medicines, About you,

Employment, Women's health questions) (Table 36: a summarised presentation of the LCQ; Appendix III; the LCQ). There was an information sheet enclosed with instructions of how to complete the questionnaire and in the last page of it there was space to add any comments or concerns.

4.2.9.2 Scottish Collaborative Group Food Frequency Questionnaire

The FFQ used in this study was the validated SCG-FFQ, Version 6.41, which has been based on an FFQ extensively used in Scottish populations (269). It has been validated against 4-day weighed diet records (270;271) and against serum phytoestrogen concentrations (272). The SCG-FFQ consisted of a list of 150 foods, divided into 20 food groups (Table 37: a summarised presentation of the FFQ; Appendix IV; the SCG-FFQ). Subjects were asked to describe the amount and frequency of each food on the list they have eaten a year prior to recruitment. Regarding frequency, subjects were asked to circle "R" (stands for rarely/ never) for those foods that were eaten either never or less than once a month. For foods that were eaten once a month or more, subjects were asked to report the amount of food eaten (counted in measures: 1 up to 5+ measures) and the number of days per week the food was usually eaten (once a month up to 7 days per week). In addition the FFQ included a field that the subjects could use to add other foods that were not listed in the FFQ and that they ate regularly (once a month or more often). Subjects were also asked to report the type and amount of vitamins, minerals and food supplements if taken, recent dietary changes and special diets or dietary restrictions. The last part of the FFQ consisted of general questions about the diet of the subjects including the amount of meals per day, the times per usual week that had fried or grilled meat and how well cooked they normally had their fried or grilled meat (lightly, medium or well browned). An FFQ information sheet that included a colour picture showing examples of the size of measures was enclosed with the FFQ.

Table 36 Lifestyle and Cancer Questionnaire sections and questions

Group	Subgroup	LCQ Questions
Medical History	-	1 – 6
Lifestyle	Cigarette smoking	7 – 15
	Cigar smoking	16 – 20
	Pipe smoking	21 – 25
Physical Activity	Occupational Physical Activity	26 – 27
	Leisure Physical Activity	28 – 30
Height and Weight	-	31 – 32
Medicines	Aspirin / Painkillers	33, 35
	Stomach medicines/tablets	34, 35
About you	Education	36
	Ethnic Origin	37
	Ancestry	38 – 44
Employment	-	45 – 54
Women's Health Questions	Menstrual Periods	55 – 57
	Hormone Replacement Therapy	58 – 62
	Reproductive History	63 – 65
	Hormonal forms of Contraception	66 – 69

Table 37 FFQ food groups and other sections

FFQ Section	Food group / Other questions
1. a – e	Breads
2. a – f	Breakfast cereals
3. a – e	Milk
4. a – e	Cream and yoghurt
5. a – e	Cheese
6. a – c	Eggs
7. a – l	Meats
8. a – l	Fish
9. a – j	Potatoes, rice and pasta
10. a – s	Savoury foods, soups and sauces
11. a – q	Vegetables
12. a – j	Fruit
13. a – h	Puddings and desserts
14. a – i	Chocolates, sweets, nuts and crisps
15. a – g	Biscuits
16. a – e	Cakes
17. a – g	Spreads
18. a – m	Beverages and soft drinks
19. a – h	Alcoholic drinks
20. a – d	Other foods and drinks
21. a – d	Vitamin, mineral and food supplements
22. a – i	Dietary restrictions and special diets
23. a – j	Other information

4.3 Collection and process of lifestyle and dietary data

This thesis was based on the analysis of the data collected from the self-administered environmental exposure questionnaires. In this part of the chapter details about the collection, storing and process of the lifestyle and dietary data are presented.

Of the 3,417 cases that were enrolled in the study: 291 cases were not asked to complete the lifestyle and food frequency questionnaires (participants recruited before September of 2001; 8.5%), 508 cases refused to complete the questionnaires (14.9%), 2,244 cases returned both questionnaires (65.7%), 64 cases returned just one questionnaire (1.9%; 52 cases returned only the LCQ and 12 cases returned only the FFQ) and 310 cases did not return any of the questionnaires (9.1%) (Table 38).

Of the 3,396 controls that were enrolled in the study: 26 controls were not asked to complete the lifestyle and food frequency questionnaires (0.8%), 33 controls refused to complete the questionnaires (1.0%), 2,850 controls returned both questionnaires (83.9%), 124 controls returned just one questionnaire (3.6%; 105 controls returned only the LCQ and 19 controls returned only the FFQ) and 363 controls did not return any of the questionnaires (10.7%) (Table 39). Distribution of cases and controls across sex, age, health board area of residence and deprivation score was examined according to the questionnaire status (not asked, refused, not returned, returned both, returned one) (Table 38, Table 39).

Table 38 Distribution of cases across sex, age, health board area of residence and deprivation examined according to the questionnaire status

Cases	Not asked	Refused	Not returned	Returned-both	Returned-one	Not asked/ refused/ not returned	Returned (one or both)	p-value (returned vs. all other)
Number	291	508	310	2244	64	1109	2308	
Sex								
Males	169 (58.1%)	312 (61.4%)	162 (52.3%)	1277 (56.9%)	38 (59.4%)	643 (58.0%)	1315 (57.0%)	
Females	122 (41.9%)	196 (38.6%)	148 (47.7%)	967 (43.1%)	26 (40.6%)	466 (42.0%)	993 (43.0%)	0.58
Age	48.1 (7.1)	62.7 (12.0)	49.2 (6.0)	62.2 (10.8)	61.59 (11.4)	55.1 (11.8)	62.2 (10.8)	$<5x10^{-5}$
Health board area								
Argyll & Clyde	23 (7.9%)	36 (7.1%)	30 (9.7%)	154 (6.9%)	6 (9.4%)	89 (8.0%)	160 (6.9%)	
Ayrshire & Arran	20 (6.9%)	29 (5.7%)	23 (7.4%)	155 (6.9%)	1 (1.6%)	72 (6.5%)	156 (6.8%)	
Borders	2 (0.7%)	6 (1.2%)	6 (1.9%)	81 (3.6%)	2 (3.1%)	14 (1.3%)	83 (3.6%)	
Dumfries & Galloway	9 (3.1%)	12 (2.4%)	6 (1.9%)	72 (3.2%)	3 (4.7%)	27 (2.4%)	75 (3.2%)	
Fife	17 (5.8%)	27 (5.3%)	29 (9.3%)	142 (6.3%)	5 (7.8%)	73 (6.6%)	147 (6.4%)	
Forth Valley	13 (4.5%)	33 (6.5%)	11 (3.5%)	128 (5.7%)	2 (3.1%)	57 (5.1%)	130 (5.6%)	
Grampian	34 (11.7%)	60 (11.8%)	40 (12.9%)	351 (15.6%)	12 (18.7%)	134 (12.1%)	363 (15.7%)	
Greater Glasgow	47 (16.1%)	121 (23.8%)	33 (10.6%)	311 (13.9%)	8 (12.5%)	201 (18.1%)	319 (13.8%)	
Highland	17 (5.8%)	29 (5.7%)	13 (4.2%)	100 (4.5%)	6 (9.4%)	59 (5.3%)	106 (4.6%)	
Lanarkshire	29 (10.0%)	72 (14.2%)	31 (10.0%)	179 (8.0%)	4 (6.2%)	132 (11.9%)	183 (7.9%)	
Lothian	47 (16.1%)	63 (12.4%)	60 (19.3%)	354 (15.8%)	9 (14.1%)	170 (15.3%)	363 (15.7%)	
Orkney	1 (0.3%)	1 (0.2%)	1 (0.3%)	8 (0.4%)	0 (0.0%)	3 (0.3%)	8 (0.3%)	
Shetland	6 (2.1%)	0 (0.0%)	2 (0.6%)	7 (0.3%)	1 (1.6%)	8 (0.7%)	8 (0.3%)	
Tayside	23 (7.9%)	17 (3.3%)	22 (7.1%)	196 (8.7%)	5 (7.8%)	62 (5.6%)	201 (8.7%)	
Western Isles	1 (0.3%)	2 (0.4%)	3 (1.0%)	6 (0.3%)	0 (0.0%)	6 (0.5%)	6 (0.3%)	

Not recorded	2 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.2%)	0 (0.0%)	<0.0005
Deprivation score								
1	27 (9.3%)	30 (5.9%)	22 (7.1%)	206 (9.2%)	2 (3.1%)	79 (7.1%)	208 (9.0%)	
2	32 (11.0%)	84 (16.5%)	65 (21.0%)	463 (20.6%)	13 (20.3%)	181 (16.3%)	476 (20.6%)	
3	73 (25.1%)	105 (20.7%)	85 (27.4%)	578 (25.8%)	19 (29.7%)	263 (23.7%)	597 (25.9%)	
4	72 (24.7%)	141 (27.7%)	81 (26.1%)	523 (23.3%)	11 (17.2%)	294 (26.5%)	534 (23.1%)	
5	33 (11.3%)	61 (12.0%)	34 (11.0%)	247 (11.0%)	14 (21.9%)	128 (11.5%)	261 (11.3%)	
6	30 (10.3%)	52 (10.2%)	11 (3.5%)	159 (7.1%)	3 (4.7%)	93 (8.4%)	162 (7.0%)	
7	22 (7.6%)	35 (6.9%)	12 (3.9%)	66 (2.9%)	2 (3.1%)	69 (6.2%)	68 (2.9%)	
Not recorded	2 (0.7%)	0 (0.0%)	0 (0.0%)	2 (0.1%)	0 (0.0%)	2 (0.2%)	2 (0.1%)	<0.0005

Table 39 Distribution of controls across sex, age, health board area of residence and deprivation examined according to the questionnaire status

Controls	**Not asked**	**Refused**	**Not returned**	**Returned-both**	**Returned-one**	**Not asked/ refused/ not returned**	**Returned (one or both)**	**p-value returned vs. all other)**
Number	26	33	363	2850	124	422	2974	
Sex								
Males	17 (65.4%)	20 (60.6%)	186 (51.2%)	1617 (56.7%)	68 (54.8%)	223 (52.8%)	1685 (56.7%)	
Females	9 (34.6%)	13 (39.4%)	177 (48.8%)	1233 (43.3%)	56 (45.2%)	199 (47.2%)	1289 (43.3%)	0.14
Age	50.5 (5.5)	67.3 (9.4)	51.0 (7.9)	62.4 (10.5)	61.4 (11.6)	52.3 (9.0)	62.4 (10.6)	$<5x10^{-5}$
Health board area								
Argyll & Clyde	2 (7.7%)	1 (3.0%)	31 (8.5%)	182 (6.4%)	8 (6.4%)	34 (8.1%)	190 (6.4%)	
Ayrshire & Arran	5 (19.2%)	1 (3.0%)	25 (6.9%)	197 (6.9%)	5 (4.0%)	31 (7.3%)	202 (6.8%)	
Borders	1 (3.8%)	1 (3.0%)	11 (3.0%)	93 (3.3%)	5 (4.0%)	13 (3.1%)	98 (3.3%)	

Dumfries & Galloway	2 (7.7%)	0 (0.0%)	9 (2.5%)	114 (4.0%)	7 (5.6%)	11 (2.6%)	121 (4.1%)	
Fife	3 (11.5%)	5 (15.1%)	33 (9.1%)	181 (6.3%)	14 (11.3%)	41 (9.7%)	195 (6.6%)	
Forth Valley	0 (0.0%)	0 (0.0%)	10 (2.7%)	171 (6.0%)	6 (4.8%)	10 (2.4%)	177 (5.9%)	
Grampian	2 (7.7%)	9 (27.3%)	51 (14.0%)	458 (16.1%)	20 (16.1%)	62 (14.7%)	478 (16.1%)	
Greater Glasgow	2 (7.7%)	3 (9.1%)	39 (10.7%)	362 (12.7%)	10 (8.1%)	44 (10.4%)	372 (12.5%)	
Highland	1 (3.8%)	2 (6.1%)	26 (7.2%)	159 (5.6%)	7 (5.6%)	29 (6.9%)	166 (5.6%)	
Lanarkshire	2 (7.7%)	3 (9.1%)	23 (6.3%)	219 (7.7%)	8 (6.4%)	28 (6.6%)	227 (7.6%)	
Lothian	2 (7.7%)	6 (18.2%)	70 (19.3%)	469 (16.5%)	21 (16.9%)	78 (14.5%)	490 (16.5%)	
Orkney	0 (0.0%)	1 (3.0%)	0 (0.0%)	13 (0.5%)	0 (0.0%)	1 (0.2%)	13 (0.4%)	
Shetland	0 (0.0%)	0 (0.0%)	1 (0.3%)	11 (0.4%)	1 (0.8%)	1 (0.2%)	12 (0.4%)	
Tayside	4 (15.4%)	1 (3.0%)	33 (9.1%)	215 (7.5%)	11 (8.9%)	38 (9.0%)	226 (7.6%)	
Western Isles	0 (0.0%)	0 (0.0%)	1 (0.3%)	6 (0.2%)	1 (0.8%)	1 (0.2%)	7 (0.2%)	
Not recorded	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.05
Deprivation score								
1	3 (11.5%)	2 (6.1%)	38 (10.5%)	263 (9.2%)	12 (9.7%)	43 (10.2%)	275 (9.2%)	
2	4 (15.4%)	5 (15.1%)	70 (19.3%)	577 (20.2%)	30 (24.2%)	79 (18.7%)	607 (20.4%)	
3	5 (19.2%)	10 (30.3%)	94 (25.9%)	782 (27.4%)	32 (25.8%)	109 (25.8%)	814 (27.4%)	
4	8 (30.8%)	9 (27.3%)	82 (22.6%)	666 (23.4%)	29 (23.4%)	99 (23.5%)	695 (23.4%)	
5	3 (11.5%)	2 (6.1%)	47 (12.9%)	300 (10.5%)	13 (10.5%)	52 (12.3%)	313 (10.5%)	
6	3 (11.5%)	2 (6.1%)	25 (6.9%)	182 (6.4%)	6 (4.8%)	30 (7.1%)	188 (6.3%)	
7	0 (0.0%)	3 (9.1%)	7 (1.9%)	80 (2.8%)	2 (1.6%)	10 (2.4%)	82 (2.8%)	0.83

4.3.1 Pre-entering (LCQ) or pre-scan (FFQ) review process

A protocol was set up to review the returned questionnaires. In the main database of the study a field was set up to record the status of the questionnaires (returned: no/ yes). When the questionnaires returned to the study office, it was recorded in the main database (returned: yes), the sex of the subject was written at the top of the LCQ and they were passed to the project co-ordinator (from 01/02/1999 to 15/01/2005) or to the author (from 16/01/2005 to 31/12/2006) for checking for any blanks, missed questions or mistakes. If only one questionnaire was returned (either the LCQ or the FFQ) this was noted on the top of the returned questionnaire and it was recorded in the field of the main database (returned: yes).

4.3.1.1 Lifestyle and Cancer Questionnaire

If there were any blanks, missed questions or mistakes in the LCQ, then it was sent back to the subject for corrections and the new corrected version was used. If the questionnaire sent for corrections was not back within three months then the uncorrected version was used. The pre-enter review checklist is presented in Box 1. After the pre-enter review the original or corrected LCQs were entered manually in the Lifestyle and Cancer database and the hard-copies were stored in filing cabinets according to their status (cases or population controls) and in numerical order.

4.3.1.2 Food frequency questionnaire

The FFQs pre-scan review was done using the FFQ review checklist (Box 2) to ensure that the FFQ was complete and ready to be scanned. For any queries regarding the "Spreads" and "Other Foods" sections the FFQ queries database was developed. In this database any queries on other foods and odd spreads or fats were stored. With the guidance of Dr Geraldine McNeill (University of Aberdeen) the "Extra Fats" guidelines and the "Other Foods" guidelines were developed and the latter queries were answered. After the pre-scan review the original or corrected FFQs were scanned using a multi-page scanner and the software scanning package TELEForm. Once the FFQs had been scanned, the scanning procedure was verified using the TELEForm Verifier. In particular, it was checked whether the FFQ data had been correctly scanned and read,

that open answer questions were correctly identified and that the chosen values for multiple response answers were correctly recognised. The FFQ data were then automatically exported and saved to an SPSS file and the hard-copies were stored in filing cabinets according to their status (cases or controls) and in numerical order.

Box 1: LCQ pre-enter review checklist

Data checks	• The whole questionnaire was checked to see if blank. • If there were any questions, where there were major parts not filled in or two or more conflicting options had been reported the questionnaire was sent back. • If there was a need to decide on conflicting answers, the first part was taken as the correct one. • If there was more than one dates entered the earliest one was used. • Physical activity section (Q28): - The items of this question form a score so if there were any missing parts, the questionnaire was sent back to the subject. - If a range instead of an absolute value was given, the average was taken. • Height and weight (Q31 and Q32a): If a range instead of an absolute value was given, the average was taken. • Waist measurement: If no waist measurement was given the clothing size was asked and the waist measurement was calculated from a clothing guide. • Medicine section (Q33-Q35): - If Q33a was YES and nothing ticked at Q33b it meant that participants did not take any medicines for at least 4 days a week for at least a month. - Any medicines ticked at Q33b and Q34b were checked that they were listed at Q35. - Number of months taken for a medicine was calculated if participants were no longer taking the medicine and it was left blank if they were still taking it. • Employment section (Q45-54): - If this section was completely blank the questionnaire was sent back for clarification. - Often self-employed people with no employees said that 0 people worked at their work, which was corrected to 1-9 employees. • Female section (Q55-Q69): - The female part of the LCQ was checked to see whether it was blank for male

	subjects and completed for female subjects. - If the whole section was blank (and the questionnaire was filled in by a female subject) the questionnaire was sent back to the subject - It was checked that for each birth given in Q64 a record was entered in Q65
Filling of LCQs	• After manually entering, the LCQs were stored in filing cabinets according to their status (cases or population controls) and in numerical order.

Box 2: FFQ pre-scan review checklist

Data checks	• The whole questionnaire was checked to see if it was blank. • For the questions 1 to 18 the following checks were done: - Number of blank lines: For up to 10 blank lines (both "measures per day" and "number of days per week" were blank) the questionnaire was not returned for completion. - Number of missing "measures per day": For up to 10 missing "measures per day" the questionnaire was not returned for completion. - Number of missing "days per week": For up to 10 missing "days per week" the questionnaire was not returned for completion. - If M (monthly) was circled together with a number of days per week then: if it was M and 1, 2 or 3 the number was just crossed out. If it was M and 4, 5, 6 or 7 the M and the number were crossed out and 1 for once a week was circled. - If R (rarely) was circled together with a number for the "measure per day", the number was crossed out leaving the R alone. - If the subject had circled M plus days or R plus measures all the way through, then the questionnaire was sent back for clarification. • In the dietary restrictions section we checked if anything not eaten did not correspond with the questions 1 to 18 and that all the fields were answered.
Coding	• Questions 17.e and 17.g were open-ended questions relating to the spreads, fats and oil consumed. Codes were entered onto the FFQ according to the type of spread/ fat/ oil. Queries were entered in the FFQ queries database and were checked with Dr G McNeill.
Other foods	• Section 20 allowed subjects to record foods and drinks that were not included in the FFQ and that they regularly ate. If the subject had reported any other food we did as follows: - We checked if the food could be easily entered in the FFQ and if so we did that by consulting the Other Food guidance and the FFQ queries database.

	- Some foods could be ignored (e.g. if less than once a week) and in that case we just scored out the food. - If guidance had a code listed for that particular food, then we wrote the details of this questionnaire (ID, food type, portion, "measures per day "and" days per week) in the FFQ other food database. - Finally, if the food was not listed and we didn't know how to deal with it, we added its details to the FFQ queries database and it was sent to Dr G McNeill for clarification.
ID number	• ID was written in the top right hand corner of each FFQ page.
Filling of FFQs	• After scanning and verifying, the FFQs were stored in filing cabinets according to their status (cases or controls) and in numerical order.

4.3.2 Quality checking of data entry

A quality checking protocol and quality checking databases were developed for all data entry of the environmental questionnaires and it was applied on a regular basis by the project co-ordinator (from 01/02/1999 to 15/01/2005) or by the author (from 16/01/2005 to 31/12/2006). This generally involved looking at 1 in 20 questionnaires and checking that they had been entered correctly. The quality checking procedure was recorded on a separate sheet for each database, noting the number of errors. Any errors found were corrected on the original databases.

4.3.3 Coding of the LCQ variables

2,296 and 2,955 LCQs were received from cases and controls respectively. Six cases (0.3%) and nine controls (0.3%) sent blank LCQs and could not be used in the analysis. In addition 13 controls (0.4%) were removed. These controls were given the same IDs with 13 withdrawn controls. However, it was not possible to distinguish whether the questionnaires were from the newly recruited or from the withdrawn controls and therefore they were not included in the analysis. The final number of LCQs that could be analysed was 2,290 for the cases and 2,933 for the controls. After the entering procedure of the data and the quality checking of it, the LCQ data were processed to form the variables used in the analysis (Table 40).

Table 40 List of variables coded from the LCQ

Category	Variables
Smoking	Smoking Status; Level of smoking; Duration of smoking; Pack-years of smoking (see Table 41)
Physical Activity	Occupational Physical Activity; Leisure time Physical Activity (recreational, household and stair climbing variables); Total Physical Activity index (see Table 45); Cambridge Physical Activity index (see Table 46); Limited Physical Activity (see Table 47)
Medicines	Mini-aspirin intake; Non steroidal anti-inflammatory drugs intake; Painkillers intake; Stomach tablets intake; Dose of intake (for mini aspirin); Duration of intake (see Table 48)
Women's Health	Hormone replacement intake; Hormonal forms of contraceptives (see Table 49)

4.3.3.1 Smoking variables

The smoking variables were coded using information from the lifestyle section of the LCQ (questions 7 to 25). Smoking status variable was coded using information from questions 7, 9, 16, 18, 21 and 23. Subjects that had never smoked regularly cigarettes (at least one per day), cigars (at least one per month) or a pipe were considered as "never smokers". Subjects that had used to smoke regularly cigarettes, cigars or a pipe, but quitted at least one year prior to recruitment were considered as "former smokers". Subjects that smoked regularly cigarettes, cigars or a pipe were considered as "current smokers" (Table 41).

The other three smoking variables (level, duration and pack-years of smoking) were based only on the smoking cigarettes questions (questions 7 to 15) and they were available for "current" and "former" smokers (Table 41). "Level of smoking" variable was about the quantity of cigarettes smoked per day and "Duration of smoking" variable was about the total years of smoking. "Pack-years of smoking" variable was coded combining information for both the quantity and duration of smoking using the following formula:

Pack-years of smoking = (n x y) / 20,

Where n was the number of cigarettes smoked per day and y was the number of years of smoking (Table 41).

Table 41 Smoking variables

Smoking variables		
All subjects	**Cases (n=2290)**	**Controls (n=2933)**
Smoking Status		
Never	965 (42.1%)	1261 (43.0%)
Former	884 (38.6%)	1110 (37.8%)
Current	394 (17.1%)	537 (18.3%)
Missing	47 (2.0%)	25 (0.8%)
Current smokers	**Cases (n=342)**	**Controls (n=467)**
Level of smoking		
0-10	43 (12.6%)	78 (16.7%)

10-20	127 (37.1%)	182 (39.0%)
≥ 20	163 (47.7%)	203 (43.5%)
missing	9 (2.6%)	4 (0.9%)
Duration of smoking		
0-15	7 (2.0%)	6 (1.3%)
15-30	42 (12.3%)	57 (12.2%)
≥ 30	291 (85.1%)	404 (86.5%)
missing	2 (0.6%)	0 (0.0%)
Pack-years of smoking		
0-10	25 (7.3%)	39 (8.3%)
10-20	46 (13.4%)	77 (16.5%)
≥ 20	261 (76.3%)	347 (74.3%)
missing	10 (2.9%)	4 (0.9%)
Former smokers	**Cases (n=900)**	**Controls (n=1127)**
Level of smoking		
0-10	116 (12.9%)	165 (14.6%)
10-20	308 (34.2%)	385 (34.2%)
≥ 20	458 (50.9%)	563 (50.0%)
missing	18 (2.0%)	14 (1.2%)
Duration of smoking		
0-15	184 (20.4%)	294 (26.1%)
15-30	339 (37.7%)	400 (35.5%)
≥ 30	350 (38.9%)	411 (36.5%)
missing	27 (3.0%)	22 (36.5%)
Pack-years of smoking		
0-10	221 (24.6%)	315 (27.9%)
10-20	180 (20.0%)	260 (23.1%)
≥ 20	465 (51.7%)	523 (46.4%)
missing	33 (3.7%)	29 (2.6%)

4.3.3.2 Physical activity variables

The physical activity part of the LCQ was the short version of the EPIC core physical activity questionnaire and we used their protocol for coding the variables. The four EPIC physical activity questions referred to activity for the year prior to diagnosis or recruitment. The first question was about the occupational physical activity and it had two parts: 1) a binary part asking for the occupation status a year prior to the recruitment (q26) and 2) a four-points, mutually-exclusive, ordered part concerning the intensity of the physical activity at work (q27). The way that the questionnaire was designed only the participants that were currently working, were asked about the type of their job. Therefore, participants that were either unemployed or retired (for at least one year prior to recruitment) did not report their physical activity at work.

The second question (recreational physical activities) asked about the amount of time spent (in hours per week) in each of the following activities: walking (separately for summer and winter), cycling (separately for summer and winter), gardening (separately for summer and winter), do-it-yourself activities, physical exercise (separately for summer and winter) and housework (q28). The third question asked whether any of the activities in question 28 were engaged in such that it caused sweating or faster heartbeat and if so, for how many hours during a typical week (q29). Finally, the fourth question asked about the amount of flights of stairs climbed per day (q30).

To be able to assess the impact of the physical activity as a whole, it was necessary to combine occupational data with the recreational, household, vigorous activity and flights of stair climbing data. We did that by using two different indexes; the Total Physical Activity index (developed by the EPIC study group) and the Cambridge Physical Activity index (developed by the Cambridge group of the EPIC study). The first index is a cross tabulation table of the occupational physical activity and a summary variable of the household, recreational and stair climbing physical activities. The second index combines the occupational physical activity with two measurements of the recreational physical activity: cycling and physical exercise. In addition, we applied a limited physical activity measurement taking into account only two recreational physical activities.

Occupational physical activity

To assess the physical activity at work a six-level categorical variable was created ("Occupational physical activity"): 1) sedentary occupation, 2) standing occupation, 3) manual work, 4) heavy manual work, 5) unemployed (included participants that reported not to have done any type of work a year prior to recruitment), 6) missing (Table 42). A limitation of the occupational part of the physical activity questionnaire was that the retired participants were misclassified as unemployed and their occupational physical activity was not reported. Therefore 48% of the cases and 47% of the controls were classified as unemployed (Table 42).

Leisure time physical activity

Regarding the leisure time physical activity 12 variables were available: 1) Walking in summer (hours/week), 2) walking in winter (hours/week), 3) cycling in summer (hours/week), 4) cycling in winter (hours/week), 5) gardening in summer (hours/week), 6) gardening in winter (hours/week), 7) doing sports in summer (hours/week), 8) doing sports in winter (hours/week), 9) housework (hours/week), 10) Do-It-Yourself (DIY) activities (hours/week), 11) engaging in vigorous activities (hours/week) and 12) number of flights of stairs climbed per day. Reasonable maximum gender-specific cut-off points were set for each activity and values above those maxima were deleted (Table 43).

To estimate the intensity of the leisure time physical activity, the hours per week of each activity were multiplied with a specific metabolic equivalent (MET) value. A MET is defined as the ratio of the metabolic rate for a specific activity compared to the resting metabolic rate. The MET values used were abstracted from the Compendium of Physical Activities (273) and were: 3.0 for walking, 6.0 for cycling, 4.0 for gardening, 6.0 for sports, 4.5 for DIY activities, 3.0 for housework and 9.0 for vigorous activities. To convert the flights of stairs into a MET-hour/week variable we used the following formula (taken from the EPIC protocol):

20 steps/flight * 1min/72 steps * 1hr/60min * #flights/day * 8 METS * 7 days/week

Means for all those variables that had been reported separately for summer and winter were created and finally by adding all the METS-hours/week variables of the leisure time, the summary variable "Leisure time physical activity" was created (Table 44).

Total Physical Activity index was the sum of "Occupational activity" and "Leisure time physical activity" (Table 45). Cambridge Physical Activity index was the sum of "Occupational activity" and two recreational physical activities (cycling and doing sports) (Table 46). Finally, for the third measurement of the physical activity only the "cycling" and "doing sports" recreational physical activities were used and the "Occupational activity" was not included. In Table 47 the distribution of the study participants according to the three different physical activity measurements is presented.

Table 42 Occupational physical activity

Occupational physical activity	Cases (n=2290)	Controls (n=2933)
Sedentary occupation	454 (19.8%)	625 (21.3%)
Standing occupation	350 (15.3%)	462 (15.7%)
Manual work	266 (11.6%)	351 (12.0%)
Heavy manual work	83 (3.6%)	72 (2.4%)
Unemployed (including retired)	1103 (48.2%)	1389 (47.4%)
Missing	34 (1.5%)	34 (1.2%)

Table 43 Maximum values for recreational physical activities, stair climbing and hours of vigorous physical activity

Leisure physical activity (hours per week)	Men		Women		Subjects with missing data (%)	Subjects with values above cut off point (%)
	median (IQ range)	Cut off point	median (IQ range)	Cut off point		
Cases (n=2290)						
Walking- summer	9 (4-16)	55	10 (5-15)	55	49 (2.1%)	38 (1.7%)
Walking- winter	7 (3-12)	50	7 (4-12)	50	56 (2.4%)	28 (1.2%)
Cycling- summer	0 (0-0)	20	0 (0-0)	20	146 (6.4%)	0 (0.0%)
Cycling- winter	0 (0-0)	15	0 (0-0)	15	157 (6.8%)	1 (0.0%)
Gardening- summer	4 (1-8)	35	3 (0-7)	30	58 (2.5%)	18 (0.8%)
Gardening- winter	0 (0-2)	30	0 (0-1)	25	97 (4.2%)	3 (0.1%)
Doing sports- summer	0 (0-2)	30	0 (0-2)	30	100 (4.4%)	2 (0.1%)
Doing sports- winter	0 (0-2)	25	0 (0-2)	25	108 (4.7%)	2 (0.1%)
Housework	3 (0-7)	30	15 (10-25)	70	65 (2.8%)	23 (1.0%)

DIY activities	2 (0-4)	30	0 (0-1)	30	120 (5.2%)	10 (0.4%)
Flights of stairs	6 (2-10)	50	5 (1-10)	50	66 (2.9%)	22 (1.0%)
Vigorous activities	4 (2-10)	40	4 (2-8)	40	50 (2.2%)	11 (0.5%)
Controls (n=2933)						
Walking- summer	10 (5-15)	55	8 (4-14)	55	41 (1.4%)	33 (1.1%)
Walking- winter	7 (3-12)	50	6 (3-12)	50	48 (1.6%)	24 (0.8%)
Cycling- summer	0 (0-0)	20	0 (0-0)	20	60 (2.0%)	2 (0.1%)
Cycling- winter	0 (0-0)	15	0 (0-0)	15	80 (2.7%)	0 (0.0%)
Gardening- summer	3 (1-8)	35	3 (0-7)	30	15 (0.5%)	23 (0. 8%)
Gardening- winter	0 (0-2)	30	0 (0-1)	25	48 (1.6%)	3 (0.1%)
Doing sports- summer	0 (0-2)	30	0 (0-3)	30	42 (1.4%)	5 (0.2%)
Doing sports- winter	0 (0-2)	25	0 (0-2)	25	58 (2.0%)	11 (0.4%)
Housework	3 (1-8)	30	15 (8-25)	70	37 (1.3%)	39 (1.3%)
DIY activities	2 (0-4)	30	0 (0-1)	30	58 (2.0%)	18 (0.6%)
Flights of stairs	6 (1-10)	50	5 (1-10)	50	49 (1.7%)	44 (1.5%)
Vigorous activities	4 (2-8)	40	4 (2-7)	40	58 (2.0%)	12 (0.4%)

Table 44 Leisure time physical activity

Leisure time physical activity (Met-hours/week)	Cases (n=2290)	Controls (n=2933)
≤61.30	498 (21.7%)	617 (21.0%)
61.30 to 101.59	460 (20.1%)	656 (22.4%)
101.59 to 159.89	474 (20.7%)	636 (21.7%)
>159.89	477 (20.8%)	636 (21.7%)
Missing	381 (16.6%)	388 (13.2%)

Table 45 Total Physical Activity Index (according to the reported occupational, recreational, household vigorous and stair climbing activities)

Occupational activity	Leisure time physical activity (Met-hours/week)			
	Low (≤61.30)	Medium (61.30 to 101.57)	High (101.57 to 159.89)	Very high (>159.89)
Sedentary	Inactive	Inactive	Moderately inactive	Moderately active
Standing	Moderately inactive	Moderately inactive	Moderately active	Active
Manual	Moderately active	Moderately active	Active	Active
Heavy manual	Moderately active	Moderately active	Active	Active
Unemployed	Moderately inactive	Moderately inactive	Moderately active	Moderately active
Unknown/ Missing	Inactive	Moderately inactive	Moderately inactive	Moderately active

Table 46 Cambridge Physical Activity Index (according to the reported occupational physical activity and two recreational physical activities: cycling and doing sports)

Occupational activity	Cycling and doing sports (hours/week)			
	Low (0)	Medium (0 to 3.5)	High (3.5 to 7)	Very high (≥7)
Sedentary	Inactive	Moderately inactive	Moderately active	Active
Standing	Moderately inactive	Moderately active	Active	Active
Manual	Moderately active	Active	Active	Active
Heavy manual	Active	Active	Active	Active

Table 47 Distribution of study participants according to the Total Physical Activity Index, the Cambridge Physical Activity Index and the limited physical activity measurement

Physical activity	Cases (n=2290)	Controls (n=2933)
Total Physical Activity Index		
Inactive	267 (11.7%)	344 (11.7%)
Moderately inactive	696 (30.4%)	928 (31.6%)
Moderately active	695 (30.3%)	963 (32.8%)
Active	251 (11.0%)	310 (10.6%)
Missing	381 (16.6%)	388 (13.2%)
Cambridge Physical Activity Index		
Inactive	220 (9.6%)	259 (8.8%)
Moderately inactive	295 (12.9%)	436 (14.9%)
Moderately active	295 (12.9%)	419 (14.3%)
Active	287 (12.5%)	348 (11.9%)
Missing	1193 (52.1%)	1471 (50.1%)
Limited physical activity measurement (hours/ week of cycling and sports)		
0 hours/week	1233 (53.8%)	1540 (52.5%)
0-3.5 hours/week	518 (22.6%)	727 (24.8%)
3.5-7 hours/week	216 (9.4%)	356 (12.1%)
>7 hours/week	145 (6.3%)	198 (6.7%)
missing	178 (7.8%)	112 (3.8%)

4.3.3.3 Consumption of analgesics (including aspirin and NSAIDs)

Information on the use of mini aspirin, NSAIDs and painkillers was ascertained by asking participants the following questions: "Up until a year ago, had you ever taken aspirin or other painkillers?" (q33a) and "Up until a year ago, had you ever taken any of the following medicines or tablets for at least 4 days per week for at least one month?" (q33b). In particular, subjects were asked to give information for the following medicines or tablets: mini-dose aspirin (75mg), normal-dose aspirin (325mg), aceclofenac, diclofenac sodium, diclofenac sodium with misoprostol, etodolac, ibuprofen, ibuprofen + codeine phosphate, indomethacin, mefenamic acid, meloxicam, nabumetone, naproxen, piroxicam, rofecoxib and any other NSAIDs or painkillers that were not included in the list. Individuals who reported regular drug use (for at least 4 days per week for at least one month) were asked to give further information regarding the started taking date, the total number of months taken and the number of days per week taken (q35). Medicine information was available for 2,279 cases (99.5%) and 2,911 (99.2%) controls and was entered in a separate Access database (Drugs database). In Table 48 the distribution of study participants according to their medicine intake is shown.

Table 48 Distribution of study participants according to intake of medicines

Categories	Cases (n=2290)	Controls (n=2933)
No*	1602 (70.0%)	1854 (63.2%)
Mini aspirin†	354 (15.5%)	527 (18.0%)
Normal aspirin‡	16 (0.7%)	19 (0.6%)
NSAIDs§	241 (10.5%)	385 (13.1%)
NSAIDs and mini aspirin**	53 (2.3%)	115 (3.9%)
NSAIDs and normal aspirin††	13 (0.6%)	11 (0.4%)
Missing	11 (0.5%)	22 (0.7%)

* Subjects with no intake of mini aspirin, normal aspirin and other NSAID drugs

† Subjects that only take mini aspirin (excluding subjects that additionally take any other NSAIDs)

‡ Subjects that only take normal aspirin (excluding subjects that additionally take any other NSAIDs)

§ Subjects that only take NSAIDs (excluding subjects that additionally take mini aspirin)

** Subjects that take both mini aspirin and other NSAIDs

†† Subjects that take both normal aspirin and other NSAIDs

4.3.3.4 Women's health variables

Female information was ascertained from the women's health part of the LCQ (questions 55 to 69). 2,259 female participants completed a LCQ and 2,255 of them completed the female section of it (99.8%). We mainly used information regarding the menstrual, hormonal replacement therapy (HRT) and oral contraception intake status. The distribution of female cases and controls for these variables is shown in Table 49.

Table 49 Distribution of female study participants along the women's health part questions

Women's health part	Cases (n=987)	Controls (n=1272)
Menstrual status		
Post-menopausal	751 (76.10%)	961 (75.5%)
Pre- / peri-menopausal	224 (22.7%)	292 (23.0%)
Missing	12 (1.2%)	19 (1.5%)
Hormonal replacement therapy		
Ever had	240 (24.3%)	421 (33.1%)
Never had	731 (74.1%)	840 (66.0%)
Missing	16 (1.6%)	11 (0.9%)
Hormonal replacement therapy (for the subjects that reported to have had HRT)		
Were on a year prior to recruitment	108	190
Were not on a year prior to recruitment	128	230
Missing	4	1
Oral contraception		
Ever used	404 (40.9%)	586 (46.1%)
Never used	556 (56.3%)	659 (51.8%)
Don't remember	4 (0.4%)	3 (0.2%)
Missing	23 (2.3%)	24 (1.9%)

4.3.3.5 Body Mass Index

Body Mass Index (BMI) was calculated using information from questions 31 and 32 and by applying the following formula:

BMI = weight (in kilograms) / height^2 (in metres)

Height information was available for 2,272 cases (99.2%) and 2,918 controls (99.5%). Weight information was available for 2,265 cases (98.9%) and 2,899 controls (98.8%). Two cases and one control were further removed due to reporting extreme values of either height (3.39 and 0.58 metres) or weight (886.2 kilos). In addition subjects that had either missing height or missing weight data could not be included in the BMI calculation. Finally, BMI was calculated for 2,257 cases (98.6%) and 2,894 controls (98.7%). BMI categories were selected according to WHO recommendations: under-weight (<18.5), average (18.5 – 24.99), over-weight (25-30), and obese (≥ 30) and the distributions of cases and controls are shown in Table 50.

Table 50 Distribution of study participants in BMI categories

BMI (kg/m^2)	Cases (n=2290)	Controls (n=2933)
Mean (SD)	26.7 (4.4)	26.7 (4.6)
BMI categories		
18.5 – 24.99 (normal weight)	856 (37.4%)	1056 (36.0%)
<18.5 (under-weight)	24 (1.0%)	42 (1.4%)
25-30 (over-weight)	949 (41.4%)	1240 (42.3%)
≥ 30 (obese)	428 (18.7%)	556 (19.0%)
Missing	33 (1.4%)	39 (1.3%)

4.3.4 Coding of the FFQ variables

2,256 and 2,851 FFQs were received from cases and controls respectively. Questionnaires that had blank values and/or blank lines were processed as described in the missing values protocol (Box 3). 241 questionnaires did not fulfil the missing values criteria (183 from cases (8.1%) and 58 from controls (2.0%)) and therefore 2,073 cases and 2,793 controls had valid FFQs for further analysis. After elaborating the FFQ data, two types of results were obtained: 1) nutrient and mineral intakes and 2) food item and food group intakes.

4.3.4.1 Nutrient intake calculations

Intakes of dietary energy, macro- and micro-nutrients were calculated using UK National Nutrient Databank, based on "McCance and Widdowson's, The Composition of Foods (5th edition)" and related supplements. Flavonoid data for the subgroups of flavones, flavonols, flavan3ols, procyanidins and flavanones and also for the individual flavonoid compounds quercetin, kaempferol, myricetin, apigenin, luteolin, catechin, epicatechin, epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, gallocatechin, naringenin and hesperetin were obtained from a nutrient database for flavonoids developed by Kyle & Duthie (274). Phytoestrogen values were derived from a database developed by Ritchie (275). Finally, specific fatty acid data (including total FAs, SFAs, MUFAs, PUFAs, ω6PUFAs, ω3PUFAs, *t*FAs and *t*MUFAs, palmitic acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid, arachidonic acid, α-linolenic acid, EPA and DHA) were obtained from both the UK food composition tables (McCance and Widdowson's The Composition of Foods, 6th summary edition) and the FOODBASE database (London, Institute of Brain Chemistry), a nutrient database for fatty acids. Although FOODBASE database contained some errors, all values were manually checked and corrected from the team of the University of Aberdeen.

Fixed ascii files (DAT files) were created from the saved SPSS scanned FFQs and sent to the University of Aberdeen for nutrient calculation. In University of Aberdeen, data were stored in an SQL Server database and then a programme written in MS Access (2000) was used to access and prepare the data. The nutrient analysis was performed

using the software Visual Basic for Applications (VBA). The nutrient intakes were calculated in three different levels (nutrients per day, nutrients per food group per day, nutrients per food per day), except for the specific fatty acid intakes, which were calculated in two levels (nutrients per day, nutrients per food group per day). Nutrient calculations were performed as described in Box 3. When data were received back from University of Aberdeen, they were saved in four different Access databases (intakes of dietary energy, macro- and micro-nutrients, intakes of flavonoids and phytoestrogens, intakes of fatty acid subgroups and intakes of specific fatty acids). The lists of calculated nutrients are presented in Table 51, Table 52 and Table 53.

Box 3: Protocol for handling missing data for nutrient and food group daily intake calculations

Less than 10 blanks:

- When the variable "measures per day" was blank, but the variable "number of days per week" had a value (either M or a number), a default value of 1 was assigned to substitute the missing value.
- When the variable "number of days per week" was blank but the variable "measures per day" had a value, a default value of 1 was assigned to substitute the missing value.
- When a whole line was blank (both the "measures per day" and the "number of days per week") then the intake of this particular food from this individual was assumed to be either null or rare and therefore, the value R was assigned to the variable "number of days per week".

More than 10 blanks:

- When a questionnaire that was sent back to the subject because it had more than 10 blanks ("measures per day", or "number of days per week", or lines) was returned with no changes/ additions then it was rejected and not used for the nutrient calculation.

Table 51 List of macro- and micro-nutrient intakes from the SCG-FFQ

Nutrient	Units
Water	g/day
Dietary energy intake	kcal/day
Dietary energy intake	kJ/day
Protein	g/day
Fat	g/day
Carbohydrate	g/day
Saturated fat	g/day
Monounsaturated fat	g/day
Polyunsaturated fat	g/day
Cholesterol	mg/day
Total sugar	g/day
Starch	g/day
Fibre	g/day
Sodium	mg/day
Potassium	mg/day
Calcium	mg/day
Magnesium	mg/day
Phosphorus	mg/day
Iron	mg/day
Copper	mg/day
Zinc	mg/day
Chloride	mg/day
Manganese	mg/day
Selenium	µg/day
Iodine	µg/day
Retinol	µg/day
Carotene equivalent	µg/day
Vitamin D	µg/day
Vitamin E	mg/day
Thiamine	mg/day
Vitamin B2	mg/day
Niacin	mg/day
Potential niacin (from tryptophan)	mg/day

Vitamin B6	mg/day
vitamin B12	µg/day
Folic acid	µg/day
Pantothenic acid	mg/day
Biotin	µg/day
Vitamin C	mg/day
Alcohol	g/day

Table 52 List of flavonoids and phytoestrogens estimated from the SCG-FFQ

Nutrient	Units
Flavonols	mg/day
Quercetin	mg/day
Kaempferol	mg/day
Myricetin	mg/day
Flavones	mg/day
Apigenin	mg/day
Luteolin	mg/day
Flavan3ols	mg/day
Epigallocatechin	mg/day
Catechin	mg/day
Epicatechin	mg/day
Epigallocatechin-3 gallate	mg/day
Epicatechin-3 gallate	mg/day
GC	mg/day
Procyanidins	mg/day
Flavanones	mg/day
Naringenin	mg/day
Hesperetin	mg/day
Phytoestrogens	µg/day

Table 53 List of total and specific fatty acid categories estimated from the SCG-FFQ

Nutrient	Units
Total fatty acids	g/day
Total saturated fatty acids	g/day
Palmitic acid	g/day
Stearic acid	g/day
Total monounsaturated fatty acids	g/day
Total poly-unsaturated fatty acids	g/day
Oleic acid	g/day
Total ω6 poly-saturated fatty acids	g/day
Linoleic acid	g/day
γ-Linolenic acid	mg/day
Arachidonic acid	mg/day
Total ω3 poly-saturated fatty acids	g/day
α-Linolenic acid	mg/day
Eicosapentaenoic acid (EPA)	mg/day
Docosahexaenoic acid (DHA)	mg/day
Total *trans* fatty acids	g/day
Total *trans* mono-unsaturated fatty acids	g/day

4.3.4.2 Food group variables

In addition to the nutrients, the FFQ food items were used to calculate food group intake data (Table 54), using a procedure that followed the same protocol as the one used in the nutrient calculations and questionnaires that had blank values and/or blank lines were processed as described in the Missing values protocol (Box 3).

In particular, the daily consumption of each individual food item (e.g. daily consumption of carrots) and of each food group (e.g. vegetables) was computed using the following formulas:

- Daily consumption of food items:
  - When the day response was one to seven days per week:
    Food item intake per day = (number of measures)*(number of days)/7
  - When the day response was "monthly" an alternative formula was used:
    Food item intake per day = (number of measures)* 1.5 measures/ 28 days
  - When the day response "Rarely" was recorded then a default value of 0 for the daily food item intake was used.
- Daily consumption of food groups:
    Food group intake = sum of food item intakes within the food group

In addition to the food items and groups consumption the grilled meat score was calculated. It combined the number of times that a subject ate grilled or fried meat with the doneness of the meat using the following formula:

Grilled meat score = [Number of times of grilled or fried meat per week]*[meat doneness]

Note: Meat doneness: 1 = lightly browned, 2 = medium browned or 3 = well browned.

Table 54 List of food group variables and other food-associated variables

Food group	FFQ food items (FFQ question number)
Total: Bread/Cereal products	Breads (qu.1), Breakfast Cereals (qu.2)
Bread products	Breads (qu.1)
Cereal products	Breakfast Cereals (qu.2)
Total: Fruit & Vegetables	Fruit (qu.12), Vegetables (qu.11)
Fruit	Fruit (qu.12)
Vegetables	Vegetables (qu.12)
Total: Meat products	Meats (qu.7), Fish (qu.8)
Meat products	Meats (qu.7)
Red meat	Meats (qu.7: a-e, g-i)
Processed meat	Meats (qu.7: b, c, i-l)
Total: Fish	Fish (qu.8)
White fish	Fish (qu.8: a-d)
Oily fish	Fish (qu.8: e-g, i)
Total: Dairy products	Milk (qu.3), Cream and Yoghurt (qu.4), Cheese (qu.5), Eggs (qu.6)
Milk products	Milk (qu.3)
Cream & yoghurts	Cream and Yoghurt (qu.4)
Cheese	Cheese (qu.5)
Eggs	Eggs (qu.6)
Total: Alcohol intake	Alcoholic drinks (qu.19) – as units
Beer & Lager	Alcoholic drinks (qu.19: a-c)
Wine	Alcoholic drinks (qu.19: d, e)
Spirits	Alcoholic drinks (qu.19: f-h)
Total: Beverages and Soft drinks	Beverages and Soft drinks (qu.18)
Beverages	Beverages and Soft drinks (qu.18: a-e)
Caffeine beverages	Beverages and Soft drinks (qu.18: a, c, e)
Non-caffeine beverages	Beverages and Soft drinks (qu.18: b, d)
Soft drinks	Beverages and Soft drinks (qu.18: f-l)
Fruit/vegetable juices	Beverages and Soft drinks (qu.18: f-i)
Fizzy drinks	Beverages and Soft drinks (qu.18: j, k)
Frequency of eating: Total number of meals and snacks per day	Other information (qu23: a-d)
Number of main meals per day	Other information (qu23: a)
Number of snack meals per day	Other information (qu23: b)
Number of snack foods per day	Other information (qu23: c)
Number of sweet drinks per day	Other information (qu23: d)

4.3.4.3 Computation of energy-adjusted nutrient and food group variables

To adjust for the potential effect of dietary energy intake on the associations between the nutrient or food intakes and colorectal cancer we used the residual method, as determined by Willet and Stampfer (276). This method estimates individual dietary intake when the energy intake remains constant (Box 4). However, to apply this method the distribution of the particular nutrient or food should be normal. Therefore, in case that a particular nutrient or food was not normally distributed (even after logarithmic or square-root transformation) then the standard method of energy adjustment was used, where dietary energy intake was added as a covariable in the logistic regression model that was used to estimate the association between the nutrient or food and colorectal cancer (Box 4).

Box 4 Procedure followed to control for the possible confounding effect of dietary energy intake (residual or standard method of energy adjustment)

For each dietary intake variable we did as follows:

1. **Check the distribution of dietary energy intake**

The distribution of dietary energy intake was checked and any outliers were identified.

2. **Check the distribution of nutrient/food intake**

The distribution of each nutrient/ food was checked. If it was normal we went to *step 5*. The nutrients or foods that were not normally distributed were transformed (logarithmic or square root transformation).

3. **Check the distribution of transformed nutrient/food intake**

The distribution of the transformed nutrient/ food was checked. If it was normal, we went to *step 5*. If it was not normally distributed, we went to *step 4*.

4. **Standard energy adjustment**

The confounding effect of energy was controlled by adding energy as a covariable in the logistic regression model with colorectal cancer as the response variable and nutrient intake as an explanatory variable.

5. **Residual energy adjustment: Simple linear regression**

Simple linear regression with dietary intake variable as response (Y variable) and energy intake as x variable was performed: $Y = a + bx$; a is the intercept and b is the slope

6. **Residual energy adjustment: Record the residuals**

Residuals from *step* 5, the intercept a and the slope b were saved.

7. Residual energy adjustment: Calculate the mean energy intake

The mean energy intake (χ) i.e. mean of x was calculated.

8. Residual energy adjustment: Calculate the expected nutrient intake, when energy intake is constant (i.e. equal to its mean)

The expected nutrient intake (y variable) was calculated using the formula: $y = a + (b^* \chi)$

Where values for *a* and *b* were from *step 5* and χ is the mean of the energy intake, from *step 7*. The value for y for each dietary intake variable was calculated.

9. Residual energy adjustment: Calculate the energy adjusted dietary intake variable

To obtain the energy adjusted dietary intake value, *y* was added to the residuals recorded in *step 5* and saved in *step 6*.

10. Residual energy adjustment: For previously transformed variables

If the dietary intakes in *step 2* have been transformed, these were reversed in this step. For example if the log transformation of a dietary intake variable had been used then the values obtained in *step 9* were to be exponentiated.

11. Residual energy adjustment: Analyse the energy adjusted variables

We looked at the mean, standard deviation, minimum and maximum of the energy adjusted variable. We compared the mean of the energy adjusted variable to that of the original variable. The means should have been similar, but the standard deviation should have been lower for the energy adjusted variable. There should have been no negative values in the energy adjusted variable. If negative values were present we went back to step 1 and step 2 and checked for outliers and ensured that the skewness of the data was between –1 and 1.

4.3.4.4 Supplements

Regular intake of dietary supplements (within the reference period) was recorded in section 21 of the FFQ and nutrient intake from these supplements was added to the daily nutrient intake from the FFQ. The supplement information (which included the brand name of the supplement, the type of the supplement, the dosage, the measures per day and the days per week) was entered in a database different to the FFQ database (Supplement database). The total number of subjects that took any kind of supplements was 1,772 (706 cases and 1,066 controls).

A database containing the vitamin, mineral and herb dosages of the products recorded by the subjects was established (Supplement reference look-up database). The necessary information regarding the composition of the supplements was collected by the manufacturer's product information, by contacting the company directly or by the internet.

The combination of the Supplement database and Supplement reference look-up database gave all the necessary information to calculate the daily nutrient intake from the supplements which was null for subjects that had not been taking any supplements. This combination was made on a supplement code that was attached on each specific supplement. This code was unique for each brand-type-dosage supplement and was entered in both tables. For example the supplement Cod Liver Oil (525 mg each capsule) that was made by the brand Seven Seas had the code SS CLO 525.

The daily intake from the supplements was added to the nutrient output from foods after the energy adjustment. The reason for this was that we were not willing to energy-adjust for the supplement intake. It is possible that the participants might have overestimated their supplement intake since they may have forgotten to take them some time or even stopped for a period. However, this overestimation probably would not be related to any overestimation of their total dietary energy intake. Therefore on balance and having consulted Dr G McNeill (University of Aberdeen), we felt it would be more accurate to adjust the nutrients from foods for total dietary energy intake and then add the estimated daily nutrient intakes from supplements to the adjusted values.

4.4 Collection and process of additional data

4.4.1 Deprivation category data

The Carstairs deprivation index (deprivation score), which was based on the 2001 Census data, was assigned to each subject at the postcode sector level. The index contained seven categories ranging from very low deprivation (deprivation score 1) to very high deprivation (deprivation score 7). The criteria that are included in the Carstairs deprivation index are presented in Table 55. In Table 56 the distribution of cases and controls along the Carstairs deprivation index categories is presented.

Table 55 Carstairs Deprivation Index criteria

Criterion	Description
Overcrowding:	Persons in private household living at a density of >1 person per room of all persons in private households
Male unemployment:	Proportion of economically active males who are seeking work
Low social class:	Proportion of all persons in private households with head of household in social class 4 or 5
No car:	Proportion of all persons in private households with no car

Table 56 Distribution of cases and controls along the categories of Carstairs deprivation index

Deprivation score	Cases (n=3417)	Controls (n=3396)
1	287 (8.4%)	318 (9.4%)
2	657 (19.2%)	686 (20.2%)
3	860 (25.2%)	923 (27.2%)
4	828 (24.2%)	794 (23.4%)
5	389 (11.4%)	365 (10.7%)
6	255 (7.5%)	218 (6.4%)
7	137 (4.0%)	92 (2.7%)
Missing	4 (0.1%)	0 (0.0%)

4.4.2 Family history risk

Family history risk was assigned according to the Scottish guidelines (see Introduction, chapter 1.5.4). The distribution of cases and controls along the family history categories are presented in Table 57.

4.4.3 Tumour related parameters

4.4.3.1 Site of cancer

Information about the site of tumour was extracted from the medical history records and from the treatment questionnaires. Distribution of the cases according to the tumour location is presented in Table 58.

4.4.3.2 Stage of cancer

During the recruitment period Duke's stage was recorded to describe the extent of the cancer in the body. In addition, by using Duke's stage information we formed the AJCC stage for each case. However, for 2,719 cases metastasis information was missing and data were requested from the Scottish regional cancer networks (SCAN, WoSCAN and NoSCAN). These data were also incomplete and therefore CT scans for all patients from the Lothian region were requested (n= 578) and individually checked for evidence of metastasis. For the WoSCAN and NoSCAN regions, the consultants of individual patients were contacted by letter requesting the staging information for their patients. Following this first round of letter to consultant surgeons, it became clear that there were inconsistencies between the staging provided by the regional databases and the death status (e.g. patients noted to have metastasis in the databases were alive several years later). A second round of letters was then sent to consultant surgeons requesting clarification of metastases status of their patients. For the remaining cases with outstanding metastasis status, individual GPs were contacted by letter. This process led to only 126 cases left without staging. The distribution of the cases according to the Duke's and AJCC staging systems is presented in Table 59.

Table 57 Distribution of cases and controls of assigned family history

Family history risk	Cases (n=3417)	Controls (n=3396)
Low	2503 (73.7%)	3084 (90.8%)
Medium	613 (18.0%)	33 (1.0%)
High	74 (2.2%)	1 (0.0%)
Unknown	135 (4.0%)	20 (0.6%)
Not given	92 (2.7%)	258 (7.6%)
Refused	61	28
Adopted	1	8
Other reason	0	1
No reason given	30	221

Table 58 Distribution of cases according to tumour location

Site of cancer	Cases (n=3417)
Colon cancer	2006 (58.7%)
Proximal	947
Distal	782
2 proximal tumours	23
2 distal tumours	8
1 proximal, 1 distal	10
Unspecified	236
Rectal cancer	1355 (39.6%)
Colon and rectal cancer	18 (0.5%)
Other (including cancer of the appendix and anus, polyps only or unknown)	30 (0.9%)
Missing	8 (0.2%)

Table 59 Distribution of the cases along the categories of the Duke's and AJCC stage systems

Stage of cancer	Cases (n=3417)
Duke's staging	
A	609 (17.8%)
B	1203 (35.2%)
C	1371 (40.1%)
D	31 (0.9%)
Missing	203 (5.9%)
Metastasis	
No	2819 (82.5%)
Yes	520 (15.2%)
Missing	78 (2.3%)
AJCC	
1	619 (18.1%)
2A	871 (25.5%)
2B	241 (7.0%)
2 (unspecified)	8 (0.2%)
3A	110 (3.2%)
3B	591 (17.3%)
3C	344 (10.1%)
4	507 (14.8%)
Missing	126 (3.7%)

4.4.4 Genetic data of specific variants

In this thesis a limited amount of genetic variants were considered for investigation. In particular the genes, which were associated with colorectal cancer were investigated, were the following: rs1801133 (*MTHFR* C677T), rs1801131 (*MTHFR* A1298C), rs1805087 (*MTR* A2756G) and rs1801394 (*MTRR* A66G) (hypothesis 3), and four *VDR* SNPs: rs10735810 (*Fok*I), rs1544410 (*Bsm*I), rs11568820 and rs7975232 (*Apa*I) (hypothesis 4).

Genotyping for *MTHFR*, *MTR* and *MTRR* SNPs was undertaken as part of an array-based candidate gene approach. Genotyping of patients aged less than 55 years old along with matched controls was undertaken together using the Illumina Infinium I Custom array platform and performed by Illumina in San Diego. DNA samples were accurately quantified by Pico-Green^{TM} and quality controlled prior to dispatch to San Diego. To avoid potential systematic batch-to-batch variation or bias, samples were anonymised as to disease status and were randomly distributed within plates. Data were subject to Illumina quality control procedures and genotypes were discarded if call rates were less than 99.5%. Genotype data for the *MTHFR*, *MTR* and *MTRR* SNPs were available for a subsample of 1001 cases and 1010 controls.

Genotyping for the four *VDR* SNPs was undertaken in two phases as part of an array-based candidate gene approach, using the Illumina Infinium I Custom array platform and performed by Illumina (San Diego). In phase I, two *VDR* gene variants (rs10735810 and rs1544410) of 1,012 cases and 1,012 controls (<55 years old) were genotyped, whereas in phase II, four VDR gene variants (rs10735810, rs1544410, rs11568820, rs7975232) of 2,013 patients and 2,071 controls (21 to 83 years old) were genotyped. DNA samples were accurately quantified by Pico-Green^{TM} and quality controlled prior to dispatch to San Diego. Case and control DNA samples were stored, genotyped and analysed in the same way. In addition to avoid potential systematic batch-to-batch variation or bias, samples were anonymised as to disease status and were randomly distributed within plates. Data were subject to Illumina quality control procedures and genotypes were discarded if call rates were less than 99.5%.

4.5 Data analysis of part 1 (Hypothesis driven analyses)

4.5.1 Introduction

In the first part of this thesis particular dietary factors were investigated in order to assess their associations with colorectal cancer in a hypothesis-driven type of analysis. In particular, four different hypotheses were tested comprising the investigation of the associations between colorectal cancer and: 1) flavonoid variables (hypothesis 1), 2) fatty acid variables (hypothesis 2), 3) nutrients involved in the one-carbon metabolic pathway (including folate, vitamin B2, vitamin B6, vitamin B12 and alcohol; hypothesis 3) and 4) vitamin D and calcium (hypothesis 4). Results of the first two hypotheses are presented in chapter 6 and results of the last two hypotheses are presented in chapter 7.

In this section the datasets that were used to investigate the aforementioned hypotheses including detailed list of the included variables will be presented. Finally, the overall descriptive statistical analysis of part 1 and the particular statistical methods that were employed will be described. All statistical analyses were conducted using the statistical package STATA IC (version 10.0, TEXAS, USA).

4.5.2 Matched and unmatched dataset

2,062 cases and 2,776 controls had complete and valid FFQ and LCQ data and were included in the analysis. Analysis was applied in two different datasets: a finely matched (1:1) dataset including 1,489 cases and 1,489 controls (used for investigation of hypotheses 1 and 2) and an unmatched dataset including 2,062 cases and 2,776 controls (used for investigation of hypotheses 3 and 4). The characteristics of both the matched and unmatched dataset are presented in the first section of chapter 6 and chapter 7, respectively. For the genetic analysis of hypothesis 3 (analysis of the following SNPs: rs1801133, rs1801131, rs1805087 and rs1801394) an unmatched dataset including 1,001 cases and 1,010 controls (aged $\leq$55 years old) was used. In addition, for the joined analysis of the genetic and dietary factors of hypothesis 3, an unmatched dataset of 468 cases and 761 controls younger than 55 years old was used. Regarding the genetic analysis of the hypothesis 4, an unmatched dataset of 2,013 cases and 2,071 controls was

used (for SNPs rs11568820, rs7975232), whereas an unmatched dataset of 3,025 cases and 3,083 controls was used (for SNPs rs10735810 and rs1544410). Finally, for the joined analysis of rs7975232, rs11568820 and the dietary factors of hypothesis 4 a dataset of 1,392 cases and 1,817 controls was used, whereas for the joined analysis of rs10735810, rs1544410 and the dietary factors of hypothesis 4 a dataset of 1,859 cases and 2,578 controls was used.

4.5.3 List of variables

The variables that were included in hypothesis 1 (association between flavonoids and colorectal cancer) were: 1) the flavonoid subgroups: flavonols, flavones, flavan3ols, procyanidins, flavanones and phytoestrogens and 2) the individual flavonoid compounds: quercetin, catechin, epicatechin, naringenin and hesperetin. The variables that were included in hypothesis 2 (association between fatty acids and colorectal cancer) were: 1) total FAs, 2) the fatty acid subgroups: SFAs, MUFAs, PUFAs, ω6PUFAs, ω3PUFAs, *t*FAs and *t*MUFAs and 3) the individual fatty acid compounds: palmitic acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid, arachidonic acid, α-linolenic acid, EPA and DHA. The variables that were included in hypothesis 3 (association between nutrients involved in one-carbon metabolic pathway and colorectal cancer) were: folate, vitamin B2, vitamin B6, vitamin B12 and alcohol and the SNPs rs1801133, rs1801131, rs1805087 and rs1801394. Finally, the variables that were included in hypothesis 4 were: vitamin D and calcium and the SNPs rs10735810, rs1544410, rs11568820 and rs7975232. The variables and potential confounding factors that were included in this part of the analysis are listed in Table 60.

Table 60 List of the variables included in the first part of the analysis (four hypotheses) and list of the potential confounding factors

Variables				**Confounders**
Matched analysis		**Unmatched analysis**		
Hypothesis 1	**Hypothesis 2**	**Hypothesis 3**	**Hypothesis 4**	**All hypotheses**
Flavonols	Total FAs	Folate	Vitamin D	Age
Flavones	SFAs	Vitamin B2	Calcium	Sex
Flavan3ols	MUFAs	Vitamin B12	rs10735810	Deprivation index
Procyanidins	PUFAs	Vitamin B6	rs1544410	Family history
Flavanones	ω6PUFAs	Alcohol	rs11568820	Body mass index
Phytoestrogens	ω3PUFAs	rs1801133	rs7975232	Physical activity
Quercetin	*t*FAs	rs1801131		Smoking
Catechin	*t*MUFAs	rs1805087		Dietary energy
Epicatechin	Palmitic acid	rs1801394		Dietary fibre
Naringenin	Stearic acid			Alcohol
Hesperetin	Oleic acid			NSAIDs*
	Linoleic acid			
	γ-Linolenic acid			
	Arachidonic acid			
	α-Linolenic acid			
	EPA			
	DHA			

* NSAIDs: Non Steroidal Anti-inflammatory Drugs

4.5.4 Statistical analysis of part 1

4.5.4.1 Descriptive analysis

The distribution of each dietary and potential confounding variable was examined. Any extreme values and outliers were noted with the view of omitting them from subsequent analysis using continuous data. Any variable that showed a skewed distribution was normalised by using appropriate transformation methods (logarithmic or square root transformation). In addition, a correlation analysis, using spearman's rank correlation was performed on the dietary variables to examine any association between these variables.

The distribution of each environmental variable and confounding factor was examined by cases versus controls. Differences in dietary intakes and confounding variables between cases and controls were tested for significance by using t-test (continuous variables) and Pearson χ^2 test (categorical variables). Finally, the Wilcoxon rank-sum test was used to test for differences in median dietary intakes.

4.5.4.2 Data categorisation

Dietary and non-dietary variables that were measured on a continuous scale were initially used as continuous variables in the statistical models. In addition they were grouped into four categories using quartiles as the cut-off points (based on the combined distribution of cases and controls).

4.5.4.3 Logistic regression analysis

The association of case/ control status with each dietary, non-dietary and confounding variable of the four hypotheses was examined by using logistic regression models. In general, logistic regression analysis is used to model dichotomous outcomes (log odds of an outcome) defined by the values of covariables in the model. For the analysis of the unmatched dataset (unconditional) logistic regression was used. For the analysis of the matched dataset, conditional logistic regression analysis was used, which is a modification of the (unconditional) logistic regression where the likelihood takes into account the fine matching.

Odds ratios and 95% CIs were obtained by comparing quartiles of each dietary variable using the lowest quarter as reference. In addition linear trend of the ORs that represents a dose-response association was examined by calculating a p-value for trend. Uni- and multi-variable conditional or unconditional logistic regression models were used to study the associations between colorectal cancer and each dietary and confounding factor.

Three main logistic regression models (conditional or unconditional) were applied: Model I was not adjusted for any confounding factors (crude analysis); Model II was corrected for dietary energy intake by using either the residual method, as determined by Willet and Stampfer (for the normal distributed variables) or the standard method including the dietary energy variable as a covariate in the regression model (for the non-normal distributed variables); Model III was corrected for family history of cancer (low, medium/high risk), BMI (kg/m^2, continuously), physical activity (hours/week of cycling and any other sport activities, 4 categories), smoking (yes vs. no), dietary energy intake (residual or standard method of adjustment), fibre intake (grams/day, energy adjusted, continuously), alcohol intake (grams/day, energy adjusted, continuously) and regular NSAIDs intake (yes vs. no) and additionally for age (continuous), sex and deprivation score for the unmatched analysis.

Two additional models were applied in hypothesis 1 (associations between colorectal cancer and intakes of flavonoids): Model IV, which was corrected for the confounding factors of model III and additionally for fruit and vegetable intake (measures/day, continuously, energy adjusted); and model V, which was corrected for the confounding factors of model III and further adjusted mutually between flavonoid categories. Two additional models were applied in hypothesis 2 (associations between colorectal cancer and intakes of fatty acids), as well: Model IV, which was corrected for the confounding factors of model III and in addition to the residual energy adjustment dietary energy intake was included as a covariate; and model V, which was corrected for the confounding factors of model III and further adjusted for total fatty acid intake. Finally, one additional model was applied in hypothesis 4 (associations between colorectal

cancer and intakes of vitamin D and calcium): Model IV, which was corrected for the confounding factors of model III and further adjusted for intake of ω3PUFAs.

In addition to the whole sample analysis, ORs and 95% CIs were calculated in stratified groups according to sex, age (≤55 years old and >55 years old) and cancer site (colon and rectal cancer) by applying model III for all four hypotheses.

4.5.4.4 Analysis of genetic data and gene-environment interactions (for hypotheses 3 and 4)

The association of case/ control status with each SNP (hypotheses 3 and 4) was examined by using logistic regression models. Each genotype (heterozygous and homozygous for the variant allele) was compared with the reference category (homozygous for the wild type allele) in order to obtain ORs and 95% CIs. Two unconditional logistic regression models were used to study the associations between colorectal cancer and each SNP: one univariable model and one simply adjusted for age, sex and deprivation score. In addition, multivariable associations between the dietary risk factors that were included in hypotheses 3 and 4, and colorectal cancer were investigated after stratification of the study sample according to the genetic factors by applying model III. In addition, interaction associations were examined by investigating the combined effects of the genotypes and nutrient intakes. Interaction was tested by examining the deviance of two different nested models; an interactive model and its nested multiplicative one. The referent category used was homozygotes of the wild type allele and being at the lower quartile of the dietary nutrient intake.

4.5.4.5 Multiple testing

For each hypothesis we corrected the observed p-values according to the number of tests that were performed in order to control for multiple testing. Correction for multiple testing was conducted in three different ways.

First, the p-values were corrected using the Bonferroni correction for the number of independent tests performed as follows: hypothesis 1 (flavonoids) was corrected for six independent tests; hypothesis 2 (fatty acids) for 14 independent tests (eight for the fatty acids making a subtotal of 14 tests including hypothesis 1); hypothesis 3 (folate, vitamin

B2, vitamin B6, vitamin B12 and alcohol) was corrected for 19 independent tests (five for the current hypothesis making a subtotal of 19 tests including hypotheses 1 and 2); and hypothesis 4 (vitamin D and calcium) was corrected for 21 independent tests (two for the current hypothesis making a subtotal of 21 tests including hypotheses 1, 2 and 3). For an original significance level (α) of 0.05, the adjusted significance level for hypothesis 1 was 0.008 (0.05 divided by 6), for hypothesis 2 was 0.004 (0.05 divided by 14), for hypothesis 3 was 0.003 (0.05 divided by 19) and for hypothesis 4 was 0.002 (0.05 divided by 21).

The second way was to account for the number of tests separately for each hypothesis but to consider each single test by including the number of models as follows: hypothesis 1 was corrected for 30 tests for the flavonoid subgroups (6 flavonoid subgroups multiplied by 5 models = 30 tests) and for 25 tests for the individual flavonoids (5 individual flavonoids multiplied by 5 models = 25 tests); hypothesis 2 was corrected for 39 tests for the fatty acid subgroups (total fatty acids multiplied by 4 models = 4 tests plus 7 fatty acid subgroups multiplied by 5 models = 35 tests) and for 45 tests for the individual fatty acids (9 individual fatty acids multiplied by 5 models = 45 tests); hypothesis 3 was corrected for 15 tests (5 nutrients multiplied by 3 models = 15 tests); and hypothesis 4 was corrected for eight tests (2 nutrients multiplied by 4 models = 8 tests). Both the Bonferroni correction method and the less conservative False Discovery Rate (FDR) method were applied.

Third, the significance level was corrected for the toal number of tests performed in all 4 hypotheses, by applying both the Bonferroni and the FDR method. In the subgroup level, we corrected for 69 independent tests (30 in hypothesis 1 and 40 in hypothesis 2), whereas in the individual nutrient level we corrected for 93 tests (25 in hypothesis 1, 45 in hypothesis 2, 15 in hypothesis 3 and 8 in hypothesis 4).

4.6 Data analysis of part 2 (Overall and stepwise regression analysis)

4.6.1 Introduction

In the second part of the thesis, an overall univariable analysis of all the collected risk factors (including demographic factors, lifestyle variables, food variables and nutrients) was conducted. In addition, stepwise regression models were applied to three different sets of variables to develop models that explain colorectal cancer risk. Results of the second part of the thesis are presented in chapter 8.

This section includes a description of the datasets used and the statistical methods. All statistical analyses were conducted using the statistical package STATA IC (version 10.0, TEXAS, USA).

4.6.2 Dataset

The dataset that was used for part 2 of the analysis was the unmatched one consisting of 2,062 cases and 2,776. Its main characteristics are presented in the first section of chapter 7 (on page 260).

4.6.3 List of variables

The variables, which were tested for an association with colorectal cancer were: 1) the demographic risk factors: age, sex, family history and deprivation score; 2) the lifestyle variables: smoking, alcohol intake, BMI, physical activity, dietary energy intake, NSAIDs intake and HRT intake (females only); 3) the food variables: breads, cereals, milk, cream, cheese, eggs, poultry, red meat, processed meat, white fish, oily fish, potatoes/ pasta/ rice, fruit, vegetables, savoury[1], sweets[2], tea, coffee, fruit/ vegetable juice, fizzy drinks; and 4) the nutrients: quercetin, catechin, epicatechin, flavones, procyanidins, flavanones, phytoestrogens, SFAs, MUFAs, ω6PUFAs, ω3PUFAs, *t*FAs, *t*MUFAs, sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, selenium, iodine, chloride, vitamin A, carotenes, vitamin D, vitamin E,

[1] Summary variable of savoury foods, soups and sauces

[2] Summary variable of puddings and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folate, pantothenic acid, potential niacin, biotin and vitamin C.

Stepwise regression was applied in three different sets of variables that are presented in Table 61. Briefly, set 1 consisted of demographic risk factors, lifestyle variables and food variables; set 2 consisted of demographic risk factors, lifestyle variables and nutrients; and set 3 consisted of demographic risk factors, lifestyle variables, food variables and nutrients. All food and nutrient variables were adjusted for dietary energy (by the residual method), except for tea and coffee (sets 1 and 3) and flavones (sets 2 and 3).

Table 61 List of the variables included in the three datasets of the second part of the analysis. All food and nutrient variables were residually adjusted for dietary energy, except for the food variables: tea and coffee and the nutrients: flavones and flavan-3-ols.

Set 1	**Set 2**	**Set 3**
Demographic risk factors	*Demographic risk factors*	*Demographic risk factors*
Age, sex, family history, deprivation score	Age, sex, family history, deprivation score	Age, sex, family history, deprivation score
Lifestyle variables	*Lifestyle variables*	*Lifestyle variables*
Smoking, alcohol intake, BMI, physical activity, dietary energy intake, NSAIDs, HRT (females only)	Smoking, alcohol intake, BMI, physical activity, dietary energy intake, NSAIDs, HRT (females only)	Smoking, alcohol intake, BMI, physical activity, dietary energy intake, NSAIDs, HRT (females only)
Food variables	*Flavonoid variables*	*Food variables*
Breads, cereals, milk, cream, cheese, eggs, poultry, red meat, processed meat, white fish, oily fish, potatoes/ pasta/ rice, fruit, vegetables, savoury*, sweets†, tea (crude intakes), coffee (crude intakes), fruit/ vegetable juice, fizzy drinks	Quercetin, catechin, epicatechin, flavones (crude intake), procyanidins, flavanones, phytoestrogens	Breads, cereals, milk, cream, cheese, eggs, poultry, red meat, processed meat, white fish, oily fish, potatoes/ pasta/ rice, fruit, vegetables, savoury, sweets, tea (crude intakes), coffee (crude intakes), fruit/ vegetable juice, fizzy drinks
	Fatty acid variables	*Flavonoid variables*
	SFAs, MUFAs, ω6PUFAs, ω3PUFAs, *t*FAs, *t*MUFAs	Quercetin, catechin, epicatechin, flavones (crude

		intake), procyanidins, flavanones, phytoestrogens
	Macronutrients	*Fatty acid variables*
	Protein, cholesterol, sugars, starch, fibre	SFAs, MUFAs, ω6PUFAs, ω3PUFAs, *t*FAs, *t*MUFAs
	Minerals	*Macronutrients*
	Sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, selenium, iodine	Protein, cholesterol, sugars, starch, fibre
	Vitamins	*Minerals*
	Vitamin A, carotenes, vitamin D, vitamin E, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folate, pantothenic acid, biotin, vitamin C	Sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, selenium, iodine
		Vitamins
		Vitamin A, carotenes, vitamin D, vitamin E, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folate, pantothenic acid, biotin, vitamin C

[*] Summary variable of savoury foods, soups and sauces

[†] Summary variable of puddings and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

4.6.4 Statistical analysis of part 2

4.6.4.1 Descriptive analysis

The distribution of each demographic, lifestyle, food and nutrient variable was examined. Any extreme values and outliers were investigated with the view of omitting them from subsequent analysis using continuous data. Any variable that showed a skewed distribution was normalised by using appropriate transformation methods (logarithmic or square root transformation).

The distribution of each variable was examined by cases versus controls and the distributions were tested for significance by using t-test (continuous variables) and Pearson χ^2 test (categorical variables). In addition, the Wilcoxon rank-sum test was used to test the median of the continuous variables. Finally, a correlation analysis, using spearman's rank correlation was performed on all continuous variables to examine any association between these variables. All food and nutrient variables were residually energy adjusted (except for tea, coffee and flavones).

4.6.4.2 Data categorisation

Dietary and non-dietary variables that were measured on a continuous scale were initially used as continuous variables in the statistical models. In addition they were standardised and changes per standard deviation were reported. Finally, they were grouped into four categories using quartiles as the cut-off points (based on the combined distribution of cases and controls).

4.6.4.3 Overall univariable logistic regression

Univariable logistic regression models were fitted for each demographic, lifestyle, dietary, food and nutrient variable. For the regression of food and nutrient variables, their residual energy adjusted form was included (except for the food groups tea and coffee and the nutrient: flavones).

4.6.4.4 Stepwise regression

Stepwise regression (both forward and backward) was applied to the three different set of variables. The p-value threshold for a variable to enter the model (forward stepwise regression) or to remain in the model (backward stepwise regression) was 0.10. In each

set of variables the quartile form of the continuous variables was included. Finally, forward and backward stepwise regression was reapplied separately for males and females for all three sets of variables using the quartile form of the continuous variables.

4.6.4.5 Bootstrap method

In order to examine the stability of the built models the bootstrap method was applied. A bootstrap sample is a sample of the same size as the original sample but where subjects have been replaced. A given subject of the original sample may occur in a specific bootstrap sample many times, only once, or not at all. 100 bootstrap samples were selected. Once a bootstrap sample was selected by the computer programme, for each set of variables forward and backward stepwise regression models were applied (in the whole sample). The p-value threshold for a variable to enter the model (forward stepwise regression) or to remain in the model (backward stepwise regression) was again 0.10. Therefore, for a given bootstrap sample and for each set of variables, two final models were obtained (1 after applying forward and 1 after applying backward stepwise regression).

For each obtained model, the selected variables were noted, results across the variable selection models were compared and this procedure was repeated for all 100 bootstrap samples. For each variable, the number of times that it was included in a regression model was calculated. In addition, the agreement between the type and number of variables included in the models after applying forward and backward stepwise regression was determined.

4.6.4.6 Multiple testing

The purpose of the overall and stepwise analysis was not to draw any certain conclusions about the strength of the associations between the risk factors and colorectal cancer. Instead, the purpose was to identify risk factors and to generate new hypotheses, which would then be tested in other prospective or retrospective studies. Therefore, no correction was made for multiple testing.

5 RESULTS: Description of the results presentation

This chapter describes the layout of the results and discussion sections of the thesis. In chapters 6 and 7, the analyses of the four "a priori" hypotheses will be described (aim 1). Chapter 6 includes the results of the matched analysis of the novel dietary risk factors (flavonoid and fatty acid subgroups and individual compounds), whereas chapter 7 includes the unmatched analysis of the additional dietary risk factors (folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium). Chapter 8 (aim 2) includes the results of the univariable overall analysis of all the explanatory variables and of the stepwise regression models.

In the first part of each chapter the study population that was included in the analysis is presented, including descriptive analysis of the main confounding factors and logistic regression analysis to investigate the association relationships between the confounding factors and colorectal cancer risk. Since the study sample that was used in chapters 7 and 8 was the same, description of its main characteristics will be presented only once, in chapter 7. In the second part of each chapter descriptive analysis of the dietary variables (chapters 6 and 7) or of all the explanatory variables (chapter 8) are presented including distribution analysis (whole sample and by case/ control status) and correlation analysis. In addition, the association relationships between colorectal cancer and each variable are investigated by applying different logistic regression models. Finally, the last part of chapter 8 includes the stepwise regression stepwise models for three different sets of explanatory variables. In the end of each chapter, a brief summary is included highlighting the main findings of each analysis, whereas further discussion of the important findings will be presented in chapter 9 (Discussion). Tables and figures of the analyses are presented at the end of each section of each chapter, as indicated in the text.

6 RESULTS: Associations between colorectal cancer and intakes of flavonoids and fatty acids (matched dataset)

6.1 Introduction

In this chapter the results of the matched analysis of the novel dietary risk factors that comprise the first two hypotheses, are presented. In particular the dietary risk factors that were analysed using the matched dataset included: 1) flavonoids (subgroups, individual compounds) and 2) fatty acids (total, subgroups, individual compounds).

In the first part, the study population included in the matched analysis is described, including descriptive analysis of the main confounding factors and logistic regression analysis to investigate their association relationships with colorectal cancer risk.

In the second part of the chapter descriptive analysis of the flavonoid and fatty acid variables are presented including distribution analysis (whole sample and by case/ control status) and correlation analysis. In addition, the association relationships between colorectal cancer and each flavonoid and fatty acid variable are investigated by applying three main and two additional conditional logistic regression models. All tables and figures are presented at the end of each section or in the Appendix, as indicated in the text.

6.2 The study sample

This section describes the characteristics of the cases and controls that were included in the matched dataset. In total 2,980 cases and controls were matched (1:1). One case had unrealistically high dietary energy and nutrient intakes and was removed from further analysis, together with its matched control.

6.2.1 Descriptive analysis of the confounding factors

The distribution of the continuous confounding factors was examined by looking at their histograms. In addition, their summary statistics are presented in Table 62 for the whole sample and also separately for cases and controls. The t-test was used to test differences between cases and controls in mean age, BMI, dietary energy intake, fibre intake (crude

and residually energy adjusted) and alcohol intake (crude and residually energy adjusted). The Pearson χ^2 test was used to test the differences in terms of sex, deprivation score, family history of cancer, physical activity (hours/ week of cycling and sport activities), smoking status and NSAIDs intake (Table 62).

6.2.2 Associations between confounding factors and colorectal cancer risk

The association relationship between each confounding factor and colorectal cancer risk was tested by applying univariable conditional logistic regression models (Table 63). Statistically significant associations were observed for the majority of the confounding factors, including:

- Family history of cancer (moderate/ high vs. low: OR (95% CI), p-value: 13.21 (7.68, 22.75), $1.25x10^{-20}$);
- Physical activity (>7 hours/week vs. 0 hours/week: OR (95% CI), p-value for trend: 0.77 (0.56, 1.04), 0.009);
- Dietary energy intake (highest vs. lowest quartile: OR (95% CI), p-value for trend: 1.34 (1.09, 1.65), 0.001);
- Residually energy adjusted fibre intake (highest vs. lowest quartile: OR (95% CI) p-value for trend) 0.67 (0.54, 0.83), 0.0001);
- Residually energy adjusted alcohol intake (highest vs. lowest quartile: OR (95% CI), p-value for trend: 0.81 (0.65, 1.00), 0.04);
- NSAIDs intake (yes vs. no: OR (95% CI) p-value: 0.74 (0.63, 0.86), 0.0001).

Table 62 Summary statistics of the confounding factors for the matched dataset

Variables	All subjects* (n=2978)	Cases* (n=1489)	Controls* (n=1489)	p-value†
Age (years)	64.0 (9.7)	63.6 (9.7)	64.4 (9.7)	0.03
Age (years)				
≤55 years	596 (20.0%)	318 (21.4%)	278 (18.7%)	
>55 years	2382 (80.0%)	1171 (78.6%)	1211 (81.3%)	0.07
Sex				
Men	1734 (58.2%)	867(58.2%)	867(58.2%)	
Women	1244 (41.8%)	622 (41.8%)	622 (41.8%)	1.00
Deprivation score‡				
1	311 (10.4%)	140 (9.4%)	171 (11.5%)	
2	650 (21.8%)	327 (22.0%)	323 (21.7%)	
3	769 (25.8%)	402 (27.0%)	367 (24.7%)	
4	713 (23.9%)	356 (23.9%)	357 (24.0%)	
5	305 (10.2%)	152 (10.2%)	153 (10.3%)	
6	162 (5.4%)	77 (5.2%)	85 (5.7%)	
7	68 (2.3%)	35 (2.4%)	33 (2.2%)	0.52
Family history risk of cancer				
Low	2664 (89.4%)	1215 (81.6%)	1449 (97.3%)	
Medium	186 (6.2%)	170 (11.4%)	16 (1.1%)	
High	22 (0.7%)	21 (1.4%)	1 (0.1%)	
Unknown	71 (2.4%)	62 (4.2%)	9 (0.6%)	<0.0005
Missing	35 (1.2%)	21 (1.4%)	14 (0.9%)	
BMI (kg/m^2)§	23.6 (4.4)	26.6 (4.3)	26.7 (4.6)	0.45
Physical activity (hours/day) *(cycling and other sport activities)*				
0	1646 (55.3%)	846 (56.8%)	800 (53.7%)	
0-3.5	692 (23.2%)	337 (22.6%)	355 (23.8%)	
3.5-7	322 (10.8%)	144 (9.7%)	178 (11.9%)	
>7	198 (6.6%)	89 (6.0%)	109 (7.3%)	0.07
Missing	120 (4.0%)	73 (4.9%)	47 (3.1%)	

Smoking				
No	1242 (41.7%)	606 (40.7%)	636 (42.7%)	
Yes[**]	1701(57.1%)	862 (57.9%)	839 (56.3%)	0.32
Missing	35 (1.2%)	21 (1.4%)	14 (0.9%)	
Dietary energy intake (MJ/day)[††]	10.9 (4.1%)	11.2 (4.2%)	10.6 (3.9%)	0.0002
Fibre intake (g/day)[‡‡]	22.4 (9.6)	22.3 (9.5)	22.4 (9.8)	0.90
Energy-adjusted fibre intake (g/day)	21.9 (6.0)	21.4 (5.8)	22.3 (6.2)	0.0001
Alcohol intake (g/day)[§§]	13.1 (15.6)	13.1 (16.1)	13.0 (15.1)	0.79
Energy-adjusted alcohol intake (g/day)[***]	12.9 (15.0)	12.8 (15.5)	13.0 (14.4)	0.36
NSAIDs intake[†††]				
No	1939 (65.1%)	1019 (68.4%)	920 (61.8%)	
Yes	1038 (34.8%)	469 (31.5%)	569 (38.2%)	<0.0005
Missing	1 (0.0%)	1 (0.1%)	0 (0.0%)	

[*] Mean values and in parentheses standard deviations for quantitative variables; number of subjects and in parenthesis percentages for categorical variables.

[†] P-values from the Pearson χ^2 for categorical variables; from t-test for continuous variables

[‡] Locally based deprivation index (Carstairs deprivation index) based on the 2001 Census data; 7 categories ranging from very low deprivation (deprivation score 1) to very high deprivation (deprivation score 7)

[§] Missing data for 15 cases and 19 controls; T-test was applied after logarithmic transformation

[**] Smokers were defined as individuals who have smoked at least one cigarette per day and/ or one cigar per month and/ or pipe.

[††] T-test was applied after logarithmic transformation

[‡‡] T-test was applied after logarithmic transformation

[§§] T-test was applied after square-root transformation

[***] T-test was applied after square-root transformation

[†††] Frequent use was defined as an intake of at least 4 days per week for at least one month.

Table 63 Association between the confounding factors and colorectal cancer risk (univariable conditional logistic regression analysis)

Confounding variables	Categories	Frequency		Univariable analysis		
		cases	***controls***	***OR***	***95% CI***	***p-value***
Family history risk of cancer	Low	1215	1449	1.00		
	Medium/ High	191	17	13.21	7.68, 22.75	$1.25x10^{-20}$
BMI (kg/m^2)	continuous	1474	1470	0.99	0.98, 1.01	0.38
BMI (kg/m^2)	18.5-25	573	531	1.00		
	<18.5	13	18	0.67	0.33, 1.38	0.28
	25-30	629	644	0.90	0.77, 1.06	0.23
	≥ 30	259	277	0.87	0.71, 1.07	0.18
				p-value for trend 0.14		
Physical activity (hours/week)	0	1646	846	1.00		
	0-3.5	692	337	0.90	0.75, 1.08	0.25
	3.5-7	322	144	0.75	0.59, 0.97	0.03
	>7	198	89	0.77	0.56, 1.04	0.09
				p-value for trend 0.009		
Smoking	No	606	636	1.00		
	Former	616	586	1.11	0.94, 1.31	0.22
	Current	246	253	1.02	0.83, 1.25	0.88
				p-value for trend 0.51		
Dietary energy intake (MJ/day)	continuous	1489	1489	1.03	1.01, 1.05	0.0005
Dietary energy intake (MJ/day)	0- 8.28	352	393	1.00		
	8.28-10.17	345	399	0.98	0.80, 1.20	0.82
	10.17- 12.73	389	356	1.25	1.02, 1.55	0.04
	>12.73	403	341	1.34	1.09, 1.65	0.006
				p-value for trend 0.001		
Fibre intake (g/day)	continuous	1489	1489	1.00	0.99, 1.01	0.83
Fibre intake (g/day)	0- 16.10	382	381	1.00		
	16.10- 20.70	371	368	1.01	0.82, 1.23	0.95
	20.70- 26.80	380	364	1.04	0.85, 1.28	0.68
	>26.80	356	376	0.94	0.77, 1.16	0.58

				p-value for trend 0.68		
Fibre intake energy adjusted (g/day)	continuous	1489	1489	0.97	0.96, 0.98	$2.3x10^{-5}$
Fibre intake	0- 17.45	392	353	1.00		
energy adjusted	17.45- 20.89	392	352	1.00	0.81, 1.22	0.97
(g/day)	20.89- 24.85	384	361	0.95	0.77, 1.17	0.61
	>25.85	321	423	0.67	0.54, 0.83	$3.6x10^{-5}$
				p-value for trend 0.0001		
Alcohol intake (g/day)	continuous	1489	1489	1.00	0.99, 1.01	0.81
Alcohol intake (g/day)	0-1.60	376	369	1.00		
	1.60-8.10	392	366	1.05	0.86, 1.28	0.63
	8.10-18.80	362	369	0.95	0.78, 1.17	0.64
	>18.80	359	385	0.89	0.72, 1.11	0.31
				p-value for trend 0.24		
Alcohol intake energy adjusted (g/day)	continuous	1489	1489	1.00	0.99, 1.00	0.76
Alcohol intake	0-1.61	384	361	1.00		
energy adjusted	1.61-8.12	387	357	1.02	0.83, 1.25	0.87
(g/day)	8.12-18.85	368	377	0.91	0.74, 1.12	0.37
	>18.85	350	394	0.81	0.65, 1.00	0.05
				p-value for trend 0.04		
NSAIDs intake	No	1939	1019	1.00		
	Yes	1038	469	0.74	0.63, 0.86	0.0001

6.3 Flavonoids

This analysis describes the distribution and correlation among subgroups of the flavonoid variables. In addition, the differences in crude and energy-adjusted flavonoid intakes between cases and controls and the unadjusted and adjusted associations between flavonoid intakes and colorectal cancer are presented.

6.3.1 Descriptive analysis

6.3.1.1 Distribution of flavonoid variables

After careful examination of the distribution of the flavonoid variables (subgroups and individual compounds) by looking at their histograms (original and transformed variables if skewed) I excluded kaempferol, myricetin, apigenin, luteolin and the gallates: epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, gallocatechin from further analysis because of their extreme patterns of distribution (perhaps due to limited compositional information). Therefore, the subclasses that were investigated were: flavonols (summary measurement of quercetin, kaempferol and myricetin), flavones (summary measurement of apigenin and luteolin), flavan3ols (summary measurement of catechin, epicatechin and gallates), procyanidins (summary measurement of procyanidin type BI - IV), flavanones (summary measurement of naringenin and hesperetin), and phytoestrogens (summary measurement of isoflavones and lignans) and the individual compounds: quercetin, catechin, epicatechin, naringenin and hesperetin (Table 64). The skewed flavonoid variables were normalised either with square root or with logarithmic transformation prior to applying parametric tests. If the distributions were not normalised after transformation (flavones, flavan3ols), only non-parametric tests were applied (Table 64).

6.3.1.2 Distribution of flavonoid variables by case control status

Evaluation of the flavonoid composition of the diet of our study population showed that the most abundant flavonoids (individual compounds) were epicatechin, quercetin and hesperetin accounting for the 11.9% and 9.0% and 7.8% of the total dietary intake of flavonoids (excluding phytoestrogens), respectively. No statistically significant differences were observed between cases and controls for crude mean and median

flavonoid intakes (Table 65). After energy adjustment (residual energy adjustment for normal distributed flavonoid variables) cases reported a lower mean intake for flavonols (p=0.02), flavanones (p=0.04), quercetin (p=0.004), catechin (p=0.003), naringenin (p=0.04), and hesperetin (p=0.04). In addition cases reported a lower median intake for flavonols (p=0.02), quercetin (p=0.004) and catechin (p=0.002) (Table 65).

6.3.1.3 Correlations between the flavonoid variables

Overall the flavonoid variables were highly correlated. The highest correlations were observed between flavonols, flavan3ols, procyanidins, quercetin, catechin and epicatechin, with r>0.7. In addition, flavanones, naringenin and hesperetin were highly correlated with r>0.7. Phytoestrogens were not correlated with any of the flavonoid subgroups or individual compounds ($r \leq 0.15$) (Table 66).

6.3.1.4 Main sources of flavonoid variables

The three main food sources (at individual food item level) of the flavonoid subgroups were: 1) for flavonols: regular tea (64.3%), onions (9.1%), and soups- home made (6.3%); 2) for flavones: soups- home made (78.2%), other salad vegetables (10.9%), and meat or chicken pies, pasties, sausage roll (4.3%); 3) for flavan3ols: regular tea (89.3%), apples (3.1%), and red wine (2.1%); 4) for procyanidins: regular tea (74.2%), apples (11.2%), and red wine (8.4%); 5) for flavanones: oranges, satsumas or grapefruits (69.3%), pure fruit juice (29.1%) and red wine (1.2%); and 6) for phytoestrogens: soya milk (26.3%), wholemeal bread (including toast and sandwiches) (18.0%), soya beans, TVP, Tofu or soya meat substitute (13.4%) (Table 67).

In addition the three main food sources of the flavonoid individual compounds were: 1) for quercetin: regular tea (50.6%), red wine (13.3%) and soups- home made (9.0%); 2) for catechin: regular tea (45.1%), red wine (16.2%) and other fruits (9.8%); 3) for epicatechin: regular tea (67.5%), apples (11.7%), chocolate (6.1%); 4) for naringenin: oranges, satsumas or grapefruits (70.5%), pure fruit juice (26.7%), red wine (2.1%); and 5) for hesperetin: oranges, satsumas or grapefruits (67.8%), pure fruit juice (31.5%) and red wine (0.6%) (Table 67).

6.3.2 Associations between flavonoid variables and colorectal cancer risk

6.3.2.1 Main conditional logistic regression models

None of the flavonoid variables were significantly associated with colorectal cancer in the crude model (Model I) (Table 68). In Model II, flavonols, procyanidins, quercetin, catechin and epicatechin were significantly associated with a decreased colorectal cancer risk (high vs. low quartile OR (95%): 0.77 (0.63-0.94), 0.80 (0.65-0.98), 0.71 (0.58-0.88), 0.68 (0.55-0.83), 0.77 (0.63-0.94); respectively), and these associations were also dose-dependant (p-value for trend: 0.02, 0.04, 0.002, 0.0001, 0.04; respectively) (Table 68). Quercetin and catechin showed also and inverse and dose-dependent association with colorectal cancer risk in Model III (p-value for trend: 0.04 and 0.02; respectively) with approximately a 25% reduction in risk for those of high versus those of low intake (OR (95% CI): 0.77 (0.60-0.99), 0.75 (0.58-0.97); respectively) (Table 68). In distinct contrast, there were no associations between flavones, flavanones and phytoestrogens and colorectal cancer risk (p-value for trend 0.28, 0.39 and 0.87 respectively in model III) (Table 68).

6.3.2.2 Additional conditional logistic regression models

Since these associations could be confounded by other compounds present in fruit and vegetables or by the intake of other flavonoids we explored these relationships further in two additional models (Model IV and V; Table 69). Model IV was corrected for the confounding factors of model III and for fruit and vegetable intake (measures/day, continuously, energy adjusted). The observed association with catechin remained significant (high vs. low quartile OR (95% CI): 0.79 (0.61-1.03); p-value for trend 0.05) (Table 69). The associations with flavonols, quercetin and epicatechin had the same direction, but were marginally not statistically significant (high vs. low quartile OR (95% CI): 0.81 (0.63-1.01), 0.82 (0.63-1.06), 0.78 (0.61-1.00), respectively) (Table 69). In model V association were corrected for the confounding factors of model III and further adjusted mutually between flavonoid categories. The observed associations between flavonols, catechin, epicatechin and colorectal cancer became stronger and

remained statistically significant (high vs. low quartile OR (95% CI), p-value for trend: 0.29 (0.16-0.54), 0.0001; 0.56 (0.37-0.86), 0.007; 0.46 (0.23-0.92), 0.03; respectively) (Table 69).

6.3.2.3 Multiple testing corrections

Bonferroni correction for multiple testing

In model II, the inverse association with catechin (p-value 0.0001) remained significant under every level of correction and the inverse association with quercetin (p-value 0.002) remained significant in the first two levels, but not after having considered all the tests conducted in all 4 hypotheses (Table 68). In model V, the inverse association with flavonols (p-value 0.0001) remained significant under every level of correction, whereas the inverse association with catechin (p-value 0.007) remained significant only in the first level of correction (Table 69).

FDR correction for multiple testing

After correcting for multiple testing using the FDR method the inverse associations that remained significant were: with catechin (p=0.0001) and quercetin (p=0.002) in model II (Table 68) and with flavonols (p=0.0001) and catechin (p=0.007) in model V (Table 69).

6.3.2.4 Associations between colorectal cancer and the main food sources of flavonols, procyanidins, quercetin, catechin and epicatechin

Intakes of the following food items were tested: regular tea, onions, apples and red wine. Results from model III, showed that comparison of highest versus lowest quartile intakes of these foods (tertiles for red wine intakes) showed ORs for colorectal cancer risk of 0.82 (95% CI 0.63, 1.06; p-value for trend 0.27) for regular tea; 0.92 (95% CI 0.72, 1.17; p-value for trend 0.44) for onions; 0.97 (95% CI 0.75, 1.25; p-value for trend 0.77) for apples; and 0.87 (95% CI 0.68, 1.11; p-value for trend 0.33) for red wine (Table 70).

6.3.2.5 Associations between the flavonoid variables and colorectal cancer after sex, age and cancer site stratification

Associations between each flavonoid variable and colorectal cancer risk were tested after sex, age and cancer site stratification by applying model III (data not shown). Sex-

specific associations were similar for almost all flavonoid subgroups and individual compounds. However, high intake of phytoestrogens was associated with a non statistically significant decrease in colorectal cancer risk for men (high vs. low intake OR (95% CI), p-value for trend: 0.80 (0.58, 1.10), 0.14), but with a not statistically significant increase in colorectal cancer risk for women (high vs. low intake OR (95% CI), p-value for trend: 1.55 (1.02, 2.36), 0.06) (data not shown). Intakes of flavonols, procyanidins, quercetin, catechin and epicatechin were significantly and dose-dependently associated with a decreased risk of colorectal cancer for the individuals older than 55 years old (data not shown). However, associations were not as clear for the individuals younger than 55 years old, with none of them reaching the 0.05 significance level (data not shown). Finally, after cancer site stratification, flavonols, procyanidins, quercetin, catechin and epicatechin were found to be inversely though not significantly associated with both colon and rectal cancer (data not shown).

6.3.3 Summary of results

Moderately strong inverse associations which showed dose response relationships were found: 1) in model II: between colorectal cancer risk and intakes of flavonols (p=0.02), procyanidins (p=0.04), quercetin (p=0.002), catechin (p=0.0001) and epicatechin (p=0.04) (Table 68); 2) in model III: between colorectal cancer and intakes of quercetin (p=0.04) and catechin (p=0.02) (Table 68); 3) in model IV between colorectal cancer and catechin (p=0.05) (Table 69); 4) in model V between colorectal cancer and intakes of flavonols (p=0.0001), catechin (p=0.007) and epicatechin (p=0.03) (Table 69). In marked contrast we showed no associations between intakes of the other four of the six flavonoid subgroups studied (flavones, flavan3ols, flavanones and phytoestrogens) and colorectal cancer risk (Table 68, Table 69).

Table 64 Flavonoid variables (subgroups and individual compounds) that were elected to be included in the analysis

Flavonoid variables included in the analysis	Transformation
Subgroups	
Flavonols	Square root
Flavones	n/a
Flavan3ols	n/a
Procyanidins	Square root
Flavanones	Square root
Phytoestrogens	Logarithmic
Individual compounds	
Quercetin	Square root
Catechin	Square root
Epicatechin	Square root
Naringenin	Square root
Hesperetin	Square root

Table 65 Descriptive report of crude and energy-adjusted flavonoid intakes

Flavonoids	All subjects (n=2978)		Cases (n=1489)		Controls (n=1489)		T-test	Wilcoxon rank test
	Mean (SD)	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*p-value*	*p-value*
Subgroups								
Flavonols (mg/day)	26.5 (13.3)	26.9 (15.4, 36.5)	26.2 (13.2)	26.8 (15.1, 36.3)	26.8 (13.3)	27.1 (15.8, 36.9)	0.28	0.28
Flavonols- energy adjusted (mg/day)	26.3 (12.5)	27.1 (15.6, 36.3)	25.8 (12.4)	26.5 (14.9, 35.9)	26.9 (12.6)	27.7 (16.1, 37.0)	0.02	0.02
Flavones (mg/day)	1.3 (1.2)	1.0 (0.5, 1.9)	1.4 (1.3)	1.1 (0.5, 1.9)	1.3 (1.1)	1 (0.5, 1.8)	n/a	0.14
Flavones- energy adjusted (mg/day)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Flavan3ols (mg/day)	105.9 (66.7)	115.0 (42.0, 161.3)	105.5 (66.3)	115.2 (42.0, 159.8)	106.3 (67.1)	114.4 (42.1, 163.7)	n/a	0.72
Flavan3ols- energy adjusted (mg/day)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Procyanidins (mg/day)	31.3 (17.7)	32.2 (16.3, 45.3)	30.9 (17.7)	31.9 (15.9, 45.0)	31.6 (17.7)	32.5 (16.7, 45.6)	0.40	0.30
Procyanidins- energy adjusted (mg/day)	31.2 (17.4)	32.3 (16.4, 45.0)	30.6 (17.2)	31.8 (15.8, 43.8)	31.7 (17.5)	33.3 (16.9, 45.7)	0.09	0.08
Flavanones (mg/day)	29.3 (31.9)	20.3 (7.5, 40.5)	28.3 (30.6)	20.1 (8.1, 39.4)	30.3 (33.1)	20.6 (6.7, 42.1)	0.51	0.61
Flavanones- energy adjusted (mg/day)	29.1 (31.0)	20.5 (7.7, 40.9)	28.0 (29.8)	19.9 (8.5, 38.1)	30.3 (32.2)	21.3 (7.2, 42.7)	0.04	0.27
Phytoestrogens (µg/day)	1075.2	596.0	981.6	593.1	1168	599.3	0.73	0.84

	(3490.3)	(393.8, 875.4)	(2674.8)	(407.2, 860.4)	(4147.5)	(384.5, 889.5)		
Phytoestrogens- energy	1059.2	581.7	925.5	570.7	1192.9	596.0	0.45	0.27
adjusted (µg/day)	(3732.6)	(401.3, 856.5)	(2345.3)	(404.1, 832.1)	(4726.3)	(400.3, 885.2)		
Individual compounds								
Quercetin (mg/day)	17.6	17.5	17.4	17.3	17.8	17.8	0.22	0.19
	(8.4)	(11.4, 22.8)	(8.4)	(11.3, 22.8)	(8.4)	(11.5, 22.8)		
Quercetin- energy	17.4	17.6	17.0	17.2	17.8	18.0	0.004	0.004
adjusted (mg/day	(7.6)	(11.5, 22.8)	(7.5)	(11.1, 22.3)	(7.7)	(12.0, 23.4)		
Catechin (mg/day)	7.5	7.1	7.4	7.0	7.6	7.2	0.12	0.08
	(4.1)	(4.7, 9.5)	(4.0)	(4.7, 9.3)	(4.2)	(4.9, 9.7)		
Catechin- energy adjusted	7.4	7.2	7.2	7.0	7.7	7.4	0.003	0.002
(mg/day)	(3.9)	(4.8, 9.4)	(3.8)	(4.6, 9.0)	(3.9)	(5.0, 9.7)		
Epicatechin (mg/day)	23.2	23.9	23.0	23.9	23.4	24.0	0.55	0.33
	(12.3)	(13.0, 32.7)	(12.3)	(12.7, 32.3)	(12.3)	(13.2, 33.0)		
Epicatechin- energy	23.1	24.0	22.7	23.4	23.5	24.3	0.07	0.06
adjusted (mg/day)	(11.9)	(12.9, 32.5)	(11.7)	(12.6, 31.8)	(12.0)	(13.2, 33.2)		
Naringenin (mg/day)	14.2	9.9	13.7	9.9	14.7	9.9	0.48	0.64
	(15.6)	(3.7, 19.9)	(14.9)	(4.0, 18.9)	(16.2)	(3.3, 21.0)		
Naringenin- energy	14.1	9.9	13.5	9.6	14.7	10.2	0.04	0.27
adjusted (mg/day)	(15.2)	(3.8, 19.7)	(14.6)	(4.0, 18.3)	(15.7)	(3.6, 20.9)		
Hesperetin (mg/day)	15.1	10.5	14.6	10.4	15.6	10.6	0.55	0.61
	(16.3)	(3.8, 20.8)	(15.6)	(4.1, 20.5)	(16.9)	(3.4, 21.5)		
Hesperetin- energy	15.0	10.6	14.4	10.3	15.6	11.0	0.04	0.29
adjusted (mg/day)	(15.9)	(3.9, 21.1)	(15.3)	(4.2, 19.9)	(16.5)	(3.6, 21.9)		

Table 66 Spearman rank correlation coefficients between flavonoid variables (n=2978, all p-values<$5x10^{-5}$)

Flavonoids	Flavonols	Flavones	Flavan3ols	Procyanidins	Flavanones	Phytoestr.	Quercetin	Catechin	Epicatechin	Naringenin	Hesperetin
Flavonols	1.00										
Flavones	0.26	1.00									
Flavan3ols	0.94	0.08	1.00								
Procyanidins	0.92	0.09	0.95	1.00							
Flavanones	0.08	0.16	0.02	0.07	1.00						
Phytoestrogens	0.13	0.12	0.08	0.09	0.11	1.00					
Quercetin	0.98	0.35	0.86	0.87	0.15	0.15	1.00				
Catechin	0.71	0.15	0.72	0.81	0.11	0.11	0.70	1.00			
Epicatechin	0.93	0.10	0.96	0.96	0.11	0.11	0.88	0.79	1.00		
Naringenin	0.08	0.16	0.02	0.07	1.00	0.11	0.15	0.23	0.11	1.00	
Hesperetin	0.08	0.16	0.02	0.06	1.00	0.10	0.15	0.21	0.11	1.00	1.00

Table 67 Three main dietary (food) sources of flavonoids in our population

Flavonoids	Main sources
Flavonols	Regular tea (62.3%)
	Onions (8.8%)
	Soups- home made (6.1%)
Flavones	Soups- home made (77.8%)
	Other salad vegetables (11.5%)
	Meat or chicken pies, pasties, sausage roll (4.1%)*
Flavan3ols	Regular tea (88.6%)
	Apples (3.0%)
	Red wine (2.0%)
Procyanidins	Regular tea (72.9%)
	Apples (12.7%)
	Red wine (8.4%)
Flavanones	Oranges, satsumas or grapefruits (69.1%)
	Pure fruit juice (29.1%)
	Red wine (1.3%)
Phytoestrogens	Soya milk (24.6%)
	Wholemeal bread (including toast and sandwiches) (18.0%)
	Soya beans, TVP, Tofu or soya meat substitute (12.5%)
Quercetin	Regular tea (50.6%)
	Onions (13.3%)
	Soups- home made (9.0%)
Catechin	Regular tea (45.1%)
	Red wine (16.2%)
	Other fruits (9.8%)
Epicatechin	Tea (67.5%)
	Apples (11.7%)
	Chocolate (6.1%)
Naringenin	Oranges, satsumas or grapefruits (70.5%)
	Pure fruit juice (26.7%)
	Red wine (2.1%)
Hesperetin	Oranges, satsumas or grapefruits (67.8%)
	Pure fruit juice (31.5%)
	Red wine (0.6%)

* Flavones probably come from suede or parsley that are usual ingredients of these foods

Table 68 Association between the flavonoid variables and colorectal cancer risk in the whole sample (3 main conditional logistic regression models; Cases and controls matched on age, gender and area of residence)

Flavonoids	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Flavonols	0 - 15.59	392	353	1.00		1.00		1.00	
(mg/day)	15.59 - 27.09	373	371	0.90	0.73, 1.11	0.90	0.74, 1.11	0.88	0.69, 1.13
	27.09 - 36.34	381	364	0.95	0.77, 1.16	0.94	0.76, 1.15	0.92	0.72, 1.17
	> 36.75	343	401	0.87	0.71, 1.07	0.77	0.63, 0.94	0.78	0.60, 0.99
	p-value for trend (quartiles)				0.27		0.02		0.08
	p-value for trend (continuous)				0.27		0.02		0.16
Flavones	0-0.5	424	427	1.00		1.00		1.00	
(mg/day)	0.5-1.0	317	332	0.96	0.78, 1.18	0.93	0.76, 1.14	0.91	0.71, 1.16
	1.0-1.9	380	403	0.95	0.78, 1.16	0.90	0.74, 1.10	1.04	0.82, 1.31
	>1.9	368	327	1.13	0.93, 1.38	0.99	0.80, 1.23	1.14	0.87, 1.48
	p-value for trend (quartiles)				0.32		0.78		0.28
	p-value for trend (continuous)				0.018		0.32		0.11
Flavan3ols	0-42	374	372	1.00		1.00		1.00	
(mg/day)	42-114.95	366	377	0.97	0.79, 1.20	0.97	0.78, 1.19	0.95	0.74, 1.22
	114.95-161.3	393	352	1.12	0.91, 1.38	1.11	0.90, 1.37	1.09	0.85, 1.40
	>161.3	356	388	0.92	0.75, 1.12	0.86	0.70, 1.05	0.81	0.63, 1.04
	p-value for trend (quartiles)				0.68		0.32		0.22
	p-value for trend (continuous)				0.74		0.34		0.30
Procyanidins	0-16.40	384	361	1.00		1.00		1.00	
(mg/day)	16.40-32.34	380	364	0.97	0.79, 1.19	0.97	0.79, 1.20	0.94	0.74, 1.21
	32.34-45.01	384	361	1.01	0.83, 1.24	1.00	0.82, 1.22	1.00	0.79, 1.27
	>45.01	341	403	0.89	0.73, 1.10	0.80	0.65, 0.98	0.82	0.64, 1.05
	p-value for trend (quartiles)				0.37		0.04		0.19

	p-value for trend (continuous)			0.31		0.09		0.31
Flavanones	0-7.69	353	392	1.00		1.00		1.00	
(mg/day)	7.69-20.51	404	340	1.36	1.11, 1.67	1.33	1.08, 1.63	1.52	1.19, 1.95
	20.51-40.86	388	357	1.26	1.03, 1.54	1.21	0.98, 1.49	1.46	1.13, 1.88
	>40.86	344	400	0.98	0.80, 1.20	0.95	0.77, 1.17	1.15	0.88, 1.51
	p-value for trend (quartiles)			0.78		0.48		0.39
	p-value for trend (continuous)			0.09		0.04		0.67
Phytoestrogens	0-401.33	368	377	1.00		1.00		1.00	
(µg/day)	401.33-581.70	403	341	1.29	1.05, 1.59	1.22	0.99, 1.50	1.18	0.92, 1.50
	581.70-856.39	372	373	1.17	0.95, 1.43	1.02	0.83, 1.26	1.12	0.87, 1.42
	>856.39	346	398	1.04	0.84, 1.28	0.90	0.73, 1.10	1.04	0.81, 1.34
	p-value for trend (quartiles)			0.97		0.11		0.87
	p-value for trend (continuous)			0.15		0.06		0.51
Quercetin	0-11.53	392	353	1.00		1.00		1.00	
(mg/day)	11.53-17.63	387	357	0.96	0.78, .18	0.98	0.80, 1.20	0.97	0.76, 1.24
	17.63-22.80	379	366	0.91	0.74, 1.11	0.93	0.76, 1.15	0.90	0.70, 1.14
	>22.80	331	413	0.93	0.75, 1.14	0.71	0.58, 0.88	0.77	0.60, 0.99
	p-value for trend (quartiles)			0.37		0.002		0.04
	p-value for trend (continuous)			0.20		0.004		0.12
Catechin	0-4.84	405	340	1.00		1.00		1.00	
(mg/day)	4.84-7.21	385	359	0.90	0.73, 1.10	0.89	0.73, 1.10	0.87	0.68, 1.11
	7.21-9.40	367	378	0.96	0.78, 1.17	0.82	0.67, 1.01	0.79	0.62, 1.00
	>9.40	332	412	0.82	0.67, 1.00	0.68	0.55, 0.83	0.75	0.58, 0.97
	p-value for trend (quartiles)			0.11		0.0001		0.02
	p-value for trend (continuous)			0.08		0.004		0.19
Epicatechin	0-12.90	385	360	1.00		1.00		1.00	
(mg/day)	12.90-24.05	374	370	0.91	0.74, 1.12	0.95	0.77, 1.17	0.95	0.74, 1.21

	24.05-32.47	396	349	1.02	0.83, 1.25	1.08	0.87, 1.33	1.10	0.86, 1.42
	>32.47	334	410	0.88	0.71, 1.07	0.77	0.63, 0.94	0.77	0.61, 0.99
	p-value for trend (quartiles)				0.40		0.04		0.12
	p-value for trend (continuous)				0.43		0.07		0.28
Naringenin	0-3.79	356	389	1.00		1.00		1.00	
(mg/day)	3.79-9.89	400	344	1.34	1.09, 1.64	1.28	1.04, 1.57	1.43	1.12, 1.83
	9.89-19.72	392	353	1.33	1.08, 1.63	1.22	0.99, 1.50	1.42	1.10, 1.84
	>19.72	341	403	0.96	0.78, 1.18	0.92	0.75, 1.13	1.11	0.85, 1.45
	p-value for trend (quartiles)				0.80		0.38		0.46
	p-value for trend (continuous)				0.08		0.04		0.66
Hesperetin	0-3.92	354	391	1.00		1.00		1.00	
(mg/day)	3.92-10.60	405	339	1.30	1.06, 1.60	1.32	1.08, 1.62	1.53	1.20, 1.97
	10.60-21.10	381	364	1.22	1.00, 1.50	1.16	0.94, 1.42	1.10	1.09, 1.80
	>21.10	349	395	0.97	0.79, 1.19	0.97	0.79, 1.20	1.18	0.90, 1.55
	p-value for trend (quartiles)				0.70		0.52		0.36
	p-value for trend (continuous)				0.09		0.04		0.68

[*]Based on the distribution of the energy adjusted variable, except for the flavonoid subgroups flavones and flavan3ols, which quartiles are based on the distribution of the crude variables

[†]Model I: Crude analysis

[‡]Model II: Adjusted for total energy intake (residual method except for the flavonoid variables flavones and flavan3ols, for which the standard energy adjustment method was used)

[§]Model III: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake, fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

Table 69 Association between the flavonoid variables and colorectal cancer risk in the whole sample (2 additional conditional logistic regression models; Cases and controls matched on age, gender and area of residence)

Flavonoids	Quartiles*	Frequency		Model IV†		Model V‡	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*
Flavonols	0 - 15.59	392	353	1.00		1.00	
(mg/day)	15.59 - 27.09	373	371	0.90	0.71, 1.15	0.63	0.45, 0.87
	27.09 - 36.34	381	364	0.94	0.74, 1.20	0.46	0.29, 0.74
	> 36.75	343	401	0.81	0.63, 1.01	0.29	0.16, 0.54
	p-value for trend (quartiles)				0.15		0.0001
	p-value for trend (continuous)				0.30		$7.1x10^{-5}$
Flavones	0-0.5	424	427	1.00		1.00	
(mg/day)	0.5-1.0	317	332	0.93	0.73, 1.19	0.93	0.73, 1.19
	1.0-1.9	380	403	1.05	0.83, 1.33	1.12	0.88, 1.44
	>1.9	368	327	1.14	0.88, 1.49	1.31	0.98, 1.75
	p-value for trend (quartiles)				0.28		0.05
	p-value for trend (continuous)				0.11		0.006
Flavan3ols	0-42	374	372	1.00		1.00	
(mg/day)	42-114.95	366	377	0.95	0.74, 1.23	1.11	0.79, 1.56
	114.95-161.3	393	352	1.09	0.85, 1.41	1.51	0.90, 2.52
	>161.3	356	388	0.81	0.63, 1.04	1.26	0.63, 2.50
	p-value for trend (quartiles)				0.22		0.51
	p-value for trend (continuous)				0.32		0.03
Procyanidins	0-16.40	384	361	1.00		1.00	
(mg/day)	16.40-32.34	380	364	0.95	0.74, 1.22	0.91	0.64, 1.28
	32.34-45.01	384	361	1.01	0.80, 1.29	0.93	0.56, 1.52
	>45.01	341	403	0.84	0.65, 1.07	0.74	0.39, 1.43
	p-value for trend (quartiles)				0.24		0.37
	p-value for trend (continuous)				0.40		0.97
Flavanones	0-7.69	353	392	1.00		1.00	
(mg/day)	7.69-20.51	404	340	1.53	1.20, 1.97	1.52	1.18, 1.95
	20.51-40.86	388	357	1.48	1.15, 1.91	1.52	1.10, 1.84
	>40.86	344	400	1.21	0.92, 1.59	1.14	0.87, 1.50
	p-value for trend (quartiles)				0.21		0.44
	p-value for trend (continuous)				0.82		0.68
Phytoestrogens	0-401.33	368	377	1.00		1.00	
(µg/day)	401.33-581.70	403	341	1.14	0.89, 1.46	1.16	0.91, 1.49
	581.70-856.39	372	373	1.03	0.81, 1.33	1.10	0.85, 1.41
	>856.39	346	398	0.93	0.72, 1.21	1.04	0.80, 1.34
	p-value for trend (quartiles)				0.46		0.92

	p-value for trend (continuous)				0.39		0.48
Quercetin	0-11.53	392	353	1.00		1.00	
(mg/day)	11.53-17.63	387	357	1.00	0.78, 1.27	0.89	0.63, 1.26
	17.63-22.80	379	366	0.92	0.72, 1.18	0.72	0.43, 1.20
	>22.80	331	413	0.82	0.63, 1.06	0.57	0.28, 1.17
	p-value for trend (quartiles)				0.10		0.10
	p-value for trend (continuous)				0.32		0.43
Catechin	0-4.84	405	340	1.00		1.00	
(mg/day)	4.84-7.21	385	359	0.88	0.69, 1.13	0.77	0.58, 1.03
	7.21-9.40	367	378	0.80	0.62, 1.02	0.65	0.45, 0.92
	>9.40	332	412	0.79	0.61, 1.03	0.56	0.37, 0.86
	p-value for trend (quartiles)				0.05		0.007
	p-value for trend (continuous)				0.41		0.14
Epicatechin	0-12.90	385	360	1.00		1.00	
(mg/day)	12.90-24.05	374	370	0.96	0.75, 1.23	0.77	0.54, 1.09
	24.05-32.47	396	349	1.13	0.88, 1.45	0.77	0.46, 1.30
	>32.47	334	410	0.78	0.61, 1.00	0.46	0.23, 0.92
	p-value for trend (quartiles)				0.15		0.03
	p-value for trend (continuous)				0.36		0.52
Naringenin	0-3.79	356	389	1.00		1.00	
(mg/day)	3.79-9.89	400	344	1.44	1.12, 1.85	1.44	1.11, 1.87
	9.89-19.72	392	353	1.45	1.12, 1.87	1.42	1.06, 1.89
	>19.72	341	403	1.17	0.89, 1.54	1.15	0.74, 1.79
	p-value for trend (quartiles)				0.25		0.14
	p-value for trend (continuous)				0.82		0.99
Hesperetin	0-3.92	354	391	1.00		1.00	
(mg/day)	3.92-10.60	405	339	1.54	1.20, 1.98	1.56	1.20, 2.02
	10.60-21.10	381	364	1.42	1.10, 1.84	1.43	1.07, 1.91
	>21.10	349	395	1.25	0.95, 1.64	1.32	0.85, 2.06
	p-value for trend (quartiles)				0.19		0.08
	p-value for trend (continuous)				0.82		0.99

[*] Based on the distribution of the energy adjusted variable, except for the flavonoid subgroups flavones and flavan3ols, which quartiles are based on the distribution of the crude variables

[†] Model IV: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake, fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake and fruit and vegetable intake (energy adjusted)

[‡] Model V: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake, fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake and mutually adjusted for other flavonoid subgroups.

Table 70 Association between intakes of tea, onions, apples and red wine and colorectal cancer risk in the whole sample (3 main conditional logistic regression models; Cases and controls matched on age, gender and area of residence)

Food items	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Regular tea	0-0.85	376	373	1.00		1.00		1.00	
(m/day**)	0.85-3	553	557	0.99	0.82, 1.20	0.99	0.82, 1.20	0.93	0.74, 1.17
	3-4	265	243	1.09	0.86, 1.37	1.05	0.83, 1.33	1.09	0.82, 1.44
	>4	295	316	0.93	0.75, 1.15	0.89	0.72, 1.10	0.82	0.63, 1.06
	p-value for trend (quartiles)				0.70		0.40		0.27
	p-value for trend (continuous)				0.90		0.58		0.36
Onions	0-0.14	553	552	1.00		1.00		1.00	
(m/day)	0.14-0.28	266	260	1.02	0.83, 1.26	1.00	0.81, 1.24	1.01	0.78, 1.29
	0.28-0.57	325	322	1.01	0.83, 1.22	0.97	0.80, 1.18	0.94	0.74, 1.19
	>0.57	345	355	0.97	0.80, 1.17	0.87	0.72, 1.07	0.92	0.72, 1.17
	p-value for trend (quartiles)				0.78		0.21		0.44
	p-value for trend (continuous)				0.02		0.0002		0.03
Apples	0-0.05	494	501	1.00		1.00		1.00	
(m/day)	0.05-0.28	423	377	1.14	0.94, 1.37	1.13	0.93, 1.36	1.16	0.92, 1.45
	0.28-0.57	267	262	1.04	0.84, 1.28	1.01	0.82, 1.25	1.07	0.83, 1.40
	>0.57	305	349	0.89	0.73, 1.08	0.84	0.69, 1.03	0.97	0.75, 1.25
	p-value for trend (quartiles)				0.23		0.09		0.77
	p-value for trend (continuous)				0.12		0.02		0.32
Red wine	0	939	877	1.00		1.00		1.00	
(m/day)	0-1.5	238	246	0.90	0.73, 1.10	0.89	0.73, 1.09	0.99	0.77, 1.25
	>1.5	312	366	0.79	0.66, 0.95	0.78	0.65, 0.93	0.87	0.68, 1.11
	p-value for trend (quartiles)				0.01		0.007		0.33

p-value for trend (continuous)	0.15	0.10	0.45

*Based on the distribution of the crude variables

†Model I: Crude analysis

‡Model II: Adjusted for total energy intake (standard method)

§Model III: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake, fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

** m/day: measures per day

6.4 Fatty acids

This analysis describes the distribution and correlation of the fatty acid variables. In addition, the differences in crude and energy-adjusted fatty acid intakes between cases and controls and the unadjusted and adjusted associations between fatty acid intakes and colorectal cancer are presented.

6.4.1 Descriptive analysis

6.4.1.1 Distribution of fatty acid variables

After careful examination of the distribution of the fatty acid variables (total, subgroups and individual compounds) by looking at their histograms (original and transformed variables if skewed) we elected to study the following variables: total FAs; seven fatty acid subgroups: SFAs, MUFAs, PUFAs, ω6PUFAs, ω3PUFAs, *t*FAs and *t*MUFAs; and nine individual fatty acid compounds: palmitic and stearic acids (SFAs), oleic acid (MUFAs), linoleic, γ-linolenic and arachidonic acids (ω6PUFAs) and α-linolenic, EPA and DHA (ω3PUFAs) (

Table 71). For total FAs, the subgroups ω6 and ω3PUFAs and the individual compounds EPA and DHA, dietary and total (diet and supplements) intakes were available.

6.4.1.2 Distribution of fatty acid variables by case control status

Evaluation of the fatty acid composition of the diet of our study population showed that the most abundant fatty acids (individual compounds) were oleic, palmitic and stearic acids, accounting for the 29.4%, 22.0% and 10.2% of the total dietary intake of fatty acids respectively. For crude fatty acid intakes, cases reported a higher mean intake for total FAs ($p<5x10^{-5}$), the subgroups: SFAs ($p<5x10^{-5}$), MUFAs ($p<5x10^{-5}$), PUFAs (p=0.004), ω6PUFAs (p=0.001), *t*FAs ($p<5x10^{-5}$), *t*MUFAs ($p<5x10^{-5}$) and the individual fatty acids: palmitic ($p<5x10^{-5}$), stearic ($p<5x10^{-5}$), oleic ($p<5x10^{-5}$), linoleic (p=0.001), γ-linolenic (p=0.007), arachidonic (p=0.0008) and α-linolenic (p=0.02) (Table 72). In addition, cases reported a higher median intake for total FAs ($p<5x10^{-5}$), the subgroups: SFAs ($p<5x10^{-5}$), MUFAs ($p<5x10^{-5}$), PUFAs (p=0.009), ω6PUFAs (p=0.004), *t*FAs ($p<5x10^{-5}$), *t*MUFAs ($p<5x10^{-5}$) and the individual fatty

acids: palmitic ($p<5x10^{-5}$), stearic ($p<5x10^{-5}$), oleic ($p<5x10^{-5}$), linoleic (p=0.0045), γ-linolenic (p=0.01), arachidonic (p=0.0025), α-linolenic (p=0.03) and a lower median intake for the individual fatty acid of EPA (p=0.05) (Table 72).

After energy adjustment (residual) cases reported a higher mean intake for total FAs (p=0.0018), the subgroups: SFAs (p=0.0001), MUFAs (p=0.03), *t*FAs (=0.0026), *t*MUFAs (p=0.0003) and the individual fatty acids: palmitic (p=0.0003), stearic (p=0.0001), oleic (p=0.0062) and a lower mean intake for the subgroup of ω3PUFAs ($p<5x10^{-5}$) and the individual fatty acids of EPA ($p<5x10^{-5}$) and DHA ($p<5x10^{-5}$) (Table 72). In addition cases reported a higher median intake for total FAs (p=0.0005), the subgroups: SFAs (p=0.0001), MUFAs (p=0.01), *t*FAs (=0.001), *t*MUFAs ($p<5x10^{-5}$) and the individual fatty acids: palmitic (p=0.0002), stearic (p=0.0001), oleic (p=0.002) and a lower median intake for the subgroup of ω3PUFAs ($p<5x10^{-5}$) and the individual fatty acids of EPA ($p<5x10^{-5}$) and DHA ($p<5x10^{-5}$) (Table 72).

6.4.1.3 Correlations between the fatty acid variables

Overall they were highly correlated. The highest correlations were observed between total FAs, SFAs, MUFAs, PUFAs, *t*FAs, *t*MUFAs, palmitic acid, stearic acid, oleic acid with r>0.7. In addition, EPA, DHA and ω3PUFAs were highly correlated with r>0.8 (Table 73).

6.4.1.4 Main sources of fatty acid variables

For fatty acid variables food sources data were not available for individual food items. The three main food sources (at food group level) of total FAs were: meat and meat products (18.0%), spreads[1] and cooking oils (13.4%) and confectionery and savoury snacks (8.3%). The three main food sources of the fatty acid subgroups were: 1) for SFAs: meat and meat products (17.5%), spreads and cooking oils (13.1%) and cheese (10.3%); 2) for MUFAs: meat and meat products (19.7%), spreads and cooking oils (13.7%), fish and fish dishes (8.5%); 3) for PUFAs: meat and meat products (15.3%), spreads and cooking oils (13.5%), confectionery and savoury snacks (11.4%); 4) for ω6PUFAs: spreads and cooking oils (15.2%), meat and meat products (14.8%), confectionery and savoury snacks (10.2%); 5) for

[1] Including butter, margarine, jam, honey, marmalade, yeast or meat extract, peanut butter, and chocolate spread

ω3PUFAs: fish and fish dishes (30.3%), meat and meat products (16.2%) and vegetables (13.1%); 6) for *t*FAs: spreads and cooking oils (20.7%), confectionery and savoury snacks (15.7%) and meat and meat products (15.4%); and 7) for *t*MUFAs: spreads and cooking oils (25.2%), meat and meat products (14.6%) and cheese (11.9%) (Table 74).

Finally, the three main food sources of the fatty acid individual compounds were: 1) for palmitic acid: meat and meat products (19.8%), spreads and cooking oils (12.8%) and cheese (8.5%); 2) for stearic acid: meat and meat products (24.8%), spreads and cooking oils (11.9%) and biscuits (8.7%); 3) for oleic acid: meat and meat products (20.6%), spreads and cooking oils (14.0%) and confectionery and savoury snacks (9.0%); 4) for linoleic acid: meat and meat products (15.9%), spreads and cooking oils (12.8%) and confectionery and savoury snacks (10.6%); 5) for γ-linolenic acid: meat and meat products (69.8%), potatoes, rice and pasta (8.6%) and fish and fish dishes (8.5%); 6) for arachidonic acid meat and meat products (62.4%), eggs (11.1%) and savoury foods, soups and sauces (10.2%); 7) for α-linolenic acid: vegetables (22.3%), spreads and cooking oils (13.0%) and savoury foods, soups and sauces (10.6%); 8) for EPA: fish and fish dishes (69.0%), meat and meat products (23.3%) and savoury foods, soups and sauces (5.7%); and 9) for DHA: fish and fish dishes (67.8%), meat and meat products (23.6%) and eggs (3.3%) (Table 74).

One thousand fifty four participants reported consumption of supplement products and 740 of them reported consumption of supplements that contributed to the fatty acid daily intake (for total FAs; the subgroups of: PUFAs, ω6PUFAs and ω3PUFAs; and the individual fatty acids of: linoleic, γ-linolenic, α-linolenic, EPA and DHA). In particular supplements that contributed to the fatty acid daily intakes included: cod or halibut liver oil (35.6% of total number of supplements taken), evening primrose oil (5.8%) and fish oils (2.5%). We identified the exact nutrient composition of these dietary supplements and added the supplement nutrients to the dietary ones.

6.4.2 Associations between fatty acid variables and colorectal cancer risk

6.4.2.1 Main conditional logistic regression models

In model I, dietary intakes of total FAs, SFAs, MUFAs, PUFAs, ω6PUFAs, *t*FAs and *t*MUFAs as well as of the individual fatty acids palmitic, stearic, oleic, linoleic, γ-

linolenic, arachidonic, EPA and DHA acid showed a dose-dependent association with colorectal cancer risk (p-value for trend fatty acid subgroups: $5.6x10^{-6}$, $4.8x10^{-6}$, $2.0x10^{-5}$, 0.01, 0.002, $3.1x10^{-6}$, $3.0x10^{-7}$; p-value for trend individual fatty acids: $1.5x10^{-7}$, $5.9x10^{-7}$, $3.1x10^{-6}$, 0.005, 0.03, 0.001, 0.05, 0.04; respectively) (Table 75). Associations between total intakes (from diet and supplements) of fatty acids and colorectal cancer were not examined in model I, since intake from supplements was added to the energy-adjusted nutrients.

In model II, a dose-dependent increase in risk was observed for dietary intake of total FAs, SFAs, MUFAs, *t*FAs and *t*MUFAs and for the individual fatty acids palmitic, stearic and oleic (high vs. low intake: OR (95% CI): 1.30 (1.06, 1.59), 1.42 (1.15, 1.75), 1.29 (1.05, 1.58), 1.38 (1.12, 1.70), 1.47 (1.19, 1.80), 1.43 (1.16, 1.75), 1.64 (1.32, 2.02), 1.38 (1.12, 1.69); respectively) (Table 75). In addition, intake of dietary ω3PUFAs, EPA and DHA was inversely and dose dependently associated with colorectal cancer (p-value for trend: $9.3x10^{-6}$, 0.0001, 0.0002, respectively) with approximately a 35% reduction in risk for those of high versus low intake (OR (95% CI): 0.65 (0.53, 0.79), 0.66 (0.54, 0.81), 0.68 (0.56, 0.84); respectively) (Table 75). Regarding total intakes, total FAs were associated with an increased and dose dependent colorectal cancer risk, whereas total intakes of ω3PUFAs, EPA and DHA were associated with a decreased and dose dependent colorectal cancer risk (p-value for trend: 0.001, $1.1x10^{-5}$, $3.4x10^{-6}$, $1.2x10^{-5}$; respectively) (Table 75).

In model III, only the association between colorectal cancer and stearic acid and the inverse associations between colorectal cancer and ω3PUFAs, EPA and DHA remained statistically significant (high vs. low intake: OR (95% CI), p-value: 1.46 (1.11, 1.91), 0.01; 0.75 (0.59, 0.97), 0.01; 0.74 (0.58, 0.95), 0.02; 0.74 (0.58, 0.95), 0.02; respectively) (Table 75). In addition, ω3PUFA, EPA and DHA total intakes were inversely and dose-dependently associated with colorectal cancer risk (p-value for trend: 0.008, 0.003, 0.003; respectively) (Table 75).

6.4.2.2 Additional conditional logistic regression models

The associations between fatty acid variables and colorectal cancer were tested in two additional models (Model IV and V): Model IV was corrected for the confounding factors of model III and in addition to the residual energy adjustment dietary energy intake was included as a covariate (suggested by Willet to reduce the

random error). In model V associations were corrected for the confounding factors of model III and further adjusted for intake of total FAs (Table 76). For the subgroups of PUFAs, ω6PUFAs and ω3PUFAs and the individual fatty acids linoleic, γ-linolenic, α-linolenic, EPA and DHA additional analyses were conducted for their total intake (intake from diet and supplements).

For both models IV and V positive statistically significant associations were observed for stearic acid (Model IV: high vs. low intake OR (95% CI), p-value: 1.38 (1.05, 1.83), 0.03; Model V: high vs. low intake OR (95% CI), p-value: 1.76 (1.18, 2.63), 0.01) and inverse significant associations were observed for ω3PUFAs, EPA and DHA (Model IV: high vs. low intake OR (95% CI), p-value: 0.75 (0.58, 0.96), 0.008; 0.74 (0.58, 0.95), 0.02; 0.73 (0.57, 0.94), 0.02; Model IV: high vs. low intake OR (95% CI), p-value: 0.69 (0.53, 0.90), 0.002; 0.72 (0.56, 0.93), 0.01; 0.71 (0.55, 0.92), 0.01; respectively) (Table 76). In addition, total intake of ω3PUFAs, EPA and DHA were inversely and dose-dependently associated with colorectal cancer risk after applying both models IV and V (p-value for trend: Model IV: 0.006, 0.003, 0.001; Model V: 0.002, 0.002, 0.001; respectively) (Table 76).

6.4.2.3 Multiple testing corrections

Bonferroni correction for multiple testing

In model I the associations between colorectal cancer and total FAs ($p=5.6x10^{-6}$), the subgroups SFAs ($p=4.8x10^{-6}$), MUFAs ($p=2.0x10^{-5}$), *t*FAs ($p=3.1x10^{-6}$), *t*MUFAs ($p=3.0x10^{-7}$), and the individual fatty acids palmitic ($p=1.5x10^{-7}$), stearic ($p=5.9x10^{-7}$) and oleic ($p=3.1x10^{-6}$) remained significant under every level of correction, whereas association with ω6PUFAs (p=0.002) and arachidonic acid (p=0.001) remained significant at the first and second level of significance, respectively (Table 75). In model II, the associations with the subgroups ω3PUFAs ($p=9.3x10^{-6}$), *t*MUFAs (p=0.0003) and the individual compounds stearic ($p=7.9x10^{-6}$), EPA (p=0.0001) and DHA (p=0.0002) remained significant under every level of correction, the associations with total FAs (p=0.001), the subgroup SFAs (p=0.001) and the individual compounds palmitic (p=0.001) and oleic (p=0.001) remained significant in the first two levels, and finally the association between colorectal cancer and *t*FAs remained significant only at the first level of correction (Table 75).

Finally, in model V, the association between ω3PUFAs (p=0.002) and colorectal cancer remained significant in the first level of correction (Table 76).

FDR correction for multiple testing

After correcting for multiple testing using the FDR method all the associations between the dietary intakes and colorectal cancer that their observed p-values were ≤0.01 remained significant (Table 75, Table 76).

6.4.2.4 Associations between colorectal cancer and main food sources of total fatty acids, SFAs, MUFAs, ω3PUFAs, *t*FAs, *t*MUFAs, palmitic acid, stearic acid, oleic acid, EPA and DHA

Intakes of the following food groups were tested: meat and meat products, confectionery and savoury snacks (including chocolates, sweets, nuts and crisps) and fish and fish dishes. Results from model III, showed that comparison of highest versus lowest quartile intakes of these foods showed ORs for colorectal cancer risk of 0.93 (95% CI 0.72, 1.21; p-value for trend 0.33) for meat and meat products; 1.47 (95% CI 0.72, 1.17; p-value for trend 0.002) for confectionery and savoury snacks; and 0.77 (95% CI 0.60, 0.99; p-value for trend 0.07) for fish and fish dishes (Table 77).

6.4.2.5 Associations between the fatty acid variables and colorectal cancer after sex, age and cancer site stratification

Associations between each fatty acid variable and colorectal cancer risk were tested after sex, age and cancer site stratification by applying model III (data not shown). Briefly, both dietary and total intakes of ω3PUFAs, EPA and DHA were inversely associated with colorectal cancer for both men and women; however associations were stronger and statistically significant for men (associations for dietary intakes for men: OR (95% CI), p-value for trend: 0.68 (0.49, 0.95), 0.02; 0.70 (0.50, 0.96), 0.02; 0.69 (0.50, 0.95), 0.02; respectively) (data not shown). In addition, dietary and total intake of total FAs, the fatty acid subgroups MUFAs, *t*FAs and *t*MUFAs and dietary intake of the individual compound oleic acid, were positively and significantly associated with colorectal cancer for women but not for men (women (dietary intakes): OR (95% CI), p-value for trend: 1.40 (0.92, 2.13), 0.03; 1.67 (1.10, 2.53), 0.008; 1.38 (0.91, 2.10), 0.05; 2.00 (1.31, 3.06), 0.002; 1.67 (1.10, 2.53), 0.007,

respectively) (data not shown). After age stratification, dietary and total intakes of ω3PUFAs, EPA and DHA were inversely associated with colorectal cancer mainly for the older study participants; with the associations for the individuals younger than 55 years old not being statistically significant (associations for dietary intakes for individuals ≥55 years old: OR (95% CI), p-value for trend: 0.75 (0.56, 0.99), 0.01; 0.70 (0.53, 0.92), 0.02; 0.71 (0.54, 0.93), 0.02; respectively) (data not shown). In addition, dietary intakes of SFAs, MUFAs, *t*FAs, *t*MUFAs and palmitic acid were positively though not significantly associated with colorectal cancer only for the individuals younger than 55 years old (data not shown). Finally, both dietary and total intakes of ω3PUFAs, EPA and DHA were inversely associated with both colon and rectal cancer (with associations with colon cancer being statistically significant) (data not shown). Dietary intakes of MUFAs, *t*FAs, *t*MUFAs and oleic acid were inversely associated only with rectal cancer, though only the positive association with *t*MUFAs was statistically significant (high vs. low intake of *t*MUFAs for rectal cancer: OR (95% CI), p-value for trend: 1.55 (1.06, 2.28), 0.02) (data not shown).

6.4.3 Summary of results

Moderately strong associations which showed dose response relationships were found: 1) in model I: between colorectal cancer and dietary intakes of total FAs ($p=5.6x10^{-6}$), SFAs ($p=4.8x10^{-6}$), MUFAs ($p=2.0x10^{-5}$), PUFAs (p=0.01), ω6PUFAs (p=0.002), *t*FAs ($p=3.1x10^{-6}$) and *t*MUFAs ($p=3.0x10^{-7}$), palmitic acid ($p=1.5x10^{-7}$), stearic acid ($p=5.9x10^{-7}$), oleic acid ($p=3.1x10^{-6}$), linoleic acid (p=0.005), γ-linolenic acid (p=0.03) and arachidonic acid (p=0.001) (high intakes increased risk) (Table 75) and between colorectal cancer and dietary intakes of EPA (p=0.05) and DHA (p=0.04) (high intakes decreased risk) (Table 75); 2) in model II: between colorectal cancer and the dietary intakes of ω3PUFAs ($p=9.3x10^{-6}$), EPA (p=0.0001), DHA (p=0.0002) (high intakes decreased risk) (Table 75) and between colorectal cancer and dietary intakes of total FAs (p=0.001), SFAs (p=0.001), MUFAs (p=0.01) *t*FAs (p=0.002), *t*MUFAs (p=0.0003), palmitic acid (p=0.001), stearic acid ($p=7.9x10^{-6}$) and oleic acid (p=0.001) (high intakes increased risk) (Table 75); 3) in model III, between colorectal cancer and dietary intakes of ω3PUFAs (p=0.01), EPA (p=0.02) and DHA (p=0.02) (high intakes decreased risk) and between colorectal cancer and stearic acid (p=0.01) (high intakes increased risk) (Table 75); 4) in model IV and V:

between colorectal cancer and stearic acid (p= 0.03 and 0.01, respectively) (high intakes increased risk) and between colorectal cancer and ω3PUFAs (p= 0.008 and 0.002, respectively), EPA (p= 0.02 and 0.01, respectively) and DHA (p= 0.02 and 0.01, respectively) (Table 76).

Table 71 Fatty acid variables (total FAs, subgroups and individual compounds) that were elected to be included in the analysis

Flavonoid variables included in the analysis	Transformation
Total FAs	
Total FAs	logarithmic
Subgroups	
SFAs	logarithmic
MUFAs	logarithmic
PUFAs	logarithmic
ω6PUFAs	logarithmic
ω3PUFAs	logarithmic
*t*FAs	logarithmic
*t*MUFAs	logarithmic
Individual compounds	
Palmitic acid	logarithmic
Stearic acid	logarithmic
Oleic acid	logarithmic
Linoleic acid	logarithmic
γ-Linolenic acid	square root
Arachidonic acid	square root
α-Linolenic acid	logarithmic
EPA	logarithmic
DHA	logarithmic

Table 72 Descriptive report of crude and energy-adjusted fatty acid intakes

Fatty acids	All subjects (n=2950)		Cases (n=1475)		Controls (n=1475)		T-test	Wilcoxon rank test
	Mean (SD)	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*p-value*	*p-value*
Total FAs								
Total FAs (g/day)	91.4 (41.9)	83.2 (64.0, 109.6)	94.6 (42.9)	86.7 (66.4, 113.4)	88.2 (40.5)	80.0 (62.5, 105.3)	$<5x10^{-5}$	$<5x10^{-5}$
Total FAs-energy adjusted (g/day)	85.1 (15.3)	86.2 (75.9, 94.9)	86.0 (14.7)	87.5 (77.1, 95.3)	84.3 (15.9)	85.2 (75.0, 94.2)	0.0018	0.0005
Subgroups								
SFAs (g/day)	40.4 (19.7)	36.5 (27.2, 49.1)	42.0 (20.2)	38.1 (28.0, 51.3)	38.7 (18.9)	34.7 (26.5, 46.8)	$<5x10^{-5}$	$<5x10^{-5}$
SFAs-energy adjusted (g/day)	37.6 (9.0)	37.2 (31.7, 43.6)	38.2 (8.7)	37.8 (32.4, 44.2)	36.9 (9.3)	36.7 (31.2, 42.7)	0.0001	0.0001
MUFAs (g/day)	34.7 (16.1)	31.5 (24.2, 41.4)	35.8 (16.3)	32.7 (25.1, 43.1)	33.6 (15.9)	30.6 (23.2, 40.5)	$<5x10^{-5}$	$<5x10^{-5}$
MUFAs-energy adjusted (g/day)	32.3 (6.0)	32.5 (28.6, 36.0)	32.5 (5.7)	32.8 (29.1, 36.2)	32.0 (6.3)	32.3 (28.1, 35.8)	0.03	0.01
PUFAs (g/day)	15.5 (7.6)	14.1 (10.5, 18.7)	15.9 (7.9)	14.4 (10.6, 19.4)	15.1 (7.3)	13.8 (10.4, 18.1)	0.0041	0.0086
PUFAs-energy adjusted (g/day)	14.5 (3.8)	14.0 (11.9, 16.6)	14.5 (3.8)	13.9 (11.8, 16.7)	14.5 (3.8)	14.1 (12.0, 16.5)	0.96	0.57
ω6PUFAs (g/day)	12.0 (6.2)	10.8 (10.5, 11.0)	12.4 (6.6)	11.1 (7.9, 15.2)	11.6 (6.6)	10.5 (7.8, 14.0)	0.001	0.004

ω6PUFAs-	11.2	10.6	11.3	10.6	11.2	10.6	0.42	0.89
energy adjusted (g/day)	(3.5)	(8.9, 13.0)	(3.6)	(8.9, 13.0)	3.4	(8.8, 13.0)		
ω3PUFAs (g/day)	2.5	2.2	2.5	2.2	2.6	2.2	0.86	0.98
	(1.4)	(1.6, 3.0)	(1.3)	(1.6, 3.0)	(1.6)	(1.6, 3.0)		
ω3PUFAs-	2.4	2.2	2.3	2.2	2.5	2.3	$<5x10^{-5}$	$<5x10^{-5}$
energy adjusted (g/day)	(0.86)	(1.8, 2.7)	(0.80)	(1.8, 2.7)	(0.91)	(1.9, 2.8)		
*t*FAs (g/day)	3.9	3.5	4.0	3.6	3.7	3.3	$<5x10^{-5}$	$<5x10^{-5}$
	(2.1)	(2.5, 4.7)	(2.1)	(2.7, 5.0)	(2.0)	(2.4, 4.5)		
*t*FAs-	3.6	3.5	3.7	3.6	3.5	3.5	0.0026	0.001
energy adjusted (g/day)	(1.1)	(2.9, 4.2)	(1.1)	(3.0, 4.3)	(1.2)	(2.8, 4.2)		
*t*MUFAs (g/day)	2.9	2.7	3.0	2.8	2.8	2.6	$<5x10^{-5}$	$<5x10^{-5}$
	(1.5)	(1.9, 3.6)	(1.5)	(2.0, 3.8)	(1.4)	(1.8, 3.4)		
*t*MUFAs-	2.7	2.7	2.8	2.8	2.7	2.7	0.0003	$<5x10^{-5}$
energy adjusted (g/day)	(0.8)	(2.2, 3.2)	(0.8)	(2.3, 3.3)	(0.8)	(2.1, 3.2)		
Individual FAs								
Palmitic acid (g/day)	20.1	18.2	20.9	19.0	19.3	17.4	$<5x10^{-5}$	$<5x10^{-5}$
	(9.6)	(13.7, 24.1)	(9.8)	(14.2, 25.2)	(9.2)	(13.3, 23.2)		
Palmitic acid-	18.7	18.7	19.0	18.9	18.4	18.4	0.0003	0.0002
energy adjusted (g/day)	(4.1)	(16.1, 21.4)	(3.9)	(16.5, 21.5)	(4.2)	(15.7, 21.1)		
Stearic acid (g/day)	9.3	8.5	9.8	8.9	8.9	8.1	$<5x10^{-5}$	$<5x10^{-5}$
	(4.6)	(6.3, 11.4)	(4.8)	(6.5, 11.9)	(4.3)	(6.1, 10.8)		
Stearic acid-	8.7	8.7	8.8	8.9	8.5	8.6	0.0001	0.0001
energy adjusted (g/day)	(2.0)	(7.4, 10.0)	(2.0)	(7.6, 10.2)	(2.1)	(7.2, 8.9)		
Oleic acid (g/day)	26.9	24.5	27.8	25.5	26.0	23.6	$<5x10^{-5}$	$<5x10^{-5}$
	(12.7)	(18.6, 32.0)	(12.9)	(19.4, 33.2)	(12.3)	(17.9, 31.3)		
Oleic acid-	25.0	25.2	25.3	25.5	24.8	24.9	0.006	0.002

energy adjusted (g/day)	(4.9)	(22.1, 28.1)	(4.6)	(22.4, 28.2)	(5.1)	(21.7, 27.9)		
Linoleic acid (g/day)	11.5	10.3	11.9	10.6	11.2	10.1	0.0011	0.0045
	(6.0)	(7.5, 14.1)	(6.4)	(7.5, 14.6)	(5.6)	(7.4, 13.5)		
Linoleic acid-	10.8	10.1	10.8	10.1	10.7	10.2	0.43	0.88
energy adjusted (g/day)	(3.5)	(8.4, 12.6)	(3.5)	(8.4, 12.6)	(3.4)	(8.4, 12.5)		
γ-Linolenic acid (mg/day)	9.3	8.0	9.6	8.0	9.0	8.0	0.0069	0.01
	(6.4)	(5.0, 12.0)	(6.5)	(5.0, 12.0)	(6.3)	(5.0, 12.0)		
γ-Linolenic acid-	8.9	8.4	8.9	8.3	8.9	8.5	0.75	0.92
energy adjusted (mg/day)	(4.4)	(5.9, 11.3)	(4.4)	(5.9, 11.3)	(4.4)	(5.8, 11.3)		
Arachidonic acid	314.3	283.0	324.3	289.0	304.4	275.5	0.0008	0.0025
(mg/day)	(167.9)	(206.7, 383.2)	(174.9)	(212.0, 394.0)	(160.0)	(200.0, 373.0)		
Arachidonic acid-	304.1	295.3	305.8	297.9	302.3	292.5	0.36	0.30
energy adjusted (mg/day)	(102.2)	(238.7, 358.6)	(101.9)	(243.8, 356.9)	(102.6)	(234.5, 360.3)		
α-Linolenic acid (mg/day)	1450.6	1298.5	1472.5	1322.0	1428.7	1265.0	0.02	0.03
	(700.1)	(990.0, 1749.0)	(699.2)	(1000.0, 1758.0)	(700.6)	(973.0, 1742.0)		
α-Linolenic acid-	1358.0	1315.8	1347.9	1314.1	1368.2	1316.3	0.11	0.35
energy adjusted (mg/day)	(346.8)	(1123.3, 1537.8)	(331.1)	(1130.5, 1522.9)	(361.7)	(1117.3, 1560.3)		
EPA (mg/day)	337.7	251.0	320.1	243.0	355.2	257.0	0.07	0.05
	(327.8)	(146.0, 418.2)	(261.4)	(144.0, 408.0)	(382.0)	(151.0, 430.0)		
EPA-	316.4	248.4	297.4	236.1	335.4	261.7	$<5x10^{-5}$	$<5x10^{-5}$
energy adjusted (mg/day)	(243.9)	(155.1, 407.8)	(223.5)	(144.8, 387.3)	(261.4)	(165.0, 429.3)		
DHA (mg/day)	463.8	350.5	440.1	338.0	487.4	360	0.06	0.07
	(439.4)	(212.0, 565.2)	(347.7)	(206.0, 555.0)	(514.1)	(216.0, 580.0)		
DHA-	435.4	346.5	410.3	326.2	460.5	360.4	$<5x10^{-5}$	$<5x10^{-5}$
energy adjusted (mg/day)	(327.0)	(220.0, 551.5)	(299.4)	(210.2, 519.1)	(350.7)	(233.6, 577.3)		

Table 73 Spearman rank correlation coefficients between fatty acid variables (all p-values<$5x10^{-5}$)

Fatty acids	FAs	SFAs	MUFAs	PUFAs	ω6PUFAs	ω3PUFAs	*t*FAs	*t*MUFAs	PA	SA	OA	LA	γLA	AA	αLA	EPA	DHA
FAs	1.00																
SFAs	0.96	1.00															
MUFAs	0.98	0.91	1.00														
PUFAs	0.84	0.67	0.85	1.00													
ω6PUFAs	0.79	0.62	0.80	0.98	1.00												
ω3PUFAs	0.71	0.57	0.76	0.76	0.67	1.00											
*t*FAs	0.89	0.89	0.85	0.68	0.62	0.52	1.00										
*t*MUFAs	0.87	0.87	0.85	0.65	0.62	0.55	0.93	1.00									
Palmitic acid (PA)	0.97	0.99	0.93	0.71	0.66	0.61	0.88	0.86	1.00								
Stearic acid (SA)	0.96	0.98	0.91	0.72	0.67	0.56	0.90	0.86	0.97	1.00							
Oleic acid (OA)	0.97	0.89	0.99	0.86	0.82	0.71	0.84	0.83	0.93	0.91	1.00						
Linoleic acid (LA)	0.78	0.61	0.79	0.98	1.00	0.66	0.61	0.61	0.65	0.65	0.81	1.00					
γ-Linolenic acid (γLA)	0.64	0.58	0.67	0.57	0.53	0.58	0.50	0.51	0.62	0.63	0.67	0.51	1.00				
Arachidonic acid (AA)	0.70	0.64	0.72	0.62	0.58	0.63	0.58	0.60	0.68	0.69	0.71	0.55	0.81	1.00			
α-Linolenic acid (αLA)	0.79	0.66	0.82	0.85	0.81	0.82	0.66	0.68	0.70	0.66	0.82	0.80	0.56	0.56	1.00		
EPA	0.40	0.32	0.45	0.43	0.33	0.82	0.24	0.28	0.33	0.30	0.37	0.32	0.40	0.50	0.40	1.00	
DHA	0.42	0.32	0.46	0.45	0.35	0.83	0.24	0.27	0.35	0.30	0.39	0.34	0.42	0.50	0.42	0.99	1.00

Table 74 Three main dietary (food) sources of fatty acids in our population

Fatty acids subgroups	Main sources (% of total intake)
Total fatty acids	Meat & meat products (18.0%)
	Spreads* & cooking oils (13.4%)
	Confectionery & savoury snacks (8.3%)
Saturated fatty acids	Meat & meat products (17.5%)
	Spreads* & cooking oils (13.1%)
	Cheese (10.3%)
Mono-unsaturated fatty	Meat & meat products (19.7%)
acids	Spreads* & cooking oils (13.7%)
	Fish & fish dishes (8.5%)
Poly-unsaturated fatty acids	Meat & meat products (15.3%)
	Spreads* & cooking oils (13.5%)
	Confectionery & savoury snacks (11.4%)
ω6 poly-unsaturated fatty	Spreads* & cooking oils (15.2%)
acids	Meat & meat products (14.8%)
	Confectionery & savoury snacks (10.2%)
ω3 poly-unsaturated fatty	Fish & fish dishes (30.3%)
acids	Meat & meat products (16.2%)
	Vegetables (13.1%)
trans fatty acids	Spreads* & cooking oils (20.7%)
	Confectionery & savoury snacks (15.7%)
	Meat & meat products (15.4%)
trans mono-unsaturated	Spreads* & cooking oils (25.2%)
fatty acids	Meat & meat products (14.6%)
	Cheese (11.9%)
Palmitic acid	Meat & meat products (19.8%)
	Spreads‡ & cooking oils (12.8%)
	Cheese (8.5%)
Stearic acid	Meat & meat products (24.8%)
	Spreads* & cooking oils (11.9%)
	Biscuits (8.7%)
Oleic acid	Meat & meat products (20.6%)
	Spreads* & cooking oils (14.0%)
	Confectionery & savoury snacks (9.0%)
Linoleic acid	Meat & meat products (15.9%)
	Spreads* & cooking oils (12.8%)
	Confectionery & savoury snacks (10.6%)

γ-Linolenic acid	Meat & meat products (69.8%)
	Potatoes, rice and Pasta (8.6%)
	Fish & fish dishes (8.5%)
Arachidonic acid	Meat & meat products (62.4%)
	Eggs (11.1%)
	Savoury foods, soups and sauces (10.2%)
α-Linolenic acid	Vegetables (22.3%)
	Spreads* & cooking oils (13.0%)
	Savoury foods, soups and sauces (10.6%)
EPA	Fish & fish dishes (69.0%)
	Meat & meat products (23.3%)
	Savoury foods, soups and sauces (5.7%)
DHA	Fish & fish dishes (67.8%)
	Meat & meat products (23.6%)
	Eggs (3.3%)

* Including butter, margarine, jam, honey, marmalade, yeast or meat extract, peanut butter, and chocolate spread

Table 75 Association between the fatty acid variables and colorectal cancer risk in the whole sample (3 main conditional logistic regression models; Cases and controls matched on age, gender and area of residence)

Fatty acids	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Total FAs	0-75.93	339	399	1.00		1.00		1.00	
(g/day)	75.93-86.21	345	392	1.09	0.89, 1.35	1.03	0.84, 1.26	0.99	0.78, 1.27
	86.21-94.91	404	334	1.37	1.11, 1.68	1.43	1.17, 1.76	1.25	0.98, 1.61
	>94.91	387	350	1.55	1.25, 1.92	1.30	1.06, 1.59	1.05	0.81, 1.36
	p-value for trend (quartiles)				$5.6x10^{-6}$		0.001		0.36
	p-value for trend (continuous)				$3.9x10^{-5}$		0.002		0.27
Total FAs	0-75.98	338	400			1.00		1.00	
(total) (g/day)	75.98-86.34	346	391			1.04	0.85, 1.28	1.01	0.79, 1.29
	86.34-94.95	404	334			1.44	1.17, 1.77	1.27	0.99, 1.34
	>95.94	387	350			1.31	1.07, 1.60	1.06	0.82, 1.37
	p-value for trend (quartiles)						0.001		0.33
	p-value for trend (continuous)						0.002		0.28
SFAs	0-31.72	336	402	1.00		1.00		1.00	
(g/day)	31.72-37.18	365	372	1.03	0.83, 1.28	1.18	0.96, 1.45	1.13	0.88, 1.45
	37.18-43.55	375	363	1.22	0.99, 1.50	1.24	1.01, 1.52	1.01	0.79, 1.30
	>43.55	399	338	1.58	1.28, 1.95	1.42	1.15, 1.75	1.19	0.91, 1.55
	p-value for trend (quartiles)				$4.8x10^{-6}$		0.001		0.34
	p-value for trend (continuous)				$4.1x10^{-6}$		0.0001		0.13
MUFAs	0-28.62	333	405	1.00		1.00		1.00	
(g/day)	28.62-32.54	377	360	1.10	0.89, 1.35	1.27	1.04, 1.56	1.27	0.99, 1.63
	32.54-36.04	385	353	1.38	1.12, 1.69	1.33	1.08, 1.64	1.23	0.96, 1.59
	>36.04	380	357	1.50	1.22, 1.86	1.29	1.05, 1.58	1.13	0.88, 1.46

	p-value for trend (quartiles)			$2.0x10^{-5}$		0.01		0.45
	p-value for trend (continuous)			0.0003		0.03		0.42
PUFAs	0-11.94	380	358	1.00		1.00		1.00	
(g/day)	11.94-14.03	370	367	1.01	0.82, 1.24	0.95	0.77, 1.17	1.11	0.87, 1.42
	14.03-16.58	348	390	0.96	0.78, 1.18	0.84	0.68, 1.03	0.94	0.74, 1.20
	>16.58	377	360	1.35	1.09, 1.66	0.98	0.80, 1.20	0.98	0.76, 1.25
	p-value for trend (quartiles)			0.01		0.62		0.54
	p-value for trend (continuous)			0.004		0.96		0.66
PUFAs	0-11.99	383	355			1.00		1.00	
(total) (g/day)	11.99-14.08	365	372			0.91	0.74, 1.12	1.04	0.82, 1.33
	14.08-16.67	350	388			0.83	0.68, 1.02	0.91	0.71, 1.17
	>16.67	377	360			0.97	0.79, 1.18	0.97	0.76, 1.24
	p-value for trend (quartiles)						0.60		0.56
	p-value for trend (continuous)						0.82		0.58
ω6PUFAs	0-8.87	365	373	1.00		1.00		1.00	
(g/day)	8.87-10.61	380	357	1.03	0.84, 1.26	1.09	0.89, 1.34	1.14	0.89, 1.45
	10.61-13.04	361	377	1.00	0.81, 1.23	0.98	0.79, 1.20	1.07	0.83, 1.37
	>13.04	369	368	1.42	1.15, 1.75	1.02	0.84, 1.26	1.02	0.80, 1.31
	p-value for trend (quartiles)			0.002			0.92		0.97
	p-value for trend (continuous)			0.001			0.42		0.77
ω6PUFAs	0-8.89	365	373			1.00		1.00	
(total) (g/day)	8.89-10.62	381	356			1.09	0.89, 1.34	1.15	0.90, 1.47
	10.62-13.06	360	378			0.97	0.79, 1.19	1.07	0.83, 1.37
	>13.06	369	368			1.02	0.84, 1.26	1.02	0.80, 1.31
	p-value for trend (quartiles)						0.89		0.94
	p-value for trend (continuous)						0.45		0.80

ω3PUFAs	0-1.82	416	322	1.00		1.00		1.00	
(g/day)	1.82-2.22	377	360	1.12	0.91, 1.38	0.82	0.66, 1.00	0.95	0.74, 1.22
	2.22-2.73	348	390	1.05	0.86, 1.28	0.70	0.57, 0.86	0.80	0.63, 1.02
	>2.73	334	403	1.07	0.87, 1.32	0.65	0.53, 0.79	0.75	0.59, 0.97
	p-value for trend (quartiles)				0.67		$9.3x10^{-6}$		0.01
	p-value for trend (continuous)				0.26		$2.8x10^{-5}$		0.004
ω3PUFAs	0-1.84	409	329			1.00		1.00	
(total) (g/day)	1.84-2.26	381	356			0.86	0.70, 1.06	0.95	0.74, 1.22
	2.26-2.79	360	378			0.77	0.63, 0.95	0.89	0.70, 1.13
	>2.79	325	412			0.64	0.52, 0.78	0.71	0.55, 0.92
	p-value for trend (quartiles)						$1.1x10^{-5}$		0.008
	p-value for trend (continuous)						$5.3x10^{-6}$		0.002
*t*FAs	0-2.87	325	413	1.00		1.00		1.00	
(g/day)	2.87-3.54	379	358	1.08	0.88, 1.32	1.34	1.09, 1.64	1.24	0.97, 1.58
	3.54-4.22	386	352	1.19	0.97, 1.47	1.38	1.13, 1.69	1.31	1.03, 1.66
	>4.22	385	352	1.63	1.32, 2.00	1.38	1.12, 1.70	1.13	0.88, 1.46
	p-value for trend (quartiles)				$3.1x10^{-6}$		0.002		0.28
	p-value for trend (continuous)				$1.1x10^{-5}$		0.003		0.41
*t*MUFAs	0-2.20	318	420	1.00		1.00		1.00	
(g/day)	2.20-2.71	378	359	1.08	0.88, 1.32	1.40	1.14, 1.73	1.39	1.08, 1.78
	2.71-3.23	390	348	1.34	1.09, 1.65	1.47	1.20, 1.80	1.35	1.05, 1.72
	>3.23	389	348	1.65	1.34, 2.02	1.47	1.19, 1.80	1.28	1.00, 1.65
	p-value for trend (quartiles)				$3.0x10^{-7}$		0.0003		0.09
	p-value for trend (continuous)				$3.1x10^{-6}$		0.0004		0.15
Palmitic acid	0-16.13	329	409	1.00		1.00		1.00	
(g/day)	16.13-18.72	369	368	1.16	0.94, 1.44	1.25	1.02, 1.53	1.16	0.91, 1.49

	18.72-21.36	384	354	1.38	1.12, 1.70	1.35	1.10, 1.66	1.22	0.95, 1.57
	>21.36	393	344	1.72	1.39, 2.12	1.43	1.16, 1.75	1.13	0.86, 1.47
	p-value for trend (quartiles)				$1.5x10^{-7}$		0.001		0.36
	p-value for trend (continuous)				$9.5x10^{-6}$		0.0003		0.21
Stearic acid	0-7.37	322	416	1.00		1.00		1.00	
(g/day)	7.37-8.71	368	369	0.95	0.77, 1.18	1.31	1.06, 1.61	1.29	1.00, 1.66
	8.71-10.03	377	361	1.27	1.03, 1.57	1.37	1.12, 1.69	1.32	1.02, 1.70
	>10.03	408	329	1.61	1.30, 1.99	1.64	1.32, 2.02	1.46	1.11, 1.91
	p-value for trend (quartiles)				$5.9x10^{-7}$		$7.9x10^{-6}$		0.01
	p-value for trend (continuous)				$8.4x10^{-7}$		$6.6x10^{-5}$		0.08
Oleic acid	0-22.06	334	404	1.00		1.00		1.00	
(g/day)	22.06-25.22	365	372	1.13	0.91, 1.39	1.19	0.97, 1.46	1.14	0.89, 1.46
	25.22-28.06	385	353	1.50	1.21, 1.85	1.32	1.08, 1.62	1.24	0.97, 1.59
	>28.06	391	346	1.56	1.25, 1.93	1.38	1.12, 1.69	1.23	0.95, 1.59
	p-value for trend (quartiles)				$3.1x10^{-6}$		0.001		0.10
	p-value for trend (continuous)				$6.9x10^{-5}$		0.006		0.15
Linoleic acid	0-8.41	367	371	1.00		1.00		1.00	
(g/day)	8.41-10.14	377	360	1.02	0.83, 1.25	1.06	0.86, 1.29	1.08	0.84, 1.38
	10.14-12.56	360	378	0.98	0.79, 1.21	0.96	0.78, 1.18	1.04	0.81, 1.34
	>12.56	371	366	1.38	1.12, 1.69	1.02	0.83, 1.25	1.00	0.78, 1.28
	p-value for trend (quartiles)				0.005		0.95		0.92
	p-value for trend (continuous)				0.001		0.43		0.77
Linoleic acid	0-8.42	368	370			1.00		1.00	
(total) (g/day)	8.42-10.15	378	359			1.06	0.86, 1.29	1.09	0.85, 1.39
	10.15-12.57	359	380			0.95	0.77, 1.17	1.02	0.80, 1.31
	>12.57	370	366			1.01	0.83, 1.24	0.99	0.78, 1.28

	p-value for trend (quartiles)						0.86		0.82
	p-value for trend (continuous)						0.46		0.80
γ-Linolenic acid	0-5.86	368	370	1.00		1.00		1.00	
(mg/day)	5.86-8.43	387	350	1.00	0.82, 1.21	1.10	0.90, 1.35	1.09	0.86, 1.38
	8.43-11.29	352	386	1.15	0.94, 1.40	0.91	0.74, 1.12	0.82	0.64, 1.05
	>11.29	368	369	1.24	1.00, 1.54	1.00	0.81, 1.23	0.98	0.76, 1.26
	p-value for trend (quartiles)				0.03		0.57		0.37
	p-value for trend (continuous)				0.01		0.74		0.99
γ-Linolenic acid	0-6.06	369	369			1.00		1.00	
(total) (mg/day)	6.06-8.69	390	347			1.11	0.91, 1.36	1.14	0.90, 1.44
	8.69-11.79	342	396			0.86	0.70, 1.06	0.79	0.61, 1.01
	>11.79	374	363			1.03	0.83, 1.26	1.05	0.82, 1.35
	p-value for trend (quartiles)						0.59		0.58
	p-value for trend (continuous)						0.23		0.58
Arachidonic acid	0-238.75	347	391	1.00		1.00		1.00	
(mg/day)	238.75-295.30	374	363	1.19	0.97, 1.46	1.17	0.95, 1.44	1.24	0.97, 1.59
	295.30-358.60	394	344	1.26	1.02, 1.55	1.29	1.05, 1.58	1.17	0.92, 1.50
	>358.60	360	377	1.42	1.15, 1.75	1.07	0.87, 1.31	1.04	0.81, 1.33
	p-value for trend (quartiles)				0.001		0.32		0.86
	p-value for trend (continuous)				0.001		0.36		0.81
α-Linolenic acid	0-1123.4	361	377	1.00		1.00		1.00	
(mg/day)	1123.4-1315.8	381	356	1.03	0.84, 1.26	1.12	0.91, 1.38	1.22	0.95, 1.57
	1315.8-1537.6	393	345	1.24	1.00, 1.52	1.19	0.97, 1.46	1.42	1.10, 1.80
	>1537.6	340	397	1.13	0.91, 1.39	0.90	0.73, 1.10	1.01	0.78, 1.30
	p-value for trend (quartiles)				0.11		0.41		0.75
	p-value for trend (continuous)				0.09		0.10		0.53

α-Linolenic acid	0-1123.6	361	377			1.00		1.00	
(total) (mg/day)	1123.6-1316.1	380	357			1.11	0.91, 1.37	1.21	0.95, 1.56
	1316.1-1538.3	394	344			1.20	0.97, 1.47	1.42	1.10, 1.83
	>1538.3	340	397			0.90	0.73, 1.10	1.01	0.78, 1.31
	p-value for trend (quartiles)						0.43		0.71
	p-value for trend (continuous)						0.09		0.54
EPA	0-155.16	409	329	1.00		1.00		1.00	
(mg/day)	155.16-248.39	371	366	0.95	0.78, 1.17	0.82	0.67, 1.01	0.92	0.72, 1.18
	248.39-407.67	363	375	0.89	0.73, 1.09	0.78	0.64, 0.96	0.87	0.68, 1.11
	>407.67	332	405	0.82	0.67, 1.01	0.66	0.54, 0.81	0.74	0.58, 0.95
	p-value for trend (quartiles)				0.05		0.0001		0.02
	p-value for trend (continuous)				0.003		$2.4x10^{-5}$		0.003
EPA	0-166.66	417	321			1.00		1.00	
(total) (mg/day)	166.66-268.42	368	369			0.77	0.63, 0.95	0.86	0.67, 1.10
	268.42-434.83	369	369			0.77	0.63, 0.95	0.85	0.67, 1.09
	>434.83	321	416			0.60	0.49, 0.73	0.67	0.52, 0.86
	p-value for trend (quartiles)						$3.4x10^{-6}$		0.003
	p-value for trend (continuous)						$3.3x10^{6}$		0.001
DHA	0-220.06	411	327	1.00		1.00		1.00	
(mg/day)	220.06-346.49	370	367	0.95	0.77, 1.16	0.81	0.66, 0.99	0.85	0.67, 1.09
	346.49-551.44	355	383	0.87	0.71, 1.06	0.74	0.60, 0.91	0.84	0.66, 1.07
	>551.44	339	398	0.82	0.67, 1.01	0.68	0.56, 0.84	0.74	0.58, 0.95
	p-value for trend (quartiles)				0.04		0.0002		0.02
	p-value for trend (continuous)				0.003		$3.0x10^{-5}$		0.002
DHA	0-233.35	413	325			1.00		1.00	
(total) (mg/day)	233.35-362.65	375	362			0.83	0.67, 1.02	0.93	0.72, 1.19

	362.65-577.16	358	380	0.74	0.61, 0.91	0.83	0.65, 1.06
	>577.16	329	408	0.64	0.52, 0.78	0.70	0.54, 0.90
	p-value for trend (quartiles)				$1.2x10^{-5}$		0.003
	p-value for trend (continuous)				$5.4x10^{-6}$		0.001

[*]Based on the distribution of the energy adjusted variable

[†]Model I: Crude analysis

[‡]Model II: Adjusted for total energy intake (residual method)

[§]Model III: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

Table 76 Association between the fatty acid variables and colorectal cancer risk in the whole sample (2 additional conditional logistic regression models; Cases and controls matched on age, gender and area of residence)

Fatty acids	Quartiles*	Frequency		Model IV†		Model V‡	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*
Total FAs	0-75.93	339	399	1.00		n/a	
(g/day)	75.93-86.21	345	392	0.97	0.76, 1.24		
	86.21-94.91	404	334	1.22	0.95, 1.58		
	>94.91	387	350	1.00	0.77, 1.29		
	p-value for trend (quartiles)				0.61		
	p-value for trend (continuous)				0.39		
Total FAs	0-75.98	338	400	1.00		n/a	
(total) (g/day)	75.98-86.34	346	391	0.99	0.77, 1.27		
	86.34-94.95	404	334	1.24	0.97, 1.60		
	>95.94	387	350	1.00	0.77, 1.30		
	p-value for trend (quartiles)				0.57		
	p-value for trend (continuous)				0.41		
SFAs	0-31.72	336	402	1.00		1.00	
(g/day)	31.72-37.18	365	372	1.12	0.87, 1.44	1.09	0.82, 1.46
	37.18-43.55	375	363	0.98	0.76, 1.27	0.97	0.70, 1.34
	>43.55	399	338	1.13	0.86, 1.47	1.11	0.74, 1.66
	p-value for trend (quartiles)				0.60		0.86
	p-value for trend (continuous)				0.24		0.27
MUFAs	0-28.62	333	405	1.00		1.00	
(g/day)	28.62-32.54	377	360	1.27	0.99, 1.64	1.21	0.90, 1.64
	32.54-36.04	385	353	1.23	0.95, 1.59	1.15	0.80, 1.65
	>36.04	380	357	1.10	0.85, 1.42	1.02	0.65, 1.60
	p-value for trend (quartiles)				0.60		0.78
	p-value for trend (continuous)				0.50		0.58
PUFAs	0-11.94	380	358	1.00		1.00	
(g/day)	11.94-14.03	370	367	1.11	0.86, 1.42	1.06	0.82, 1.37
	14.03-16.58	348	390	0.92	0.72, 1.18	0.88	0.68, 1.14
	>16.58	377	360	0.97	0.76, 1.24	0.89	0.67, 1.17
	p-value for trend (quartiles)				0.48		0.20
	p-value for trend (continuous)				0.70		0.25
PUFAs	0-11.99	383	355	1.00		1.00	
(total) (g/day)	11.99-14.08	365	372	1.04	0.81, 1.33	1.00	0.77, 1.28
	14.08-16.67	350	388	0.90	0.70, 1.15	0.85	0.65, 1.10
	>16.67	377	360	0.96	0.75, 1.23	0.88	0.66, 1.16
	p-value for trend (quartiles)				0.51		0.21
	p-value for trend (continuous)				0.61		0.20
ω6PUFAs	0-8.87	365	373	1.00		1.00	

(g/day)	8.87-10.61	380	357	1.14	0.89, 1.47	1.10	0.86, 1.42
	10.61-13.04	361	377	1.06	0.83, 1.36	1.03	0.79, 1.33
	>13.04	369	368	1.03	0.80, 1.32	0.97	0.74, 1.26
	p-value for trend (quartiles)				0.96		0.63
	p-value for trend (continuous)				0.71		0.85
ω6PUFAs	0-8.89	365	373	1.00		1.00	
(total) (g/day)	8.89-10.62	381	356	1.16	0.90, 1.49	1.11	0.87, 1.43
	10.62-13.06	360	378	1.06	0.82, 1.36	1.02	0.79, 1.33
	>13.06	369	368	1.03	0.80, 1.32	0.96	0.74, 1.26
	p-value for trend (quartiles)				0.91		0.59
	p-value for trend (continuous)				0.74		0.81
ω3PUFAs	0-1.82	416	322	1.00		1.00	
(g/day)	1.82-2.22	377	360	0.97	0.75, 1.25	0.92	0.72, 1.19
	2.22-2.73	348	390	0.80	0.63, 1.03	0.76	0.59, 0.97
	>2.73	334	403	0.75	0.58, 0.96	0.69	0.53, 0.90
	p-value for trend (quartiles)				0.008		0.002
	p-value for trend (continuous)				0.004		0.001
ω3PUFAs	0-1.84	409	329	1.00		1.00	
(total) (g/day)	1.84-2.26	381	356	0.96	0.74, 1.23	0.91	0.71, 1.18
	2.26-2.79	360	378	0.89	0.69, 1.13	0.84	0.65, 1.07
	>2.79	325	412	0.71	0.55, 0.91	0.66	0.50, 0.86
	p-value for trend (quartiles)				0.006		0.002
	p-value for trend (continuous)				0.001		0.0002
*t*FAs	0-2.87	325	413	1.00		1.00	
(g/day)	2.87-3.54	379	358	1.22	0.96, 1.56	1.22	0.94, 1.57
	3.54-4.22	386	352	1.27	0.99, 1.62	1.27	0.96, 1.66
	>4.22	385	352	1.10	0.85, 1.43	1.08	0.79, 1.48
	p-value for trend (quartiles)				0.41		0.61
	p-value for trend (continuous)				0.54		0.90
*t*MUFAs	0-2.20	318	420	1.00		1.00	
(g/day)	2.20-2.71	378	359	1.38	1.07, 1.77	1.38	1.07, 1.79
	2.71-3.23	390	348	1.33	1.04, 1.71	1.34	1.02, 1.77
	>3.23	389	348	1.23	0.96, 1.59	1.28	0.94, 1.74
	p-value for trend (quartiles)				0.18		0.21
	p-value for trend (continuous)				0.23		0.34
Palmitic acid	0-16.13	329	409	1.00		1.00	
(g/day)	16.13-18.72	369	368	1.14	0.88, 1.46	1.12	0.84, 1.49
	18.72-21.36	384	354	1.18	0.91, 1.52	1.15	0.82, 1.61
	>21.36	393	344	1.08	0.82, 1.41	1.04	0.68, 1.59
	p-value for trend (quartiles)				0.58		0.93
	p-value for trend (continuous)				0.33		0.55
Stearic acid	0-7.37	322	416	1.00		1.00	
(g/day)	7.37-8.71	368	369	1.24	0.96, 1.60	1.41	1.06, 1.87

	8.71-10.03	377	361	1.29	0.99, 1.67	1.50	1.08, 2.09
	>10.03	408	329	1.38	1.05, 1.83	1.76	1.18, 2.63
	p-value for trend (quartiles)				0.03		0.01
	p-value for trend (continuous)				0.13		0.12
Oleic acid	0-22.06	334	404	1.00		1.00	
(g/day)	22.06-25.22	365	372	1.15	0.90, 1.48	1.17	0.88, 1.56
	25.22-28.06	385	353	1.24	0.96, 1.59	1.29	0.93, 1.79
	>28.06	391	346	1.22	0.94, 1.58	1.30	0.87, 1.94
	p-value for trend (quartiles)				0.12		0.21
	p-value for trend (continuous)				0.16		0.33
Linoleic acid	0-8.41	367	371	1.00		1.00	
(g/day)	8.41-10.14	377	360	1.09	0.85, 1.39	1.05	0.82, 1.34
	10.14-12.56	360	378	1.03	0.80, 1.33	1.00	0.77, 1.30
	>12.56	371	366	1.01	0.79, 1.29	0.95	0.72, 1.23
	p-value for trend (quartiles)				0.91		0.58
	p-value for trend (continuous)				0.70		0.86
Linoleic acid	0-8.42	368	370	1.00		1.00	
(total) (g/day)	8.42-10.15	378	359	1.10	0.86, 1.40	1.06	0.82, 1.36
	10.15-12.57	359	380	1.02	0.79, 1.30	0.98	0.76, 1.27
	>12.57	370	366	1.00	0.78, 1.28	0.94	0.72, 1.22
	p-value for trend (quartiles)				0.80		0.49
	p-value for trend (continuous)				0.74		0.83
γ-Linolenic acid	0-5.86	368	370	1.00		1.00	
(mg/day)	5.86-8.43	387	350	1.12	0.88, 1.42	1.08	0.85, 1.36
	8.43-11.29	352	386	0.85	0.66, 1.09	0.80	0.62, 1.02
	>11.29	368	369	0.99	0.77, 1.28	0.94	0.72, 1.22
	p-value for trend (quartiles)				0.44		0.23
	p-value for trend (continuous)				0.86		0.78
γ-Linolenic acid	0-6.06	369	369	1.00		1.00	
(total) (mg/day)	6.06-8.69	390	347	1.16	0.91, 1.47	1.12	0.88, 1.42
	8.69-11.79	342	396	0.81	0.63, 1.04	0.78	0.61, 1.00
	>11.79	374	363	1.06	0.82, 1.36	1.02	0.79, 1.32
	p-value for trend (quartiles)				0.61		0.42
	p-value for trend (continuous)				0.52		0.57
Arachidonic acid	0-238.75	347	391	1.00		1.00	
(mg/day)	238.75-295.30	374	363	1.29	1.01, 1.66	1.24	0.97, 1.58
	295.30-358.60	394	344	1.20	0.94, 1.54	1.15	0.90, 1.47
	>358.60	360	377	1.04	0.81, 1.34	1.02	0.79, 1.31
	p-value for trend (quartiles)				0.88		0.99
	p-value for trend (continuous)				0.81		0.97
α-Linolenic acid	0-1123.4	361	377	1.00		1.00	
(mg/day)	1123.4-1315.8	381	356	1.24	0.97, 1.60	1.19	0.92, 1.54
	1315.8-1537.6	393	345	1.40	1.08, 1.80	1.37	1.05, 1.79

	>1537.6	340	397	1.00	0.77, 1.30	0.96	0.73, 1.27
	p-value for trend (quartiles)				0.82		0.90
	p-value for trend (continuous)				0.51		0.22
α-Linolenic acid	0-1123.6	361	377	1.00		1.00	
(total) (mg/day)	1123.6-1316.1	380	357	1.24	0.96, 1.59	1.19	0.92, 1.53
	1316.1-1538.3	394	344	1.40	1.09, 1.81	1.37	1.05, 1.79
	>1538.3	340	397	1.01	0.78, 1.31	0.97	0.73, 1.28
	p-value for trend (quartiles)				0.78		0.94
	p-value for trend (continuous)				0.52		0.22
EPA	0-155.16	409	329	1.00		1.00	
(mg/day)	155.16-248.39	371	366	0.93	0.72, 1.18	0.92	0.72, 1.17
	248.39-407.67	363	375	0.88	0.69, 1.13	0.86	0.68, 1.10
	>407.67	332	405	0.74	0.58, 0.95	0.72	0.56, 0.93
	p-value for trend (quartiles)				0.02		0.01
	p-value for trend (continuous)				0.003		0.001
EPA	0-166.66	417	321	1.00		1.00	
(total) (mg/day)	166.66-268.42	368	369	0.86	0.67, 1.11	0.86	0.67, 1.10
	268.42-434.83	369	369	0.86	0.67, 1.10	0.84	0.66, 1.07
	>434.83	321	416	0.67	0.52, 0.86	0.66	0.51, 0.84
	p-value for trend (quartiles)				0.003		0.001
	p-value for trend (continuous)				0.001		0.0004
DHA	0-220.06	411	327	1.00		1.00	
(mg/day)	220.06-346.49	370	367	0.85	0.67, 1.09	0.85	0.66, 1.08
	346.49-551.44	355	383	0.84	0.66, 1.08	0.83	0.65, 1.06
	>551.44	339	398	0.73	0.57, 0.94	0.71	0.55, 0.92
	p-value for trend (quartiles)				0.02		0.01
	p-value for trend (continuous)				0.002		0.001
DHA	0-233.35	413	325	1.00		1.00	
(total) (mg/day)	233.35-362.65	375	362	0.94	0.73, 1.21	0.92	0.72, 1.18
	362.65-577.16	358	380	0.83	0.65, 1.07	0.81	0.64, 1.04
	>577.16	329	408	0.70	0.55, 0.90	0.67	0.52, 0.87
	p-value for trend (quartiles)				0.004		0.002
	p-value for trend (continuous)				0.001		0.0003

[*] Based on the distribution of the energy adjusted variable

[†] Model IV: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake and for total energy intake (included as a covariate)

[‡] Model V: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake and for total fatty acids intake (energy-adjusted)

Table 77 Association between meat and meat products, confectionery and savoury snacks, fish and fish dishes and colorectal cancer risk in the whole sample (3 main conditional logistic regression models; Cases and controls matched on age, gender and area of residence)

Food groups	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Meat and meat products (m/day**)	0-1.71	363	375	1.00		1.00		1.00	
	1.71-2.31	380	357	1.14	0.94, 1.40	1.10	0.90, 1.35	1.03	0.81, 1.32
	2.31-3.05	360	378	1.08	0.88, 1.34	0.98	0.80, 1.20	0.85	0.66, 1.08
	>3.05	372	365	1.35	1.09, 1.68	1.05	0.85, 1.30	0.93	0.72, 1.21
	p-value for trend (quartiles)				0.02		0.89		0.33
	p-value for trend (continuous)				0.005		0.40		0.89
Confectionery & savoury snacks (m/day)	0-0.42	339	399	1.00		1.00		1.00	
	0.42-0.93	342	395	1.08	0.88, 1.32	1.02	0.83, 1.25	1.08	0.85, 1.37
	0.93-1.75	380	358	1.29	1.05, 1.59	1.27	1.04, 1.56	1.26	0.99, 1.60
	>1.75	414	323	1.57	1.28, 1.93	1.55	1.25, 1.91	1.47	1.14, 1.90
	p-value for trend (quartiles)				$7.4x10^{-6}$		$9.4x10^{-6}$		0.002
	p-value for trend (continuous)				$9.0x10^{-5}$		0.002		0.02
Fish and fish dishes (m/day)	0-0.42	391	347	1.00		1.00		1.00	
	0.42-0.73	373	364	1.07	0.87, 1.31	0.91	0.74, 1.11	0.93	0.73, 1.18
	0.73-1.17	378	360	0.96	0.78, 1.19	0.94	0.76, 1.15	0.96	0.75, 1.21
	>1.17	333	404	0.92	0.76, 1.14	0.73	0.60, 0.90	0.77	0.60, 0.99
	p-value for trend (quartiles)				0.30		0.006		0.07
	p-value for trend (continuous)				0.09		0.002		0.05

*Based on the distribution of the energy adjusted variable

†Model I: Crude analysis

‡Model II: Adjusted for total energy intake (residual method)

§Model III: Adjusted for family history of cancer, BMI, physical activity, smoking, total energy intake, fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

** m/d: measures per day

6.5 Summary of results of chapter 6

In this chapter the results of the matched analysis of the novel dietary risk factors (flavonoid and fatty acid subgroups and individual compounds) that comprised the first two hypotheses, were presented. In particular, one crude and four multivariable conditional logistic regression models were applied in the whole sample, whereas one conditional multivariable model adjusted for the main potential confounding factors was applied after sex, age and cancer site stratification.

6.5.1 Flavonoids

Moderately strong inverse associations which showed dose response relationships were found in the energy-adjusted conditional logistic regression model (model II) between colorectal cancer risk and the intake of the subgroups flavonols (p=0.02) and procyanidins (p=0.04) and the individual flavonoid compounds quercetin (p=0.002), catechin (p=0.0001) and epicatechin (p=0.04) (Table 68). After adjusting for the main potential confounding factors (model III), only the inverse associations between colorectal cancer, quercetin (p=0.04) and catechin (p=0.02) remained statistically significant (Table 68). Results from model IV, which was corrected for the confounding factors of model III and for fruit and vegetable intake showed an inverse association between catechin and colorectal cancer (p=0.05) (Table 69). Finally, results in model V, which was corrected for the confounding factors of model III and further adjusted mutually between flavonoid categories, showed inverse associations between colorectal cancer and flavonols (p=0.0001), catechin (p=0.007) and epicatechin (p=0.03). In marked contrast we showed no associations between intakes of the other four of the six flavonoid subgroups studied (flavones, flavan3ols, flavanones and phytoestrogens) and colorectal cancer risk (Table 68, Table 69). In addition, results of the analysis of the main food sources (regular tea, onions, apples and red wine) of the flavonoid variables that were found to be significantly associated with colorectal cancer, suggest that there is some evidence in favour of an inverse association but this is less well defined than in the analysis of the association of flavonol, procyanidin, quercetin, catechin or epicatechin intakes and colorectal cancer risk (Table 70).

6.5.2 Fatty acids

After residual energy-adjustment (model II) significant inverse dose-dependent associations were observed between colorectal cancer and the dietary intakes of the fatty acid subgroup ω3PUFAs ($p=9.3x10^{-6}$) and the individual compounds EPA (p=0.0001) and DHA (p=0.0002) (Table 75). In contrast, a dose-dependent increase in risk was observed for intake of dietary total FAs (p=0.001), SFAs (p=0.001), MUFAs (p=0.01) *t*FAs (p=0.002) and *t*MUFAs (p=0.0003) and for the individual fatty acids palmitic (p=0.001), stearic ($p=7.9x10^{-6}$) and oleic (p=0.001) (Table 75). In model III, only the positive association between colorectal cancer and stearic acid (p=0.01) and the inverse associations between colorectal cancer and dietary ω3PUFAs (p=0.01), EPA (p=0.02) and DHA (p=0.02) remained statistically significant (Table 75). For both model IV (further adjusted for total fatty acid intake) and model V (further adjusted for energy, in addition to the residual energy adjustment) positive significant associations were observed for stearic acid (p= 0.03 and 0.01, respectively) and inverse significant associations were observed for ω3PUFAs, EPA and DHA (model IV: p=0.008, p=0.02 and p=0.02; model IV: p=0.002, p=0.01, p=0.01; respectively) (Table 76). In marked contrast, the subgroups of PUFAs, ω6PUFAs and the individual fatty acids linoleic, γ-linolenic, arachidonic and α-linolenic were not associated with colorectal cancer risk in any of the adjusted logistic regression models (Table 75). Finally, results of the analysis of the main food sources (meat and meat products, confectionery and savoury snacks and fish and fish dishes) of the fatty acids that were found to be significantly associated with colorectal cancer, suggest that there is some evidence in favour of a statistically significant association (Table 77).

7 RESULTS: Associations between colorectal cancer and intakes of folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium (unmatched dataset)

7.1 Introduction

In this chapter the results of the unmatched analysis of the additional dietary risk factors that comprise the last two hypotheses are presented. Specifically, the dietary risk factors that were analysed using the unmatched dataset included: a) folate, vitamin B2, vitamin B6, vitamin B12, alcohol and b) vitamin D and calcium.

In the first part of this chapter, the study population used in the unmatched analysis is presented, including descriptive analysis of the main confounding factors and logistic regression analysis to investigate their association relationships with colorectal cancer risk. In the second part of the chapter descriptive analysis of the dietary risk factors are presented including distribution analysis (whole sample and by case/ control status) and correlation analysis. In addition, the association relationships between colorectal cancer and each nutrient are investigated by applying three main unconditional logistic regression models and one additional unconditional logistic regression model (for the analysis of vitamin D and calcium). All tables and figures are presented at the end of each section or in the Appendix, as indicated in the text.

7.2 The study sample

This analysis describes the characteristics of the cases and controls that were included in the unmatched dataset. In total 2,062 cases and 2,776 controls were included. One case reported very high dietary energy and nutrient intakes and therefore it was removed from the analysis.

7.2.1 Descriptive analysis of the confounding factors

The distribution of the continuous confounding factors was examined by looking at their histograms. In addition, their summary statistics are presented in Table 78 for the whole sample and also separately for cases and controls. The t-test was used to test differences

between cases and controls in mean age, BMI, dietary energy, fibre intake (crude and residually energy adjusted) and alcohol intake (crude and residually energy adjusted). The Pearson χ^2 test was used to test the differences in terms of sex, deprivation score, family history of cancer, physical activity (hours/ week of cycling and sport activities), smoking status and NSAIDs intake.

7.2.2 Associations between confounding factors and colorectal cancer risk

The association relationship between each confounding factor and colorectal cancer risk was tested by applying univariable logistic regression models (Table 79). Statistically significant associations were observed for the majority of the confounding factors, including:

- Age (>55 years old vs. ≤55 years old: OR (95%CI), p-value: 0.85 (0.75, 0.97), 0.01);
- Family history of cancer (moderate/ high vs. low: OR (95% CI), p-value: 18.58 (12.72, 27.13), 1.12×10^{-51});
- Dietary energy intake (highest vs. lowest quartile: OR (95% CI), p-value for trend: 1.37 (1.17, 1.61), 2.2×10^{-5});
- Residually energy adjusted fibre intake (highest vs. lowest quartile: OR (95% CI), p-value for trend: 0.71 (0.60, 0.84), 3.3×10^{-5});
- NSAIDs intake (yes vs. no: OR (95% CI), p-value: 0.73 (0.65, 0.83), 7.3×10^{-7}).

Table 78 Summary statistics of the confounding factors for the unmatched dataset

Variables	All subjects* (n=4837)	Cases* (n=2061)	Controls* (n=2776)	p-value†
Age (years)	62.2 (10.6)	62.0 (10.8)	62.4 (10.5)	0.14
Age (years)				
≤55 years	1392 (28.8%)	632 (30.7%)	760 (27.4%)	
>55 years	3443 (71.2%)	1429 (69.3%)	2014 (72.6%)	0.01
Sex				
Men	2762 (57.1%)	1180 (57.2%)	1582 (57.0%)	
Women	2075 (42.9%)	881 (42.8%)	1194 (43.0%)	0.85
Deprivation score‡				
1	452 (9.3%)	194 (9.4%)	258 (9.3%)	
2	1002 (20.7%)	434 (21.1%)	568 (20.5%)	
3	1290 (26.7%)	532 (25.8%)	758 (27.3%)	
4	1133 (23.4%)	488 (23.7%)	645 (23.2%)	
5	511 (10.6%)	218 (10.6%)	293 (10.6%)	
6	318 (6.6%)	140 (6.8%)	178 (6.4%)	
7	130 (2.7%)	54 (2.6%)	76 (2.7%)	0.95
Family history risk of cancer				
Low	4305 (89.0%)	1610 (78.1%)	2695 (97.1%)	
Medium	328 (6.8%)	299 (14.5%)	29 (1.0%)	
High	35 (0.72%)	34 (1.6%)	1 (0.0%)	
Unknown	108 (2.2%)	91 (4.4%)	17 (0.61%)	<0.0005
Missing	61 (1.3%)	27 (1.3%)	34 (1.2%)	
BMI (kg/m^2)§	26.7 (4.5)	26.6 (4.4)	26.7 (4.6)	0.41
Physical activity (hours/day) *(cycling and other sport activities)*				
0	2595 (53.6%)	1139 (55.3%)	1456 (52.4%)	
0-3.5	1189 (24.6%)	486 (23.6%)	703 (25.3%)	
3.5-7	544 (11.2%)	205 (9.9%)	339 (12.2%)	
>7	318 (6.6%)	133 (6.5%)	185 (6.7%)	0.04
Missing	191 (3.9%)	98 (4.8%)	93 (3.4%)	

Smoking				
No	2074 (42.9%)	874 (42.4%)	1200 (43.2%)	
Yes**	2719 (56.2%)	1161 (56.3%)	1558 (56.1%)	0.70
Missing	44 (0.9%)	26 (1.3%)	18 (0.65%)	
Dietary energy intake (MJ/day)	10.91 (4.1)	11.26 (4.4)	10.66 (3.95)	$<5x10^{-5}$
Fibre intake (g/day)	22.4 (9.8)	22.5 (9.8)	22.3 (9.9)	0.64
Energy-adjusted fibre intake (g/day)	21.4 (6.0)	20.9 (5.7)	21.7 (6.2)	$<5x10^{-5}$
Alcohol intake (g/day)	13.2 (15.8)	13.2 (16.0)	13.2 (15.6)	0.98
Energy-adjusted alcohol intake (g/day)	13.0 (15.1)	12.8 (15.3)	13.2 (15.0)	0.44
NSAIDs intake††				
No	3206 (66.3%)	1449 (70.3%)	1757 (63.3%)	
Yes	1605 (33.2%)	605 (29.4%)	1000 (36.0%)	<0.0005
Missing	26 (0.5%)	7 (0.3%)	19 (0.7%)	

* Mean values and in parentheses standard deviations for quantitative variables; number of subjects and in parenthesis percentages for categorical variables.

† P-values from the Pearson χ^2 for categorical variables; from t-test for continuous variables

‡ Locally based deprivation index (Carstairs deprivation index) based on the 2001 Census data; 7 categories ranging from very low deprivation (deprivation score 1) to very high deprivation (deprivation score 7). Missing data for one case

§ Missing data for 21 cases and 34 controls

** Smokers were defined as individuals who have smoked at least one cigarette per day and/ or one cigar per month and/ or pipe.

†† Frequent use was defined as an intake of at least 4 days per week for at least one month.

Table 79 Association between the confounding factors and colorectal cancer risk (univariable logistic regression analysis)

Confounding variables	Categories	Frequency		Univariable analysis		*p-value*
		cases	***controls***	***OR***	***95% CI***	
Age (years)		2061	2774	0.99	0.99, 1.00	0.14
Age (years)	≤55 years	632	760	1.00		
	>55 years	1429	2014	0.85	0.75, 0.97	0.01
Sex	Men	1180	1582	1.00		
	Women	881	1194	0.99	0.88, 1.11	0.85
Deprivation score	1	194	258	1.00		
	2	434	568	1.02	0.81, 1.27	0.89
	3	532	758	0.96	0.75, 1.16	0.53
	4	488	645	1.01	0.81, 1.25	0.96
	5	218	293	0.99	0.77, 1.28	0.94
	6	140	178	1.05	0.78, 1.40	0.76
	7	54	76	0.94	0.64, 1.40	0.78
				p-value for trend 0.94		
Family history risk of cancer	Low	1610	2695	1.00		
	Medium/ High	333	30	18.58	12.72, 27.13	$1.12x10^{-51}$
BMI (kg/m^2)	Continuous	2040	2742	0.99	0.98, 1.01	0.42
BMI (kg/m^2)	18.5-25	778	1000	1.00		
	<18.5	21	38	0.71	0.41, 1.22	0.22
	25-30	862	1173	0.94	0.83, 1.07	0.38
	≥ 30	379	531	0.92	0.78, 1.08	0.30
				p-value for trend 0.27		
Physical activity (hours/week)	0	1139	1456	1.00		
	0-3.5	486	703	0.88	0.77, 1.02	0.08
	3.5-7	205	339	0.77	0.64, 0.93	0.008
	>7	133	185	0.92	0.73, 1.16	0.48
				p-value for trend 0.02		
Smoking	No	874	1200	1.00		
	Former	818	1049	1.07	0.94, 1.21	0.29
	Current	343	509	0.93	0.79, 1.09	0.35
				p-value for trend 0.63		

Dietary energy intake (MJ/day)	continuous	2061	2776	1.04	1.02, 1.05	$7.4x10^{-7}$
Dietary energy intake (MJ/day)	0- 8.25	478	733	1.00		
	8.25-10.17	483	725	1.02	0.87, 1.20	0.80
	10.17- 12.73	529	680	1.19	1.01, 1.40	0.03
	>12.73	571	638	1.37	1.17, 1.61	0.0001
				p-value for trend $2.2x10^{-5}$		
Fibre intake (g/day)	continuous	2061	2776	1.00	0.99, 1.01	0.64
Fibre intake (g/day)	0- 15.90	508	712	1.00		
	15.90- 20.70	527	690	1.07	0.91, 1.26	0.41
	20.70- 26.90	510	685	1.04	0.89, 1.23	0.61
	>26.90	516	689	1.05	0.89, 1.23	0.56
				p-value for trend 0.64		
Fibre intake energy adjusted (g/day)	continuous	2061	2776	0.98	0.97, 0.99	$2.8x10^{-6}$
Fibre intake energy adjusted (g/day)	0- 17.34	549	661	1.00		
	17.34-20.97	542	667	0.98	0.83, 1.15	0.79
	20.97-24.94	521	688	0.91	0.78, 1.01	0.26
	>24.94	449	760	0.71	0.60, 0.84	$4.0x10^{-5}$
				p-value for trend $3.3x10^{-5}$		
Alcohol intake (g/day)	continuous	2061	2776	1.00	0.99, 1.00	0.98
Alcohol intake (g/day)	0-1.70	526	696	1.00		
	1.70-8.10	534	692	1.02	0.87, 1.20	0.80
	8.10-19.2	501	681	0.97	0.83, 1.14	0.74
	>19.20	500	707	0.94	0.80, 1.10	0.42
				p-value for trend 0.34		
Alcohol intake energy adjusted (g/day)	continuous	2061	2776	1.00	0.99, 1.00	0.44
Alcohol intake energy adjusted (g/day)	0-1.84	526	684	1.00		
	1.84- 8.07	539	670	1.05	0.89, 1.23	0.58
	8.07-18.99	509	700	0.95	0.80, 1.11	0.50
	>18.99	487	722	0.88	0.75, 1.03	0.11
				p-value for trend 0.06		
NSAIDs intake	No	1449	1757	1.00		
	Yes	605	1000	0.73	0.65, 0.83	$7.3x10^{-7}$

7.3 Folate, vitamin B2, vitamin B6, vitamin B12 and alcohol

This analysis describes the distribution and correlation of the nutrients involved in one-carbon metabolic pathway. In addition, differences in crude and energy-adjusted nutrient intakes between cases and controls and the unadjusted and adjusted associations between nutrient intakes and colorectal cancer are presented.

7.3.1 Descriptive analysis

7.3.1.1 Distribution of nutrients

Distribution of the nutrients (folate, vitamin B2, vitamin B6, vitamin B12 and alcohol) was examined by looking at their histograms (original and transformed variables if skewed). The distributions of the nutrients under study were skewed and they were normalised either with square root or with logarithmic transformation (Table 80).

7.3.1.2 Distribution of nutrients by case control status

Cases reported higher mean crude intakes for folate (p=0.05), vitamin B2 (p=0.0018) and vitamin B6 (p=0.03). In addition cases reported higher median crude intakes for vitamin B2 (p=0.005) (Table 81). After energy adjustment (residual energy adjustment) cases reported lower mean intakes for folate (p=0.0002), vitamin B6 ($<5x10^{-5}$) and vitamin B12 (0.0047). In addition cases reported lower median intakes for folate (p=0.0003), vitamin B6 ($<5x10^{-5}$) and vitamin B12 (0.02) (Table 81).

7.3.1.3 Correlations between the nutrients

The highest correlations were observed between folate, vitamin B2 and vitamin B6 with r>0.7. Vitamin B12 was moderately correlated with folate (r=0.56), vitamin B2 (r=0.69) and vitamin B6 (r=0.62). Alcohol was not correlated with any of the nutrients (r<0.15) (Table 82).

7.3.1.4 Main sources of folate, vitamin B2, vitamin B6, vitamin B12 and alcohol

The three main food sources (at individual food item level) were: 1) for folate: boiled or baked potatoes (10.0%), bran flakes and sultana bran and All Bran (4.9%) and regular tea (3.7%); 2) for vitamin B2: semi-skimmed milk (14.0%), full fat milk (4.5%) and corn flakes, Special K and Rice Krispies (4.1%); 3) for vitamin B6:

boiled or baked potatoes (14.4%), bananas (4.9%) and mixed vegetable dishes (4.3%); 4) for vitamin B12: fried oily fish (12.9%), liver, liver sausage or liver pate (8.4%) and semi-skimmed milk (8.3%). The three alcoholic drinks that were the main sourced of the total grams of consumed alcohol were: spirits or liqueurs (28.2%), red wine (23.7%) and white wine (17.3%) (Table 83).

One thousand six hundred and sixty participants reported consumption of supplement products and 461 of them reported consumption of supplements that contributed to the daily intake of the nutrients involved in the one-carbon metabolic pathway (433 reported intakes of supplements that contributed in the folate dietary intake, 429 in the B2 dietary intake, 445 in the B6 dietary intake and 411 in the B12 intake). We identified the exact nutrient composition of these dietary supplements and added the supplement nutrients to the dietary ones.

7.3.2 Associations between folate, vitamin B2, vitamin B6, vitamin B12, alcohol and colorectal cancer risk

7.3.2.1 Main logistic regression models

In model I, dietary intake of vitamin B2 was positively associated with colorectal cancer (high vs. low intake: OR (95% CI), p-value: 1.21 (1.03, 1.42), 0.02) (Table 84). Associations between total intakes (from diet and supplements) of the nutrients and colorectal cancer were not examined in model I, since intake from supplements was added to the energy-adjusted nutrients. After energy adjustment (Model II), both dietary and total intakes of folate, vitamin B6 and vitamin B12 were significantly and inversely associated with colorectal cancer (high vs. low dietary intake: OR (95% CI), p-value: 0.80 (0.68, 0.94), 0.003; 0.71 (0.60, 0.83), $7.1x10^{-6}$; 0.80 (0.68, 0.95), 0.02; respectively) (Table 84). In model III dietary and total vitamin B12 was inversely associated with colorectal cancer risk (high vs. low dietary intake: OR (95% CI), p-value: 0.80 (0.67, 0.96), 0.04). In addition, an inverse marginally non-significant association between dietary vitamin B6 and colorectal cancer was observed (high vs. low intake: OR (95% CI), p-value: 0.85 (0.69, 1.04), 0.09) (Table 84). Regarding alcohol intake, when divided in quartiles, a significant inverse and dose-dependent association was observed when applying model III (high vs. low intake: OR (95% CI), p-value: 0.83 (0.68, 1.00), 0.03) (Table 84). However, when alcohol was divided into categories according to the level of intake, individuals with

an intake of more than 60g/day were associated with a non dose-dependent increased colorectal cancer risk, which was statistically significant only when applying model I (high vs. low dietary intake: OR (95% CI), p-value for trend: 1.70 (1.11, 2.60), 0.28) (Table 84).

7.3.2.2 Multiple testing corrections

Bonferroni correction for multiple testing

The adjusted level of significance after having controlled for multiple testing was: a) 0.003 using the Bonferroni correction for 19 independent tests, b) 0.003 using the Bonferroni correction for 15 tests conducted in hypothesis 3 and c) 0.0005 for the individual compound analysis after having corrected for 93 tests conducted in all 4 hypotheses. Here we report only associations between dietary intakes and colorectal cancer. In model II the associations between colorectal cancer and vitamin B6 ($p=7.1x10^{-6}$) remained significant at all three levels of correction, whereas associations with folate (p=0.001) remained significant at the second level of significance (Table 84).

FDR correction for multiple testing

After correcting for multiple testing using the FDR method by taking into account the number of tests that were conducted in hypothesis 3 (15 tests) or the number of tests that were conducted in all 4 hypotheses (93 tests in the individual compound analysis), model II associations between dietary intakes of vitamin B6 ($p=7.1x10^{-6}$) and folate (p=0.001) and colorectal cancer remained significant (Table 84).

7.3.2.3 Associations between colorectal cancer and main food sources of folate, vitamin B6 and vitamin B12

Intakes of the following food groups were tested: boiled or baked potatoes, bran flakes, bananas, fried oily fish and liver, liver sausage or liver pate. Results from model III, showed that comparison of highest versus lowest quartile (tertile) intakes of these foods showed ORs for colorectal cancer risk of 1.13 (95% CI 0.93, 1.37; p-value for trend 0.38) for boiled or baked potatoes; 1.15 (95% CI 0.98, 1.35; p-value for trend 0.17) for bran flakes; 0.82 (95% CI 0.67, 0.99; p-value for trend 0.06) for bananas; 0.74 (95% CI 0.61, 0.91; p-value for trend 0.20) for fried oily fish; and 0.98 (95% CI 0.81, 1.18; p-value for trend 0.86) for liver, liver sausage or liver pate (Table 85).

7.3.2.4 Associations between folate, vitamin B2, vitamin B6, vitamin B12, alcohol and colorectal cancer after sex, age and cancer site stratification

Associations between each nutrient and colorectal cancer risk were tested after sex, age and cancer site stratification by applying model III (data not shown) for both dietary and total intakes. Sex-specific associations were similar for almost all nutrients and for alcohol. However, high intakes of both dietary and total vitamin B6 and B12 were associated with a stronger decrease in colorectal cancer risk for women (high vs. low dietary intake OR (95% CI), p-value for trend: 0.75 (0.53, 1.05), 0.08; 0.75 (0.56, 0.99), 0.04, respectively), than for men (data not shown). Regarding age-specific differences, high intakes of vitamin B6 (dietary and total) and alcohol (when divided in quartiles) was significantly and dose-dependently associated with a decreased risk of colorectal cancer for the individuals younger than 55 years old (high vs. low dietary intake: OR (95% CI), p-value for trend: 0.59 (0.39, 0.89), 0.005; 0.63 (0.43, .93), 0.006; respectively), whereas high intake of both dietary and total vitamin B12 was associated with a significant and dose-dependent decreased risk of colorectal cancer for the individuals older than 55 years old (high vs. low dietary intake: OR (95% CI), p-value for trend: 0.80 (0.64, 0.98), 0.05) (data not shown). Finally, after cancer site stratification, the relationships of all the nutrients with colon and rectal cancer were similar. Regarding alcohol, intake when divided into quartiles, it was inversely associated with colon cancer (high vs. low intake: OR (95% CI), p-value: 0.72 (0.57, 0.91), 0.003) but not with rectal cancer. In contrast, when divided into categories according to the level of intake, high intake of alcohol (>60 g/day) was positively associated only with rectal cancer (OR (95% CI): 1.81 (0.99, 3.29)) (data not shown).

7.3.2.5 Interaction relationships with variants of genes involved in the one-carbon metabolic pathway

The genotypic effects of 3 polymorphic genes involved in the one-carbon metabolic pathway on colorectal cancer risk were examined. In particular the genetic variants that were examined were rs1801133 (*MTHFR* C677T), rs1801131 (*MTHFR* A1298C), rs1805087 (*MTR* A2756G) and rs1801394 (*MTRR* A66G). The variant

allele frequencies of the four polymorphisms in the control sample were under Hardy-Weinberg equilibrium (rs1801133 11.6%, rs1801131, 10.0%, rs1805087 19.6%, rs1801394 3.0%).

The associations between colorectal cancer risk and each of the four SNPs were tested by applying one unadjusted and one simply adjusted (for age, sex and deprivation score) logistic regression model (data not shown). In addition, ORs and 95% CI for dietary intakes of the nutrients were calculated in stratified groups according to the rs1801133, rs1801131, rs1805087 and rs1801394 genotypes by applying the multivariable model III adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, dietary energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake (data not shown). Finally, interaction associations were examined by investigating the combined effects of the genotypes and nutrient intakes. Interaction was tested by examining the deviance of two different nested models; an interactive model and its nested multiplicative one. The referent category used was homozygotes of the wild type allele and of the lowest dietary nutrient intake quartile (data not shown).

None of the four examined SNPs was significantly associated with colorectal cancer risk (data not shown). However, a not statistically significant increased risk was observed for the GG genotype of the rs1805087 (crude model: OR (95% CI), p-value for trend: 1.30 (0.79, 2.12), 0.19) (data not shown). In addition, there was no clear trend for the associations between colorectal cancer and folate, vitamin B2, vitamin B6, vitamin B12 and alcohol after stratification according to the genotypes of rs1801133, rs1801131, rs1805087 or rs1801394 (data not shown). Finally, our data did not support the hypothesis that folate or any of the vitamins B2, B6, B12 interacts with the rs1801133 (*MTHFR* 677TT) variant or with any of the rs1801131 (*MTHFR* A1298G), rs1805087 (*MTR* A2756G) or rs1801339 (*MTRR* A66G) variants (data not shown).

7.3.3 Summary of results

Inverse associations which showed dose response relationships were found: 1) in model II: between colorectal cancer risk and the dietary intakes of folate (p=0.003), vitamin B6 ($p=7.1x10^{-6}$) and vitamin B12 (p=0.02) (Table 84); 2) in model III:

between colorectal cancer and vitamin B12 (p=0.05) and alcohol (p=0.03) (Table 84). When alcohol intakes were divided in six categories (instead of quartiles), no association was observed for an intake of less than 60 g/day, whereas a positive non-significant association was observed for an alcohol intake of more than 60 g/day (Table 84). Regarding the analysis of the main food sources of folate, vitamin B6 and vitamin B12, results suggest that there is some evidence in favour of a significant inverse association between colorectal cancer and intakes of bananas (dietary source of vitamin B6) and fried oily fish (dietary source of vitamin B12) (Table 85). Finally, regarding the genetic analysis, none of the four examined SNPs was significantly associated with colorectal cancer risk. Furthermore, there was no clear trend for the associations between colorectal cancer and folate, vitamin B2, vitamin B6, vitamin B12 and alcohol after stratification according to the genotypes of the aforementioned variants (data not shown).

Table 80 Nutrients involved in the one-carbon metabolic pathway that were elected to be included in the analysis

Nutrients included in the analysis	Transformation
Individual compounds	
Folate	logarithmic
Vitamin B2	logarithmic
Vitamin B6	logarithmic
Vitamin B12	logarithmic
Alcohol	square root

Table 81 Descriptive report of crude and energy-adjusted nutrients involved in the one-carbon metabolic pathway

Nutrients	All subjects (n=4837)		Cases (n=2061)		Controls (n=2776)		T-test	Wilcoxon rank test
	Mean (SD)	***Median (IQR)***	***Mean (SD)***	***Median (IQR)***	***Mean (SD)***	***Median (IQR)***	***p-value***	***p-value***
Folate (µg/day)	343.2 (131.1)	322.0 (256.0, 400.0)	346.1 (128.0)	324.0 (260.0, 402.0)	341.1 (133.2)	321.0 (253.0, 399.0)	0.05	0.10
Energy-adjusted folate (µg/day)	329.3 (71.5)	325.9 (282.6, 370.8)	324.9 (68.2)	321.3 (280.5, 365.7)	332.7 (73.6)	328.9 (283.8, 374.7)	0.0002	0.0003
Vitamin B2 (mg/day)	2.2 (0.9)	2.1 (1.6, 2.6)	2.3 (0.9)	2.1 (1.7, 2.7)	2.2 (0.9)	2.1 (1.6, 2.6)	0.0018	0.005
Energy-adjusted vitamin B2 (mg/day)	2.1 (0.5)	2.1 (1.8, 2.4)	2.1 (0.5)	2.1 (1.8, 2.4)	2.1 (0.5)	2.1 (1.8, 2.4)	0.09	0.13
Vitamin B6 (mg/day)	3.0 (1.2)	2.8 (2.2, 3.5)	3.0 (1.2)	2.8 (2.2, 3.5)	3.0 (1.2)	2.8 (2.2, 3.5)	0.03	0.15

Energy-adjusted	2.8	2.8	2.8	2.8	2.9	2.9	$<5x10^{-5}$	$<5x10^{-5}$
vitamin B6 (mg/day)	(0.6)	(2.5, 3.2)	(0.5)	(2.4, 3.1)	(0.6)	(2.5, 3.2)		
Vitamin B12	8.2	6.9	8.2	7.0	8.2	6.8	0.13	0.20
(µg/day)	(5.4)	(4.9, 9.9)	(5.2)	(5, 9.9)	(5.5)	(4.8, 9.8)		
Energy-adjusted	7.7	7.0	7.5	6.9	7.8	7.0	0.06	0.02
vitamin B12* (µg/day)	(3.6)	(5.3, 9.2)	(3.4)	(5.2, 9.0)	(3.7)	(5.3, 9.4)		
Alcohol	13.2	8.1	13.2	7.7	13.2	8.1	0.84	0.64
(g/day)	(15.8)	(1.7, 19.2)	(16.0)	(1.7, 18.8)	(15.6)	(1.7, 19.4)		
Energy-adjusted	13.0	8.1	12.8	7.7	13.2	8.4	0.44	0.15
alcohol (g/day)	(15.1)	(1.8, 19.0)	(15.3)	(1.7, 18.3)	(15.0)	(1.9, 19.9)		

* Logarithmic transformed values were used for calculating the t-test due to skewed distribution

Table 82 Spearman rank correlation coefficients between nutrients involved in the one-carbon metabolic pathway (all p-values<$5x10^{-5}$)

Nutrients	folate	B2	B6	B12	alcohol
folate	1.00				
vitamin B2	0.82	1.00			
vitamin B6	0.92	0.79	1.00		
vitamin B12	0.56	0.69	0.62	1.00	
alcohol	0.14	0.08	0.16	0.15	1.00

Table 83 Three main dietary (food) sources of nutrients involved in the one-carbon metabolic pathway in our population

Nutrients	Main sources
Folate	Boiled or baked potatoes (10.0%)
	Bran flakes, Sultana Bran and All Bran (4.9%)
	Tea (3.7%)
Vitamin B2	Semi-skimmed milk (14.0%)
	Full fat milk (4.5%)
	Corn Flakes, Special K and Rice Krispies (4.1%)
Vitamin B6	Boiled or baked potatoes (14.4%)
	Bananas (4.9%)
	Mixed vegetable dishes (4.3%)
Vitamin B12	Fried oily fish (12.9%)
	Liver, liver sausage or liver pate (8.4%)
	Semi-skimmed milk (8.3%)
Alcohol	Spirits or liqueurs (28.2%)
	Red wine (23.7%)
	White wine (17.3%)

Table 84 Association between the nutrients involved in the one-carbon metabolic pathway and colorectal cancer risk in the whole sample (3 main unconditional logistic regression models)

Nutrients	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Folate	0-282.65	533	677	1.00		1.00		1.00	
(µg/day)	282.65-325.89	546	663	1.17	0.99, 1.37	1.05	0.89, 1.23	1.22	1.01, 1.46
	325.89-370.81	515	694	1.13	0.96, 1.33	0.94	0.80, 1.11	1.14	0.94, 1.39
	≥370.81	467	742	1.15	0.98, 1.35	0.80	0.68, 0.94	1.03	0.82, 1.29
	p-value for trend (quartiles)				0.14		0.003		0.92
	p-value for trend (continuous)				0.19		0.0002		0.73
Folate	0-286.24	532	678			1.00		1.00	
(total) (µg/day)	286.24-332.60	566	643			1.12	0.96, 1.32	1.31	1.09, 1.58
	332.60-386.03	488	721			0.86	0.73, 1.01	1.07	0.88, 1.30
	≥386.03	475	734			0.82	0.70, 0.97	1.06	0.85, 1.31
	p-value for trend (quartiles)						0.001		0.84
	p-value for trend (continuous)						0.42		0.36
Vitamin B2	0-1.80	522	688	1.00		1.00		1.00	
(mg/day)	1.80-2.10	537	672	1.03	0.88, 1.22	1.05	0.90, 1.24	1.06	0.88, 1.26
	2.10-2.42	511	698	1.11	0.94, 1.30	0.96	0.82, 1.13	1.00	0.83, 1.20
	≥2.42	491	718	1.21	1.03, 1.42	0.90	0.77, 1.06	0.87	0.72, 1.05
	p-value for trend (quartiles)				0.02		0.13		0.12
	p-value for trend (continuous)				0.006		0.09		0.13
Vitamin B2	0-1.83	519	691			1.00		1.00	
(total) (mg/day)	1.83-2.15	536	673			1.06	0.90, 1.24	1.04	0.87, 1.24

	2.15-2.53	510	699			0.97	0.83, 1.14	0.97	0.81, 1.17
	≥2.53	496	713			0.93	0.79, 1.09	0.93	0.77, 1.17
	p-value for trend (quartiles)						0.22		0.35
	p-value for trend (continuous)						0.63		0.81
Vitamin B6	0-2.47	547	663	1.00		1.00		1.00	
(mg/day)	2.47-2.83	555	654	1.17	1.00, 1.38	1.03	0.88, 1.21	1.14	0.95, 1.37
	2.83-3.21	514	695	1.05	0.89, 1.24	0.90	0.76, 1.05	1.04	0.86, 1.26
	≥3.21	445	764	1.12	0.95, 1.31	0.71	0.60, 0.83	0.85	0.69, 1.04
	p-value for trend (quartiles)				0.38		$7.1x10^{-6}$		0.09
	p-value for trend (continuous)				0.10		$2.9x10^{-5}$		0.13
Vitamin B6	0-2.51	555	655			1.00		1.00	
(total) (mg/day)	2.51-2.90	541	668			0.96	0.81, 1.12	1.08	0.90, 1.30
	2.90-3.32	494	715			0.81	0.69, 0.96	1.00	0.82, 1.21
	≥3.32	471	738			0.75	0.64, 0.89	0.91	0.74, 1.11
	p-value for trend (quartiles)						0.0001		0.25
	p-value for trend (continuous)						0.10		0.43
Vitamin B12	0-5.27	538	672	1.00		1.00		1.00	
(µg/day)	5.27-6.96	516	693	1.05	0.89, 1.23	0.93	0.79, 1.09	0.95	0.79, 1.14
	6.96-9.21	533	676	1.20	1.02, 1.41	0.98	0.84, 1.16	1.00	0.84, 1.20
	≥9.21	474	735	1.12	0.95, 1.31	0.80	0.68, 0.95	0.80	0.67, 0.97
	p-value for trend (quartiles)				0.06		0.02		0.05
	p-value for trend (continuous)				0.73		0.005		0.003
Vitamin B12	0-5.35	543	667			1.00		1.00	
(total) (µg/day)	5.35-7.09	515	694			0.91	0.78, 1.07	0.90	0.75, 1.08

	7.09-9.41	527	682			0.95	0.81, 1.11	0.95	0.80, 1.14
	≥9.41	476	733			0.80	0.68, 0.94	0.80	0.67, 0.96
	p-value for trend (quartiles)						0.01		0.04
	p-value for trend (continuous)						0.71		0.81
Alcohol	0-1.70	526	696	1.00		1.00		1.00	
(g/day)	1.70-8.10	534	692	1.02	0.87, 1.20	1.05	0.89, 1.22	1.07	0.89, 1.28
	8.10-19.2	501	681	0.97	0.83, 1.14	0.95	0.80, 1.11	0.94	0.78, 1.13
	>19.20	500	707	0.94	0.80, 1.10	0.88	0.75, 1.03	0.83	0.68, 1.00
	p-value for trend (quartiles)				0.34		0.06		0.03
	p-value for trend (continuous)				0.98		0.44		0.24
Alcohol	0	291	427	1.00		1.00		1.00	
(g/day)	0-15	1125	1473	1.12	0.95, 1.33	1.09	0.92, 1.29	1.10	0.91, 1.33
	15-30	393	548	1.05	0.86, 1.28	1.00	0.82, 1.23	1.02	0.81, 1.29
	30-45	139	202	1.01	0.78, 1.31	0.94	0.72, 1.22	0.97	0.72, 1.32
	45-60	61	81	1.11	0.77, 1.59	1.00	0.69, 1.44	0.97	0.65, 1.46
	>60	52	45	1.70	1.11, 2.60	1.47	0.96, 2.27	1.37	0.84, 2.22
	p-value for trend				0.28		0.89		0.91

[*]Based on the distribution of the energy adjusted variable

[†]Model I: Crude analysis

[‡]Model II: Adjusted for total energy intake (residual method)

[§]Model III: Adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

Table 85 Association between boiled or baked potatoes, bran flakes, bananas, fried oily fish, liver sausage or liver pate and colorectal cancer risk in the whole sample (3 main unconditional logistic regression models)

Food sources	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Baked or boiled potatoes (m/day**)	0-0.42	583	888	1.00		1.00		1.00	
	0.42-0.85	702	845	1.27	1.09, 1.46	1.21	1.05, 1.40	1.22	1.04, 1.44
	0.85-1.28	197	427	1.06	0.88, 1.27	1.00	0.83, 1.20	1.08	0.88, 1.32
	>1.28	479	616	1.18	101, 1.39	1.06	0.90, 1.25	1.13	0.93, 1.37
	p-value for trend (quartiles)				0.14		0.93		0.38
	p-value for trend (continuous)				0.53		0.31		0.82
Bran flakes (m/day)	0	1533	2079	1.00		1.00		1.00	
	0-0.05	54	97	0.75	0.54, 1.06	0.75	0.54, 1.06	0.72	0.49, 1.07
	>0.05	474	600	1.07	0.93, 1.23	1.06	0.93, 1.22	1.15	0.98, 1.35
	p-value for trend (quartiles)				0.51		0.56		0.17
	p-value for trend (continuous)				0.47		0.65		0.31
Bananas (m/day)	0-0.14	645	845	1.00		1.00		1.00	
	0.14-0.42	621	735	1.11	0.95, 1.28	1.09	0.94, 1.26	1.13	0.96, 1.33
	0.42-0.71	400	511	1.02	0.87, 1.21	0.99	0.83, 1.17	1.07	0.89, 1.30
	>0.71	395	685	0.75	0.64, 0.89	0.70	0.60, 0.83	0.82	0.67, 0.99
	p-value for trend (quartiles)				0.001		$3.6x10^{-5}$		0.06
	p-value for trend (continuous)				0.0003		$2.5x10^{-6}$		0.02
Fried oily fish (m/day)	0	1148	1524	1.00		1.00		1.00	
	0-0.14	659	832	1.05	0.92, 1.19	1.05	0.92, 1.19	1.09	0.94, 1.26

	>0.14	254	420	0.80	0.67, 0.95	0.73	0.61, 0.87	0.74	0.61, 0.91
	p-value for trend (quartiles)				0.20		0.05		0.20
	p-value for trend (continuous)				0.02		0.001		0.001
Liver, liver sausage	0	1450	1990	1.00		1.00		1.00	
or liver pate (m/day)	0-0.05	299	417	0.98	0.84, 1.16	0.98	0.83, 1.15	1.00	0.83, 1.20
	>0.05	312	369	1.16	0.98, 1.37	1.07	0.91, 1.27	0.98	0.81, 1.18
	p-value for trend (quartiles)				0.18		0.58		0.86
	p-value for trend (continuous)				0.33		0.92		0.29

[*]Based on the distribution of the crude variable

[†]Model I: Crude analysis

[‡]Model II: Adjusted for total energy intake (standard method)

[§]Model III: Adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

[**] m/day: measures per day

7.4 Vitamin D and calcium

This analysis describes the distribution and correlation of vitamin D and calcium. In addition, the differences in crude and energy-adjusted nutrient intakes between cases and controls and the unadjusted and adjusted association between nutrient intakes and colorectal cancer are presented.

7.4.1 Descriptive analysis

7.4.1.1 Distribution of vitamin D and calcium intakes

Distributions of vitamin D and calcium were examined by looking at their histograms (original and transformed variables if skewed). The distributions of the nutrients under study were skewed and they were normalised either with square root or with logarithmic transformation (Table 86).

7.4.1.2 Distribution of vitamin D and calcium intakes by case control status

For crude nutrient intakes, cases reported statistically significant higher mean and median intakes of calcium (p-values: 0.0002 and 0.0005, respectively) than controls. After residual energy adjustment cases reported lower mean and median vitamin D intakes (p-values: 0.001 and 0.001 respectively) than controls (Table 87).

7.4.1.3 Correlations between vitamin D and calcium

The Spearman rank correlation coefficient (r) was used to test the correlation between vitamin D and calcium (Table 88) and they were found to be moderately correlated ($r<0.50$).

7.4.1.4 Main sources of vitamin D and calcium

The three main food sources (at individual food item level) were: 1) for vitamin D fried oily fish (22.9%), smoked oily fish (10.0%) and grilled, poached, baked or pickled oily fish (6.7%); and 2) for calcium: semi-skimmed milk (17.8%), full fat hard cheese (8.7%) and full fat milk (5.9%) (Table 89).

One thousand six hundred and sixty participants reported consumption of supplement products and 1,255 of them reported consumption of supplements that contributed to the daily intake of vitamin D (1,212 participants) and calcium (260 participants). The exact nutrient composition of these dietary supplements was identified and added to the dietary ones.

7.4.2 Associations between vitamin D, calcium and colorectal cancer risk

7.4.2.1 Main logistic regression models

In model I, intakes of calcium were positively associated with colorectal cancer (high vs. low intake: OR (95% CI), p-value: 1.33 (1.13, 1.57), 0.001) (Table 90). Associations between total intakes (from diet and supplements) of vitamin D and calcium and colorectal cancer were not examined in model I, since intake from supplements was added to the energy-adjusted nutrients. After energy adjustment (Model II), both dietary and total vitamin D intakes were significantly and inversely associated with colorectal cancer (high vs. low dietary intake: OR (95% CI), p-value for trend: 0.83 (0.70, 0.97), 0.01; high vs. low total intake: OR (95% CI), p-value for trend: 0.80 (0.68, 0.95), 0.003) (Table 90). Finally, in model III an inverse statistically significant association between dietary vitamin D and colorectal cancer (high vs. low dietary intake: OR (95% CI), p-value for trend: 0.83 (0.69, 0.99), 0.03) was observed, whereas association with total vitamin D and colorectal cancer was marginally not statistically significant (high vs. low total intake: OR (95% CI), p-value for trend: 0.88 (0.73, 1.06), 0.14) (Table 90).

7.4.2.2 Additional logistic regression models

The associations between vitamin D, calcium and colorectal cancer were tested in one additional model (Model IV). Model IV was corrected for the confounding factors of model III and further adjusted ω3PUFAs intake, since ω3PUFAs share the same main food source with vitamin D (Table 91). The inverse association between vitamin D intakes (dietary and total) and colorectal cancer was diluted and was no longer statistically significant after adjusting for ω3PUFAs intake (p- value for trend: 0.51 and 0.41 respectively) (Table 91).

7.4.2.3 Multiple testing corrections

Bonferroni correction for multiple testing

The adjusted level of significance after having controlled for multiple testing was: 1) 0.002 using the Bonferroni correction for 21 independent tests, 2) 0.006 using the Bonferroni correction for 8 tests conducted in hypothesis 4 and 3) 0.0005 for the individual compound analysis after having corrected for 93 tests conducted in all 4

hypotheses. Here we report only associations between dietary intakes and colorectal cancer. In model I, the association between colorectal cancer and calcium (p=0.001) remained significant at the first and second level of significance (Table 90).

FDR correction for multiple testing

After correcting for multiple testing using the FDR method by taking into account the number of tests that were conducted in hypothesis 4 (8 tests) or the number of tests that were conducted in all 4 hypotheses (93 tests in the individual compound analysis), associations between dietary intakes of calcium (p= 0.001; model I) and vitamin D (p=0.01; model II) remained statistically significant (Table 90).

7.4.2.4 Associations between colorectal cancer and main food sources of vitamin D and calcium

Intakes of the following food groups were tested: fried oily fish, smoked oily fish, semi-skimmed milk and full fat hard cheese. Results from model III, showed that comparison of highest versus lowest tertile intakes of these foods showed ORs for colorectal cancer risk of 0.74 (95% CI 0.61, 0.91; p-value for trend 0.20) for fried oily fish; 0.85 (95% CI 0.73, 1.00; p-value for trend 0.07) for smoked oily fish; 0.93 (95% CI 0.76, 1.14, p-value for trend 0.48); and 1.23 (95% CI 1.01, 1.49, p-value for trend 0.009) for full fat hard cheese (Table 92).

7.4.2.5 Associations between vitamin D, calcium and colorectal cancer after sex, age and cancer site stratification

Associations between vitamin D, calcium and colorectal cancer risk were tested after sex, age and cancer site stratification by applying model III (data not shown). Sex-specific associations were similar for dietary vitamin D intake and dietary and total calcium intakes, with dietary vitamin D being inversely, but not significantly associated with both male and female colorectal cancer (data not shown). In addition, total vitamin D intake was associated with a decreased colorectal cancer risk (marginally not statistically significant) for men, but not for women (data not shown). Regarding age-specific differences, high intake of dietary and total vitamin D was significantly and dose-dependently associated with a decreased risk of colorectal cancer for the individuals older than 55 years old (high vs. low dietary intake: OR (95% CI), p-value for trend: 0.80 (0.65, 0.99), 0.05), but not for the ones younger than 55 years old (data not shown). Finally, after cancer site stratification,

both colon and rectal cancer were similarly associated with vitamin D (dietary and total). Regarding calcium, high intakes of both dietary and total calcium were inversely but not statistically significantly associated only with rectal cancer (high vs. low intake: OR (95% CI), p-value: 0.83 (0.65, 1.07), 0.21) (data not shown).

7.4.2.6 Interaction relationships with variants of vitamin D receptor gene

The genotypic effect of four SNPs of *VDR* (*Fok*I (rs10735810), *Bsm*I (rs1544410), rs11568820 and *Apa*I (rs7975232)) on colorectal cancer risk was examined (data not shown). The variant allele frequencies in the control sample of three of the four SNPs (*Fok*I (rs10735810), *Apa*I (rs7975232) and rs11568820) were under Hardy-Weinberg equilibrium ($p>0.05$), but *Bsm*I (rs1544410) was not ($p= 0.01$).

The associations between colorectal cancer risk and each of the four SNPs were tested by applying one unadjusted and one simply adjusted (for age, sex and deprivation score) logistic regression model (data not shown). ORs and 95% CI for vitamin D and calcium dietary intakes were calculated in stratified groups according to the rs10735810, rs1544410, rs11568820 and rs7975232 genotypes by applying the multivariable adjusted model III (adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, dietary energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake) (data not shown). In addition, interaction associations were examined by investigating the combined effects of the genotypes and nutrient intakes. Interaction was tested by examining the deviance of two different nested models; an interactive model and its nested multiplicative one. The referent category used was homozygotes of the wild type allele being at greatest risk (low dietary nutrient intake).

None of the four examined SNPs was associated with colorectal cancer (data not shown). The inverse association between vitamin D and colorectal cancer was more profound for individuals of the rs10735810 CC genotype than for individuals of the CT or TT genotypes (data not shown). Furthermore, calcium intake was inversely though not significantly associated with colorectal cancer for the rs10735810 CC individuals, whereas it was positively associated for the TT individuals (data not shown). Finally, there was some evidence that rs10735810 interacts with dietary

vitamin D (p for interaction 0.06) and calcium intakes (p for interaction 0.13) (data not shown).

7.4.3 Summary of results

Significant dose-dependent associations were observed: 1) in model I: between colorectal cancer and dietary calcium (p=0.001); 2) in model II: between colorectal cancer and dietary vitamin D (p=0.01); 3) in model III: between colorectal cancer and dietary vitamin D (p=0.03) (Table 90). Regarding the analysis of the main food sources of vitamin D and calcium, there is some evidence in favour of a significant inverse association between colorectal cancer and intakes of fried and smoked oily fish and a positive association between colorectal cancer and intakes of full fat hard cheese (Table 92). In addition, none of the four examined SNPs was associated with colorectal cancer (data not shown). Finally, there was some evidence that rs10735810 interacts with vitamin D (p for interaction 0.06) and calcium dietary intakes (p for interaction 0.13) (data not shown).

Table 86 Vitamin D and calcium transformation

Nutrients included in the analysis	Transformation
Individual compounds	
Vitamin D	logarithmic
Calcium	logarithmic

Table 87 Descriptive report of crude and energy-adjusted intakes of vitamin D and calcium

Nutrients	All subjects (n=4837)		Cases (n=2061)		Controls (n=2776)		T-test	Wilcoxon rank test
	Mean (SD)	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*p-value*	*p-value*
Vitamin D (µg/day)	4.8 (3.7)	3.9 (2.5, 5.8)	4.8 (3.4)	3.9 (2.54, 5.8)	4.8 (3.9)	3.9 (2.5, 5.8)	0.53	0.44
Energy-adjusted vitamin D* (µg/day)	4.5 (2.7)	3.9 (2.7, 5.5)	4.3 (2.5)	3.8 (2.7, 5.4)	4.6 (2.8)	3.9 (2.8, 5.6)	0.007	0.009
Calcium (mg/day)	1158.3 (461.3)	1089.0 (840.0, 1391.0)	1183.3 (460.8)	1114.0 (860.0, 1424.0)	1139.7 (461.0)	1074.0 (824.0, 1365.5)	0.0002	0.0005
Energy-adjusted calcium (g/day)	1108.2 (270.6)	1091.1 (924.4, 1269.7)	1105.3 (260.9)	1091.5 (926.6, 1268.3)	1110.3 (277.6)	1091.0 (923.3, 1270.9)	0.53	0.81

Table 88 Spearman rank correlation coefficients between nutrients (p-values<$5x10^{-5}$)

Nutrients	vitamin D	calcium
vitamin D	1.00	
calcium	0.45	1.00

Table 89 Three main dietary (food) sources of vitamin D and calcium in our population

Nutrients	Main sources
Vitamin D	Fried oily fish (22.9%)
	Smoked oily fish (10.0%)
	Grilled, poached, baked or pickled oily fish (6.7%)
Calcium	Semi-skimmed milk (17.8%)
	Full fat hard cheese (8.7%)
	Full fat milk (5.9%)

* Logarithmic transformed values were used for calculating the t-test due to skewed distribution

Table 90 Association between vitamin D, calcium and colorectal cancer risk in the whole sample (3 main unconditional logistic regression models)

Nutrients	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Vitamin D	0-2.74	538	672	1.00		1.00		1.00	
(µg/day)	2.74-3.86	535	674	0.94	0.80, 1.11	0.99	0.84, 1.16	1.00	0.83, 1.20
	3.86-5.47	506	703	1.04	0.89, 1.23	0.90	0.77, 1.06	0.93	0.77, 1.11
	≥5.47	482	727	0.98	0.84, 1.16	0.83	0.70, 0.97	0.83	0.69, 0.99
	p-value for trend (quartiles)				0.83		0.01		0.03
	p-value for trend (continuous)				0.58		0.001		0.002
Vitamin D	0-3.03	528	682			1.00		1.00	
(total) (µg/day)	3.03-4.64	554	655			1.09	0.93, 1.28	1.06	0.88, 1.27
	4.64-7.48	515	694			0.96	0.82, 1.13	1.00	0.84, 1.20
	≥7.48	464	745			0.80	0.68, 0.95	0.88	0.73, 1.06
	p-value for trend (quartiles)						0.003		0.14
	p-value for trend (continuous)						$8.7x10^{-5}$		0.008
Calcium	0-924.53	511	699	1.00		1.00		1.00	
(mg/day)	924.53-1091.09	519	690	1.16	0.99, 1.36	1.03	0.88, 1.21	0.93	0.78, 1.12
	1091.09-1269.60	520	689	1.12	0.95, 1.32	1.03	0.88, 1.21	0.97	0.81, 1.17
	≥1269.60	511	698	1.33	1.13, 1.57	1.00	0.85, 1.18	0.96	0.80, 1.15
	p-value for trend (quartiles)				0.001		0.98		0.76
	p-value for trend (continuous)				0.001		0.53		0.53
Calcium	0-931.59	511	699			1.00		1.00	
(total) (mg/day)	931.59-1100.64	521	688			1.04	0.88, 1.22	0.94	0.78, 1.13
	1100.64-1284.65	530	679			1.07	0.91, 1.25	1.00	0.83, 1.20
	≥1284.65	499	710			0.96	0.82, 1.13	0.93	0.77, 1.12
	p-value for trend (quartiles)						0.74		0.59
	p-value for trend (continuous)						0.32		0.46

[*]Based on the distribution of the energy adjusted variable

[†]Model I: Crude analysis

[‡]Model I: Adjusted for total energy intake

[§]Model III: Adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

Table 91 Association between vitamin D, calcium and colorectal cancer risk in the whole sample (additional unconditional logistic regression models)

Nutrients	Quartiles*	Frequency		Model IV†	
		cases	*controls*	*OR*	*95% CI*
Vitamin D	0-2.74	538	672	1.00	
(µg/day)	2.74-3.86	535	674	1.05	0.87, 1.26
	3.86-5.47	506	703	1.04	0.86, 1.26
	≥5.47	482	727	1.10	0.86, 1.41
	p-value for trend (quartiles)				0.51
	p-value for trend (continuous)				0.71
Vitamin D	0-3.03	528	682	1.00	
(total) (µg/day)	3.03-4.64	554	655	1.13	0.94, 1.36
	4.64-7.48	515	694	1.14	0.94, 1.38
	≥7.48	464	745	1.09	0.88, 1.36
	p-value for trend (quartiles)				0.41
	p-value for trend (continuous)				0.66
Calcium	0-924.53	511	699	1.00	
(mg/day)	924.53-1091.09	519	690	0.95	0.79, 1.13
	1091.09-1269.60	520	689	1.00	0.84, 1.20
	≥1269.60	511	698	0.96	0.80, 1.16
	p-value for trend (quartiles)				0.87
	p-value for trend (continuous)				0.60
Calcium	0-931.59	511	699	1.00	
(total) (mg/day)	931.59-1100.64	521	688	0.95	0.80, 1.14
	1100.64-1284.65	530	679	1.03	0.86, 1.23
	≥1284.65	499	710	0.94	0.78, 1.13
	p-value for trend (quartiles)				0.70
	p-value for trend (continuous)				0.55

* Based on the distribution of the energy adjusted variable

† Model IV: Adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake, ω3PUFAs (energy adjusted)

Table 92 Association between fried oily fish, smoked oily fish, semi-skimmed milk and full fat hard cheese and colorectal cancer risk in the whole sample (3 main unconditional logistic regression models)

Food sources	Quartiles*	Frequency		Model I†		Model II‡		Model III§	
		cases	*controls*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Fried oily fish (m/day**)	0	1148	1524	1.00		1.00		1.00	
	0-0.14	659	832	1.05	0.92, 1.19	1.05	0.92, 1.19	1.09	0.94, 1.26
	>0.14	254	420	0.80	0.67, 0.95	0.73	0.61, 0.87	0.74	0.61, 0.91
	p-value for trend (quartiles)				0.20		0.05		0.20
	p-value for trend (continuous)				0.02		0.001		0.001
Smoked oily fish (m/day)	0	1258	1628	1.00		1.00		1.00	
	0-0.05	324	446	0.94	0.80, 1.10	0.95	0.81, 1.11	0.97	0.81, 1.16
	>0.05	479	702	0.88	0.77, 1.01	0.82	0.72, 0.95	0.85	0.73, 1.00
	p-value for trend (quartiles)				0.07		0.01		0.07
	p-value for trend (continuous)				0.003		$5.0x10^{-5}$		0.001
Semi-skimmed milk (m/day)	0	687	905	1.00		1.00		1.00	
	0-1	555	800	0.91	0.79, 1.06	0.94	0.81, 1.09	0.91	0.77, 1.08
	1-2	509	670	1.00	0.86, 1.16	1.00	0.86, 1.16	0.95	0.80, 1.12
	>2	310	401	1.02	0.85, 1.22	0.96	0.81, 1.15	0.93	0.76, 1.14
	p-value for trend (quartiles)				0.74		0.84		0.48
	p-value for trend (continuous)				0.78		0.71		0.30
Full fat hard cheese (m/day)	0-0.05	462	754	1.00		1.00		1.00	
	0.05-0.28	533	769	1.13	0.97, 1.33	1.13	0.96, 1.32	1.11	0.93, 1.33
	0.28-0.71	550	646	1.39	1.18, 1.63	1.34	1.14, 1.58	1.31	1.09, 1.57

>0.71	516	607	1.39	1.18, 1.64	1.26	1.07, 1.50	1.23	1.01, 1.49
p-value for trend (quartiles)				$6.2x10^{-6}$		0.001		0.009
p-value for trend (continuous)				0.04		0.68		0.85

[*]Based on the distribution of the energy adjusted variable

[†]Model I: Crude analysis

[‡]Model II: Adjusted for total energy intake, standard method

[§]Model III: Adjusted for age, sex, deprivation score, family history of cancer, BMI, physical activity, smoking, total energy intake (residual method), fibre intake (energy adjusted), alcohol intake (energy adjusted), NSAIDs intake

[**] m/d: measure per day

7.5 Summary of results of chapter 7

In this chapter the results of the unmatched analysis of the additional dietary risk factors (folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium) that comprised the last two hypotheses, were presented. In particular, one crude and three multivariable unconditional logistic regression models were applied in the whole sample, whereas one unconditional multivariable model adjusted for the main potential confounding factors was applied after sex, age and cancer site stratification.

7.5.1 Folate, vitamin B2, vitamin B6, vitamin B12 and alcohol

Inverse associations, which showed dose response relationships, were found in the energy-adjusted conditional logistic regression model (model II) between colorectal cancer risk and the dietary intakes of folate (p=0.003), vitamin B6 ($p=7.1x10^{-6}$) and vitamin B12 (p=0.02) (Table 84). After adjusting for the main potential confounding factors (model III), only the inverse associations between colorectal cancer and vitamin B12 (p=0.05) remained statistically significant (Table 84). Alcohol intake when divided in quartiles was associated with a decreased colorectal cancer risk, and the association was statistically significant in model III (p=0.03) (Table 84). However, when divided into categories, no association was observed for an intake of less than 60 g/day, whereas a positive not statistically significant association was observed for an alcohol intake of more than 60 g/day (Table 84). Regarding the analysis of the main food sources of folate, vitamin B6 and vitamin B12, results suggest that there is some evidence in favour of a significant inverse association between colorectal cancer and intakes of bananas (dietary source of vitamin B6) and fried oily fish (dietary source of vitamin B12) (Table 85). Finally, regarding the genetic analysis, none of the four examined SNPs was significantly associated with colorectal cancer risk (data not shown). Furthermore, there was no clear trend for the associations between colorectal cancer and folate, vitamin B2, vitamin B6, vitamin B12 and alcohol after stratification according to the aforementioned genotypes (data not shown).

7.5.2 Vitamin D and calcium

Regarding vitamin D, significant inverse dose-dependent associations were observed between colorectal cancer and dietary vitamin D in both models II (p=0.01) and III (p=0.03) (Table 90). In marked contrast, dietary and total calcium intakes were not associated with colorectal cancer risk in any of the adjusted models, whereas high dietary calcium intake was associated with a significant increased colorectal cancer risk (p=0.001) in model I (Table 90). Regarding the analysis of the main food sources of vitamin D and calcium, there is some evidence in favour of a significant inverse association between colorectal cancer and intakes of fried and smoked oily fish and a positive association between colorectal cancer and intakes of full fat hard cheese (Table 92). None of the four examined SNPs was associated with colorectal cancer (data not shown), but there was some evidence that rs10735810 interacts with vitamin D (p for interaction 0.06) and calcium dietary intakes (p for interaction 0.13) (data not shown).

8 RESULTS: Overall and stepwise regression analysis

8.1 Introduction

This analysis describes the overall analysis as well as the application of forward and backward stepwise regression. The study sample included in this analysis is the same as the sample that was included in the unmatched analysis of the additional dietary factors. Therefore, the presentation of the study sample will be omitted, since it has been described in detail in the first part of Chapter 7 (on page 260).

The explanatory variables that were included in the overall and stepwise regression models consist of demographic factors, lifestyle variables, foods and nutrients. In the first part of the chapter, distributions and correlations of all the explanatory variables, as well as univariable logistic regression of colorectal cancer on each explanatory variable are presented (overall analysis). In the second part of the chapter, results of the forward and backward stepwise regression applied in three different sets of explanatory variables are presented for the whole sample and separately for males and females.

8.2 Overall analysis

8.2.1 Distribution of explanatory variables by case control status

Numbers and percentages of all categorical explanatory variables as well as mean (with standard deviations) and median intakes (with interquartile ranges) of all continuous explanatory variables are presented in Table 93 and Table 94. The tests chi-square (categorical variables), t-test (continuous variables) and the Wilcoxon rank-sum (continuous variables) were used to test for differences between cases and controls.

Regarding the categorical explanatory variables, significant differences were observed for family history of colorectal cancer ($p<0.0005$), physical activity ($p=0.04$), NSAIDs intake ($p<0.0005$) and HRT intake ($p<0.0005$) (Table 93). Regarding the continuous explanatory variables, cases when compared to controls

reported higher mean intakes of dietary energy ($p<5x10^{-5}$), eggs ($p<5x10^{-5}$), sweets[1] (p=0.0001), fruit/ vegetable juice ($p<5x10^{-5}$), SFAs ($p<5x10^{-5}$), MUFAs (p=0.04), *t*FAs (p=0.002), *t*MUFAs (p=0.0004), cholesterol (p=0.0001), starch (p=0.04) and vitamin A (p=0.0001) (Table 94). In addition cases reported higher median intakes of dietary energy ($p<5x10^{-5}$), breads (p=0.05), eggs ($p<5x10^{-5}$), sweets ($p<5x10^{-5}$), fruit/ vegetable juice ($p<5x10^{-5}$), SFAs ($p<5x10^{-5}$), MUFAs (p=0.01), *t*FAs (p=0.0004), *t*MUFAs ($p<5x10^{-5}$), cholesterol ($p<5x10^{-5}$), starch (p=0.05) and vitamin A (p=0.0001) (Table 94).

On the other hand, cases when compared to controls reported lower mean intakes of oily fish ($p<5x10^{-5}$), fruits (p=0.006), vegetables ($p<5x10^{-5}$), savoury foods[2] (p=0.02), coffee (p=0.001), ω3PUFAs ($p<5x10^{-5}$), quercetin (p=0.0006), catechin (p=0.03), protein (p=0.004), fibre ($p<5x10^{-5}$), calcium ($p<5x10^{-5}$), magnesium ($p<5x10^{-5}$), phosphorus (p=0.0002), iron (p=0.0004), copper ($p<5x10^{-5}$), zinc (0.003), manganese ($p<5x10^{-5}$), selenium (p=0.0002), carotenes (p=0.0007), vitamin D (p=0.007), vitamin B1 (p=0.03), potential niacin (p=0.004), niacin ($p<5x10^{-5}$), vitamin B6 ($p<5x10^{-5}$), folate (0.0002), biotin ($p<5x10^{-5}$), vitamin C (p=0.001) (Table 94).

In addition cases reported lower median intakes of oily fish (p=0.0003), fruits (p=0.004), vegetables ($p<5x10^{-5}$), savoury foods (p=0.01), coffee (p=0.001), ω3PUFAs ($p<5x10^{-5}$), quercetin (p=0.001), catechin (p=0.008), phytoestrogens (p=0.04), protein (p=0.0004), fibre ($p<5x10^{-5}$), calcium ($p<5x10^{-5}$), magnesium ($p<5x10^{-5}$), phosphorus (p=0.0001), iron ($p<5x10^{-5}$), copper ($p<5x10^{-5}$), zinc (0.0001), manganese ($p<5x10^{-5}$), selenium (p=0.005), carotenes ($p<5x10^{-5}$), vitamin D (p=0.001), vitamin B1 (p=0.01), potential niacin (p=0.0006), niacin ($p<5x10^{-5}$), vitamin B6 ($p<5x10^{-5}$), vitamin B12 (p=0.02), folate (0.0003), pantothenic acid (p=0.006), biotin (p=0.0001), vitamin C (p=0.0001) (Table 94).

[1] Sweets: Summary variable of puddings and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

[2] Savoury foods: Summary variable of savoury foods, soups and sauces

Table 93 Descriptive report of all explanatory variables (categorical variables)

	All subjects (n=4837)		Cases (n=2061)		Controls (n=2776)		χ^2-test
Demographic factors	***Number***	***%***	***Number***	***%***	***Number***	***%***	***p-value***
Sex							
Males	2762	57.1%	1180	57.2%	1582	57.0%	
Females	2075	42.9%	881	42.8%	1194	43.0%	0.85
Family history*							
Low	4305	92.2%	1610	82.9%	2695	98.9%	
Medium/ high	363	7.8%	333	17.1%	30	1.1%	<0.0005
Deprivation score							
1	452	9.3%	194	9.4%	258	9.3%	
2	1002	20.7%	434	21.1%	568	20.5%	
3	1290	26.7%	532	25.8%	758	27.3%	
4	1133	23.4%	488	23.7%	645	23.2%	
5	511	10.6%	218	10.6%	293	10.6%	
6	318	6.6%	140	6.8%	178	6.4%	
7	130	2.7%	54	2.6%	76	2.7%	0.95
Lifestyle variables							
Smoking							
Never	2074	43.3%	874	42.9%	1200	43.5%	
Former	1867	38.9%	818	40.2%	1049	38.0%	
Current	852	17.8%	343	16.9%	509	18.5%	0.20
Alcohol (g/day)							
0	718	14.8%	291	14.1%	427	15.4%	
0-15	2598	53.7%	1125	54.6%	1473	53.1%	
15-30	941	19.4%	393	19.1%	548	19.7%	
30-45	341	7.1%	139	6.7%	202	7.3%	
45-60	142	2.9%	61	3.0%	81	2.9%	
>60	97	2.0%	52	2.5%	45	1.6%	0.20
Physical activity (hours/week)							
0	2595	53.6%	1139	55.3%	1456	52.4%	
0-3.5	1189	24.6%	486	23.6%	703	25.3%	
3.5-7	544	11.2%	205	9.9%	339	12.2%	
>7	318	6.6%	133	6.5%	185	6.7%	0.04
NSAIDs							
No	3206	66.3%	1449	70.3%	1757	63.3%	
Yes	1605	33.2%	605	29.4%	1000	36.0%	<0.0005
HRT†							
No	1487	73.6%	666	77.8%	821	70.5%	
Yes	533	26.4%	190	22.2%	343	29.5%	<0.0005

* High/moderate vs. low family history risk

† HRT: Hormone Replacement Therapy; Intake of 6 months or more vs. no intake/ intake of less than 6 months

Table 94 Descriptive report of all explanatory variables (continuous variables)

	All subjects (n=4837)		Cases (n=2061)		Controls (n=2776)		t test	rank test
Demographic factors	***Mean (SD)***	***Median (IQR)***	***Mean (SD)***	***Median (IQR)***	***Mean (SD)***	***Median (IQR)***	***p-value***	***p-value***
Age	62.2 (10.6)	63.0 (55.0, 71.0)	62.0 (10.8)	63.0 (54.0, 71.0)	62.4 (10.5)	63.0 (55.0, 71.0)	0.14	0.25
Lifestyle variables								
BMI	26.7 (4.5)	26.1 (23.7, 29.1)	26.6 (4.4)	26.1 (23.6, 29.0)	26.7 (4.6)	26.1 (23.7, 29.1)	0.41	0.68
Dietary energy (MJ/day)	10.9 (4.1)	10.2 (8.2, 12.7)	11.3 (4.4)	10.5 (8.4, 13.1)	10.7 (3.9)	9.9 (8.1, 12.5)	$<5x10^{-5}$	$<5x10^{-5}$
Foods (m/day[*])								
Breads	2.8 (1.3)	2.7 (1.9, 3.6)	2.8 (1.2)	2.7 (1.9, 3.6)	2.7 (1.3)	2.6 (1.8, 3.6)	0.15	0.05
Cereals[†]	1.2 (0.9)	1.1 (0.4, 1.7)	1.2 (0.9)	1.0 (0.5, 1.6)	1.2 (1.0)	1.1 (0.4, 1.8)	0.21	0.78
Milk	2.0 (1.2)	1.9 (1.1, 2.4)	2.0 (1.3)	1.9 (1.1, 2.5)	1.9 (1.2)	1.8 (1.1, 2.4)	0.45	0.46
Cream†	0.5 (0.6)	0.3 (0.0, 0.8)	0.5 (0.6)	0.3 (0.0, 0.8)	0.5 (0.7)	0.3 (0.0, 0.8)	0.21	0.47
Cheese†	0.8 (0.8)	0.6 (0.3, 1.1)	0.8 (0.7)	0.6 (0.3, 1.1)	0.8 (0.8)	0.6 (0.3, 1.1)	0.59	0.16
Eggs†	0.5 (0.4)	0.4 (0.2, 0.7)	0.5 (0.4)	0.5 (0.2, 0.7)	0.5 (0.4)	0.4 (0.2, 0.6)	$<5x10^{-5}$	$<5x10^{-5}$
Poultry†	0.4 (0.3)	0.3 (0.2, 0.6)	0.4 (0.3)	0.3 (0.2, 0.5)	0.4 (0.4)	0.3 (0.1, 0.6)	0.16	0.12
Red meat†	1.3 (0.8)	1.3 (0.8, 1.7)	1.4 (0.8)	1.3 (0.8, 1.7)	1.3 (0.8)	1.2 (0.8, 1.8)	0.14	0.20
Processed meat†	1.0 (0.8)	0.9 (0.5, 1.4)	1.0 (0.7)	0.9 (0.5, 1.4)	1.0 (0.8)	0.9 (0.5, 1.4)	0.63	0.72
White fish†	0.3 (0.3)	0.3 (0.2, 0.5)	0.4 (0.3)	0.3 (0.2, 0.5)	0.3 (0.3)	0.3 (0.1, 0.5)	0.25	0.09
Oily fish†	0.2 (0.3)	0.1 (0.0, 0.3)	0.2 (0.3)	0.1 (0.0, 0.3)	0.2 (0.3)	0.1 (0.0, 0.3)	$<5x10^{-5}$	0.0003
Potatoes/ Pasta/ Rice	2.4 (1.0)	2.3 (1.7, 2.9)	2.4 (1.0)	2.3 (1.7, 2.9)	2.4 (1.0)	2.3 (1.7, 2.9)	0.96	0.77
Fruit†	2.9 (2.2)	2.5 (1.4, 3.8)	2.8 (2.1)	2.4 (1.4, 3.7)	3.0 (2.2)	2.5 (1.5, 4.0)	0.006	0.004
Vegetables†	5.6 (3.4)	4.9 (3.3, 7.0)	5.2 (3.1)	4.6 (3.1, 6.5)	5.9 (3.6)	5.1 (3.4, 7.4)	$<5x10^{-5}$	$<5x10^{-5}$
Savoury†[‡]	2.8 (1.4)	2.5 (1.8, 3.4)	2.7 (1.4)	2.5 (1.8, 3.3)	2.8 (1.5)	2.6 (1.8, 3.5)	0.02	0.01
Sweets[§]	4.7 (2.5)	4.4 (3.0, 6.1)	4.9 (2.5)	4.3 (2.9, 6.0)	4.6 (2.5)	4.6 (3.1, 6.3)	0.0001	$<5x10^{-5}$

Tea**	2.7 (1.8)	3.0 (1.0, 4.0)	2.6 (1.8)	3.0 (1.0, 4.0)	2.7 (1.8)	3.0 (1.0, 4.0)	0.70	0.55
Coffee**	1.6 (1.7)	1.0 (0.1, 3.0)	1.5 (1.7)	1.0 (0.0, 2.4)	1.7 (1.7)	1.0 (0.1, 3.0)	0.001	0.001
fruit/ vegetable juice †	1.0 (1.1)	0.8 (0.1, 1.4)	1.1 (1.2)	0.9 (0.2, 1.6)	1.0 (1.1)	0.8 (0.1, 1.4)	$<5x10^{-5}$	$<5x10^{-5}$
Fizzy drinks†	0.4 (0.8)	0.0 (0.0, 0.4)	0.4 (0.8)	0.0 (0.0, 0.4)	0.3 (0.8)	0.0 (0.0, 0.4)	0.11	0.13
Fatty acids (g/day)								
SFAs	37.4 (9.1)	37.0 (31.5, 43.3)	38.1 (8.7)	37.7 (32.3, 43.9)	36.9 (9.3)	36.6 (30.9, 42.7)	$<5x10^{-5}$	$<5x10^{-5}$
MUFAs	32.3 (6.1)	32.6 (28.6, 36.2)	32.5 (5.7)	32.8 (29.0, 36.3)	32.1 (6.4)	32.4 (28.2, 36.1)	0.04	0.01
ω6PUFAs	11.3 (3.5)	10.7 (8.9, 13.2)	11.4 (3.6)	10.7 (8.9, 13.2)	11.3 (3.5)	10.7 (8.9, 13.1)	0.58	0.81
ω3PUFAs	2.4 (0.9)	2.2 (1.8, 2.7)	2.3 (0.8)	2.2 (1.8, 2.7)	2.4 (0.9)	2.3 (1.8, 2.8)	$<5x10^{-5}$	$<5x10^{-5}$
*t*FAs	3.6 (1.1)	3.5 (2.9, 4.2)	3.7 (1.1)	3.6 (3.0, 4.3)	3.6 (1.2)	3.5 (2.8, 4.2)	0.002	0.0004
*t*MUFAs	2.7 (0.8)	2.7 (2.2, 3.2)	2.8 (0.8)	2.7 (2.2, 3.2)	2.7 (0.8)	2.6 (2.1, 3.2)	0.0004	$<5x10^{-5}$
Flavonoids (mg/day)								
Quercetin	17.2 (7.7)	17.4 (11.2, 22.6)	16.8 (7.5)	16.9 (10.9, 22.1)	17.5 (7.8)	17.6 (11.4, 22.1)	0.0006	0.001
Catechin†	7.4 (3.8)	7.2 (4.8, 9.4)	7.3 (3.8)	7.0 (4.6, 9.1)	7.5 (3.8)	7.4 (4.9, 9.5)	0.03	0.008
Epicatechin	22.9 (11.9)	23.3 (12.7, 32.3)	22.6 (11.6)	23.0 (12.6, 31.8)	23.1 (12.0)	23.6 (12.8, 32.8)	0.12	0.13
Flavones	1.3 (1.2)	1.0 (0.5, 1.8)	1.3 (1.2)	1.0 (0.5, 1.8)	1.3 (1.1)	1.0 (0.5, 1.8)	n/a	0.55
Procyanidins	30.8 (17.3)	31.7 (16.0, 44.7)	30.3 (17.0)	31.3 (15.8, 43.6)	31.1 (17.5)	32.0 (16.1, 45.5)	0.09	0.09
Flavanones†	29.1 (31.1)	20.6 (7.4, 40.6)	28.3 (29.8)	20.2 (8.5, 39.1)	29.7 (32.0)	20.9 (6.7, 41.2)	0.55	0.82
Phytoestrogens†† (μg/day)	1058.1 (3644.0)	575.3 (400.0, 846.2)	917.6 (2311.9)	561.5 (397.8, 821.6)	1162.4 (4375.9)	585.5 (401.7, 869.8)	0.15	0.04
Macronutrients (g/day)								
Protein	101.3 (17.5)	101.3 (90.6, 112.3)	100.5 (17.1)	100.3 (89.9, 111.0)	101.9 (17.7)	102.1 (91.3, 113.1)	0.004	0.0004
Cholesterol	369.2 (111.8)	362.0 (296.2, 430.1)	376.5 (107.3)	367.2 (306.7, 438.3)	363.7 (114.8)	358.3 (290.2, 424.6)	0.0001	$<5x10^{-5}$
Sugars††	137.5 (46.1)	132.7 (109.4, 158.5)	136.8 (41.8)	132.2 (109.9, 157.8)	138.0 (43.9)	132.9 (109.2, 159.6)	0.56	0.63
Starch	163.3 (33.2)	164.6 (143.3, 184.5)	164.4 (31.0)	165.6 (145.5, 184.1)	162.5 (34.8)	163.7 (141.7, 184.9)	0.04	0.05
Fibre	21.4 (6.0)	21.0 (17.3, 24.9)	20.9 (5.7)	20.5 (17.1, 24.4)	21.7 (6.2)	21.3 (17.6, 25.4)	$<5x10^{-5}$	$<5x10^{-5}$

Minerals (mg/day)								
Sodium	3462.8	3450.9	3466.7	3470.4	3459.9	3436.5	0.72	0.50
	(638.9)	(3065.8, 3849.0)	(618.5)	(3086.1, 3843.3)	(653.8)	(3048.3, 3853.6)		
Potassium	4284.2	4274.8	4208.5	4185.4	4340.3	4331.9	$<5x10^{-5}$	$<5x10^{-5}$
	(793.4)	(3789.2, 4760.3)	(751.3)	(3747.7, 4668.4)	(818.8)	(3827.6, 4830.8)		
Calcium	1108.2	1091.1	1105.3	1091.5	1110.3	1091.0	0.53	0.80
	(270.6)	(924.4, 1269.7)	(260.9)	(926.6, 1268.3)	(277.6)	(923.3, 1270.9)		
Magnesium	384.6 (67.7)	383.3 (340.3, 428.4)	376.8 (64.4)	375.6 (335.4, 418.3)	390.4 (69.6)	390.2 (344.6, 334.3)	$<5x10^{-5}$	$<5x10^{-5}$
Phosphorus	1748.1	1750.5	1730.9	1728.0	1760.9	1763.3	0.0002	0.0001
	(280.8)	(1573.9, 1931.4)	(270.1)	(1561.4, 1906.7)	(287.9)	(1579.2, 1947.8)		
Iron	15.4 (2.9)	15.3 (13.6, 17.1)	15.2 (2.8)	15.1 (13.3, 16.9)	15.5 (2.9)	15.4 (13.7, 17.3)	0.0004	$<5x10^{-5}$
Copper	1.6 (0.4)	1.5 (1.4, 1.8)	1.6 (0.3)	1.5 (1.3, 1.7)	1.6 (0.4)	1.6 (1.4, 1.8)	$<5x10^{-5}$	$<5x10^{-5}$
Zinc	12.0 (2.4)	11.3 (10.5, 13.4)	11.9 (2.3)	11.7 (10.4, 13.2)	12.1 (2.4)	12.1 (10.6, 13.5)	0.003	0.0001
Chloride	5290.6	5285.5	5288.5	5297.4	5292.2	5276.0	0.90	0.90
	(957.7)	(4705.0, 5872.5)	(930.1)	(4726.2, 5858.0)	(977.9)	(4691.3, 5883.6)		
Manganese	3.9 (1.2)	3.8 (3.0, 4.7)	3.8 (1.2)	3.7 (3.0, 4.5)	4.0 (1.2)	3.9 (3.1, 4.7)	$<5x10^{-5}$	$<5x10^{-5}$
Selenium (µg/day)	83.1 (32.4)	79.0 (63.2, 96.4)	81.1 (29.4)	78.0 (61.8, 95.0)	84.6 (34.4)	79.9 (63.9, 97.5)	0.0002	0.005
Iodine†† (µg/day)	202.9 (77.8)	190.2 (149.8, 240.7)	200.3 (73.7)	188.6 (149.8, 237.7)	204.7 (80.6)	191.4 (149.8, 243.5)	0.19	0.19
Vitamins (mg/day)								
Vitamin A†† (Retinol)	642.3	496.5	654.6	518.1	633.2	480.4	0.0001	0.0001
(µg/day)	(530.2)	(348.1, 752.6)	(482.9)	(363.4, 758.0)	(562.6)	(336.1, 749.3)		
Carotenes†† (µg/day)	3973.6	3519.5	3800.7	3352.8	4102.1	3628.4	0.0007	$<5x10^{-5}$
	(2399.3)	(2405.8, 4953.3)	(2237.6)	(2330.1, 4712.9)	(2505.4)	(2464.1, 127.5)		
Vitamin D†† (µg/day)	4.5 (2.7)	3.9 (2.7, 5.5)	4.3 (5.5)	3.8 (2.7, 5.4)	4.6 (2.8)	3.9 (2.8, 5.6)	0.007	0.001
Vitamin E††	9.1 (3.5)	8.3 (6.8, 10.4)	9.1 (3.5)	8.2 (6.8, 10.4)	9.1 (3.4)	8.3 (6.8, 10.5)	0.87	0.54
Vitamin $B1^{††}$ (Thiamine)	2.1 (0.8)	2.0 (1.8, 2.3)	2.1 (0.6)	2.0 (1.8, 2.3)	2.1 (0.8)	2.0 (1.8, 2.3)	0.03	0.01

Vitamin B2	2.1 (0.5)	2.1 (1.8, 2.4)	2.1 (0.5)	2.1 (1.8, 2.4)	2.1 (0.5)	2.1 (1.8, 2.4)	0.09	0.13
Potential niacin	21.1 (3.6)	21.1 (18.8, 23.4)	20.9 (3.5)	20.9 (18.7, 23.1)	21.2 (3.7)	21.3 (19.9, 23.5)	0.004	0.0006
Niacin	24.2 (5.3)	24.0 (20.6, 27.5)	23.8 (5.3)	23.5 (20.3, 26.9)	24.5 (5.4)	24.3 (20.8, 27.9)	$<5x10^{-5}$	$<5x10^{-5}$
Vitamin B6	2.8 (0.6)	2.8 (2.5, 3.2)	2.8 (0.5)	2.8 (2.4, 3.1)	2.9 (0.6)	2.9 (2.5, 3.2)	$<5x10^{-5}$	$<5x10^{-5}$
Vitamin B12†† (µg/day)	7.7 (3.6)	7.0 (5.3, 9.2)	7.5 (3.4)	6.9 (5.2, 9.0)	7.8 (3.7)	7.0 (5.3, 9.4)	0.06	0.02
Folic acid (µg/day)	329.4 (71.5)	325.9 (282.6, 370.8)	324.9 (68.2)	321.3 (280.5, 365.7)	332.7 (73.6)	328.9 (283.8, 374.7)	0.0002	0.0003
Pantothenic acid	7.8 (5.3)	6.6 (5.9, 7.6)	7.8 (5.5)	6.6 (5.8, 7.5)	7.8 (5.2)	6.7 (5.9, 7.6)	0.26	0.006
Biotin (µg/day)	49.6 (10.0)	49.3 (43.4, 55.7)	48.9 (9.7)	48.9 (42.9, 55.0)	50.2 (10.3)	49.8 (43.6, 56.1)	$<5x10^{-5}$	0.0001
Vitamin C	125.3 (63.1)	114.2 (81.2, 156.1)	120.5 (58.5)	111.2 (78.8, 147.8)	128.9 (66.2)	117.6 (83.0, 161.4)	0.001	0.0001

* m/d: measures per day

† Square root transformed values were used for calculating the t-test due to skewed distribution

‡ Savoury foods: Summary variable of savoury foods, soups and sauces

§Sweets: Summary variable of pudding and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

** Not energy adjusted

†† Logarithmic transformed values were used for calculating the t-test due to skewed distribution

8.2.2 Correlation matrix for the explanatory variables

Correlation coefficients that were ≥0.70 are highlighted in Table 95 (divided in three categories: 0.70-0.80, 0.80-0.90, ≥0.90) The variables that were highly correlated were: 1) oily fish consumption with ω3PUFAs and vitamin D intakes; ω3PUFAs with vitamin D intakes; and vitamin D with vitamin B12 intakes; 2) tea consumption with quercetin, epicatechin and procyanidin intakes; quercetin with epicatechin and procyanidin intakes; catechin with epicatechin and procyanidin intakes; and epicatechin with procyanidin intakes 3) vegetable consumption with carotene intakes; 4) fruit consumption with vitamin C intakes; and vitamin C with flavanone intakes; 5) SFA with *t*FA intakes, and *t*FA with *t*MUFA intakes; 6) protein with phosphorus, zinc, potential niacin and niacin intakes; magnesium with phosphorus and iron intakes; phosphorus with zinc, vitamin B2 and potential niacin intakes; and zinc with potential niacin intakes 7) fibre with potassium and magnesium intakes; potassium with magnesium, vitamin B6 and folic acid intakes; vitamin B6 with folic acid intakes; and vitamin B6 with thiamine intakes; 8) sodium with chloride intakes; 9) calcium with phosphorus and vitamin B2 intakes (Table 95).

Table 95 Correlation matrix of the explanatory variables (demographic factors, lifestyle factors, foods and nutrients)

Variables	age	deprivat.	alcohol	BMI	energy	breads	cereals	milk	cream	cheese	eggs
age	1.00										
deprivation	-0.06	1.00									
alcohol	-0.14	-0.08	1.00								
BMI	-0.04	0.10	0.01	1.00							
energy	-0.11	0.02	0.21	0.08	1.00						
breads	0.10	0.04	-0.07	0.03	0.07	1.00					
cereals	0.18	-0.03	-0.13	-0.02	0.03	-0.03	1.00				
milk	0.03	0.00	-0.13	-0.01	0.02	0.02	0.24	1.00			
cream	-0.03	-0.12	-0.07	0.00	0.04	-0.12	0.13	0.07	1.00		
cheese	0.00	-0.10	0.05	-0.03	0.04	-0.04	-0.03	0.01	0.12	1.00	
eggs	0.11	0.09	0.04	0.06	0.00	0.04	-0.06	0.01	-0.07	0.06	1.00
poultry	-0.20	-0.06	0.06	0.05	0.02	-0.10	-0.02	-0.03	0.09	-0.02	-0.08
processed meat	-0.18	0.10	0.07	0.13	0.04	0.09	-0.14	-0.07	-0.11	-0.04	0.11
red meat	0.00	0.08	0.09	0.11	0.04	0.00	-0.17	-0.09	-0.18	-0.04	0.24
white fish	0.19	-0.01	-0.02	0.01	0.02	-0.05	0.08	-0.01	0.05	0.02	0.11
oily fish	0.16	-0.11	0.08	-0.05	0.05	-0.10	0.09	-0.04	0.16	0.08	0.02
potatoes/pasta/rice	-0.12	0.02	0.05	0.02	0.07	-0.02	-0.07	-0.08	-0.11	-0.07	-0.05
savoury	-0.10	-0.04	0.07	0.00	0.05	-0.10	-0.05	-0.07	0.03	0.04	-0.05
sweets	0.10	0.00	-0.21	0.02	0.08	-0.12	-0.05	-0.06	-0.02	-0.02	-0.08
tea	0.10	0.02	-0.10	-0.07	0.10	0.11	0.08	0.10	-0.03	-0.05	0.02
coffee	-0.06	-0.11	0.12	0.02	0.08	-0.07	-0.04	0.03	0.09	0.13	-0.04
fruit/ vegetable juice	-0.07	-0.09	-0.01	0.02	0.04	-0.06	0.06	0.03	0.15	0.05	-0.08
fizzy drinks	-0.24	0.09	0.03	0.18	0.05	-0.07	-0.11	-0.10	0.01	-0.06	0.00
vegetables	-0.10	-0.08	0.01	0.00	0.04	-0.14	-0.01	-0.08	0.14	0.05	-0.11
fruit	0.10	-0.10	-0.17	-0.02	0.01	-0.12	0.17	0.02	0.23	0.01	-0.16
SFAs	0.05	0.01	-0.10	-0.04	0.17	0.04	-0.13	0.11	-0.01	0.27	0.18

Variables	age	deprivat.	alcohol	BMI	energy	breads	cereals	milk	cream	cheese	eggs
MUFAs	-0.01	0.01	-0.05	-0.01	0.23	0.06	-0.18	-0.04	-0.05	0.17	0.22
ω6PUFAs	-0.12	-0.01	-0.04	0.00	0.15	0.11	-0.06	-0.13	-0.01	0.00	0.01
ω3PUFAs	0.00	-0.10	0.04	-0.01	0.13	-0.07	0.00	-0.11	0.14	0.08	0.01
*t*FAs	0.00	-0.01	-0.09	-0.07	0.13	0.15	-0.11	0.09	-0.01	0.22	0.11
*t*MUFAs	0.11	-0.02	-0.07	-0.07	0.13	0.26	-0.06	0.14	0.01	0.30	0.17
Quercetin	0.13	-0.03	-0.10	-0.06	0.03	0.03	0.08	0.05	-0.01	-0.04	-0.05
Catechin	0.00	-0.13	0.21	-0.10	0.04	-0.08	0.04	0.00	0.06	0.01	-0.10
Epicatechin	0.09	-0.03	-0.09	-0.08	0.02	0.06	0.09	0.08	-0.03	-0.06	-0.05
Flavones	0.10	-0.03	0.03	0.01	0.36	-0.05	0.05	-0.06	0.10	0.06	-0.03
Procyanidins	0.10	-0.04	0.01	-0.07	0.03	0.07	0.08	0.07	-0.03	-0.03	-0.02
Flavanones	0.01	-0.09	-0.04	0.00	0.02	-0.06	0.09	0.00	0.14	0.05	-0.09
Phytoestrogens	0.09	-0.04	-0.09	-0.01	0.01	0.59	0.03	-0.01	0.03	0.01	-0.05
Protein	0.10	0.02	-0.10	-0.07	0.10	0.11	0.08	0.10	-0.03	-0.05	0.02
Cholesterol	0.14	0.06	0.01	0.04	0.11	-0.02	-0.12	0.04	-0.05	0.13	**0.73***
Sugars	-0.03	-0.05	-0.17	0.03	0.12	-0.18	0.12	0.13	0.23	-0.10	-0.20
Starch	0.04	0.04	-0.16	0.03	0.17	0.47	0.20	-0.05	-0.18	-0.13	-0.08
Fibre	0.01	-0.11	-0.14	-0.02	0.11	0.04	0.24	-0.04	0.13	-0.02	-0.22
Na	-0.02	0.05	-0.02	0.09	0.21	0.32	0.11	0.03	-0.05	0.10	0.16
K	-0.03	-0.11	-0.02	0.00	0.17	-0.16	0.14	0.18	0.19	-0.02	-0.15
Ca	0.03	-0.08	-0.15	-0.03	0.13	0.00	0.22	0.67	0.33	0.36	0.00
Mg	-0.08	-0.13	0.06	-0.02	0.18	-0.01	0.27	0.18	0.19	0.04	-0.18
P	0.00	-0.09	-0.07	0.02	0.21	-0.05	0.26	0.44	0.30	0.24	0.03
Fe	0.05	-0.11	0.05	-0.02	0.18	0.05	0.44	-0.03	0.10	-0.01	-0.06
Cu	-0.13	-0.08	0.11	0.04	0.18	-0.02	0.01	-0.14	0.04	0.00	-0.09
Zn	0.01	-0.05	-0.01	0.05	0.18	-0.01	0.13	0.17	0.10	0.12	0.07
Cl	-0.03	0.04	-0.01	0.08	0.21	0.35	0.13	0.06	-0.03	0.11	0.14
Mn	0.12	-0.11	-0.12	-0.06	0.08	0.25	0.22	0.02	0.09	0.00	-0.18

Variables	age	deprivat.	alcohol	BMI	energy	breads	cereals	milk	cream	cheese	eggs
Se	0.00	-0.01	-0.02	0.04	0.09	0.27	-0.04	-0.03	0.05	0.06	0.08
I	0.11	-0.08	-0.06	-0.01	0.08	-0.11	0.14	0.31	0.39	0.14	0.15
Retinol	0.12	-0.03	-0.02	-0.02	0.08	0.01	-0.06	0.12	0.06	0.21	0.28
Carotenes	-0.06	-0.10	-0.01	-0.01	0.07	-0.12	0.00	-0.06	0.12	0.04	-0.11
Vitamin D	0.12	-0.06	0.02	-0.02	0.07	-0.08	0.11	-0.02	0.09	0.06	0.19
Vitamin E	-0.07	-0.04	-0.11	-0.02	0.12	-0.03	0.06	-0.06	0.07	0.02	-0.07
Thiamine	0.10	-0.04	-0.13	0.04	0.17	0.13	0.36	0.11	0.03	-0.08	-0.05
Vitamin B2	0.03	-0.06	-0.10	-0.01	0.12	-0.09	0.45	0.62	0.33	0.12	0.06
Pot Niacin	-0.04	-0.03	0.01	0.08	0.21	0.00	0.05	0.22	0.13	0.21	0.18
Niacin	-0.13	-0.04	0.09	0.08	0.15	0.05	0.22	-0.03	0.04	-0.03	-0.08
Vitamin B6	-0.01	-0.05	0.00	0.04	0.18	-0.10	0.31	0.09	0.06	-0.05	-0.09
Vitamin B12	0.13	-0.06	0.03	0.01	0.08	-0.12	0.10	0.17	0.14	0.12	0.18
Folic acid	0.03	-0.09	-0.03	-0.01	0.15	0.05	0.36	0.13	0.14	0.06	-0.03
Pantoth. acid	0.06	-0.05	0.15	0.02	0.15	-0.03	0.14	0.27	0.04	0.01	0.15
Biotin	0.10	-0.12	0.08	-0.05	0.13	-0.08	0.25	0.29	0.17	0.09	0.20
Vitamin C	-0.02	-0.14	-0.08	-0.02	0.04	-0.15	0.10	-0.02	0.22	0.05	-0.17

Variables	poultry	process. meat	red meat	white fish	oily fish	potatoes pasta rice	savoury	sweets	tea	coffee	fruit/ vegetable juice	fizzy	Vegs	fruit
poultry	1.00													
processed meat	0.03	1.00												
red meat	-0.02	0.41	1.00											
white fish	0.04	-0.02	0.04	1.00										
oily fish	0.06	-0.13	-0.09	0.27	1.00									
potatoes/pasta/rice	0.09	0.09	0.13	-0.01	-0.11	1.00								
savoury	0.06	0.01	-0.01	0.00	0.01	0.15	1.00							
sweets	-0.12	-0.06	-0.13	-0.09	-0.14	-0.18	-0.13	1.00						
tea	-0.05	-0.03	0.00	0.05	0.02	0.00	-0.01	0.05	1.00					
coffee	0.06	-0.04	-0.04	-0.02	0.07	-0.03	0.04	0.00	-0.43	1.00				
fruit/ vegetable juice	0.04	-0.07	-0.10	0.01	0.08	-0.04	0.05	-0.01	-0.09	0.06	1.00			
fizzy drinks	0.06	0.14	0.04	-0.08	-0.12	0.01	0.00	0.02	-0.13	0.01	-0.02	1.00		
vegetables	0.18	-0.11	-0.15	0.04	0.16	0.12	0.35	-0.19	0.00	0.08	0.11	-0.05	1.00	
fruit	0.09	-0.23	-0.28	0.08	0.20	-0.13	0.03	-0.01	0.03	0.03	0.20	-0.09	0.35	1.00
SFAs	-0.18	0.11	0.24	-0.05	-0.12	-0.12	-0.12	0.27	0.03	0.02	-0.09	-0.08	-0.29	-0.26
MUFAs	-0.07	0.20	0.30	0.06	0.08	-0.08	-0.03	0.19	0.03	0.03	-0.10	-0.09	-0.18	-0.26
ω6PUFAs	0.08	0.12	0.02	-0.01	-0.03	0.01	0.14	0.10	0.00	0.04	0.00	-0.03	0.10	-0.06
ω3PUFAs	0.21	-0.01	0.00	0.29	**0.70***	0.00	0.21	-0.16	0.02	0.07	0.05	-0.12	0.38	0.17
*t*FAs	-0.17	0.11	0.18	-0.10	-0.17	-0.08	-0.08	0.14	0.00	0.03	-0.07	-0.05	-0.27	-0.26
*t*MUFAs	-0.19	0.09	0.20	-0.05	-0.12	-0.06	-0.05	0.01	0.06	0.02	-0.06	-0.13	-0.23	-0.24
Quercetin	0.01	-0.10	-0.05	0.06	0.08	0.02	0.15	-0.06	**0.80****	-0.32	-0.03	-0.15	0.29	0.25
Catechin	0.06	-0.14	-0.13	0.03	0.13	-0.06	0.02	-0.05	0.58	-0.18	0.06	-0.13	0.17	0.32
Epicatechin	-0.03	-0.08	-0.07	0.03	0.03	-0.04	-0.03	0.06	**0.87****	-0.37	-0.02	-0.13	0.04	0.20
Flavones	0.04	-0.08	-0.04	0.10	0.13	0.01	0.35	-0.08	0.06	0.05	0.06	-0.04	0.29	0.16

Variables	poultry	process. meat	red meat	white fish	oily fish	potatoes pasta rice	savoury	sweets	tea	coffee	fruit/ vegetable juice	fizzy	Vegs	fruit
Procyanidins	-0.01	-0.05	-0.04	0.05	0.05	-0.01	-0.01	-0.01	**0.87****	-0.35	-0.08	-0.14	0.06	0.15
Flavanones	0.08	-0.13	-0.14	0.05	0.14	-0.07	0.03	-0.05	-0.04	0.07	0.47	-0.06	0.23	0.55
Phytoestrogens	-0.06	-0.05	-0.15	-0.02	0.02	-0.06	0.01	-0.11	0.07	-0.02	0.01	-0.12	0.06	0.07
Protein	0.33	0.17	0.37	0.31	0.25	0.13	0.19	-0.30	0.06	0.03	-0.03	-0.15	0.25	0.06
Cholesterol	0.01	0.19	0.47	0.20	0.08	-0.04	-0.04	-0.05	0.04	-0.02	-0.11	-0.08	-0.16	-0.22
Sugars	0.01	-0.17	-0.31	-0.07	-0.05	-0.18	0.01	0.29	-0.03	0.02	0.23	0.25	0.14	0.55
Starch	-0.04	0.08	-0.05	-0.05	-0.22	0.48	0.04	0.08	0.12	-0.08	-0.10	-0.10	-0.07	-0.15
Fibre	0.11	-0.21	-0.31	0.03	0.10	0.16	0.26	-0.11	0.06	0.05	0.13	-0.16	0.60	0.59
Na	0.03	0.31	0.20	0.15	0.01	0.00	0.31	-0.12	0.07	-0.02	-0.04	-0.08	0.10	-0.13
K	0.18	-0.15	-0.14	0.12	0.16	0.28	0.26	-0.24	0.08	0.11	0.16	-0.18	0.55	0.53
Ca	-0.02	-0.14	-0.21	0.04	0.05	-0.15	0.03	-0.04	0.07	0.07	0.07	-0.17	0.11	0.18
Mg	0.16	-0.18	-0.26	0.08	0.19	0.12	0.21	-0.24	0.03	0.16	0.14	-0.20	0.45	0.43
P	0.19	-0.03	-0.03	0.22	0.24	0.02	0.15	-0.21	0.05	0.09	0.05	-0.17	0.29	0.21
Fe	0.12	-0.08	-0.05	0.09	0.20	0.09	0.26	-0.20	0.04	0.06	0.09	-0.22	0.40	0.27
Cu	0.12	-0.03	-0.04	0.06	0.17	0.20	0.25	-0.14	0.06	0.02	0.03	-0.04	0.34	0.29
Zn	0.16	0.11	0.43	0.11	0.09	0.17	0.20	-0.27	0.03	0.04	-0.03	-0.17	0.24	0.05
Cl	0.03	0.29	0.15	0.13	0.01	0.02	0.30	-0.18	0.08	-0.02	-0.03	-0.09	0.12	-0.11
Mn	0.01	-0.22	-0.31	0.03	0.10	0.03	0.11	-0.03	0.38	-0.07	0.07	-0.25	0.32	0.35
Se	0.19	0.06	0.07	0.30	0.35	0.04	0.09	-0.23	0.05	0.01	-0.02	-0.12	0.18	0.09
I	0.07	-0.10	-0.08	0.55	0.42	-0.09	0.00	-0.13	0.05	0.05	0.07	-0.16	0.15	0.21
Retinol	-0.14	0.03	0.21	0.06	0.07	-0.09	-0.05	0.02	0.01	0.04	-0.02	-0.09	-0.15	-0.13
Carotenes	0.16	-0.13	-0.14	0.03	0.10	0.11	0.35	-0.17	0.00	0.07	0.13	-0.08	**0.77***	0.27
Vitamin D	0.07	0.00	0.09	0.25	**0.78***	-0.06	0.02	-0.12	0.02	0.04	0.02	-0.14	0.12	0.10
Vitamin E	0.11	-0.06	-0.21	0.06	0.04	-0.04	0.19	0.03	0.02	0.05	0.08	-0.07	0.37	0.27

Variables	poultry	process. meat	red meat	white fish	oily fish	potatoes pasta rice	savoury	sweets	tea	coffee	fruit/ vegetable juice	fizzy	Vegs	fruit
Thiamine	0.09	-0.02	0.07	0.09	0.04	0.35	0.23	-0.20	0.12	-0.05	0.05	-0.17	0.31	0.20
Vitamin B2	0.07	-0.09	-0.06	0.12	0.15	-0.10	0.07	-0.15	0.18	-0.01	0.06	-0.19	0.15	0.23
Pot Niacin	0.31	0.19	0.36	0.27	0.20	0.13	0.15	-0.29	0.01	0.09	-0.05	-0.15	0.21	0.00
Niacin	0.44	0.18	0.21	0.17	0.25	0.17	0.19	-0.33	-0.05	0.13	0.06	-0.09	0.33	0.15
Vitamin B6	0.22	0.00	0.04	0.15	0.13	0.40	0.17	-0.29	0.05	-0.01	0.08	-0.14	0.41	0.31
Vitamin B12	0.08	0.01	0.23	0.36	0.67	-0.07	0.05	-0.20	0.02	0.04	0.01	-0.15	0.11	0.10
Folic acid	0.09	-0.13	-0.15	0.09	0.11	0.24	0.18	-0.25	0.17	-0.03	0.15	-0.19	0.52	0.38
Pantoth. acid	0.15	0.06	0.16	0.14	0.16	0.11	0.07	-0.26	0.12	0.01	0.01	-0.21	0.22	0.13
Biotin	0.04	-0.14	-0.07	0.28	0.28	-0.13	0.02	-0.15	0.18	0.21	0.06	-0.27	0.17	0.22
Vitamin C	0.16	-0.20	-0.24	0.05	0.19	0.06	0.17	-0.13	-0.01	0.08	0.44	-0.10	0.62	**0.73***

Variables	SFAs	MUFAs	ω6PUFAs	ω3PUFAs	*t*FAs	*t*MUFAs	Quercetin	Catechin	Epicatechin	Flavones	Procyanid.	Flavan.	Phytoestr.
SFAs	1.00												
MUFAs	0.67	1.00											
ω6PUFAs	-0.01	0.42	1.00										
ω3PUFAs	-0.08	0.34	0.30	1.00									
*t*FAs	**0.74***	0.59	0.10	-0.07	1.00								
*t*MUFAs	0.68	0.59	0.10	-0.01	**0.83****	1.00							
Quercetin	-0.12	-0.10	-0.01	0.12	-0.11	-0.03	1.00						
Catechin	-0.09	-0.12	-0.06	0.10	-0.06	-0.11	0.65	1.00					
Epicatechin	-0.01	-0.05	-0.03	0.01	0.02	-0.02	**0.87****	**0.77***	1.00				
Flavones	-0.07	-0.04	0.03	0.21	0.00	0.07	0.26	0.04	0.02	1.00			
Procyanidins	-0.04	-0.05	-0.02	0.04	-0.05	0.00	**0.87****	**0.79***	**0.96*****	0.03	1.00		
Flavanones	-0.17	-0.16	-0.03	0.13	-0.15	-0.13	0.12	0.19	0.09	0.12	0.05	1.00	
Phytoestrogens	-0.07	0.00	0.16	0.07	0.02	0.11	0.06	0.03	0.05	0.01	0.06	0.06	1.00
Protein	0.05	0.23	0.12	0.50	0.01	0.10	0.15	0.01	0.02	0.23	0.06	0.06	0.04
Cholesterol	0.51	0.51	0.02	0.16	0.33	0.40	-0.04	-0.12	-0.05	0.05	-0.02	-0.13	-0.13
Sugars	-0.09	-0.20	-0.04	-0.05	-0.09	-0.22	0.07	0.14	0.12	0.04	0.02	0.28	-0.05
Starch	-0.04	0.05	0.22	-0.06	0.03	0.11	0.06	-0.12	0.05	0.03	0.06	-0.12	0.32
Fibre	-0.36	-0.25	0.13	0.28	-0.27	-0.23	0.31	0.23	0.16	0.29	0.15	0.37	0.35
Na	0.11	0.30	0.29	0.30	0.12	0.23	0.06	-0.10	-0.02	0.08	0.02	-0.07	0.25
K	-0.31	-0.21	0.02	0.34	-0.27	-0.22	0.32	0.28	0.16	0.29	0.17	0.33	0.03
Ca	0.18	0.05	-0.05	0.08	0.15	0.22	0.08	0.05	0.07	0.05	0.06	0.11	0.05
Mg	-0.30	-0.16	0.12	0.34	-0.23	-0.19	0.22	0.26	0.11	0.21	0.12	0.29	0.32
P	0.00	0.10	0.09	0.41	-0.02	0.07	0.15	0.07	0.05	0.21	0.07	0.14	0.13
Fe	-0.23	-0.06	0.16	0.39	-0.17	-0.09	0.19	0.23	0.06	0.32	0.10	0.20	0.31
Cu	-0.19	0.03	0.20	0.38	-0.12	-0.14	0.20	0.24	0.11	0.26	0.11	0.16	0.17

Variables	SFAs	MUFAs	ω6PUFAs	ω3PUFAs	*t*FAs	*t*MUFAs	Quercetin	Catechin	Epicatechin	Flavones	Procyanid.	Flavan.	Phytoestr.
Zn	0.05	0.16	0.10	0.32	0.06	0.14	0.15	0.02	0.01	0.26	0.04	0.04	0.10
Cl	0.09	0.26	0.28	0.29	0.12	0.23	0.06	-0.08	-0.01	0.07	0.03	-0.05	0.29
Mn	-0.22	-0.13	0.17	0.20	-0.15	-0.07	0.49	0.41	0.43	0.17	0.44	0.20	0.56
Se	-0.06	0.20	0.22	0.48	-0.06	0.01	0.10	0.04	0.03	0.09	0.06	0.08	0.28
I	0.03	0.11	-0.03	0.46	-0.05	0.02	0.09	0.08	0.05	0.10	0.06	0.13	-0.05
Retinol	0.53	0.36	-0.05	0.05	0.36	0.45	-0.05	-0.07	-0.05	0.05	-0.03	-0.06	-0.06
Carotenes	-0.22	-0.14	0.08	0.34	-0.17	-0.13	0.23	0.11	0.02	0.37	0.03	0.18	0.04
Vitamin D	0.04	0.30	0.08	**0.74***	-0.07	-0.02	0.05	0.05	0.00	0.07	0.03	0.08	0.00
Vitamin E	-0.17	0.10	0.67	0.29	-0.12	-0.12	0.14	0.07	0.06	0.15	0.05	0.17	0.11
Thiamine	-0.19	-0.08	0.11	0.22	-0.15	-0.05	0.22	0.04	0.09	0.22	0.10	0.16	0.25
Vitamin B2	0.02	-0.01	-0.05	0.21	-0.01	0.06	0.21	0.15	0.18	0.08	0.18	0.12	0.02
Pot Niacin	0.08	0.24	0.12	0.44	0.04	0.13	0.08	-0.04	-0.04	0.19	0.00	0.02	0.01
Niacin	-0.23	0.03	0.17	0.49	-0.21	-0.15	0.07	0.03	-0.05	0.15	-0.01	0.14	0.15
Vitamin B6	-0.29	-0.16	0.03	0.32	-0.27	-0.19	0.21	0.09	0.06	0.22	0.09	0.19	-0.02
Vitamin B12	0.07	0.25	0.01	0.65	-0.01	0.06	0.07	0.05	0.00	0.15	0.03	0.07	-0.07
Folic acid	-0.28	-0.20	0.05	0.25	-0.24	-0.14	0.33	0.21	0.19	0.22	0.20	0.34	0.21
Pantoth. acid	-0.06	0.04	0.03	0.27	-0.07	0.04	0.20	0.12	0.11	0.16	0.15	0.12	0.03
Biotin	-0.01	0.14	0.14	0.32	-0.07	0.02	0.26	0.29	0.22	0.10	0.26	0.18	0.14
Vitamin C	-0.30	-0.26	-0.01	0.28	-0.27	-0.24	0.25	0.30	0.12	0.26	0.09	**0.70***	0.07

Variables	Protein	Cholest.	Sugars	Starch	Fibre	Na	K	Ca	Mg	P	Fe	Cu	Zn	Cl	Mn	Se	I
Protein	1.00																
Cholesterol	0.43	1.00															
Sugars	-0.10	-0.24	1.00														
Starch	0.05	-0.11	-0.14	1.00													
Fibre	0.21	-0.31	0.33	0.27	1.00												
Na	0.48	0.24	-0.15	0.34	0.14	1.00											
K	0.47	-0.13	0.33	0.12	**0.73***	0.15	1.00										
Ca	0.37	0.08	0.29	-0.04	0.16	0.24	0.37	1.00									
Mg	0.42	-0.21	0.27	0.15	**0.77***	0.23	**0.79***	0.38	1.00								
P	**0.77***	0.20	0.18	0.04	0.42	0.42	0.62	**0.73***	**0.70***	1.00							
Fe	0.45	-0.03	0.06	0.28	0.68	0.40	0.56	0.16	**0.70***	0.53	1.00						
Cu	0.32	-0.04	0.16	0.17	0.53	0.20	0.51	0.02	0.61	0.39	0.58	1.00					
Zn	**0.84****	0.34	-0.08	0.08	0.31	0.36	0.46	0.31	0.49	**0.70***	0.56	0.41	1.00				
Cl	0.47	0.19	-0.14	0.36	0.18	**0.98*****	0.20	0.28	0.29	0.44	0.42	0.22	0.35	1.00			
Mn	0.14	-0.26	0.13	0.33	0.67	0.21	0.43	0.15	0.67	0.36	0.57	0.41	0.25	0.25	1.00		
Se	0.55	0.21	-0.14	0.11	0.20	0.35	0.23	0.08	0.33	0.40	0.31	0.39	0.39	0.36	0.24	1.00	
I	0.53	0.28	0.14	-0.18	0.11	0.23	0.38	0.51	0.32	0.62	0.19	0.12	0.30	0.25	0.07	0.39	1.00
Retinol	0.09	0.51	-0.13	-0.09	-0.21	0.09	-0.14	0.18	-0.16	0.10	-0.02	0.17	0.10	0.07	-0.15	0.04	0.16
Carotenes	0.26	-0.11	0.14	-0.01	0.58	0.16	0.54	0.16	0.43	0.31	0.45	0.34	0.27	0.17	0.30	0.13	0.14
Vitamin D	0.44	0.37	-0.07	-0.13	0.05	0.18	0.15	0.07	0.15	0.33	0.27	0.18	0.25	0.16	0.03	0.46	0.46
Vitamin E	0.13	-0.10	0.19	0.08	0.43	0.20	0.32	0.11	0.33	0.22	0.29	0.25	0.10	0.21	0.30	0.18	0.12
Thiamine	0.47	-0.02	0.06	0.48	0.56	0.44	0.62	0.21	0.55	0.49	0.61	0.37	0.51	0.47	0.43	0.27	0.18
Vitamin B2	0.52	0.15	0.24	-0.05	0.24	0.27	0.48	**0.75***	0.49	**0.78***	0.40	0.20	0.48	0.30	0.21	0.18	0.56
Pot Niacin	**0.98*****	0.47	-0.11	0.03	0.16	0.47	0.44	0.42	0.41	**0.78***	0.39	0.29	**0.83****	0.47	0.08	0.51	0.52

Variables	Protein	Cholest.	Sugars	Starch	Fibre	Na	K	Ca	Mg	P	Fe	Cu	Zn	Cl	Mn	Se	I
Niacin	**0.71***	0.09	-0.05	0.14	0.38	0.40	0.48	0.04	0.53	0.52	0.59	0.43	0.61	0.42	0.24	0.54	0.26
Vitamin B6	0.54	-0.02	0.14	0.26	0.55	0.25	**0.80****	0.19	0.60	0.52	0.57	0.41	0.51	0.30	0.28	0.28	0.31
Vitamin B12	0.63	0.42	-0.09	-0.21	0.01	0.20	0.24	0.26	0.21	0.52	0.29	0.33	0.48	0.19	-0.01	0.49	0.60
Folic acid	0.37	-0.09	0.18	0.27	0.67	0.22	**0.72***	0.30	0.66	0.51	0.62	0.41	0.39	0.28	0.48	0.23	0.26
Pantoth. acid	0.54	0.25	0.03	0.02	0.27	0.25	0.50	0.34	0.48	0.58	0.37	0.31	0.55	0.28	0.21	0.28	0.37
Biotin	0.42	0.25	0.09	-0.11	0.31	0.19	0.44	0.42	0.64	0.64	0.44	0.36	0.42	0.21	0.43	0.32	0.49
Vitamin C	0.19	-0.21	0.41	-0.08	0.69	-0.04	0.69	0.18	0.55	0.32	0.41	0.38	0.18	0.00	0.38	0.14	0.22

Variables	Retinol	Carotenes	Vitamin D	Vitamin E	Thiamine	Riboflav.	Pot Niacin	Niacin	Vitamin B6	Vitamin B12	Folic acid	Pantoth. acid	Biotin	Vitamin C
Retinol	1.00													
Carotenes	-0.07	1.00												
Vitamin D	0.19	0.09	1.00											
Vitamin E	-0.10	0.35	0.10	1.00										
Thiamine	-0.11	0.31	0.12	0.19	1.00									
Vitamin B2	0.19	0.17	0.26	0.12	0.40	1.00								
Pot Niacin	0.11	0.23	0.40	0.11	0.43	0.54	1.00							
Niacin	-0.14	0.29	0.42	0.21	0.52	0.38	0.68	1.00						
Vitamin B6	-0.14	0.41	0.23	0.24	**0.72***	0.45	0.52	0.67	1.00					
Vitamin B12	0.38	0.09	**0.77***	0.03	0.16	0.47	0.60	0.46	0.29	1.00				
Folic acid	-0.08	0.45	0.15	0.29	0.68	0.54	0.35	0.51	**0.77***	0.19	1.00			
Pantoth. acid	0.10	0.22	0.23	0.09	0.42	0.49	0.56	0.43	0.52	0.38	0.44	1.00		
Biotin	0.16	0.13	0.32	0.19	0.27	0.54	0.44	0.32	0.29	0.42	0.39	0.52	1.00	
Vitamin C	-0.14	0.51	0.11	0.34	0.36	0.22	0.13	0.29	0.49	0.11	0.59	0.24	0.25	1.00

8.2.3 Univariable logistic regression of the explanatory variables

Univariable logistic regression models were fitted for each explanatory variable. Odds ratios and 95% CI were calculated for each quartile of the continuous variables and each category of the categorical variables (Table 96). P-values for trend were calculated for the quartile form of the quantitative explanatory variables (Table 96). For the regression of food and nutrient variables, their residual energy adjusted form was used (except for the food groups: tea and coffee and the nutrient flavones).

For the demographic and lifestyle factors significant (at a $p \leq 0.05$ level) associations were observed between colorectal cancer and family history of cancer ($p=1.1x10^{-51}$), NSAIDs intake ($p=7.3x10^{-7}$), dietary energy intake ($p=2.0x10^{-5}$), HRT intake ($p=0.0003$) and physical activity ($p=0.02$) (Table 96). For the food group variables significant associations were observed between colorectal cancer and intakes of vegetables ($p=2.4x10^{-8}$), eggs ($p=4.0x10^{-7}$), sweets ($p=7.9x10^{-7}$), fruit/ vegetable juice ($p=1.7x10^{-6}$), oily fish ($p=0.001$), coffee ($p=0.001$), fruit ($p=0.009$), savoury foods ($p=0.009$) and white fish ($p=0.04$) (Table 96). For the nutrient variables significant associations were observed between colorectal cancer and intakes of the fatty acids: *t*MUFAs ($p=6.7x10^{-6}$), ω3PUFAs ($p=1.3x10^{-5}$), SFAs ($p=0.0001$), *t*FAs ($p=0.001$) and MUFAs ($p=0.01$); of the flavonoids: quercetin ($p=0.001$), catechin ($p=0.001$) and phytoestrogens ($p=0.04$); of the macronutrients: cholesterol ($p=1.4x10^{-5}$), fibre ($p=3.3x10^{-5}$), protein ($p=0.001$) and starch ($p=0.05$); of the minerals: magnesium ($p=2.7x10^{-11}$), potassium ($p=9.1x10^{-8}$), manganese ($p=1.8x10^{-7}$), copper ($p=2.0x10^{-6}$), iron ($p=1.3x10^{-5}$), zinc ($p=4.6x10^{-5}$), phosphorus ($p=0.0001$), selenium ($p=0.009$); and of the vitamins: niacin ($p=8.2x10^{-7}$), vitamin B6 ($p=7.1x10^{-6}$), carotenes ($p=2.6x10^{-5}$), vitamin C ($p=4.6x10^{-5}$), vitamin A ($p=0.001$), potential niacin ($p=0.001$), biotin ($p=0.001$), folate ($p=0.003$), pantothenic acid ($p=0.006$), vitamin D ($p=0.01$), vitamin B1 ($p=0.02$) and vitamin B12 ($p=0.02$) (Table 96).

Table 96 Univariable logistic regression of colorectal cancer on each explanatory variable included in the stepwise regression (2061 cases; 2776 controls)

Variables	Quartiles	Frequency		Model II		
		Cases	*Controls*	*OR*	*95% CI*	*p-value for trend*
Demographic factors						
Age (years)	21-55	632	760	1.00		
	55-63	401	627	0.77	0.65, 0.91	
	63-71	583	761	0.92	0.79, 1.07	
	>71	445	626	0.85	0.73, 1.00	0.18
Sex	males	1180	1582	1.00		
	females	881	1194	0.99	0.88, 1.11	0.85
Family history	low	1610	2695	1.00		
	moderate/ high	333	30	18.58	12.72, 27.13	$1.1x10^{-51}$
Deprivation score	1	194	258	1.00		
	2	434	568	1.02	0.81, 1.27	
	3	532	758	0.96	0.75, 1.16	
	4	488	645	1.01	0.81, 1.25	
	5	218	293	0.99	0.77, 1.28	
	6	140	178	1.05	0.78, 1.40	
	7	54	76	0.94	0.64, 1.40	0.94
Lifestyle variables						
Smoking	No	874	1200	1.00		
	Former	818	1049	1.07	0.94, 1.21	
	Current	343	509	0.93	0.79, 1.09	0.63
Alcohol (g/day)	0-1.70	526	696	1.00		
	1.70-8.10	534	692	1.02	0.87, 1.20	
	8.10-19.2	501	681	0.97	0.83, 1.14	
	>19.20	500	707	0.94	0.80, 1.10	0.34
Alcohol (g/day)	0	291	427	1.00		
	0-15	1125	1473	1.12	0.95, 1.33	
	15-30	393	548	1.05	0.86, 1.28	
	30-45	139	202	1.01	0.78, 1.31	
	45-60	61	81	1.11	0.77, 1.59	
	>60	52	45	1.70	1.11, 2.60	0.28
BMI (kg/m^2)	<23.71	523	673	1.00		
	23.71-26.11	499	702	0.91	0.78, 1.07	
	26.11-29.09	515	675	0.98	0.83, 1.15	

	>29.09	503	692	0.94	0.79, 1.10	0.62
Physical activity	0	1139	1456	1.00		
	0-3.5	486	703	0.88	0.77, 1.02	
	3.5-7	205	339	0.77	0.64, 0.93	
	>7	133	185	0.92	0.73, 1.16	0.02
Dietary energy	0- 8.25	478	733	1.00		
intake* (KJ/day)	8.25-10.17	483	725	1.02	0.87, 1.20	
	10.17- 12.73	529	680	1.19	1.01, 1.40	
	>12.73	571	638	1.37	1.17, 1.61	$2x10^{-5}$
NSAIDs	no	1449	1757	1.00		
	yes	605	1000	0.73	0.65, 0.83	$7.3x10^{-7}$
HRT	no	666	821	1.00		
	yes	190	343	0.68	0.56, 0.84	0.0003
Foods (m/day)						
Breads	0-1.89	475	735	1.00		
	1.89-2.66	518	691	1.16	0.99, 1.36	
	2.66-3.59	563	646	1.35	1.15, 1.58	
	>3.59	505	704	1.11	0.94, 1.31	0.08
Cereals	0-0.45	457	753	1.00		
	0.45-1.06	584	625	1.54	1.31, 1.81	
	1.06-1.71	534	675	1.30	1.11, 1.53	
	>1.71	486	723	1.11	0.94, 1.30	0.62
Milk	0-1.08	511	699	1.00		
	1.08-1.86	495	714	0.95	0.81, 1.11	
	1.86-2.44	528	681	1.06	0.90, 1.25	
	>2.44	527	682	1.06	0.90, 1.24	0.28
Cream	0-0.18	501	709	1.00		
	0.18-0.34	529	680	1.10	0.94, 1.29	
	0.34-0.81	555	654	1.20	1.02, 1.41	
	>0.81	476	733	0.92	0.78, 1.08	0.53
Cheese	0-0.28	472	738	1.00		
	0.28-0.60	530	679	1.22	1.04, 1.43	
	0.60-1.07	550	659	1.30	1.11, 1.53	
	>1.07	509	700	1.14	0.97, 1.34	0.09
Eggs	0-0.22	452	758	1.00		
	0.22-0.43	504	705	1.20	1.02, 1.41	
	0.43-0.68	533	676	1.32	1.12, 1.56	
	>0.68	572	637	1.51	1.28, 1.77	$4.0x10^{-7}$

Poultry	0-0.16	508	702	1.00		
	0.16-0.30	571	638	1.24	1.05, 1.45	
	0.30-0.56	495	714	0.96	0.81, 1.13	
	>0.56	487	722	0.93	0.79, 1.10	0.07
Red meat	0-0.82	490	720	1.00		
	0.82-1.26	517	692	1.10	0.93, 1.29	
	1.26-1.75	544	665	1.20	1.02, 1.41	
	>1.75	510	699	1.07	0.91, 1.26	0.25
Processed meat	0-0.50	502	708	1.00		
	0.50-0.88	523	686	1.08	0.92, 1.26	
	0.88-1.38	526	683	1.09	0.92, 1.28	
	>1.38	510	699	1.03	0.87, 1.21	0.71
White fish	0-0.16	492	718	1.00		
	0.16-0.29	521	688	1.10	0.94, 1.30	
	0.29-0.47	494	715	1.01	0.86, 1.19	
	>0.47	554	655	1.23	1.05, 1.45	0.04
Oily fish	0-0.01	524	686	1.00		
	0.01-0.13	570	639	1.17	0.99, 1.37	
	0.13-0.31	507	702	0.95	0.80, 1.12	
	>0.31	460	749	0.80	0.68, 0.95	0.001
Potatoes/ Pasta/	0-1.69	518	692	1.00		
Rice	1.69-2.27	525	684	1.02	0.87, 1.20	
	2.27-2.94	515	694	0.99	0.84, 1.16	
	>2.94	503	706	0.95	0.81, 1.12	0.48
Fruit	0-1.43	528	682	1.00		
	1.43-2.47	544	665	1.06	0.90, 1.24	
	2.47-3.84	520	689	0.97	0.83, 1.14	
	>3.84	469	740	0.82	0.70, 0.96	0.009
Vegetables	0-3.31	569	641	1.00		
	3.31-4.92	537	672	0.90	0.77, 1.06	
	4.92-7.03	526	683	0.87	0.74, 1.02	
	>7.03	429	780	0.62	0.53, 0.73	2.4×10^{-8}
Savoury†	0-1.82	524	686	1.00		
	1.82-2.55	549	660	1.09	0.93, 1.28	
	2.55-3.44	523	686	1.00	0.85, 1.17	
	>3.44	465	744	0.82	0.70, 0.96	0.009
Sweets‡	0-2.98	454	756	1.00		
	2.98-4.44	498	711	1.17	0.99, 1.37	

	4.44-6.10	544	665	1.36	1.16, 1.60	
	>6.10	565	644	1.46	1.24, 1.72	$7.9x10^{-7}$
Tea§	0-1	527	683	1.00		
	1-3	509	700	0.97	0.84, 1.12	
	3-4	534	675	1.03	0.87, 1.23	
	>4	491	718	0.91	0.78, 1.07	0.38
Coffee§	0-0.1	567	691	1.00		
	0.1-1	571	714	0.97	0.83, 1.14	
	1-3	550	769	0.87	0.75, 1.02	
	>3	373	602	0.75	0.64, 0.89	0.001
Fruit/ vegetable	0-0.14	439	771	1.00		
juice	0.14-0.82	532	677	1.38	1.17, 1.62	
	0.82-1.45	527	682	1.36	1.15, 1.60	
	>1.45	563	646	1.53	1.30, 1.80	$1.7x10^{-6}$
Fizzy drinks	0-0.01	495	715	1.00		
	0.01-0.02	515	694	1.07	0.91, 1.26	
	0.02-0.38	522	687	1.10	0.93, 1.29	
	>0.38	529	680	1.12	0.96, 1.32	0.15
Nutrients						
Fatty acids (g/day)						
SFAs	0-31.48	466	739	1.00		
	31.48-37.03	512	692	1.17	1.00, 1.38	
	37.03-43.30	523	681	1.22	1.04, 1.43	
	>43.30	560	644	1.38	1.17, 1.62	0.0001
MUFAs	0-28.57	467	738	1.00		
	28.57-32.56	529	675	1.24	1.05, 1.46	
	32.56-36.18	539	665	1.28	1.09, 1.51	
	>36.18	526	678	1.23	1.04, 1.44	0.01
ω6PUFAs	0-8.92	515	690	1.00		
	8.92-10.73	519	685	1.02	0.86, 1.19	
	10.73-13.19	510	694	0.98	0.84, 1.16	
	>13.19	517	687	1.01	0.86, 1.18	0.98
ω3PUFAs	0-1.83	557	648	1.00		
	1.83-2.22	538	666	0.94	0.80, 1.10	
	2.22-2.74	513	691	0.86	0.73, 1.01	
	>2.74	453	751	0.70	0.60, 0.83	$1.3x10^{-5}$
*t*FAs	0-2.86	453	752	1.00		
	2.86-3.53	526	678	1.29	1.09, 1.52	

	3.53-4.24	552	652	1.40	1.19, 1.65	
	>4.24	530	674	1.30	1.11, 1.54	0.001
*t*MUFAs	0-2.17	448	757	1.00		
	2.17-2.68	505	699	1.22	1.04, 1.44	
	2.68-3.19	565	639	1.49	1.27, 1.76	
	>3.19	543	661	1.39	1.18, 1.63	$6.7x10^{-6}$
Flavonoids (mg/day)						
Quercetin	0-11.20	541	669	1.00		
	11.20-17.38	528	681	0.96	0.82, 1.13	
	17.38-22.65	538	671	0.99	0.84, 1.16	
	>22.65	454	755	0.74	0.63, 0.87	0.001
Catechin	0-4.81	548	662	1.00		
	4.81-7.21	535	674	0.96	0.82, 1.13	
	7.21-9.39	504	705	0.86	0.73, 1.01	
	>9.39	474	735	0.78	0.66, 0.92	0.001
Epicatechin	0-12.71	522	688	1.00		
	12.71-23.34	528	681	1.02	0.87, 1.20	
	23.34-32.28	534	675	1.04	0.89, 1.22	
	>32.28	477	732	0.86	0.73, 1.01	0.10
Flavones§	0-0.5	607	806	1.00		
	0.5-1	460	621	0.94	0.80, 1.10	
	1-1.8	481	698	0.85	0.73, 1.00	
	>1.8	513	651	0.90	0.76, 1.07	0.12
Procyanidins	0-15.98	523	687	1.00		
	15.98-31.72	527	682	1.02	0.86, 1.19	
	31.72-44.66	534	675	1.04	0.88, 1.22	
	>44.66	477	732	0.86	0.73, 1.01	0.09
Flavanones	0-7.42	478	732	1.00		
	7.42-20.57	563	646	1.33	1.14, 1.57	
	20.57-40.61	526	683	1.18	1.00, 1.39	
	>40.61	494	715	1.06	0.90, 1.24	0.87
Phytoestrogens	0-400.1	522	688	1.00		
(µg/day)	400.1-575.3	551	658	1.10	0.94, 1.30	
	575.3-845.7	504	705	0.94	0.80, 1.11	
	>845.7	484	725	0.88	0.75, 1.03	0.04
*Macronutrients (g/da*y)						
Protein	0-90.60	554	656	1.00		
	90.60-101.3	526	683	0.91	0.78, 1.07	

	101.3-112.3	510	699	0.86	0.74, 1.01	
	>112.3	471	738	0.76	0.64, 0.89	0.001
Cholesterol	0-296.3	453	757	1.00		
	296.3-362.0	533	676	1.32	1.12, 1.55	
	362.0-430.1	500	709	1.18	1.00, 1.39	
	>430.1	575	634	1.52	1.29, 1.78	$1.4x10^{-5}$
Sugars	0-109.4	507	703	1.00		
	109.4-132.7	535	674	1.10	0.94, 1.29	
	132.7-158.5	519	690	1.04	0.89, 1.22	
	>158.5	500	709	0.98	0.83, 1.15	0.64
Starch	0-143.3	463	747	1.00		
	143.3-164.6	537	672	1.29	1.10, 1.52	
	164.6-184.5	554	655	1.36	1.16, 1.60	
	>184.5	507	702	1.16	0.99, 1.37	0.05
Fibre	0- 17.34	549	661	1.00		
	17.34-20.97	542	667	0.98	0.83, 1.15	
	20.97-24.94	521	688	0.91	0.78, 1.01	
	>24.94	449	760	0.71	0.60, 0.84	$3.3x10^{-5}$
Minerals (mg/day)						
Sodium	0-3065.8	496	714	1.00		
	6065.8-3450.9	514	695	1.06	0.91, 1.25	
	3450.9-3848.8	543	666	1.17	1.00, 1.38	
	>3848.8	508	701	1.04	0.89, 1.23	0.39
Potassium	0-3789.3	557	653	1.00		
	3789.3-4274.8	567	642	1.03	0.88, 1.21	
	4274.8-4759.8	493	716	0.81	0.69, 0.95	
	>4759.8	444	765	0.68	0.58, 0.80	$9.1x10^{-8}$
Calcium	0-924.5	511	699	1.00		
	924.5-1091.1	519	690	1.03	0.88, 1.21	
	1091.1-1269.6	520	689	1.03	0.88, 1.21	
	>1269.6	511	698	1.00	0.85, 1.18	0.98
Magnesium	0-340.36	578	632	1.00		
	340.36-383.26	558	651	0.94	0.80, 1.10	
	383.26-428.36	499	710	0.77	0.65, 0.90	
	>428.36	426	783	0.59	0.50, 0.70	$2.7x10^{-11}$
Phosphorus	0-1574.1	545	665	1.00		
	1574.1-1750.5	544	665	1.00	0.85, 1.17	
	1750.5-1931.4	518	691	0.91	0.78, 1.07	

	>1931.4	454	755	0.73	0.62, 0.86	0.0001
Iron	0-13.56	567	643	1.00		
	13.56-15.28	525	684	0.87	0.74, 1.02	
	15.28-17.15	509	700	0.82	0.70, 0.97	
	>17.15	460	749	0.70	0.59, 0.82	$1.3x10^{-5}$
Copper	0-1.37	573	637	1.00		
	1.37-1.54	536	673	0.88	0.75, 1.04	
	1.54-1.76	484	725	0.74	0.63, 0.87	
	>1.76	468	741	0.70	0.60, 0.82	$2.0x10^{-6}$
Zinc	0-10.48	556	654	1.00		
	10.48-11.94	548	661	0.97	0.83, 1.14	
	11.94-13.37	485	724	0.79	0.67, 0.93	
	>13.37	472	737	0.75	0.64, 0.88	$4.6x10^{-5}$
Chloride	0-4705.2	499	711	1.00		
	4705.2-5285.5	517	692	1.06	0.91, 1.25	
	5285.5-5872.4	540	669	1.15	0.98, 1.35	
	>5872.4	505	704	1.02	0.87, 1.20	0.58
Manganese	0-3.04	573	637	1.00		
	3.04-3.79	542	667	0.90	0.77, 1.06	
	3.79-4.67	490	719	0.76	0.64, 0.89	
	>4.67	456	753	0.67	0.57, 0.79	$1.8x10^{-7}$
Selenium	0-63.25	542	668	1.00		
(µg/day)	63.25-78.96	529	680	0.96	0.82, 1.13	
	78.96-96.43	509	700	0.90	0.76, 1.05	
	>96.43	481	728	0.81	0.69, 0.96	0.009
Iodine (µg/day)	0-149.78	515	695	1.00		
	149.78-190.19	540	669	1.09	0.93, 1.28	
	190.19-240.72	516	693	1.00	0.85, 1.18	
	>242.72	490	719	0.92	0.78, 1.08	0.20
Vitamins (mg/day)						
Vitamin A	0-348.35	461	749	1.00		
(µg/day)	348.35-496.55	499	710	1.14	0.97, 1.34	
	496.55-752.52	579	630	1.49	1.27, 1.76	
	>752.52	522	687	1.23	1.04, 1.45	0.001
Carotenes	0-2406.1	544	666	1.00		
(µg/day)	2406.1-3519.5	556	653	1.04	0.89, 1.22	
	3519.5-4952.1	510	699	0.89	0.76, 1.05	
	>4952.1	451	758	0.73	0.62, 0.86	$2.6x10^{-5}$

Vitamin D	0-2.74	538	672	1.00		
(µg/day)	2.74-3.86	535	674	0.99	0.84, 1.16	
	3.86-5.47	506	703	0.90	0.76, 1.06	
	>5.47	482	727	0.83	0.70, 0.97	0.01
Vitamin E	0-6.83	532	678	1.00		
	6.83-8.26	510	699	0.93	0.79, 1.09	
	8.26-10.42	511	698	0.93	0.79, 1.10	
	>10.42	508	701	0.92	0.79, 1.08	0.37
Vitamin B1	0-1.80	537	673	1.00		
	1.80-2.03	526	683	0.96	0.82, 1.13	
	2.03-2.28	523	686	0.95	0.81, 1.12	
	>2.28	475	734	0.81	0.69, 0.95	0.02
Vitamin B2	0-1.80	522	688	1.00		
	1.80-2.10	537	672	1.05	0.90, 1.24	
	2.10-2.42	511	698	0.96	0.82, 1.13	
	>2.42	491	718	0.90	0.77, 1.06	0.13
Potential niacin	0-18.82	543	667	1.00		
	18.82-21.11	542	667	1.00	0.85, 1.17	
	21.11-23.37	507	702	0.89	0.75, 1.04	
	>23.37	469	740	0.78	0.66, 0.91	0.001
Niacin	0-20.64	566	644	1.00		
	20.64-23.98	550	659	0.95	0.81, 1.11	
	23.98-27.47	484	725	0.76	0.65, 0.89	
	>27.47	461	748	0.70	0.60, 0.82	$8.2x10^{-7}$
Vitamin B6	0-2.47	547	663	1.00		
	2.47-2.83	555	654	1.03	0.88, 1.21	
	2.83-3.21	514	695	0.90	0.76, 1.05	
	>3.21	445	764	0.71	0.60, 0.83	$7.1x10^{-6}$
Vitamin B12	0-5.27	538	672	1.00		
(µg/day)	5.27-6.96	516	693	0.93	0.79, 1.09	
	6.96-9.21	533	676	0.98	0.84, 1.16	
	>9.21	474	735	0.80	0.68, 0.95	0.02
Folic acid	0-282.65	533	677	1.00		
(µg/day)	282.65-325.89	546	663	1.05	0.89, 1.23	
	325.89-370.81	515	694	0.94	0.80, 1.11	
	>370.81	467	742	0.80	0.68, 0.94	0.003
Pantothenic acid	0-5.90	454	665	1.00		
	5.90-6.65	534	675	0.96	0.82, 1.13	

	6.65-7.58	494	715	0.84	0.72, 0.99	
	>7.58	488	721	0.83	0.70, 0.97	0.006
Biotin (µg/day)	0-43.38	549	661	1.00		
	43.38-49.35	529	680	0.94	0.80, 1.10	
	49.35-55.69	519	690	0.91	0.77, 1.06	
	>55.69	464	745	0.75	0.64, 0.88	0.001
Vitamin C	0-81.20	553	657	1.00		
	81.20-114.17	528	681	0.91	0.78, 1.08	
	114.17-156.03	534	675	0.94	0.80, 1.10	
	>156.03	446	763	0.69	0.59, 0.82	$4.6x10^{-5}$

[*] Intakes divided into quartiles

[†] Summary variable of savoury foods, soups and sauces

[‡] Summary variable of pudding and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

[§] Not energy adjusted

8.3 Stepwise regression analysis

Stepwise regression (both forward and backward) was applied to three different set of variables: 1) Set 1 consisted of the demographic factors, lifestyle variables and foods; 2) Set 2 consisted of the demographic factors, lifestyle variables and nutrients; and 3) Set 3 consisted of the demographic factors, lifestyle variables, foods and nutrients. The p-value threshold for a variable to enter the model (forward stepwise regression) or to remain in the model (backward stepwise regression) was 0.10. Forward and backward stepwise regression for all three sets of variables using the quartile form of continuous variables was initially applied in the whole sample (Tables 97-101) and then separately for females and males (data not shown). In the female datasets the HRT lifestyle variable was included.

In order to examine the stability of the selected models (of the whole sample only), the bootstrap method was used. In particular, 100 bootstrap samples were randomly drawn from the original sample. Then, each bootstrap sample was used to apply forward and backward stepwise regression for each set of variables (set 1, 2 and 3).

8.3.1 Set 1: Demographic factors, lifestyle variables and foods

The explanatory factors that were included in set 1 were the demographic risk factors (age, sex, family history and deprivation score), the lifestyle variables (smoking, alcohol, BMI, physical activity, dietary energy, NSAIDs and HRT only for the female analysis) and the food variables (breads, cereals, milk, cream, cheese, eggs, poultry, red meat, processed meat, white fish, oily fish, potatoes/ pasta/ rice, fruit, vegetables, savoury, sweets, tea, coffee, fruit/ vegetable juice and fizzy drinks) (30 risk factors in total). Forward and backward stepwise regression was applied in the whole sample and separately for males and females.

8.3.1.1 Whole sample (Set 1)

Findings from the original sample

Forward and backward stepwise regression using the quartile form of the continuous variables resulted in two identical models, which included the following 13 risk factors: family history ($p=3.6x10^{-49}$), sweets ($p=4.4x10^{-8}$), eggs ($p=1.7x10^{-7}$), NSAIDs ($p=1.3x10^{-5}$), fruit/ vegetable juice ($p=1.0x10^{-5}$), dietary energy (p=0.001),

coffee (p=0.001), white fish (p=0.001), vegetables (p=0.004), tea (p=0.006), physical activity (p=0.01), breads (p=0.02) and oily fish (p=0.07) (Table 97). The risk factors family history, sweets, eggs, fruit/ vegetable juice, dietary energy intake, white fish and breads were associated with an increased colorectal cancer risk, whereas NSAIDs, coffee, physical activity, tea, vegetables and oily fish were associated with a decreased risk (Table 97).

Findings from the 100 bootstrap samples

The main findings after applying forward and backward stepwise regression to the 100 bootstrap samples were:

- Forward stepwise regression applied to 100 bootstrap samples resulted in 100 unique regression models (i.e. all 100 models were chosen only once).
- Backward stepwise regression applied to 100 bootstrap samples resulted also in 100 unique regression models.
- Over the 100 bootstrap samples, the variables selected by backward selection were identical to those selected by forward selection in 65 of the bootstrap samples. For 25 bootstrap samples the agreement between the variables selected by backward and forward selection was over 90%, whereas for the remaining five bootstrap samples the agreement was between 84% and 88% (mean percentage of agreement (SD): 96.97% (4.56%)).
- Forward and backward stepwise regression resulted in a final model with 11-20 variables (mean (SD): 15.43 (1.89), median (IQR): 15 (14, 17)) and 12-20 variables (mean (SD): 16.01 (1.86), median (IQR): 16 (15, 17)) respectively in the 100 samples. Furthermore, the distribution of the number of variables in the resultant models is close to normal, with the mean of the number of the included variables in backward regression models to be significantly larger than the mean of the number of the included variables in forward regression models (t-test p-value: 0.03).
- The variables: family history, NSAIDs, eggs and sweets were selected to be included in built models of all 100 bootstrap samples using either forward or backward stepwise regression. The variables dietary energy, and fruit/ vegetable juice were selected to be included in the 98% of the built models of the 100 bootstrap samples using each of the two methods. The variables coffee and white

fish were selected to be included in 92-94% of the built models of the 100 bootstrap samples using each of the two methods. Finally, the remaining 21 variables were selected in <90% of the bootstrap samples using either selection method.

8.3.1.2 Females (Set 1)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following nine risk factors: family history ($p=1.9x10^{-25}$), fruit/ vegetable juice (p=0.0002), sweets (p=0.0003), vegetables (p=0.002), breads (p=0.002), NSAIDs (p=0.002), white fish (p=0.01), eggs (p=0.06) and HRT (p=0.09) (data not shown). Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 10 risk factors: family history ($p=1.3x10^{-25}$), fruit/ vegetable juice (p=0.0002), sweets (p=0.0002), vegetables (p=0.002), breads (p=0.002), white fish (p=0.007), NSAIDs (p=0.001), coffee (p=0.04), tea (p=0.05) and eggs (p=0.08) (data not shown). In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in similar models, with the common variables being family history, fruit/ vegetable juice, sweets, vegetables, breads, NSAIDs and white fish (data not shown).

8.3.1.3 Males (Set 1)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following eight risk factors: family history ($p=4.0x10^{-25}$), eggs ($p=8.3x10^{-8}$), dietary energy ($p=3.1x10^{-5}$), sweets (p=0.0002), NSAIDs (p=0.006), fruit/ vegetable juice (p=0.008), savoury (p=0.02) and physical activity (p=0.02) (data not shown). Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 10 risk factors: family history ($p=7.4x10^{-25}$), eggs ($p=1.0x10^{-7}$), dietary energy ($p=4.8x10^{-6}$), sweets (p=0.0001), NSAIDs (p=0.005), fruit/ vegetable juice (p=0.01), savoury (p=0.02), coffee (p=0.02), physical activity (p=0.02) and tea (p=0.04) (data not shown). In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in almost identical models, with the

common variables being family history, eggs, dietary energy, sweets, NSAIDs, fruit/ vegetable juice, savoury and physical activity (data not shown).

8.3.1.4 Summary (Set 1)

Original sample

Briefly, the variables of set 1 that were selected to be included in all six resultant models after application of forward and backward stepwise regression in the whole, female and male samples were: family history (p-value range: $3.6x10^{-49}$ to $7.4x10^{-25}$), NSAIDs (p-value range: $1.3x10^{-5}$ to 0.006), eggs (p-value range: $8.3x10^{-8}$ to 0.08), sweets (p-value range: $4.4x10^{-8}$ to 0.0003) and fruit/ vegetable juice (p-value range: $1.0x10^{-5}$ to 0.01) (Table 97). In contrast, the variables vegetables (p-value range: 0.001 to 0.002) and white fish (p-value range: 0.001 to 0.01) were only included in the models derived from the whole and female samples (Table 97), the variables dietary energy intake (p-value range: $4.8x10^{-6}$ to 0.001) and physical activity (p-value range: 0.01 to 0.02) were included only in the models derived from the whole and male samples (Table 97), and the variable savoury intake was included only in the models derived from the male sample (p-value range: 0.02) (data not shown). Regarding the direction of the associations, the risk factors family history, sweets, eggs, fruit/ vegetable juice, white fish and dietary energy intake were associated with an increased colorectal cancer risk, whereas NSAIDs, vegetables, physical activity and savoury were associated with a decreased risk (Table 97). Finally, a matrix of the selected variables of the set 1 after applying forward and backward stepwise regression in the whole, female and male samples is presented in Table 102 (in the end of this chapter).

Bootstrap samples

Results from the bootstrap method (whole sample) showed that all 100 resultant models after applying forward stepwise regression were chosen only once and the same after applying backward stepwise regression. Within the same bootstrap sample application of either forward or backward stepwise regression resulted in the same model in 65 cases. Regarding the number of the included variables, it ranged from 11 to 20 and application of the backward stepwise regression resulted in models with slightly more variables. In addition, the variables family history, NSAIDs, eggs and sweets were included in the models derived either from forward or backward

stepwise regression, in all 100 bootstrap samples. Furthermore, the variables energy, fruit/ vegetable juice, coffee and white fish were included in more than 90% of the built models. These results are in accordance with the findings of the analysis of the original sample, which suggested that the risk factors of set 1 more strongly associated with colorectal cancer were family history, NSAIDs, eggs, sweets and fruit/ vegetable juice (Table 97).

Table 97 Set 1: Stepwise regression built model[*] using the quartile form of the continuous variables (Whole sample; forward and backward stepwise regression resulted to the same model)

Included variables	Number (%) or median (IQR)		OR	95% CI	p-value
	Cases	*Controls*			
Family history					
Low	1610 (82.9%)	2695 (98.9%)			
Medium/high	333 (17.1%)	30 (1.1%)	19.66	13.22, 29.21	$3.6x10^{-49}$
Sweets[†] (m/day[‡])	4.3 (2.9, 6.0)	4.6 (3.1, 6.3)	1.19	1.12, 1.27	$4.4x10^{-8}$
Eggs (m/day)	0.5 (0.2, 0.7)	0.4 (0.2, 0.6)	1.17	1.10, 1.24	$1.7x10^{-7}$
NSAIDs					
No	3206 (66.3%)	1449 (70.3%)			
Yes	1605 (33.2%)	605 (29.4%)	0.73	0.64, 0.84	$1.3x10^{-5}$
fruit/ vegetable juice (m/day)	0.9 (0.2, 1.6)	0.8 (0.1, 1.4)	1.14	1.08, 1.21	$1.0x10^{-5}$
Dietary energy (MJ/day[§])	10.5 (8.4, 13.1)	9.9 (8.1, 12.5)	1.11	1.05, 1.18	0.001
Coffee (m/day)	1.0 (0.0, 2.4)	1.0 (0.1, 3.0)	0.90	0.84, 0.96	0.001
White fish (m/day)	0.3 (0.2, 0.5)	0.3 (0.1, 0.5)	1.11	1.04, 1.17	0.001
Vegetables (m/day)	4.6 (3.1, 6.5)	5.1 (3.4, 7.4)	0.92	0.86, 0.97	0.004
Tea (m/day)	3.0 (1.0, 4.0)	3.0 (1.0, 4.0)	0.91	0.86, 0.97	0.006
Physical activity (h/week[**])	0.0 (0.0, 2.0)	0.0 (0.0, 3.0)	0.91	0.85, 0.98	0.01
Breads (m/day)	2.7 (1.9, 3.6)	2.6 (1.8, 3.6)	1.08	1.01, 1.14	0.02
Oily fish (m/day)	0.1 (0.0, 0.3)	0.1 (0.0, 0.3)	0.95	0.89, 1.01	0.07

[*] McFadden's pseudo R^2 for the model: 0.099

[†] Summary variable of puddings and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

[‡] m/day: measures/day

[§] MJ/day: 1000 Joules/day

[**] h/week: hours/week

8.3.2 Set 2: Demographic factors, lifestyle variables and nutrients

The explanatory factors that were included in set 2 were the demographic risk factors (age, sex, family history and deprivation score), the lifestyle variables (smoking, alcohol, BMI, physical activity, dietary energy, NSAIDs and HRT only for the female analysis) and the nutrients (SFAs, MUFAs, ω6PUFAs, ω3PUFAs, *t*FAs, *t*MUFAs, quercetin, catechin, epicatechin, flavones, procyanidins, flavanones, phytoestrogens, protein, cholesterol, sugars, starch, fibre, sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, selenium, iodine, vitamin A, carotenes, vitamin D, vitamin E, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folate, pantothenic acid, biotin and vitamin C) (52 risk factors in total). Chloride and potential niacin intakes were excluded from the stepwise regression, since they were very highly correlated with other nutrients ($r>0.95$). Forward and backward stepwise regression was applied in the whole sample and separately for males and females.

8.3.2.1 Whole sample (Set 2)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 15 risk factors: family history ($p=1.8x10^{-49}$), dietary energy ($p=1.1x10^{-5}$), cholesterol ($p=1.5x10^{-5}$), NSAIDs ($p=2.3x10^{-5}$), magnesium ($p=3.8x10^{-5}$), protein ($p=0.01$), starch ($p=0.01$), flavanones ($p=0.02$), ω3PUFAs ($p=0.02$), fibre ($p=0.02$), iodine ($p=0.02$), quercetin ($p=0.03$), copper ($p=0.06$), physical activity ($p=0.07$) and alcohol ($p=0.07$) (Table 98). The risk factors family history, cholesterol, dietary energy, fibre, starch, iodine and flavanones were associated with an increased colorectal cancer risk, whereas NSAIDs, magnesium, protein, ω3PUFAs, copper, quercetin and physical activity were associated with a decreased risk (Table 98).

Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 14 risk factors: family history ($p=3.3x10^{-49}$), dietary energy ($p=4.6x10^{-7}$), NSAIDs ($p=2.4x10^{-5}$), cholesterol ($p=6.4x10^{-5}$), magnesium ($p=0.0002$), *t*MUFAs ($p=0.002$), zinc ($p=0.002$), flavanones ($p=0.004$), fibre ($p=0.004$), ω3PUFAs ($p=0.005$), quercetin ($p=0.01$), *t*FAs ($p=0.01$), vitamin C

(p=0.05) and physical activity (p=0.07) (Table 99). The risk factors family history, dietary energy, *t*MUFAs, cholesterol, fibre and flavanones were associated with an increased colorectal cancer risk, whereas NSAIDs, magnesium, *t*FAs, zinc, vitamin C, ω3PUFAs, physical activity and quercetin were associated with a decreased risk (Table 99).

In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in similar models, with the common variables including family history, dietary energy, cholesterol, NSAIDs, magnesium, flavanones, ω3PUFAs, fibre, quercetin and physical activity (Table 98, Table 99).

Findings from the 100 bootstrap samples

The main findings after applying forward and backward stepwise regression to the 100 bootstrap samples were:

- Forward stepwise regression applied to 100 bootstrap samples resulted in 100 unique regression models (i.e. all 100 models were chosen only once).
- Backward stepwise regression applied to 100 bootstrap samples resulted also in 100 unique regression models.
- Over the 100 bootstrap samples, the variables selected by backward selection were identical to those selected by forward selection in two of the bootstrap samples. For 30 bootstrap samples the agreement between the variables selected by backward and forward selection was over 90%, whereas for the remaining 68 bootstrap samples the agreement was between 58% and 89% (mean percentage of agreement (SD): 84.36% (9.08%)).
- Forward and backward stepwise regression resulted in a final model with 10-27 variables (mean (SD): 19.29 (3.34), median (IQR): 19 (17, 22)) and 16-31 variables (mean (SD): 22.18 (3.25), median (IQR): 22 (20, 25)) respectively in the 100 samples. Furthermore, the mean of the number of the included variables in backward regression models was significantly larger than the mean of the number of the included variables in forward regression models (t-test p-value: $<5x10^{-5}$).
- Only the variable family history was selected to be included in built models of all 100 bootstrap samples using either forward or backward stepwise regression. The variables dietary energy and NSAIDs were selected to be included in the 99% of

the built models of the 100 bootstrap samples using either of the two methods. For forward stepwise regression models, the variables cholesterol and magnesium were selected to be included in 91% and 90% of the built models, respectively, whereas for backward stepwise regression models, the variables cholesterol and fibre were selected to be included in 94% and 90% of the built models, respectively. Finally, the remaining 47 variables were selected in <90% of the bootstrap samples using either selection.

8.3.2.2 Females (Set 2)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 10 risk factors: family history ($p=2.3x10^{-25}$), *t*MUFAs (p=0.0001), zinc (p=0.001), NSAIDs (p=0.008), cholesterol (p=0.01), starch (p=0.02), sugars (p=0.04), HRT (p=0.05), ω3PUFAs (p=0.06) and *t*FAs (0.08) (data not shown). Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 13 risk factors: family history ($p=7.2x10^{-26}$), *t*MUFAs ($p=3.9x10^{-5}$), NSAIDs (p=0.004), sodium (p=0.005), fibre (p=0.01), magnesium (p=0.01), niacin (p=0.02), iodine (p=0.03), sugars (p=0.04), carotenes (p=0.05), calcium (p=0.06), ω3PUFAs (p=0.06), *t*FAs (p=0.08) and HRT (p=0.08) (data not shown). In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in similar models, with the common variables being family history, *t*MUFAs, NSAIDs, sugars, HRT, ω3PUFAs and *t*FAs (data not shown).

8.3.2.3 Males (Set 2)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 10 risk factors: family history ($p=1.8x10^{-24}$), dietary energy ($p=1.2x10^{-8}$), magnesium ($p=4.6x10^{-5}$), cholesterol (p=0.004), NSAIDs (p=0.007), flavanones (p=0.009), quercetin (p=0.02), *t*MUFAs (p=0.02), zinc (p=0.06) and physical activity (p=0.07) (data not shown). Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 16 risk factors: family history ($p=1.6x10^{-24}$), dietary energy ($p=2.6x10^{-8}$), cholesterol (p=0.004), NSAIDs (p=0.006), magnesium (p=0.01),

phosphorus (p=0.01), vitamin D (p=0.02), copper (p=0.02), biotin (p=0.02), flavanones (p=0.02), starch (p=0.03), quercetin (p=0.05), *t*MUFAs (p=0.05), manganese (p=0.06), fibre (p=0.06) and vitamin B12 (p=0.06) (data not shown). In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in similar models, with the common variables being family history, dietary energy, magnesium, cholesterol, NSAIDs, flavanones, quercetin and *t*MUFAs (data not shown).

8.3.2.4 Summary (Set 2)

Original sample

Briefly, the variables of set 2 that were included in all six resultant models after application of forward and backward stepwise regression in the whole, female and male samples were: family history (p-value range: $1.8x10^{-49}$ to $1.8x10^{-24}$) and NSAIDs (p-value range: $2.3x10^{-5}$ to 0.008), whereas the variables cholesterol (p-value range: $1.5x10^{-5}$ to 0.01) and magnesium (p-value range: $3.8x10^{-5}$ to 0.01) were included in five of the six resultant models (Table 98, Table 99). In marked contrast, the variable ω3PUFAs (p-value range: 0.005 to 0.06) was only included in the models derived from the whole and female samples (Table 98, Table 99), the variables dietary energy intake (p-value range: $1.2x10^{-8}$ to $1.1x10^{-5}$) and quercetin intake (p-value range: 0.01 to 0.05) were included only in the models derived from the whole and male samples (Table 98, Table 99), the variable *t*MUFA intake (p-value range: $3.9x10^{-5}$ to 0.05) was included only in the models derived from the female and male samples (data not shown) and the variables *t*FA intake (p-value range: 0.08) and sugar intake (p-value range: 0.04 to 0.08) were included only in the models derived from the female sample (data not shown). Regarding the direction of the associations, the risk factors family history, cholesterol and dietary energy were associated with an increased colorectal cancer risk, whereas NSAIDs, magnesium, ω3PUFAs, quercetin, *t*FAs and sugars were associated with a decreased risk (Table 98, Table 99). The variable *t*MUFAs was associated with an increased colorectal cancer risk in the whole and female sample analysis, whereas it was associated with a decreased risk in the male sample analysis. A matrix of the selected variables of the set 2 after applying forward and backward stepwise regression in the whole, female and male samples is presented in Table 102 (in the end of this chapter).

Bootstrap samples

Results from the bootstrap method (whole sample) showed that all 100 resultant models after applying forward stepwise regression were chosen only once and the same after applying backward stepwise regression. Within the same bootstrap sample application of either forward or backward stepwise regression resulted in the same model in only two cases. Regarding the number of the included variables, it ranged from 10 to 27 for forward and from 16 to 31 for backward stepwise regression (p-value of difference of number of variables selected from forward and backward stepwise regression: $<5x10^{-5}$) (data not shown). In addition, only the variable family history was selected to be included in the models derived either from forward or backward stepwise regression in all 100 bootstrap samples (data not shown). Furthermore, the variables energy, NSAIDs, cholesterol, magnesium and fibre were included in more than 90% of the built models (data not shown). These results are in accordance with the findings of the analysis of the original sample, which suggested that the risk factors of set 2 more strongly associated with colorectal cancer were family history, NSAIDs, cholesterol and magnesium (Table 98, Table 99).

Table 98 Set 2: Forward stepwise regression built model[*] using the quartile form of the continuous variables (Whole sample)

Included variables	Number (%) or median (IQR)		OR	95% CI	p-value
	Cases	*Controls*			
Family history					
Low	1610 (82.9%)	2695 (98.9%)			
Medium/high	333 (17.1%)	30 (1.1%)	19.75	13.30, 29.33	$1.8x10^{-49}$
Dietary energy (MJ/day[†])	10.5 (8.4, 13.1)	9.9 (8.1, 12.5)	1.15	1.08, 1.22	$1.1x10^{-5}$
Cholesterol (g/day[‡])	367.2 (306.7, 438.3)	358.3 (290.2, 424.6)	1.17	1.09, 1.25	$1.5x10^{-5}$
NSAIDs					
No	3206 (66.3%)	1449 (70.3%)			
Yes	1605 (33.2%)	605 (29.4%)	0.74	0.65, 0.85	$2.3x10^{-5}$
Magnesium (mg/day[§])	375.6 (335.4, 418.3)	390.2 (344.6, 334.3)	0.81	0.73, 0.90	$3.8x10^{-5}$
Protein (g/day)	100.3 (89.9, 111.0)	102.1 (91.3, 113.1)	0.90	0.83, 0.97	0.01
Starch (g/day)	165.6 (145.5, 184.1)	163.7 (141.7, 184.9)	1.09	1.02, 1.16	0.01
Flavanones (mg/day)	20.2 (8.5, 39.1)	20.9 (6.7, 41.2)	1.08	1.0, 1.15	0.02
ω3PUFAs (g/day)	2.2 (1.8, 2.7)	2.3 (1.8, 2.8)	0.92	0.86, 0.99	0.02
Fibre (g/day)	20.5 (17.1, 24.4)	21.3 (17.6, 25.4)	1.12	1.02, 1.24	0.02
Iodine (µg/day[**])	188.6 (149.8, 237.7)	191.4 (149.8, 243.5)	1.09	1.02, 1.17	0.02
Quercetin (mg/day)	16.9 (10.9, 22.1)	17.6 (11.4, 22.1)	0.93	0.88, 0.99	0.03
Copper (mg/day)	1.5 (1.3, 1.7)	1.6 (1.4, 1.8)	0.93	0.86, 1.00	0.06
Physical activity (h/week[††])	0.0 (0.0, 2.0)	0.0 (0.0, 3.0)	0.94	0.87, 1.01	0.07
Alcohol (g/day)	7.7 (1.7, 18.3)	8.4 (1.9, 19.9)	1.06	0.99, 1.14	0.07

[*] McFadden's pseudo R^2 for model: 0.096

[†] MJ/day: 1000 Joules/day

[‡] g/day: grams/day

[§] mg/day: milligrams/day

[**] µg/day: micrograms/day

[††] h/week: hours/week

Table 99 Set 2: Backward stepwise regression built model[*] using the quartile form of the continuous variables (Whole sample)

Included variables	Number (%) or median (IQR)		OR	95% CI	p-value
	Cases	*Controls*			
Family history					
Low	1610 (82.9%)	2695 (98.9%)			
Medium/high	333 (17.1%)	30 (1.1%)	19.61	13.21, 29.13	$3.3x10^{-49}$
Dietary energy (MJ/day[†])	10.5 (8.4, 13.1)	9.9 (8.1, 12.5)	1.17	1.10, 1.24	$4.6x10^{-7}$
NSAIDs					
No	3206 (66.3%)	1449 (70.3%)			
Yes	1605 (33.2%)	605 (29.4%)	0.74	0.65, 0.85	$2.4x10^{-5}$
Cholesterol (g/day[‡])	367.2 (306.7, 438.3)	358.3 (290.2, 424.6)	1.15	1.08, 1.24	$6.4x10^{-5}$
Magnesium (mg/day[§])	375.6 (335.4, 418.3)	390.2 (344.6, 334.3)	0.84	0.76, 0.92	0.0002
*t*MUFAs (g/day)	2.7 (2.2, 3.2)	2.6 (2.1, 3.2)	1.17	1.06, 1.29	0.002
Zinc (mg/day)	11.7 (10.4, 13.2)	12.1 (10.6, 13.5)	0.89	0.83, 0.96	0.002
Flavanones (mg/day)	20.2 (8.5, 39.1)	20.9 (6.7, 41.2)	1.12	1.03, 1.21	0.004
Fibre (g/day)	20.5 (17.1, 24.4)	21.3 (17.6, 25.4)	1.15	1.05, 1.26	0.004
ω3PUFAs (g/day)	2.2 (1.8, 2.7)	2.3 (1.8, 2.8)	0.91	0.86, 0.97	0.005
Quercetin (mg/day)	16.9 (10.9, 22.1)	17.6 (11.4, 22.1)	0.93	0.87, 0.98	0.01
*t*FAs (g/day)	3.6 (3.0, 4.3)	3.5 (2.8, 4.2)	0.88	0.80, 0.97	0.01
Vitamin C (mg/day)	111.2 (78.8, 147.8)	117.6 (83.0, 161.4)	0.91	0.83, 1.00	0.05
Physical activity (h/week[**])	0.0 (0.0, 2.0)	0.0 (0.0, 3.0)	0.93	0.87, 1.00	0.07

[*] McFadden's pseudo R^2 for model: 0.096

[†] MJ/day: 1000 Joules/day

[‡] g/day: grams/day

[§] mg/day: milligrams/day

[**] h/week: hours/week

8.3.3 Set 3: Demographic factors, lifestyle variables, foods and nutrients

The explanatory factors that were included in set 3 were the demographic risk factors (age, sex, family history and deprivation score), the lifestyle variables (smoking, alcohol, BMI, physical activity, dietary energy, NSAIDs and HRT for the female analysis), the foods (breads, cereals, milk, cream, cheese, eggs, poultry, red meat, processed meat, white fish, oily fish, potatoes/ pasta/ rice, fruit, vegetables, savoury, sweets, tea, coffee, fruit/ vegetable juice, fizzy drinks) and the nutrients (SFAs, MUFAs, ω6PUFAs, ω3PUFAs, *t*FAs, *t*MUFAs, quercetin, catechin, epicatechin, flavones, procyanidins, flavanones, phytoestrogens, protein, cholesterol, sugars, starch, fibre, sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, selenium, iodine, vitamin A, carotenes, vitamin D, vitamin E, vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folate, pantothenic acid, biotin, vitamin C) (82 risk factors in total). Chloride and potential niacin intakes were excluded from the stepwise regression, since they were very highly correlated with other nutrients ($r>0.95$). Forward and backward stepwise regression was applied in the whole sample and separately for males and females.

8.3.3.1 Whole sample (Set 3)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 19 risk factors: family history ($p=3.6x10^{-50}$), sweets ($p=1.1x10^{-6}$), eggs ($p=3.9x10^{-6}$), fruit/ vegetable juice ($p=3.9x10^{-6}$), NSAIDs ($p=6.4x10^{-6}$), magnesium ($p=1.6x10^{-5}$), white fish ($p=5.4x10^{-5}$), dietary energy ($p=0.0001$), *t*MUFAs ($p=0.0005$), fibre ($p=0.001$), alcohol ($p=0.003$), quercetin ($p=0.003$), coffee ($p=0.01$), cereals ($p=0.02$), ω3PUFAs ($p=0.02$), *t*FAs ($p=0.02$), iron ($p=0.04$), breads ($p=0.07$) and physical activity ($p=0.08$) (Table 100). The risk factors family history, *t*MUFAs, fibre, sweets, eggs, fruit/ vegetable juice, white fish, dietary energy, alcohol, cereals and breads were associated with an increased colorectal cancer risk, whereas NSAIDs, magnesium, *t*FAs, quercetin, iron, ω3PUFAs, coffee and physical activity were associated with a decreased risk (Table 100).

Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 21 risk factors: family history ($p=3.1x10^{-}$

[50]), fruit/ vegetable juice ($p=1.3x10^{-6}$), sweets ($p=1.5x10^{-6}$), eggs ($p=3.4x10^{-6}$), NSAIDs ($p=6.1x10^{-6}$), magnesium ($p=6.1x10^{-5}$), white fish (p=0.0001), fibre (p=0.0003), *t*MUFAs (p=0.001), dietary energy (p=0.002), quercetin (p=0.002), alcohol (p=0.003), coffee (p=0.01), cereals (p=0.02), ω3PUFAs (p=0.02), *t*FAs (p=0.02), iron (p=0.02), physical activity (p=0.08), breads (p=0.10), vitamin C (p=0.10) and flavones (p=0.10) (Table 101). The risk factors family history, fibre, fruit/ vegetable juice, *t*MUFAs, sweets, eggs, white fish, dietary energy, alcohol, cereals, flavones and breads were associated with an increased colorectal cancer risk, whereas NSAIDs, magnesium, *t*FAs, quercetin, iron, ω3PUFAs, coffee, vitamin C and physical activity were associated with a decreased risk (Table 101).

In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in almost identical models, with the common variables being family history, sweets, eggs, fruit/ vegetable juice, NSAIDs, magnesium, white fish, dietary energy, *t*MUFAs, fibre, alcohol, quercetin, coffee, cereals, ω3PUFAs, *t*FAs, iron, breads and physical activity (Table 100, Table 101).

Findings from the 100 bootstrap samples

The main findings after applying forward and backward stepwise regression to the 100 bootstrap samples were:

- Forward stepwise regression applied to 100 bootstrap samples resulted in 100 unique regression models (i.e. all 100 models were chosen only once).
- Backward stepwise regression applied to 100 bootstrap samples resulted also in 100 unique regression models.
- Over the 100 bootstrap samples, the variables selected by backward selection were identical to those selected by forward selection in five of the bootstrap samples. For 17 bootstrap samples the agreement between the variables selected by backward and forward selection was over 90%, whereas for the remaining 78 bootstrap samples the agreement was between 58% and 89% (mean percentage of agreement (SD): 83.12% (9.38%)).
- Forward and backward stepwise regression resulted in a final model with 15-34 variables (mean (SD): 25.34 (3.87), median (IQR): 25 (23, 28)) and 19-39 variables (mean (SD): 29.43 (4.17), median (IQR): 30 (27, 32)) respectively in the 100 samples. Furthermore, the distribution of the number of variables in the resultant models was close to normal, with the mean of the included variables in

backward regression models to be significantly larger than the mean of the included variables in forward regression models (t-test p-value: $<5x10^{-5}$)

- Only the variable family history was selected to be included in built models of all 100 bootstrap samples using either forward or backward stepwise regression. The variables fruit/ vegetable juice, and NSAIDs were selected to be included in more than 99% of the built models of the 100 bootstrap samples using either of the two methods (with the fruit/ vegetable juice variable being included in 100% of the backward stepwise regression models). For forward stepwise regression models, the variables energy, sweets, white fish and eggs were selected to be included in 97-98% of the built models, whereas for backward stepwise regression models, the variables sweets, white fish, energy, fibre and eggs were selected to be included in 91-99% of the built models, respectively. Finally, the remaining 64 variables were selected in <90% of the bootstrap samples using either selection method.

8.3.3.2 Females (Set 3)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 14 risk factors: family history ($p=1.0x10^{-25}$), fruit/ vegetable juice ($p=2.7x10^{-5}$), *t*MUFAs (p=0.0005), white fish (p=0.002), NSAIDs (p=0.002), fibre (p=0.003), sweets (p=0.005), biotin (p=0.007), niacin (p=0.02), *t*FAs (p=0.03), vitamin C (p=0.04), cholesterol (p=0.04), vegetables (p=0.09) and HRT (p=0.09) (data not shown). Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 13 risk factors: family history ($p=5.6x10^{-26}$), *t*MUFAs ($p=4.1x10^{-6}$), fruit/ vegetable juice ($p=1.7x10^{-5}$), sweets (p=0.001), white fish (p=0.001), NSAIDs (p=0.002), fibre (p=0.004), vitamin C (p=0.005), ω3PUFAs (p=0.02), magnesium (p=0.03), *t*FAs (p=0.04), coffee (p=0.06) and tea (p=0.06) (data not shown).

In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in similar models, with the common variables being family history, fruit/ vegetable juice, *t*MUFAs, white fish, NSAIDs, fibre, sweets *t*FAs and vitamin C (data not shown).

8.3.3.3 Males (Set 3)

Findings from the original sample

Forward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 17 risk factors: family history ($p=7.8x10^{-25}$), dietary energy ($p=9.3x10^{-9}$), eggs ($p=2.5x10^{-6}$), sweets ($p=6.9x10^{-5}$), magnesium (p=0.003), fruit/ vegetable juice (p=0.003), sugars (p=0.003), NSAIDs (p=0.006), white fish (p=0.02), fruit (p=0.02), MUFAs (p=0.04), cereals (p=0.04), vitamin D (p=0.05), quercetin (p=0.06), manganese (p=0.06), coffee (p=0.08) and physical activity (p=0.09) (data not shown). Backward stepwise regression using the quartile form of the continuous variables resulted in a model including the following 19 risk factors: family history ($p=3.0x10^{-25}$), dietary energy ($p=5.4x10^{-9}$), eggs ($p=3.8x10^{-6}$), sweets ($p=5.3x10^{-5}$), manganese (p=0.0001), fruit/ vegetable juice (p=0.005), NSAIDs (p=0.009), phosphorus (p=0.01), white fish (p=0.01), MUFAs (p=0.02), sugars (p=0.02), vitamin C (p=0.02), copper (p=0.06), fibre (p=0.04), coffee (p=0.06), fruit (p=0.08), physical activity (p=0.09), cheese (p=0.09) and flavanones (p=0.10) (data not shown).

In summary, forward and backward stepwise regression using the quartile form of the continuous variables resulted in similar models, with the common variables being family history, dietary energy, eggs, sweets, fruit/ vegetable juice, sugars, NSAIDs, white fish, fruit, MUFAs, manganese, coffee and physical activity (data not shown).

8.3.3.4 Summary (Set 3)

Original sample

Briefly, the variables of set 3 that were included in all six resultant models after application of forward and backward stepwise regression in the whole, female and male samples were: family history (p-value range: $3.1x10^{-50}$ to $7.8x10^{-25}$), NSAIDs (p-value range: $6.1x10^{-6}$ to 0.009), white fish (p-value range: $5.4x10^{-5}$ to 0.02), sweets (p-value range: $1.1x10^{-5}$ to 0.005) and fruit/ vegetable juice (p-value range: $1.3x10^{-6}$ to 0.005), whereas coffee (p-value range: 0.01 to 0.08) and fibre (p-value range: 0.0003 to 0.01) were included in five of the six resultant models (Table 100, Table 101). In contrast, the variables dietary energy intake (p-value range: $5.4x10^{-9}$ to 0.002) and physical activity (p-value range: 0.08 to 0.09) were included only in the models derived from the whole and male samples (Table 100, Table 101) and the variables fruit (p-value range: 0.02 to 0.08), MUFA intake (p-value range: 0.02 to 0.04), sugar intake (p-value range: 0.003 to 0.02) and manganese intake (p-value range: 0.0001 to 0.06) were included only in the models derived from the male

sample (data not shown). Regarding the direction of the associations, the risk factors family history, white fish, sweets, fruit/ vegetable juice, fibre, dietary energy and fruit were associated with an increased colorectal cancer risk, whereas NSAIDs, coffee, physical activity, sugars, manganese and MUFAs were associated with a decreased risk (Table 100, Table 101). A matrix of the selected variables of the set 2 after applying forward and backward stepwise regression in the whole, female and male samples is presented in Table 102 (in the end of the chapter).

Bootstrap samples

Results from the bootstrap method (whole sample) showed that all 100 resultant models after applying forward stepwise regression were chosen only once and the same after applying backward stepwise regression. Within the same bootstrap sample application of either forward or backward stepwise regression resulted in the same model in only five cases. The number of the included variables ranged from 15 to 34 for forward and from 19 to 39 for backward stepwise regression (p-value of difference of number of variables selected from forward and backward stepwise regression: $<5x10^{-5}$) (data not shown). In addition, only the variable family history was selected to be included in the models derived either from forward or backward stepwise regression in all 100 bootstrap samples (data not shown). Furthermore, the variables fruit/ vegetable juice, NSAIDs, dietary energy, sweets, white fish, eggs and fibre were included in more than 90% of the built models (data not shown). These results are in accordance with the findings of the analysis of the original sample, which suggested that the risk factors of set 3 more strongly associated with colorectal cancer were family history, NSAIDs, white fish, sweets, fruit/ vegetable juice, coffee and fibre (Table 100, Table 101).

Table 100 Set 3: Forward stepwise regression built model[*] using the quartile form of the continuous variables

Included variables	Number (%) or median (IQR)		OR	95% CI	p-value
	Cases	***Controls***			
Family history					
Low	1610 (82.9%)	2695 (98.9%)			
Medium/high	333 (17.1%)	30 (1.1%)	20.59	13.83, 30.65	$3.6x10^{-50}$
Sweets[†] (m/day[‡])	4.3 (2.9, 6.0)	4.6 (3.1, 6.3)	1.17	1.10, 1.25	$1.1x10^{-6}$
Eggs (m/day)	0.5 (0.2, 0.7)	0.4 (0.2, 0.6)	1.15	1.09, 1.23	$3.9x10^{-6}$
fruit/ vegetable juice (m/day)	0.9 (0.2, 1.6)	0.8 (0.1, 1.4)	1.15	1.08, 1.22	$3.9x10^{-6}$
NSAIDs					
No	3206 (66.3%)	1449 (70.3%)			
Yes	1605 (33.2%)	605 (29.4%)	0.73	0.63, 0.83	$6.4x10^{-6}$
Magnesium (mg/day[§])	375.6 (335.4, 418.3)	390.2 (344.6, 334.3)	0.81	0.74, 0.89	$1.6x10^{-5}$
White fish (m/day)	0.3 (0.2, 0.5)	0.3 (0.1, 0.5)	1.14	1.07, 1.21	$5.4x10^{-5}$
Dietary energy (MJ/day[**])	10.5 (8.4, 13.1)	9.9 (8.1, 12.5)	1.13	1.06, 1.21	0.0001
*t*MUFAs (g/day[††])	2.7 (2.2, 3.2)	2.6 (2.1, 3.2)	1.19	1.08, 1.32	0.0005
Fibre (g/day)	20.5 (17.1, 24.4)	21.3 (17.6, 25.4)	1.18	1.07, 1.30	0.001
Alcohol (g/day)	7.7 (1.7, 18.3)	8.4 (1.9, 19.9)	1.11	1.04, 1.20	0.003
Quercetin (mg/day)	16.9 (10.9, 22.1)	17.6 (11.4, 22.1)	0.90	0.85, 0.96	0.003
Coffee (m/day)	1.0 (0.0, 2.4)	1.0 (0.1, 3.0)	0.92	0.86, 0.98	0.01
Cereals (m/day)	1.0 (0.5, 1.6)	1.1 (0.4, 1.8)	1.08	1.01, 1.16	0.02
ω3PUFAs (g/day)	2.2 (1.8, 2.7)	2.3 (1.8, 2.8)	0.92	0.86, 0.98	0.02
*t*FAs (g/day)	3.6 (3.0, 4.3)	3.5 (2.8, 4.2)	0.89	0.81, 0.98	0.02
Iron (mg/day)	15.1 (13.3, 16.9)	15.4 (13.7, 17.3)	0.91	0.83, 1.00	0.04
Breads (m/day)	2.7 (1.9, 3.6)	2.6 (1.8, 3.6)	1.06	1.00, 1.13	0.07
Physical activity (h/week[‡‡])	0.0 (0.0, 2.0)	0.0 (0.0, 3.0)	0.94	0.87, 1.01	0.08

[*] McFadden's pseudo R^2 for model: 0.108

[†] Summary variable of puddings and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

[‡] m/day: measures/day

[§] mg/day: milligrams/day

[**] MJ/day: 1000 Joules/day

[††] g/day: grams/day

[‡‡] h/week: hours/week

Table 101 Set 3: Backward stepwise regression built model[*] using the quartile form of the continuous variables

Included variables	Number (%) or median (IQR)		OR	95% CI	p-value
	Cases	*Controls*			
Family history					
Low	1610 (82.9%)	2695 (98.9%)			
Medium/high	333 (17.1%)	30 (1.1%)	20.67	13.88, 30.78	$3.1x10^{-50}$
fruit/ vegetable juice (m/day[†])	0.9 (0.2, 1.6)	0.8 (0.1, 1.4)	1.18	1.10, 1.26	$1.3x10^{-6}$
Sweets[‡] (m/day)	4.3 (2.9, 6.0)	4.6 (3.1, 6.3)	1.17	1.10, 1.25	$1.5x10^{-6}$
Eggs (m/day)	0.5 (0.2, 0.7)	0.4 (0.2, 0.6)	1.16	1.09, 1.23	$3.4x10^{-6}$
NSAIDs					
No	3206 (66.3%)	1449 (70.3%)			
Yes	1605 (33.2%)	605 (29.4%)	0.72	0.63, 0.83	$6.1x10^{-6}$
Magnesium (mg/day[§])	375.6 (335.4, 418.3)	390.2 (344.6, 334.3)	0.82	0.75, 0.90	$6.1x10^{-5}$
White fish (m/day)	0.3 (0.2, 0.5)	0.3 (0.1, 0.5)	1.13	1.06, 1.20	0.0001
Fibre (g/day[**])	20.5 (17.1, 24.4)	21.3 (17.6, 25.4)	1.22	1.09, 1.35	0.0003
*t*MUFAs (g/day)	2.7 (2.2, 3.2)	2.6 (2.1, 3.2)	1.18	1.07, 1.30	0.001
Dietary energy (MJ/day[††])	10.5 (8.4, 13.1)	9.9 (8.1, 12.5)	1.11	1.04, 1.19	0.002
Quercetin (mg/day)	16.9 (10.9, 22.1)	17.6 (11.4, 22.1)	0.90	0.84, 0.96	0.002
Alcohol (g/day)	7.7 (1.7, 18.3)	8.4 (1.9, 19.9)	1.11	1.04, 1.19	0.003
Coffee (m/day)	1.0 (0.0, 2.4)	1.0 (0.1, 3.0)	0.92	0.86, 0.98	0.01
Cereals (m/day)	1.0 (0.5, 1.6)	1.1 (0.4, 1.8)	1.09	1.01, 1.16	0.02
ω3PUFAs (g/day)	2.2 (1.8, 2.7)	2.3 (1.8, 2.8)	0.92	0.87, 0.99	0.02
*t*FAs (g/day)	3.6 (3.0, 4.3)	3.5 (2.8, 4.2)	0.89	0.81, 0.98	0.02
Iron (mg/day)	15.1 (13.3, 16.9)	15.4 (13.7, 17.3)	0.90	0.82, 0.98	0.02
Physical activity (h/week[‡‡])	0.0 (0.0, 2.0)	0.0 (0.0, 3.0)	0.94	0.87, 1.01	0.08
Breads (m/day)	2.7 (1.9, 3.6)	2.6 (1.8, 3.6)	1.06	0.99, 1.12	0.09
Vitamin C (mg/day)	111.2 (78.8, 147.8)	117.6 (83.0, 161.4)	0.93	0.85, 1.01	0.10
Flavones (mg/day)	1.0 (0.5, 1.8)	1.0 (0.5, 1.8)	1.06	0.99, 1.13	0.10

[*] McFadden's pseudo R^2 for model: 0.109

[†] m/d: measures/day

[‡] Summary variable of puddings and deserts; chocolates, sweets, nuts and crisps; biscuits; and cakes

[§] mg/day: milligrams/day

[**] g/day: grams/day

[††] MJ/day: 1000 Joules/day

[‡‡] h/week: hours/week

Table 102 Matrix of the variables included in the three sets and finally selected into the forward or backward stepwise regression models in the whole sample and after sex stratification (original sample)

Variables	Set 1						Set 2						Set 3					
	Whole sample		Female sample		Male sample		Whole sample		Female sample		Male sample		Whole sample		Female sample		Male sample	
	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B
Demographic																		
Sex																		
Age		x						x						x				
Family history	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Deprivation score																		
Lifestyle																		
BMI																		
Dietary energy	x	x			x	x	x	x			x	x	x	x			x	x
Smoking																		
Alcohol							x						x	x				
Physical activity	x	x			x	x	x				x		x	x			x	x
NSAIDs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
HRT			x						x	x					x			
Foods																		
Breads	x		x	x									x					
Cereals													x				x	
Milk																		
Cream																		
Cheese																		x
Eggs	x	x	x	x	x	x							x				x	x
Poultry																		
Red meat																		
Processed meat																		
White fish	x	x	x	x									x	x	x	x	x	x
Oily fish	x	x												x				
Potatoes/ Pasta/ Rice																		
Fruit																	x	x
Vegetables	x	x	x	x										x	x			
Savoury					x	x												
Sweets	x	x	x	x	x	x							x	x	x	x	x	x
Tea	x	x		x		x										x		
Coffee	x	x		x		x							x	x		x	x	x
fruit/ vegetable juice	x	x	x	x	x	x							x	x	x	x	x	x
Fizzy drinks																		
Nutrients																		
SFAs																		

MUFAs																	x	x
ω6PUFAs																		
ω3PUFAs							x	x	x	x			x			x		
*t*FAs									x	x			x		x	x		
*t*MUFAs									x	x	x	x	x		x	x		
Quercetin							x	x			x	x	x				x	
Catechin								x										
Epicatechin														x				
Flavones								x										
Procyanidins																		
Flavanones							x				x	x						x
Phytoestrogens																		
Protein							x											
Cholesterol							x	x	x		x	x		x	x			
Sugars									x	x							x	x
Starch							x	x	x			x						
Fibre							x			x			x	x	x	x		x
Sodium										x								
Potassium																		
Calcium										x				x				
Magnesium							x	x		x	x	x	x	x		x	x	
Phosphorus												x						x
Iron													x					
Copper							x	x				x		x				x
Zinc									x		x							
Manganese												x					x	x
Selenium																		
Iodine							x			x								
Vitamin A																		
Carotenes										x								
Vitamin D												x					x	
Vitamin E								x						x				
Vitamin B1																		
Vitamin B2																		
Niacin										x					x			
Vitamin B6																		
Vitamin B12																	x	
Folate																		
Pantothenic acid																		
Biotin												x			x			
Vitamin C															x	x		x

8.4 Summary of results of chapter 8

In this chapter the overall and stepwise regression analysis of the study was presented. The explanatory variables that were investigated in the overall analysis and included in the stepwise regression models consisted of demographic factors, lifestyle variables, food variables and nutrients. The overall analysis was conducted for the quartile, standardised and continuous forms of the continuous variables. Finally stepwise regression analysis was conducted for the quartile form of the continuous variables in the whole sample and then separately for men and women.

8.4.1 Overall analysis

In the overall analysis of the study, distributions and correlations of all the explanatory variables, as well as univariable logistic regression of colorectal cancer on each explanatory variable were investigated.

The risk factors that were significantly associated with colorectal cancer, according to the results of the univariable logistic regression were: 1) the demographic and lifestyle factors: family history of cancer, NSAIDs intake, dietary energy intake, HRT intake and physical activity (Table 96); 2) the food group variables: vegetables, eggs, sweets, fruit/ vegetable juice, oily fish, coffee, fruit, savoury foods and white fish (Table 96); and 3) the nutrient variables: *t*MUFAs, ω3PUFAs, SFAs, *t*FAs and MUFAs (fatty acids); quercetin, catechin and phytoestrogen (flavonoids); cholesterol, fibre, protein and starch (macronutrients); magnesium, potassium, manganese, copper, iron, zinc, phosphorus, selenium (minerals); niacin, vitamin B6, carotenes, vitamin C, vitamin A, potential niacin, biotin, folate, pantothenic acid, vitamin D, vitamin B1 and vitamin B12 (vitamins) (Table 96).

8.4.2 Stepwise regression analysis

8.4.2.1 Original sample

Forward and backward stepwise regression models were applied in three different sets of variables using the quartile form of the continuous variables in the whole sample (Tables 97-101) and after sex stratification (data not shown). In Table 102, a matrix of the variables included in the three different sets and finally selected into the forward or backward stepwise regression models for the whole, female and male sample is presented. The variables that were included in 100% of the models derived

from the whole, female and male analysis of all three sets were: family history, NSAIDs, sweets and fruit/ vegetable juice. The following variables were included in models derived from the female sample, but not in models derived from the male samples: *t*FAs (100% of the models), vegetables (75%), ω3PUFAs (75%), HRT (67%), breads (50%) and niacin (50%). In addition, the following variables were included in models derived from the male sample, but not in models derived from the female sample: dietary energy intake (100% of models), physical activity (83%), quercetin (75%), flavanones (75%), manganese (75%), fruit (50%), savoury (50%), MUFAs (50%), phosphorus (50%), copper (50%) and vitamin D (50%) (Table 102).

8.4.2.2 Bootstrap samples

The bootstrap method was applied to investigate the stability of the models and it was applied for forward and backward stepwise regression of all three sets of variables (whole sample). In particular, 100 bootstrap samples were randomly drawn from the original sample. Then, each bootstrap sample was used to apply forward and backward stepwise regression for each set of variables (set 1, 2 and 3).

According to the findings of this analysis, all 100 models derived after forward stepwise regression were chosen once (for all sets of variables), and the same was observed for the 100 models derived after applying backward stepwise regression. The agreement between the models derived from forward and backward stepwise regression within the same bootstrap sample was high for the analysis of the set 1 variables (mean percentage of agreement (SD): 96.97% (4.56%)), whereas it was lower for the analysis of the set 2 and set 3 variables (mean percentage of agreement (SD): 84.36% (9.08%), 83.12% (9.38%); respectively). Furthermore, the number of variables that were selected to be included in the models of the 100 bootstrap samples was smaller for the set 1 analysis (11-20 variables), than for the set 2 and set 3 analysis (10-31 and 15-39 variables respectively) (data not shown). In addition, for set 1, 2 and 3, more variables were selected to be included in models derived from backward stepwise regression (mean number of selected variables (SD): 22.54 (6.37)) than in models derived from forward stepwise regression (mean number of selected variables (SD): 20.02 (5.15)). Finally, the variables that were selected to be included in models for the majority of the bootstrap samples (more than 90%) were: 1) family history, NSAIDs and dietary energy, if we consider all three sets of

variables; 2) family history, NSAIDs, dietary energy, eggs, sweets, fruit/ vegetable juice and white fish, if we consider set 1 and set 3; and 3) family history, NSAIDs and dietary energy, if we consider set 2 and 3 (data not shown).

9 DISCUSSION

9.1 Introduction

In the first three chapters of this thesis background information regarding colorectal cancer and its main risk factors was given and the aims and objectives of the current thesis were presented. In chapter four, all the aspects regarding the design of the study this thesis was based on and the applied analytical methods were described. Finally, in the four following chapters the results of the dietary analysis of the SOCCS study were presented, with the most important findings being summarised in the end of each chapter.

In this chapter, which is divided in three parts, the main issues of this thesis will be described. In the first part of the discussion, issues regarding the methodological and analytical aspects of this thesis will be presented. In particular, the strengths and limitations of the study design and of the employed analytical methods will be addressed and evaluated. In the second part of the study the most important findings and principal results of the analysis will be discussed and compared with findings from previous published studies. Finally, in the last part of the discussion, the main conclusions that are drawn from this thesis as well as suggestions for future research will be presented.

9.2 Methodological and analytical issues

In this part of the chapter the following issues will be presented and discussed: 1) epidemiological issues, including description of the main study designs of observational analytical epidemiology together with their main advantages and disadvantages (bias and confounding); 2) nutritional issues, including methods of diet assessment, diet validation and energy adjustment; and 3) analytical issues including study power calculations (for the matched and the unmatched datasets), methods of multiple testing correction and issues regarding the stepwise regression and bootstrap sampling methods. The main strengths and limitations of the current study regarding the above methodological and analytical issues will also be summarised and discussed.

9.2.1 Epidemiological issues

Epidemiological studies are used in order to investigate the distribution and the main determinants of a particular disease in different populations. They are divided in experimental (like randomised clinical trials) and observational studies. Observational studies can then further divided according to whether the unit of the study is a population (ecological studies) or an individual (descriptive: case series; analytical: cross-sectional, case-control, cohort studies).

Large cohort studies of diet and colorectal cancer

Some of the main cohort studies that have investigated associations between specific nutrients, food items or food groups and colorectal cancer, include:

1) Cohort studies conducted in the USA and Canada:

- Women's Health Study (USA): 37,547 female participants
- New York's University Health Study (USA): 14,727 female participants
- Iowa's Women's Health Study (USA): 32,215 female participants
- Nurses' Health Study (USA): 87,733 female participants
- Health Professionals Follow-up Study (USA): 47,949 male participants
- Physicians' Health Study (USA): 22,071 male participants
- NHANES I Epidemiologic Follow-up Study (USA): 14,407 male and female participants
- Multiethnic cohort study (USA): 191,011 male and female participants
- Cancer Prevention Study II Nutrition (USA): 127,749 male and female participants
- Breast Cancer Detection Demonstration Project (USA): 45,354 female participants
- Canadian National Breast Screening Study (Canada): 5,629 female participants

2) Cohort studies conducted in Europe:

- European Prospective Investigation into Cancer and Nutrition (Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom): 520,000 male and female participants
- Netherlands Cohort Study (The Netherlands): 120,852 male and female participants
- Finnish Mobile Clinic Health Examination Survey (Finland): 9,959 male and female participants

- Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Finland): 27,111 male participants
- Swedish Mammography Cohort Study (Sweden): 61,433 female participants
- Cohort of Swedish Men (Sweden): 45,306 male participants
- Cohort Study in Norway (Norway): 50,535 male and female participants

3) Cohort studies conducted in Asia:

- Japan Public Health Centre-based Study (Japan): 95,376 male and female participants
- Shanghai Women's Health Study (Shanghai): 73,314 female participants

Large case-control studies of diet and colorectal cancer

Some of the main and largest case-control studies that have investigated associations between specific nutrients, food items or food groups and colorectal cancer, include:

1) Case-control studies conducted in the USA and Canada:

- Ontario Familial Colon Cancer Registry (Canada): 2,985 male and female participants
- A population-based case-control study of colon cancer conducted in Northern California, Utah, and the 'Twin Cities' area of Minnesota (USA): 4,403 male and female participants
- Oahu (Hawaii) case-control study (USA): 2,384 male and female participants
- A population-based case-control study in Massachusetts (USA): 2,394 male and female participants
- A population-based case-control study of rectal cancer conducted in Northern California and Utah (USA): 1,730 male and female participants
- North Carolina Colon Cancer Study (USA): 1,609 male and female participants

2) Case-control studies conducted in Europe:

- Scottish Colorectal Cancer Study (current study; Scotland): 4,837 male and female participants
- A multi-centre Italian study (Italy): 6,107 male and female participants

3) Case-control studies conducted in Asia and Australia:

- Fukuoka Colorectal Cancer Study (Japan): 1,575 male and female participants

- Hospital-based Epidemiologic Research Program at Aichi Cancer Centre (Japan): 2,535 male and female participants
- Melbourne Colorectal Cancer Study (Australia): 1,442 male and female participants

9.2.1.1 Strengths and limitations of the current study (epidemiological issues)

Strengths

Case and control selection

One of the main strengths of this colorectal cancer study is its careful design regarding the selection and recruitment of the study participants (cases and controls). A careful recruitment strategy both for cases and controls was developed, which involved the set up of a firm recruitment protocol covering all the main steps of the participant's study entry.

In particular, regarding case recruitment, strict criteria were applied in order to avoid misclassification bias and only incident cases of colorectal adenocarcinoma were included in the study. In addition, correct diagnosis was ensured by careful examination of the pathological reports and was histologically confirmed. An attempt was made to keep the time between diagnosis and recruitment short by developing a recruiting network of well-trained nurses, by placing recruitment staff in hospitals and by establishing close cooperation with clinical staff. From data provided from the Scottish Cancer Registry, we were able to calculate that the median time between date of recruitment and date of cancer diagnosis (incidence) was 150 days. Incidence date was reported as the date of the first pathology report for the particular colorectal cancer and often pre-dates the date of hospital admission. We also compared basic information on age, gender and place of residence of the cases included in our study with data aggregated over a five year period (1999-2003) from the Scottish Cancer Registry. There was a slight over-representation of male cases but the distribution among the 15 boards of Scotland was similar to that from the Cancer Registry.

Controls were randomly selected from the CHI, which is a national register of all individuals who are registered with a GP in Scotland and represents an ideal sampling frame for the selection of population based controls (95% completeness). In addition,

controls were closely matched to cases by age, sex, and area of residence. In particular, strict matching criteria were applied and selected controls were individually matched to cases according to sex, age (+/- 1 year) and health board area. The main advantages of a matched case-control study are that cases and controls are more comparable, that confounding from the matched factors is accounted for and usually study precision and power is increased.

Confounding factors selection

An attempt was made to minimise the confounding effect by careful selection of the confounding factors, which were chosen according to findings of previous studies. In particular, we chose to adjust the observed associations (of the four hypotheses of the first part of the thesis) for the following factors: family history of cancer, BMI, physical activity (hours of cycling and of doing other sport activities per week; proxy of total leisure physical activity), smoking (never and ever smokers), energy intake, fibre intake (energy adjusted), alcohol intake (energy adjusted) and NSAIDs intake. In addition, age, sex, deprivation score (proxy of the social-economic status) were included as confounding factors in the analysis of the unmatched dataset, but not in the analysis of the matched dataset, since age, sex and health board area were the matching risk factors.

Univariable analysis of the confounding factors showed that the following factors were not statistically significant with colorectal cancer in our study population: for the matched dataset BMI (p=0.38) and smoking (p=0.51) (Table 63) and for the unmatched dataset sex (p=0.85), deprivation score (p=0.94), BMI (p=0.42) and smoking (p=0.63) (Table 79). The selection of the confounding factors was made prior to any analyses and therefore even the factors that were found not to be significantly associated with colorectal cancer were included in the multivariable analyses. However, results from multivariable regression analysis models that did not include BMI and smoking (matched analysis) and sex, deprivation score, BMI and smoking (unmatched analysis) were similar to the ones that included these confounding factors.

Limitations

Case and control selection

It is inevitable that this study, despite close cooperation with clinical staff, was unable to recruit patients who died soon after diagnosis or who were seriously ill at presentation or in the post-operative period. There is, therefore, an under-representation of cases that were very ill at time of presentation to hospital, which might affect the external validity of the study. In addition, there might be a possibility that early stage of disease may be due to more frequent screening of more health conscious cases, which also had a healthier lifestyle. We were not given ethical approval to collect data from the participants that refused or were not able to be included in the study and therefore we cannot identify whether there are any significant differences. Regarding the matching procedure, even if 2,062 cases and 2,776 controls had complete and valid FFQ and LCQ data and could be included in the analysis, for some cases no controls that fulfilled all the matching criteria were identified. Therefore when the fine matching was kept, 573 cases and 1,287 controls needed to be excluded from the analysis (1,489 matched pairs were included in the matched analysis).

Despite the careful design of this case-control study, many cases and controls refused to take part in the study and participation rates were 52% for cases and 39% for controls. Participation rates for both cases and controls differ according to area of residence and age. In particular, subjects from the Health Boards of Grampian, Highland and Lothian were more likely to participate, whereas subjects from Greater Glasgow Health Board were less likely to participate (Table 33, Table 35). In addition, both cases and controls that refused to participate were significantly older than the ones that agreed to participate ($p<5x10^{-5}$) (Table 33, Table 35), which is in accordance with findings from other population-based case-control studies suggesting that younger individuals are more likely to participate (277;278). Furthermore, participation rates in our study differ according to disease status with fewer controls having agreed to participate than cases (39% vs. 52%). This is a common problem of population-based case-control studies, with other case-control studies also reporting lower participation rates for controls than for cases (277-279). The difference in participation rates between cases and controls might be due to the fact that cases are more eager than population controls to take part in a study that investigates their disease. Therefore in our case, the controls that agreed to

participate might have had a healthier diet and lifestyle and therefore were more eager to participate in a case-control study asking about their lifestyle choices and dietary habits (participation bias). This fact is also supported by the lower participation rates of controls with high deprivation (deprivation scores of 6 and 7; Table 35).

A direct comparison of participation rates in our study and similar population-based case-control studies may not be straight forward mainly due to not reporting or inconsistently reporting of participation rates from case-control studies (280). In particular, a recent review demonstrated that more than 50% of published case-control studies failed to report any information regarding participation rates (280). Generally, it has been observed, that participation rates of population-based case-control studies conducted after 1990 were considerably lower than those of studies conducted between 1970 and 1990 (280). Although the exact reasons for this decline are not fully understood, possible explanations include changes in study design and in methods of recruitment, as well as differences in social and lifestyle factors (280). An additional explanation might be that many recent case-control studies include collection of biological specimens, such as blood (like in our study), which may also have an effect on participation rates (280). Median participation rates of 34 population-based case-control studies conducted from January 1 to April 30, 2003 and published in 10 high impact epidemiology, public health and medical journals was 84% (range: 44%-99%) for cases and 74% (range: 41%-88%) for controls (280). In addition, participation rates of a large population-based case-control study of colon cancer conducted in USA were approximately 76% and 64% for cases and controls, respectively (278). Therefore participation rates of both cases and controls in our study were lower than the aforementioned, with one possible explanation being that collection of biological specimens was required. In addition, lower participation rates in controls might be due to the recruitment procedure. In particular, eligible controls were contacted only via mail by their GPs, since we did not have ethical approval to contact them directly by phone. It has been shown that population-based case-control studies that use letters as the only contact mode have lower participation rates (279). In addition, in case of no reply, we had ethical approval to contact GPs or controls only once more. It has been suggested

though that in order to obtain high control participation rates a number of contacts as high as 14 may be required (281). Given these lower than usual participation rates for both cases and controls of our study, participation bias cannot be ruled out and therefore results should be interpreted with caution.

An alternative case-control design that tends to overcome the low participation problem is the hospital-based case-control study, where controls are selected from hospitals (patients with a disease other than the one under investigation). Hospital-based case-control studies have usually higher participation rates than the population-based ones and in some cases they can be as high as 95% (282). Median participation rates of 33 hospital-based case-control studies conducted from January 1 to April 30, 2003 and published in 10 high impact epidemiology, public health and medical journals was 92% (range: 74%-99%) for cases and 86% (range: 60%-99%) for controls (280). In addition, participation rate of a large hospital-based multi-centre case-control study of colorectal cancer conducted in Italy was approximately 96% for both cases and controls (282). However, the hospital based design is usually not preferred, mainly because hospital controls may have a condition that is also influenced by the risk factor under investigation or because they may come from different populations, which will affect the representativeness of the case-control study.

Finally, when no ideal control group is identified, then a possible strategy is to have more than one control groups (i.e. one hospital-based and one population-based) and compare results obtained from different control groups.

Bias and confounding

Since cases were aware of their disease status when completed the questionnaires, it is likely that they recalled their dietary and lifestyle habits differently than controls (recall bias). In addition, completion rates in cases were lower than completion rate in controls (65.7% vs. 83.9%), which is likely to be due to cases being re-admitted to hospital or otherwise too ill to fully cooperate in the study.

Regarding confounding, we tried to minimise the confounding effects by measuring and adjusting for the majority of the potential confounding factors. However, the possibility of residual confounding due to not controlling for unknown or unmeasured confounding

factors or due to measurement errors of the accounted confounding factors can not be ruled out.

9.2.2 Nutritional epidemiology issues

Nutritional epidemiology is based on the application of experimental or observational epidemiological studies in order to investigate the effects of particular nutrients, food items or food groups on a disease. Even if randomised clinical trials are considered as the gold standard in order relationships between nutritional factors and diseases to be established, there are many cases where only observational studies can be applied (283). In the following sections issues regarding diet assessment, validation and energu adjustment methods of the observational studies will be presented and discussed. Weight will be given to the description of the FFQ, since this was the diet assessment method used in the current study.

9.2.2.1 Diet assessment

The main diet assessment methods are Diet Recalls, Food Records, Diet Histories and FFQs. Diet Recalls and Food Records methods are based on recording the foods that are consumed by the individual at one or more days, whereas Diet Histories and FFQs are used in order to measure long-term dietary intakes.

Diet Recalls and Food Records

Diet Recalls are usually based on a 24-hour level and are normally conducted by a trained interviewer. The interviewer asks the participant about the foods and drinks that he/ she consumed during the previous day as well as details about the used food preparation methods. This method is relatively quick, but it relies on the short term memory abilities of each individual. On the other hand, Food Records are based on the recording of consumed foods and drinks at the actual time of the consumption. Therefore, this method does not rely on the individual's memory abilities, but it requires more time and effort than the Diet Recall method. The main advantages of these two methods are that they can be used in order to estimate absolute intakes of foods, energy, macro- and micro-nutrients and that due to the fact that they contain open-ended questions they can be very specific regarding the consumed food types and the food preparation methods. The main disadvantage of these methods is that they do not capture

the usual dietary habits of the individuals, unless multiple recalls or records are to be collected, which is not an efficient process for large epidemiological studies due to the effort and cost that are required (283).

Diet Histories and Food Frequency Questionnaires

The main characteristic of the Diet History is that it captures quantitative information about the individual's usual diet using a fixed food item list, but information regarding frequencies and portions are obtained from the individual. Whereas the first Diet History developed by Burke in 1947 was menu-based, the most recent ones are initially list-based and then the individual reports frequencies and portion of only the foods that he/ she usually consumes (284).

Food frequency questionnaire, which is the most widely used diet assessment method, measures long-term dietary intakes like Diet History, but their main difference is that in addition to the fixed food list it also has a fixed frequency. FFQs can be administered by interview (personal or by telephone) or they can be completed by the study participants (self-administered). The food list section of the FFQs can be long or short depending on the purpose of the study and the hypothesis that is to be tested, but generally a comprehensive assessment of the diet by including a wide range of foods and drinks is preferred. In addition, the food-list should consist of foods that are relevant to the usual diet of the particular population that the study results will be applied to. The frequency section usually has a multiple-choice format and the individuals can choose how often they consume the reported food item (never, once a month, two days per week, etc.). Finally, the portion section is optional and when portion information is recorded (semi-quantitative FFQ) the individuals report how often they consume a specific portion of the food item (rather than reporting how often they consume a food item). For some food items that come in natural portions (such as milk or bread) adding portions is straightforward and also sometimes adds clarity. For food items that do not come in natural portions (such as meat or rice) a typical portion can be specified and subjects are expected to double the frequency of consumption when their usual portion is twice the specified one. However, that practice might introduce bias if not all the participants adjust the consumption frequency according to their usual portion (283;284).

The FFQ method is relatively easy (even when the FFQ is self-administered), fully computerised, inexpensive and quick making it one of the most popular ways to assess the usual and long-term dietary intakes, especially for studies that include large numbers of study participants. However, there are limitations of the FFQ method, with one of them being that the derived quantitative estimates of the food and nutrient intakes cannot be used as absolute intakes and should only be used to rank individuals into categories (e.g. quartiles of intakes). In addition, the participants are required to report their usual intakes of generally more than 100 different foods for a specific past period of time. This task, which relies on the memory abilities of each individual, might be complex for some participants (especially ones with particular disabilities or of an advanced age). Finally, since the FFQ has a certain list of foods, a particular questionnaire can not be applied in different populations or different times and therefore results from studies using different FFQs are not always comparable (285).

Nutrient assessment

The immediate outcome of all the diet assessment methods is data about the food group and item intakes. However, many hypotheses are about the investigation of the associations between intakes of particular nutrients and disease. In order to convert food intakes to nutrient intakes, a nutrient database and an analysis programme are necessary. Regarding the conversion of foods measured by an FFQ in nutrients, if portion sizes have been specified (semi-quantitative FFQ), the nutrient values can be estimated according to that portion sizes. However, if no portion sizes have been specified, then a typical or average portion size is used in order to estimate the nutrient intakes. Finally, for open-ended questions, specific data for each reported response need to be obtained (283).

The nutrient databases used by nutritional observational studies are normally nationally based. For the estimation of total energy and the main macro- and micro-nutrients the most commonly used database in the UK is the McCance and Widdowson's Composition of Foods (5th Edition plus related supplements). For specific nutrients (such as flavonoids, specific fatty acids, etc.) supplementary nutrient databases need to be used. For example for estimating flavonoid intakes a flavonoid composition database

containing entries from fruit, vegetables, beverages, jams, chocolate and herbs was developed in Scotland and was used for estimating flavonoid intakes in the current study (274).

Measurement error at diet assessment

When assessing diet two types of measurement error can occur: random or systematic. When assessing diet by using either Diet Recalls or Food Records, within-person random errors reflect the day-to-day variations in dietary intakes and they can usually be accounted for and corrected by using two or more dietary measurements for each participant. On the other hand, when assessing diet by using either Diet Histories or FFQs, within-person random errors can occur due to true changes of diet over time, which is particularly important for a disease that has a long latent period (e.g. cancer). To be more specific, usually cases of a case-control study are asked to complete an FFQ for a reference period of approximately a year prior to their diagnosis. However, their dietary habits for even up to 10 years prior to their diagnosis might have affected initiation and progression of a disease with a long latent period, and therefore true changes (that are not captured by the FFQ) within this 10 year period can lead to measurement errors. Within-person systematic errors mainly occur when a participant deliberately over- or under-reported the intake of a particular food (when Diet Recalls or Food Records are used) or when an important food for one or more participants (but not for others) has been omitted from the fixed food list of a Diet History or an FFQ. Between-person random error happens when for example there is a random over-reporting of a food item from some participants and a random under-reporting of the same food item from some other participants. In that case, the mean of the intake of the food item will be correct, but there will be an over-estimation of the standard deviation. Finally, between-person systematic error occurs when the over- or under-reporting is not random and some examples are the omission of an important food item from the FFQ, inaccurate compositional databases, or under- or over reporting according to the disease status in a case-control study (recall bias). Usually random errors (both within- and between-persons) tend to attenuate the relationships between nutrients and disease. However, effects of systematic errors on observed associations are generally

unpredictable and can bias the results of a study. Measurement errors when assessing diet with an FFQ can be derived by the fixed food list, by the memory abilities of the participants and by wrong interpretation of the food portions (for a semi-quantitative FFQ) (283;286).

9.2.2.2 Diet validation

As we described in the previous sections each diet assessment method has specific strengths and limitations. Whichever method is chosen, validation of its performance in assessing dietary intakes is required. In the following chapter, we will discuss about reproducibility and validity methods of the FFQ.

Reproducibility

Reproducibility of a questionnaire is estimated by administering the same questionnaire to a specific number of participants in two or more different occasions and then examining the consistency of the measurements. The interval between the two different administrations should be neither too short, since then participants will probably remember their previous responses, nor too long, since true changes in dietary habits may decrease the questionnaire's reproducibility. Finally, whereas a questionnaire with low reproducibility should not be considered as a valid method of assessing long-term diet, a questionnaire with high reproducibility does not necessarily mean that is a diet assessment method of high validity (283).

Reproducibility is also a way to account for the random measurement errors. For categorical variables, it is usually addressed by calculating the kappa or the weighed kappa statistic, which is equivalent to the measurement of the proportion of agreement between the measurements in the two different time points. For continuous variables, reproducibility is usually estimated by calculating the interclass correlation coefficient, which represents the reliability of a measurement (286).

Validity

On the other hand the relative validity of a questionnaire is estimated by comparing nutrient intakes measured by the FFQ with intakes measured using another diet assessment method (external standard method). It is preferred to use a method that its limitations (errors) are of different type than the errors produced by the FFQ. Usually,

the validation method that is used for an FFQ is diet assessment by Food Records, since these two methods have different types of limitations (FFQ: fixed food, frequency and portion questions, rely on memory, rely on the way a question is interpreted vs. Food Records: open-ended questions, do not rely on memory, no questions). However, in order to represent average dietary intakes, multiple Food Records need to be obtained. In addition, 24-hour Diet Recalls can be used to validate FFQs. Even though, these two methods share similar sources of measurement errors (both depend on memory), validation using Diet Recalls might be the only option in cases of illiterate or less motivated participants (283).

Alternatively, an FFQ can be validated by comparing nutrient intakes estimated by the FFQ with measurements of an appropriate biomarker of the particular nutrient. The advantage of this validation method is that FFQ and biomarker errors are not correlated and therefore spurious validation results can be avoided. However, there are specific limitations of applying this validation method. In particular, usually biomarker levels of a particular nutrient do not depend only on dietary intakes, but also on other lifestyle choices, physiological characteristics and genetic variants. In addition, biomarker measurements are subject to laboratory and technical errors as well as to daily dietary intake variations. Generally, the effect of these limitations is to attenuate the correlations between the questionnaire and biomarker measurements, a fact that should be accounted for at the interpretation of the results. Finally, appropriate biomarkers are only available for a few specific nutrients and therefore, by applying this validation method intakes of several nutrients can not be validated (283).

Validation studies are usually conducted in a subset of the study population and the usual size of the subset lies between 100 and 200 individuals. The two main methods that are used to assess the validity of the FFQ are calculation of the Pearson correlation coefficient (for normally distributed variables) and the Spearman rank correlation coefficient (for not normally distributed variables) between the FFQ and the validation method measurements. Alternatively, the kappa and weighed kappa statistics can be calculated in order to measure misclassification when measurements of both methods are divided into different intake categories. It has been suggested that: 1) correlations of 0.5-

0.7 between the FFQ and the validation method's measurements, 2) more than 50% of subjects classified in the correct category (tertile, quartile, etc.) and less than 10% of subjects classified into a wrong category (tertile, quartile, etc.) and 3) weighed kappa values above 0.4 indicate that the FFQ has the ability to correctly rank individuals according to their dietary intakes (270).

9.2.2.3 Energy adjustment

Analysis of nutritional observational studies require controlling for dietary energy intake in order to ensure that observed associations are not due to a higher or lower total energy intake between cases and controls. This requirement is more important when energy intake is highly correlated with both the nutrient under investigation and the disease. The main energy-adjustment methods are: the residual energy adjustment method, the standard multivariable method, the energy partition method, the nutrient density method and the multivariable nutrient density method.

Residual energy adjustment method

This method is based on the regression of the nutrient on total energy intake and then inclusion of the residuals of this regression in the model with disease as the dependent variable. This method has been thought to be an equivalent of a study that examines the effect of particular nutrients by keeping total energy intake constant. In the case that total energy intake is also an important and recognised risk factor of the disease, it has been suggested that total energy intake should also be included in the disease-model (as a co-variable together with the nutrient residuals variable).

Standard multivariable method

The standard multivariable method is based on the inclusion of total energy and the nutrient intakes in the same model. The residual energy adjustment method and the standard multivariable method give usually similar coefficients for the association between the nutrient and the disease. However, the main difference between these two models is about the interpretation of the coefficient of the total energy intake term. In the residual method the coefficient of this term represents the association between energy intake and the disease, whereas in the multivariable method the coefficient represents the

association between energy intake from other nutrients than the one under examination and disease.

Energy partition method

For the partition method of energy adjustment, energy intake from the nutrient under investigation and energy intake from other sources are entered in the model separately. Using this method, the association between the particular nutrient and the disease are protected from the confounding effect of energy intake from other sources. However, any observed association might be due to the energy contribution of the nutrient on the total energy intake. Another limitation of this method is that it can not be directly applied for nutrients that do not contribute to total energy intake.

Nutrient density methods

The simple nutrient density method is based on directly dividing the nutrient intakes with total energy intake. This is a convenient method that provides simplicity and practicality, especially when somebody needs to describe food or diets in a comparable way. However, this method does not protect from the confounding effect of total energy. In particular when energy intake is associated with the disease, then nutrient densities (nutrient/ total energy) tend to be associated with disease in the opposite way to total energy. On the other hand, if energy intake is not associated with the disease and it is only weakly correlated with the nutrient intakes, then by dividing nutrient intakes with total energy, variation might be added in the nutrient densities. Increased variation will be added to the nutrient densities, also when energy intake is measured inaccurately. A way to use the nutrient densities, without having to deal with the limitations mentioned above, is to include together total energy and nutrient densities as covariables in a model with disease as dependent variable (Multivariable nutrient density method).

9.2.2.4 Strengths and limitations of the current study (issues regarding collection of nutritional, lifestyle and other data)

Strengths

Diet assessment, nutrient assessment and diet validation

To assess dietary intakes in the current study a semi-quantitative FFQ listing 150 food items was employed (Scottish Collaborative Group FFQ, Version 6.41), which also

included images of portion sizes and careful instructions in order to improve accuracy of diet reporting. In addition, this questionnaire was developed for studies of diet and health in Scotland containing the vast majority of food items that are frequently consumed from the Scottish population.

Further more, the nutrient databases that were used for estimating nutrient intakes were of UK (McCance and Widdowson's Composition of Foods) or Scottish level (flavonoid database). In addition, for some nutrients (e.g. specific fatty acids), food preparation and method of cooking (e.g. frying, grilling, oven-baking etc) could affect the amount of nutrient that was actually ingested. It is worth saying therefore that foods on this questionnaire were grouped with consideration of their fat content and method of cooking (e.g. oven chips are separate from home-cooked chips, and grilled fish is separate from fried fish). Furthermore, for foods which were home-cooked, the oil used for nutrient calculations was the one that was listed by the subject, whereas for foods cooked outside home an average of commonly-used fats was assumed. For bread, the spread(s) listed by the subject were used, taking into consideration the thickness of the spread (a scrape, a thin layer or a thick layer) as selected by the subject, with the aid of a colour photograph illustrating a thin layer.

Relative validity of the current FFQ was also assessed by comparing its nutrient intakes (total energy, main macro- and micro-nutrients and flavonoid subgroups) with those obtained from 4-day weighed Diet Records, in a sample of 41 men (mean age 36 years old) and 40 women (mean age 33 years old) (270;271).

Energy adjustment

We tried to minimise the confounding effect of total energy by carefully adjusting each nutrient intakes using the residual method. The alternative standard energy adjustment method was used for nutrients that were not normally distributed (even after transformation), since linear regression (first step of residual energy adjustment) between the nutrient (dependent variable) and total energy (independent variable) could not be applied.

Lifestyle and cancer questionnaire

Regarding the Lifestyle and Cancer Questionnaire, it was made up from questions from other standard questionnaires and we sought to employ validated instruments, where possible. In particular, the questions about physical exercise derived from the short version of the EPIC questionnaire. Reproducibility and relative validity of this questionnaire were checked in two different studies and it was found that although this physical activity index is not suitable for estimating energy expenditure at an absolute level, it can successfully rank participants according to their activity and cardio-respiratory fitness (287;288). Regarding other parts of the Lifestyle and Cancer Questionnaire, the questions about Women's reproduction history came from the Million Women Study's Questionnaire and the Employment section was based on the Census 2001 questions.

Limitations

We attempted to limit the common problems of nutritional epidemiology by adopting identical study procedures in cases and controls, use of validated questionnaires, use of images of portion sizes, use of careful instructions to improve accuracy of reporting diet and lifestyle habits and adoption of a recall period one year before diagnosis or recruitment date to reduce recall bias. However, recognised limitations of case-control studies employing questionnaires including recall bias, misclassification bias due to imprecise measurements (random measurement errors) and residual confounding after attempts to control for confounders might have affected the current study.

Diet assessment, nutrient assessment and diet validation

Variation due to random measurement error tends to attenuate the true associations between the risk factor and colorectal cancer risk, a bias called regression dilution. In order to account for regression dilution bias, dietary and other measurements should be obtained more than once for at least a subsample of the study sample. Interclass correlation coefficients between measurements can then be used to adjust the regression coefficients. In our study, dietary measurements were obtained only once for the majority of the study participants, whereas we obtained a second measurement of the diet for 44 population controls. The size of the subsample with duplicate dietary data was not large enough to accurately estimate the size of random measurement error and

to check the reproducibility of the questionnaire. Given the available resources, we were not able to collect duplicate data for more population controls and therefore we were not able to correct the regression coefficients for the effect of regression dilution bias. However, this type of error would have probably led to reporting underestimated rather than biased associations.

Regarding the FFQ validation studies, we cannot be sure of the exact validity of the estimate of nutrient intakes in our age group as the validity study was carried out in younger subjects (270). Furthermore, results of validation of this FFQ for ranking individuals according to specific fatty acid intakes were not available at the time that analysis of this thesis was conducted.

Energy adjustment

Regarding confounding, although adjusting for energy by using the residual method should have reduced the confounding effect of total energy, probably it would not eliminate it, since measurements of both energy and the nutrient would be subject to measurement error. On the other hand, for nutrients highly correlated with total energy intake, such as fatty acid intakes, the application of the residual adjustment method could have led to over-adjustment and this could have masked significant associations between these nutrients and colorectal cancer. In order to investigate this further, associations between colorectal cancer and intakes of subgroup and individual fatty acids obtained from multivariable logistic regression models (model III) using different energy adjustment methods were compared (residual, standard, simple nutrient density and multivariable nutrient density methods). Models using either method of energy adjustment produced similar findings, with high intakes of ω3PUFAs, EPA and DHA being associated with a statistically significant and dose-dependent decreased colorectal cancer risk. However, associations derived from models using the multivariable nutrient density method were slightly stronger with lower p-values. The only difference between findings after applying different energy adjustment methods was for stearic acid intakes. In particular, high intakes of stearic acid were found to be significantly and dose-dependently associated with an increased colorectal cancer risk, when applying the residual, the standard and the simple nutrient density methods, but not when applying

the multivariable nutrient density method. Regarding the other fatty acid subgroups and individual compounds, there were no differences in the observed associations no matter what energy adjustment method was used.

Lifestyle and cancer questionnaire

Regarding the Lifestyle and Cancer questionnaire, measurements were also obtained only once and therefore random measurement errors due to within-subject variation could not be estimated and reproducibility of the questionnaire was not measured. In addition, a limitation of the occupational part of the physical activity questionnaire was that it was designed for younger individuals than the participants of the current study with no proper separation for retired and unemployed individuals. Therefore, the retired participants of our study were misclassified as unemployed and their occupational physical activity was not reported (48% of the cases and 47% of the controls were classified as unemployed; Table 42). In order, to account for this limitation, we decided not to use data on occupational physical activity and use a physical activity measurement based only on leisure time activities. In addition, in order to reduce the number of individuals that should be omitted due to missing data, we chose to use information only for two leisure time activities: cycling and other physical exercise. It has been suggested, that higher-intensity physical activities are reported with greater accuracy (288) and therefore we believe that this limited physical activity measurement will be an acceptable approximation of the general physical activity status of the study participants for the purposes of providing a rank distribution of physical activity in the study sample.

9.2.3 Issues on applied analytical methods

In this section, we will briefly describe the main issues about the applied analytical methods and the way they could have influenced the results of the current thesis. In particular, issues regarding the power of the study (matched and unmatched dataset), the effect of multiple testing and the stepwise regression methods will be presented.

9.2.3.1 Power calculation

Power, sample size and hypothesis tests

"The power of a test is equal to the probability that a study of a given sample size can detect an effect size of a particular magnitude as statistically significant" (definition

taken from (289)). In order to calculate the power of a test we need to know the level of significance (α, usually 0.05), the size of the study and the size of the effect that we want to detect. The power calculation can be carried out either *a priori* (during the design stage of the study) or *post hoc* (after the end of the study). *A priori* power analysis is usually preferable, since it determines from the beginning the scale of effect sizes the particular study can detect. *Post hoc* power analysis is usually conducted in order to explain the inability of a particular study to detect statistically significant results. The power of a test can be increased by: 1) increasing the sample size, 2) increasing the significance level (i.e. from α=0.05 to 0.10), 3) aiming to detect larger effect sizes and 4) decreasing the measurement error (and therefore decreasing the standard deviations) (286).

Power calculations of the current study (matched and unmatched datasets)

As it has been already described, in the end of the study 2,062 cases and 2,776 controls had complete and valid FFQ and LCQ data and could be included in the analysis (unmatched dataset). However, for some cases no controls that fulfilled all the matching criteria were identified. Therefore, when the fine matching was kept 573 cases needed to be excluded from the analysis, leaving the matched dataset with 1,489 cases and 1,489 controls.

Power calculations were conducted using the software NQuery Advisors (version 6.0). The formulas that the power calculations were based on are presented in Appendix V. The matched dataset of 1,489 pairs of cases and controls had a 97% power to detect an effect size of 0.1 per SD at a significance level of 0.05 (paired 2-sided t-test). On the other hand the unmatched dataset (2,062 cases and 2,776 controls) had a 93% power to detect an effect size of 0.1 per SD at a significance level of 0.05 (student's 2-sided t-test). In addition, power calculations for weak, moderate and strong effect sizes (measured by the OR) showed that the matched dataset of 1,489 pairs of cases and controls had 44%, 78% and more than 99% power to detect ORs of 0.93, 0.85 and 0.43, respectively (McNemar's chi-square test). On the other hand, the unmatched dataset of

2,062 cases and 2,776 controls had 27%, 78% and more than 99% power to detect ORs of 0.93, 0.85 and 0.43, respectively (normal chi-square test).

Therefore, according to the above calculations, the matched analysis had slightly greater power to detect weaker associations, whereas both the matched and unmatched dataset had the same power to detect moderate and strong associations. Even if a study with a 44% power is not considered as sufficiently powered to detect a particular effect size, we decided to use the matched dataset for the analysis of the first two hypotheses (flavonoids and fatty acids), in case the associations between these novel dietary risk factors and colorectal cancer were weak. For the analysis of the additional risk factors (folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium), where we expected slightly larger ORs we chose to use the unmatched dataset. The main reason for this choice was that we wished to include all cases with environmental and genetic data, since for the additional hypotheses specific gene-environment interactions as well as stratified analyses according to genotypes of particular SNPs were selected to be investigated.

9.2.3.2 Multiple testing

Multiple testing methods

The possibility of finding significant results by chance increases, when in a single dataset multiple tests are performed (Type I error). Therefore it is necessary to correct the p-value significance level according to the number of performed tests. Different types of multiple testing correction have been developed and they can be roughly divided in the traditional methods and a more recent alternative one, known as the False Discovery Rate (FDR) method (290).

Bonferroni correction and similar methods

The Bonferroni method is the most simple though the most conservative method and it is based on setting a new level of significance by dividing the initial p-value level (α, usually 0.05) with the total number of tests performed (new p-value threshold = α/n, where n is the total number of performed tests). The null hypotheses are then rejected according to the new significance level. Similar methods based on the Bonferroni

method have been developed, which tend to be less conservative (e.g. the Holm's method, the Hochberg's method) (290).

False discovery rate

A quite different and far less conservative approach was introduced by Benjamini and Hochberg in 1995. The method was based on the fact that false positive results will occur in every study, but they tried to develop a method that identifies false positives without failing to reject false null hypotheses. This method is a three-step procedure, with the first step being the ascending ranking of the *k* observed p-values. The adjusted level of significance is then calculated separately for each p-value according to the formula: $\alpha^{*}i/k$, with i=1, 2, 3, …, k (the ranking position of the unadjusted p-value). Finally, each null hypothesis, for which corresponding unadjusted p-value is smaller than the new individual significance level, is rejected (290;291).

Multiple testing corrections in the current study

Part 1 of the study (aim 1, hypotheses 1-4): Bonferroni correction and FDR

For the first part (aim 1, hypotheses 1-4) of the current study, we corrected the observed p-values for multiple testing using the Bonferroni correction in three different ways.

Initially, the p-values were corrected according to the number of the performed independent tests (of the current and previous hypotheses). In particular, for the analysis of the first hypothesis (flavonoids), p-values were corrected for six independent tests (new level of significance 0.008); for the analysis of the second hypothesis (fatty acids) p-values were corrected for 14 independent tests (new level of significance 0.004); for the analysis of the third hypothesis (folate, vitamin B2, vitamin B6, vitamin B12 and alcohol) p-values were corrected for 19 independent tests (new level of significance 0.003); and finally for the analysis of the fourth hypothesis (vitamin D and calcium) p-values were corrected for 21 independent tests (new level of significance 0.002).

The second way that was used in order to account for multiple testing was by adjusting the p-values according to the number of tests conducted in each hypothesis (using both the Bonferroni correction and the less conservative FDR method). In particular, for hypothesis 1, we corrected the flavonoid subgroup p-values for 30 tests and the individual flavonoid p-values for 25 tests. For hypothesis 2, we corrected the fatty acid

subgroup p-values for 39 tests and the individual fatty acid p-values for 45 tests. For hypothesis 3, we corrected the observed p-values for 15 tests and finally, for hypothesis 4, we corrected the observed p-values for eight tests.

Finally, the third way that we used in order to correct for the number of performed tests was to correct for the total number of tests performed in all four hypotheses, applying both the Bonferroni and the FDR method. In the subgroup level, we corrected for 69 independent tests, whereas in the individual nutrient level we corrected for 93 tests.

Part 2 of the study (aim 2, overall and stepwise regression analysis)

The purpose of the overall and stepwise analysis was not to draw any specific conclusions about the strength of the associations between the risk factors and colorectal cancer. Instead, the purpose was to identify risk factors that seemed to be associated with the disease in order to generate new hypotheses, which could be then properly checked in other prospective or retrospective studies. Therefore, we thought that we would not need to correct for multiple testing, but we would take care to interpret these present findings appropriately within this context.

9.2.3.3 Stepwise regression

Forward and backward stepwise regression

The simplest data-driven model building approach is the forward stepwise regression. In this approach, variables are added to the model one at a time, and at each step each variable that is not already included in the model is tested for inclusion. The most significant of these variables is added to the model, as long as its p-value is below some pre-set significance level. Thus the first variable to be included in the model is the one that was the most significant in the initial analysis. The procedure of adding variables continues until all the variables are added in the model or none of the remaining variables has a p-value below the pre-set level when added to the model (292).

However, forward stepwise regression has drawbacks, including the fact that each addition of a new variable may render one or more of the already included variables non-significant or that one variable might be significantly associated with the outcome only when a group of other variables is also in the model. An alternative approach, which avoids these limitations, is backward stepwise regression. Under this approach, all

the variables of interest are fitted in the model and the least significant variable is dropped, as long as it is not significant at our chosen pre-set significance level. Reduced models are successively re-fitted and the same rule is applied until all remaining variables are statistically significant. Backward stepwise regression has also drawbacks. For instance, variables that may be dropped could have been significant if added to the final reduced model. In addition, backward stepwise regression should not be used when the sample size is small considering the number of independent variables that are included or when there might be issues of multicollinearity. Since in backward stepwise regression all variables are included in the initial model, an unstable initial model (either due to small sample size or multicollinearity) might produce spurious results (292).

In general both forward and backward stepwise regression methods are not used in cases when there is a clear hypothesis with already selected confounding factors. In contrast, they are mainly used in two other research settings: 1) To predict the likelihood of a particular outcome using several explanatory variables, when the predictive accuracy of the constructed model is more important than the risk factors that were chosen to be entered in the model; 2) To construct regression models that generate new hypotheses (explanatory analysis), which can then be tested as prior hypotheses in future studies (293). However, the models that derive from stepwise regression will possibly contain either variables, for which associations with the outcome are genuine or variables that have wrongly been identified as significant risk factors of the outcome (false positives). Therefore, to draw specific conclusions and to avoid reporting spurious findings, it is necessary to investigate the accuracy of the models, which are produced, either by comparing the final model with other models reported in the literature or by validating it in an independent dataset (293).

Bootstrap sampling method

An alternative method to explore the stability of the selected model is to apply the bootstrap sampling method. A bootstrap sample is a sample of the same size as the original sample chosen with replacement. Thus, a given subject in the original sample may occur multiple times, only once, or not at all in a specific bootstrap sample. This method is commonly used to estimate the sampling distribution of a particular test

statistic. In 2004, Austin and Tu proposed to use bootstrap sampling in order to evaluate the models produced by either forward or backward stepwise regression, by estimating the likelihood that a candidate variable is indeed an independent risk factor for a particular outcome (294).

Application of stepwise regression and bootstrap sampling method in the current study

In the current study we applied forward and backward stepwise regression models in three different sets of variables in the whole sample and after sex stratification, in order to investigate which of the explanatory factors were more strongly associated with colorectal cancer. All three sets included the main demographic and lifestyle variables. In addition, set 1 included food variables, set 2 included nutrient variables and set 3 included both food and nutrient variables. However, as already mentioned the goal of this part of the study was not to draw any specific conclusions about the risk factors identified, but instead to generate new hypotheses that could be studied in more detail in future observational studies.

Having identified the instability and general limitations of the stepwise regression models, we tested the reproducibility of the selected models by applying the bootstrap sampling method in the whole sample. One hundred bootstrap samples were selected and on each one forward and backward stepwise regression models were applied. Findings of the above analysis will be discussed in detail below.

Usually, when the bootstrap sampling method is employed, at least 1,000 to 10,000 bootstrap samples are produced. Therefore, the number of 100 bootstrap samples that was used in the current study is possibly not large enough to draw any specific conclusions about the stability of the selected models. However, the computing power and available time, when this thesis was conducted, did not allow us to perform this analysis in a larger number of samples. For future purpose and beyond the scope of the current PhD study we are planning to write a specific programme to perform the bootstrap sampling analysis in 1,000 to 10,000 samples.

9.3 Main findings

In this part of this chapter the main findings of the hypothesis driven analysis as well as of the overall and stepwise regression analysis will be discussed. In addition, results of the current study will be presented in relation to previous findings of other studies.

9.3.1 Main findings of part 1: Hypothesis driven analysis

9.3.1.1 Introduction

In this part of the chapter the main results of the matched analysis of the novel dietary risk factors (flavonoid and fatty acid subgroups and individual compounds) that comprised the first two hypotheses, and the main results of the unmatched analysis of the additional dietary risk factors (folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium) that comprised the last two hypotheses, will be presented and discussed.

In addition, results of this study will be compared with findings from previous studies in order to investigate the current causal evidence for each particular nutrient. As it has been suggested by Hill (295), to draw causal conclusions for a particular risk factor, nine criteria should be fulfilled that are related to:

1) Consistency of association across populations, study designs and statistical methods;

2) Strength of association (with a 20% change in risk to be considered as a positive association and a more than 40% change to be considered as a strong association);

3) Dose response (with greater amounts of a substance giving more protection/ risk and less amounts less protection/ risk);

4) Biological plausibility (i.e. existence of a plausible biological mechanism that explains an observed association; evidence usually collected from animal, *in vitro* and clinical studies);

5) Temporality (with the exposure to the risk factor preceding the onset of the disease);

6) Experimentation (i.e. evidence from randomised clinical trials);

7) Analogy (i.e. similar associations to be observed for similar diseases);

8) Specificity (i.e. the particular risk factor raises the risk of the particular disease and not generally of any disease);

9) Coherence (i.e. the possibility of causation of one risk factor is in accordance with other known facts).

However, in nutritional epidemiology a subset of the above criteria (consistency, strength, dose response, biological plausibility and temporality) is usually used in order to form specific nutrition recommendations and to draw causal conclusions. In addition, failure to fulfil a particular criterion does not always reflects to a lack of an association (296). In particular, lack of consistency might be due to different levels of intakes across the studies or due to noncomparability of the dietary assessment methods. Weak associations might be due to attenuation of true stronger effects by measurement errors. Lack of dose response might be due to lack of variation of intake or due to a threshold effect. Biological plausibility can not be always ascertained especially for novel dietary factors or for diseases that are not well described. Finally, temporality is sometimes difficult to be established. For cohort studies exposure assessment precedes the diagnosis, but it is possible that disease was already present when long latency diseases (e.g. cancer) are investigated. On the other hand, in case-control studies diagnosis precedes exposure assessment and therefore cases and controls are asked to report their dietary habits for a specific time period before diagnosis. However, for diseases of long latency, this time period might not be long enough. If evidence for the association between a particular dietary factor and a disease is in conflict with all five criteria, then public recommendation and causal conclusions for this dietary factor are not suggested. On the other hand, if evidence is in accordance with all five criteria then one can argue that this particular dietary factor is likely to be causally related with the disease and a public recommendation regarding its intake is desirable. However, it is unlikely in nutritional epidemiology to report a perfect agreement or disagreement with all five criteria and sometimes dietary recommendations can be made even if some of the aforementioned criteria are not perfectly fulfilled (296).

9.3.1.2 Flavonoids

Introduction

The recent increase in published data on flavonoid content of foods has enabled the development of databases which can be linked to FFQs and provided us with

the opportunity to investigate the flavonoid chemoprotective effects, which have been reported *in vitro* and animal *in vivo* studies. In this study the 150 foods listed in the FFQ included all the most important sources of flavonoids. A number of different foods contributed to the intake of the five flavonoid subgroups and phytoestrogens in our study and the results were not determined by one major food category (major sources included: regular tea, onions, soups- home made, apples, oranges, satsumas or grapefruits and soya milk; Table 67). In addition, the main sources found in our population were similar to the main sources found from the flavonoid validation study (274). Median and range estimations of flavonoid intakes in the Scottish population as they were estimated from the 4-day weighed record data validation study (271), were: 18.9 mg/day (range: 1.9 - 58.0 mg/day) for flavonols and flavones, 59.0 mg/day (range: 1.8 - 263.3 mg/day) for flavan3ols, 22.5 mg/day (range 0-144.5 mg/day) for procyanidins and 1.2 mg/day (range: 0 – 238.6 mg/day) for flavanones. Finally, the estimates of this FFQ for flavonol, flavan3ol and procyanidin dietary intakes have been shown to be strongly correlated (r=0.70, 0.94 and 0.73 respectively) with 4 day weighed record estimates in the Scottish population, whereas FFQ estimates for flavones and flavanones were only poorly correlated (0.12, 0.33, respectively) (271).

Main findings

Regarding the main findings of the flavonoid analysis of the current thesis, whereas no statistically significant associations were observed in the crude model (model I), moderately strong inverse associations that showed dose response relationships were found in the energy-adjusted conditional logistic regression model II between colorectal cancer risk and intakes of the subgroups flavonols (p=0.02) and procyanidins (p=0.04) and the individual flavonoid compounds quercetin (p=0.002), catechin (p=0.0001) and epicatechin (p=0.04) (Table 68). After adjusting for the main potential confounding factors (model III), only the inverse associations between colorectal cancer and intakes of quercetin (p=0.04) and catechin (p=0.02) remained statistically significant (Table 68). We investigated the existence of collinearity effects by correcting for overall fruit and vegetable intake and for intakes of other individual flavonoids and the observed associations became more clearly defined (especially for model V, which was further

corrected for intakes of other flavonoids) (Table 69). According to our results the direction of the associations of flavonols, procyanidins, quercetin, catechin and epicatechin remained similar in all four models, although the effect sizes changed. It is difficult to be certain about which model shows the true associations, since there is limited knowledge on the biological mechanism of flavonoids. Therefore it might be possible that the very strong associations reported in model V were due to instability because of the highly correlated variables.

After correcting for multiple testing using either the Bonferroni or the FDR method, the inverse associations that remained significant were: with catechin (p=0.0001) and quercetin (p=0.002) in model II (Table 68) and with flavonols (p=0.0001) and catechin (p=0.007) in model V (Table 69). Therefore for the flavonoid subclass flavonols and for the individual compounds quercetin and catechin the direction of the associations remained constant in all five models and in addition their associations with colorectal cancer remained statistically significant after correcting for multiple testing.

In marked contrast there were no statistically significant associations between colorectal cancer and intakes of the other four of the six flavonoid subgroups (flavones, flavan3ols, flavanones and phytoestrogens; Table 68, Table 69). The association with catechin and epicatechin but the lack of association with the flavan3ol subgroup (comprising catechin, epicatechin and gallates) may be explained by our inability to study the other main representatives of flavan3ols – the gallates (epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, and gallocatechin) as described previously. The lack of association between colorectal cancer and the other three subgroups (flavones, flavanones and phytoestrogens) could be explained by different biological action of these flavonoid subgroups, limited dietary sources (celery and herbs for flavones, citrus fruit for flavanones and soya products for phytoestrogens), low levels of dietary intake of these subgroups in Scotland across all population groups (e.g. soya and soya products are not commonly consumed in Scotland) leading to insufficient variation in intake across the population to permit their study, or less complete nutritional database information on these subgroups leading to greater misclassification and loss of study power. In addition, results from the flavonoid validation study showed that FFQ

estimates for flavones and flavanones did not correlate closely (r=0.12 and 0.33, respectively) with results from 4 day weighed records (271) and so interpretation of the findings for these compounds is problematic and results may represent false negative findings.

We also explored associations between colorectal cancer risk and the intakes of foods that were the main sources of the flavonoids with statistically significant associations (regular tea, onions, apples and red wine). Comparison of the highest versus the lowest quartile of intake of these foods suggested that there is some evidence in favour of an inverse association but this is less well defined than in the analysis of the association of flavonol, procyanidins, quercetin, catechin or epicatechin intakes and colorectal cancer risk (Table 70).

Findings from the current study in relation to previous studies

Most of previous cohort studies reporting associations between colorectal cancer and flavonoids were either much smaller in scale (118;128;129;134) or did not investigate all six subgroups of flavonoids (125;132;133;139) (Table 4). In a recent analysis of the Iowa Women's Health study, associations between the main subgroups (flavonols, flavones, flavan3ols, anthocyanidins, procyanidins, flavanones and isoflavones) and incidence of several types of cancer (including colorectal) were examined, but neither intakes of total flavonoids nor intakes of any of the main subgroups were found to be significantly associated with colorectal cancer (131).

On the other hand, three of the four identified case-control studies reported significant inverse associations between flavonoid subgroups or compounds and colorectal cancer (Table 5). The Canadian and Chinese case-control studies examined the associations between colorectal cancer and only specific flavonoids, with the Canadian study reporting statistically significant and dose-dependent associations with lignans, isoflavones and phytoestrogens (137;138). In the Italian case-control study the effect of the main six flavonoid subgroups was examined and the authors have reported a significant inverse association for flavonols, flavones, anthocyanidins, and isoflavones (135). Associations between colorectal cancer and intakes of flavonols and anthocyanidins were similar in strength to the associations reported from the current

study (results published in (136)). However, the inverse association for flavones and isoflavones, which was reported in the Italian case-control study, was not replicated from our study. This may be due to the lower validity of our questionnaire for flavones and the fact that we studied phytoestrogens rather than isoflavones which represent a subgroup of phytoestrogens. In addition the main differences between our and the Italian study were that the controls that were included in our study were closely matched population-based rather than hospital-based controls. In addition FFQ flavonoid estimations were calculated from a nutrient database developed for this study population, whereas in the Italian study the flavonoid database of U.S. Department of Agriculture was utilised (135).

Some animal and cell-line studies have reported chemoprotective effects of flavonoids, with possible biological mechanisms being inhibition of DNA oxidation (297;298), alteration of phase I and II drug metabolising enzymes (299-301), inhibition of protein kinases, blocking of receptor-mediated functions, alteration of cell-cycle checkpoints apoptosis, inhibition of angiogenesis, invasion and metastasis and epigenetic changes in promoter methylation and chromatin remodelling (302). An alternative theory for the protective effect of flavonoids is through their regulation of the *COX-2* gene. Increased expression of COX-2 enzyme provides survival advantage to cancer cells through high cell proliferation and angiogenesis. Results from recent laboratory and mechanistic studies show that flavonoids inhibit the expression of COX-2 both on mRNA and protein levels by inhibit signalling of the ERK and Akt pathways (303).

For quercetin in particular, which is the major representative of flavonols in diet, several animal and cell line studies have demonstrated that it might have certain anticarcinogenic effects. Possible mechanisms of actions might be the inhibition of the β-catenin/ Tcf signalling via the decrease of nuclear β-catenin/ Tcf-4 proteins (304). In other studies quercetin has been found to inhibit cell growth and to induce apoptosis in colon cancer cells, by downregulating the Akt pathway and ErbB2/ ErbB3 (receptor tyrosine kinases) signalling (305;306).

Summary

A few observational studies have investigated the associations between colorectal cancer and intakes of flavonoids. The null and inconsistent findings from cohort studies provided no evidence for an inverse association. However, the majority of the cohort studies were possibly underpowered to detect a significant association (<150 cases). On the other hand, results from case-control studies were more consistent reporting statistically significant and dose dependent associations of moderate strength for some flavonoid subgroups. In addition, there is some biological evidence mainly supporting the inverse association with flavonols (and quercetin in particular). However, taking into consideration the null findings from the cohort studies, conclusions of a causal effect of flavonoids can not be drawn and their associations with colorectal cancer should be further studied in large prospective studies.

9.3.1.3 Fatty acids

Introduction

Results from ecological studies indicated that fats from different sources might affect colorectal carcinogenesis in opposite directions, with diets high in animal fat increasing colorectal cancer risk and diets high in fish-derived fat reducing risk (146). The development of a database, which was linked to the FFQ used in the current study, enabled us to investigate how different types of fatty acids are associated with colorectal cancer. All the main food sources of the fatty acids were included in the 150-item FFQ list, and each food and drink item was assessed, manually checked and corrected in order to estimate its fatty acid contribution. A number of different foods contributed to the intake of the fatty acid subgroups and the results were not determined by one main food category (major sources included: meat and meat products, spreads and cooking oils, fish and fish dishes and confectionery and savoury snacks; Table 74). Median and range estimations of fatty acid intakes in the Scottish population as they were estimated from the population-based controls that participated in the current study were: 80.0 mg/day (62.5 - 105.3 mg/day) for total FAs, 34.7 mg/day (26.5 - 46.8 mg/day) for SFAs, 30.6 mg/day (23.2 - 40.5 mg/day) for MUFAs, 13.8 mg/day (10.4 - 18.1 mg/day) for PUFAs, 10.5 mg/day (7.8 - 14.0 mg/day) for ω6PUFAs, 2.2 mg/day (1.6 - 3.0 mg/day) for ω3PUFAs, 3.3 mg/day (2.4 - 4.5 mg/day) for *t*FAs and 2.6 mg/day (1.8 - 3.4 mg/day)

for *t*MUFAs (Table 72). In addition, nutrient data from supplements were extracted for the subgroups ω6PUFAs and ω3PUFAs and the individual compounds linoleic, γ-linolenic, α-linolenic, EPA and DHA and they were added to the daily dietary intakes (after energy adjustment) of total FAs, of the subgroups PUFAs, ω6PUFAs and ω3PUFAs and of the individual fatty acids linoleic, γ-linolenic, α-linolenic, EPA and DHA. Finally, we can not be sure about the accuracy of the FFQ estimates for the intakes of the specific fatty acid subgroups and individual compounds since this validation had not been finished by the time the thesis was written. However, the FFQ estimates of saturated, mono-unsaturated and poly-unsaturated fat have been compared with 4 day weighed record estimates in the Scottish population and the Spearman rank correlations were: 0.59, -0.07 and 0.36 for men, and 0.71, 0.58 and 0.66 for women, respectively (270).

Main findings

Regarding the main findings of the fatty acid analysis of the current thesis, in the crude model high intakes of total FAs, SFAs, MUFAs, ω6PUFAs, *t*FAs, *t*MUFAs and the individual fatty acids palmitic, stearic and oleic were associated with an increased colorectal cancer risk (Table 75). After residual energy-adjustment (model II) a dose-dependent increase in risk was observed for intake of total FAs (p=0.001), SFAs (p=0.001), MUFAs (p=0.01) *t*FAs (p=0.002) and *t*MUFAs (p=0.0003) and for the individual fatty acids palmitic (p=0.001), stearic ($p=7.9x10^{-6}$) and oleic (p=0.001). In contrast, significant inverse dose-dependent associations were observed between colorectal cancer and the dietary intakes of the fatty acid subgroup ω3PUFAs ($p=9.3x\ 10^{-6}$) and the individual compounds EPA (p=0.0001) and DHA (p=0.0002) (Table 75). However after further adjustment for potential confounding factors (model III), only the positive association between colorectal cancer and stearic acid (p=0.01) and the inverse associations between colorectal cancer and dietary ω3PUFAs (p=0.01), EPA (p=0.02) and DHA (p=0.02) remained significant (Table 75). Fatty acid intakes are highly correlated with dietary energy intake and as suggested by Willet to adjust more efficiently for energy intake, dietary energy intake was also added as a covariable together with the residually energy-adjusted variables and the potential confounding

factors (model IV). In that model only intakes of stearic acid were positively associated with colorectal cancer, whereas intakes of ω3PUFAs, EPA and DHA were negatively associated with colorectal cancer (Table 76). In marked contrast, the subgroup of PUFAs, and the individual fatty acids linoleic, γ-linolenic, arachidonic and α-linolenic were not associated with colorectal cancer risk in any of the adjusted logistic regression models (Table 75, Table 76). After correcting for multiple testing using the FDR method and taking into account either the tests that were conducted in hypothesis 2 (39 tests for the subgroup analysis and 45 tests in the individual compound analysis), or taking into account the tests that were conducted in all 4 hypotheses (69 tests for the subgroup analysis and 93 tests in the individual compound analysis) all the associations between the dietary intakes and colorectal cancer that their observed p-values were ≤0.01 remained significant (Table 75, Table 76).

Furthermore intakes of fatty acids from dietary supplements and diet were investigated for the following variables: total FAs, ω6PUFAs, ω3PUFAs, linoleic, γ-linolenic, α-linolenic, EPA and DHA and the reported associations were of similar direction and size as for the dietary variables (Table 75 and Table 76). Finally, we also explored associations between colorectal cancer risk and the intakes of the foods that were the main sources of the fatty acids with the significant associations (meat and meat products, confectionery and savoury snacks, fish and fish dishes). Results showed that comparison of highest versus lowest quartile intakes of confectionery and savoury snacks (main sources of *t*FAs and *t*MUFAs) were associated with an increased risk of colorectal cancer (model III: p=0.002), whereas high intakes of fish and fish dishes (main source of ω3PUFAs) were associated with a decreased colorectal cancer risk (model III: p=0.07) (Table 77). Associations between colorectal cancer and the food group spreads (including butter, margarine, jam, honey, marmalade, yeast or meat extract, peanut butter, and chocolate spread) were not investigated. The main reason was that intakes of margarine and cooking oils, which would be the food items of this food group that contributed the most in fatty acid intake, could not be estimated.

Findings from the current study in relation to previous studies

Saturated and mono-unsaturated fatty acids

It has been suggested that red and processed meat (one of the main sources of saturated and mono-unsaturated fat) as well as animal fat (which consists mainly of cholesterol, saturated and mono-unsaturated fat), may increase colorectal cancer risk (30). However, results from studies included in our literature review, which investigated the associations between saturated fat (or SFAs), mono-unsaturated fat (or MUFAs) and colorectal cancer provided little evidence that these particular types of fat are linked with colorectal cancer risk (Table 6, Table 7, Table 8, and Table 9). The reported associations from the majority of the studies were with energy-adjusted variables. Therefore the lack of statistically significant associations might be due to the fact that both saturated and mono-unsaturated fats are highly correlated with dietary energy intake with energy adjustment causing fat intakes to be over-controlled for. Findings of the current study suggest that there might be a positive association between colorectal cancer and intakes of SFAs and MUFAs, however these associations were diluted in the multivariable models.

Regarding potential biological mechanisms of SFAs and MUFAs affecting colorectal carcinogenesis, experimental data support the hypothesis of an increased colorectal cancer risk due to high intakes of SFAs. Some of the reported tumour enhancing effects of SFAs include alteration of the hormonal status and modification of cell membranes structure and function (307). On the other hand, experimental data regarding the effect of MUFAs are not as conclusive. In particular it has been shown that MUFAs and especially oleic acid may enhance oxidative stress and/ or disturb the membrane enzymes. However, oleic acid has also been found to improve the secondary bile acid patterns in the colon, which probably leads to a decreased colorectal cancer risk (142).

The current and previous studies do not support a direct effect of SFAs and MUFAs on colorectal cancer (after adjustment for various confounding factors). However, these two types of fat are mainly found in red and processed meat and they also contribute greatly to the dietary energy intake. Increased intakes of red and processed meat as well as of dietary energy have been linked to colorectal carcinogenesis. Particularly for red and processed meat public recommendations for low intakes have been made (30). Therefore

high intakes of SFAs or MUFAs should still be considered as important risk factors for colorectal carcinogenesis.

Omega-3 and omega-6 poly-unsaturated fatty acids

Regarding the two classes of PUFAs, ω3 and ω6, it has been suggested that they play an important though opposite role in colorectal carcinogenesis, with ω3PUFAs decreasing and ω6PUFAs increasing colorectal cancer risk (169). Regarding previous studies on ω3PUFAs, the results of a recent systematic review of clinical trials and cohort studies for their effect on cardiovascular risk and cancer indicated that these fatty acids have no effect on either diseases (308). However, the design of the systematic review had several limitations (309). With respect to cancer most of the studies had very small numbers of cancer cases and did not distinguish between types of cancer. The two largest studies (310;311) were originally designed to examine the effect of ω3PUFAs on cardiovascular mortality and did not have cancer as a primary study outcome. Results from a recent meta-analysis of prospective studies that investigated the associations between fish (19 prospective studies) and/ or marine ω3PUFA intakes (three prospective studies) and colorectal cancer, suggested a statistically significant inverse association between fish intake and colorectal cancer (combined RR (95% CI): 0.88 (0.78, 1.00)), and a not statistically significant inverse association between marine ω3PUFA intakes and colorectal cancer (combined RR (95% CI): 0.91 (0.70, 1.19)) (312). Finally results from both cohort and case-control studies as summarised in Table 12 and Table 13 regarding the effect of ω3PUFAs are inconsistent. In particular, from the identified prospective studies only the Health Professional's Study (2008) reported a statistically significant and dose dependent inverse association of moderate strength between colorectal cancer and marine ω3PUFAs intakes in male individuals (170), whereas other large cohort studies (including the Women's Health Study, the Japan Public Health Centre-based Study, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study and the Iowa's Women's Health Study) reported null associations (Table 12). Results from case-control studies were more consistent. In particular, most of the studies reported inverse associations of similar magnitude as the ones observed in the current study (40-30% reduction in risk) and four studies reported dose dependent and statistically significant

associations (158;161;164;172) (Table 13). This inconsistency regarding the ω3PUFAs associations especially between studies might be due to different habits of the populations regarding the amount and duration of fish intake. It has also been proposed that ω3PUFA significant inverse associations might be confounded by a vitamin D effect, since these two nutrients share common sources. However, when we further adjusted the associations of the current study for vitamin D intakes, high intakes of ω3PUFAs were still associated with a reduced colorectal cancer risk though not statistically significant (high vs. low quartile of intake OR (95% CI), p-value for trend: 0.84 (0.61, 1.18), 0.18).

Regarding the ω6PUFAs, results from animal studies showing an increase in colorectal cancer incidence, led to the investigation of the hypothesis that high intakes of ω6PUFAs are associated with a high colorectal cancer risk. However, according to findings of the literature review intakes of ω6PUFAs were not associated with colorectal cancer in prospective studies (Table 14). In addition, the majority of case-control studies (including ours) reported null associations with ω6PUFAs, whereas a small number of retrospective studies reported inverse not statistically significant or dose-dependent associations of moderate strength between high intakes of ω6PUFAs and colorectal cancer (Table 15).

The significant association between colorectal cancer and ω3PUFAs and on the other hand the lack of any association between ω6PUFAs and colorectal cancer that were observed in the current and previous studies can be explained by the different biological action of the ω3 and the ω6PUFAs. Omega 3 PUFAs have been found to rapidly incorporate into cell membranes and affect several anti-carcinogenic biological responses (313-315). The main biological mechanism of ω3PUFAs has been suggested to be the inhibition of the COX-2 enzyme and the production of eicosanoids that have anti-inflammatory and antiproliferative properties. In addition, several other mechanisms by which ω3PUFAs may decrease the risk of colorectal cancer have been proposed, including inhibition of bile acid excretion, altered protein kinase C activity, decreased NFκB activity, activation of PPARα and γ and decreased nitric oxide production (169). Regarding ω6PUFAs, it has been suggested that they enhance the production of

eicosanoids that promote inflammation and carcinogenesis by using the same enzymatic system as ω3PUFAs. Therefore, changes in the ω3/ ω6 ratio may contribute to the early stages of carcinogenesis (316). In addition, other studies report that ω6PUFAs promote colorectal carcinogenesis by influencing the protein kinase C pathway (141).

Evidence from the current and previous studies suggest that ω3PUFAs operate differently than the other types of fat, decreasing colorectal cancer risk. However, results from prospective studies are not as consistent as results from case-control studies. In addition ω3PUFAs share common sources (main food source: oily fish) with other nutrients that may affect colorectal carcinogenesis (vitamin B12, vitamin D) and therefore these inverse associations might be confounded. Therefore, specific recommendations regarding intakes ω3PUFAs are not suggested. In contrast further investigation of their associations with colorectal cancer in large prospective observational studies is proposed.

Trans fatty acids

Trans fatty acids are unsaturated fatty acids that are formed during hydrogenation and instead of the natural occurring *cis* form, they have a *trans* form (176). It has been reported that *t*FAs increase the risk of various chronic diseases, including ischemic heart disease, diabetes and obesity (317). Due to the fact that *t*FAs might be causally link with several chronic diseases, the major UK retailers announced that they will stop adding *t*FAs in their products by the end of 2007 (British Retail Consortium, 2007). Regarding colorectal cancer, a limited amount of observational studies (3 cohort and 3 case-control studies) have investigated the associations between *t*FAs and colorectal cancer risk (**Error! Reference source not found.**, Table 17). None of the cohort studies reported statistically significant associations. However, two of the three cohort studies were not large enough (in terms of cases) and therefore might have been underpowered to detect a significant association (141;162). Regarding case-control studies, results from the current study and from one more case-control study suggested a positive association especially among females with a 40 and 50% increase in risk, respectively (164;175).

Regarding biological mechanisms of *t*FAs, it has been suggested that some of their properties can affect colorectal cancer carcinogenesis. In particular, it has been

suggested that high consumption of *t*FAs may alter bile acid and other fatty acid concentration of the large bowel, which can then lead to increased mucosa inflammation and oxidative stress (318). Indeed, *t*FAs have been found to be associated with markers of oxidative stress and inflammation (319;320). In addition, some studies have reported that high consumption of *t*FAs is associated with insulin resistance, which may enhance colorectal carcinogenesis due to increased cell proliferation (318).

To draw specific causal conclusions about *t*FAs, further investigation regarding their association with colorectal cancer is necessary. However, this type of fat has been recognised as an important risk factor for other chronic diseases (ischemic heart disease, diabetes and obesity) and action has already been taken by reducing its amounts in several products.

Summary

To summarise, according to the findings of the current study different types of fatty acids were found to be associated differently with colorectal cancer. In particular, SFAs, MUFAs, *t*FAs and *t*MUFAs were positively associated with colorectal cancer (though not in all multivariable models), ω3PUFAs were inversely associated with colorectal cancer and ω6PUFAs were not associated with colorectal cancer (in any of the adjusted models). When considering other retrospective and large prospective studies, findings regarding intakes of SFAs, MUFAs and *t*FAs were generally consistent with null associations. However, since null associations might be due to over-correction after energy adjustment and since these fatty acids are found in foods that have been linked to colorectal cancer, it is recommended that high intakes should be avoided. On the other hand, findings regarding intakes of ω3PUFAs are more consistent with a statistically significant and dose-dependent decreased colorectal cancer risk. It has been suggested though, that these inverse associations might be confounded by other nutrients like vitamin D, since they share common food sources. Evidence from large prospective studies might be therefore necessary in order to further investigate the ω3PUFAs effect on colorectal cancer. However, application of alternative study designs (e.g. Mendelian randomisation, described below) might be required in order to be able to isolate the effect of ω3PUFAs from the effect of other nutrients.

9.3.1.4 Folate, vitamin B2, vitamin B6, vitamin B12 and alcohol

Introduction

Folate, vitamin B2, vitamin B6 and vitamin B12 have important roles in the one-carbon metabolic pathway, which is essential for DNA synthesis, repair and methylation. In addition, alcohol may have an indirect effect on the one-carbon pathway via its own metabolic pathway. The role of folate against the NTD syndrome is well established and in order to reduce the amount of newborns with this disease mandatory folic acid fortification has been introduced in several countries (including the USA and Canada). However, folic acid fortification in the UK has been suspended in order to better investigate the folic acid effects on cancer (including colorectal cancer). In this study the 150 food and drink items listed in the FFQ included all the most important sources of folate as well as of vitamin B2, vitamin B6, vitamin B12 and alcohol. A number of different foods contributed to the intake of the four nutrients in our study and the results were not determined by one major food category including baked or boiled potatoes, bran flakes, bananas and fried oily fish (Table 83). Median and range estimations of these nutrients and alcohol in the Scottish population as they were estimated from the population-based controls that participated in the current study were: 321.0 µg/day (253.0 - 399.0 µg/day) for folate, 2.1 mg/day (1.6 - 2.6 mg/day) for vitamin B2, 2.8 mg/day (2.2 - 3.5 mg/day) for vitamin B6, 6.8 µg/day (4.8 - 9.8 µg/day) for vitamin B12 and 8.1 g/day (1.7 - 19.4 g/day) for alcohol (Table 81). In addition, nutrient data from supplements were extracted for folate, vitamin B2, vitamin B6 and vitamin B12 and they were added to their daily dietary intakes (after energy adjustment). Finally, the FFQ estimates for folate, vitamin B2, vitamin B6, vitamin B12 and alcohol intakes have been compared with 4 day weighed record estimates in the Scottish population and the Spearman rank correlations were: 0.55, 0.69, 0.33, 0.25 and 0.72 for men, and 0.73, 0.69, 0.48, 0.31 and 0.79 for women, respectively (270).

Main findings

Regarding the main findings of the analyses of folate, vitamin B2, vitamin B6, vitamin B12 and alcohol, inverse associations which showed dose response relationships were observed in the energy-adjusted logistic regression model (model II) between colorectal

cancer risk and the dietary intakes of folate (p=0.003), vitamin B6 ($p=7.1x10^{-6}$) and vitamin B12 (p=0.02) (Table 84). After adjusting for the main potential confounding factors (model III), only the inverse associations between colorectal cancer and vitamin B12 (p=0.05) remained statistically significant, with the vitamin B6 associations being of similar direction as in model II though borderline not statistically significant (p=0.09) (Table 84). In contrast, the association between folate and colorectal cancer followed a bell-shaped pattern with individuals of the second quartile of intake having the greatest colorectal cancer risk (Table 84). After correcting for multiple testing using the FDR method by taking into account the tests that were conducted in hypothesis 3 (15 tests) or the tests that were conducted in all 4 hypotheses (93 tests in the individual compound analysis), model II associations between dietary intakes of vitamin B6 ($p=7.1x10^{-6}$) and folate (p=0.001) and colorectal cancer remained statistically significant (Table 84).

Regarding the analysis of the main food sources of folate, vitamin B6 and vitamin B12, results suggest that there is some evidence in favour of a significant inverse association between colorectal cancer and intakes of bananas (dietary source of vitamin B6) and fried oily fish (dietary source of vitamin B12) (Table 85).

Alcohol intake when divided in quartiles was associated with a decreased colorectal cancer risk, and this association was statistically significant in model III (p=0.03) (Table 84), which was in the opposite direction when compared to previous findings (83). However, it has been proposed that alcohol intakes of less than 30 g/day are either weakly or not at all associated with colorectal cancer (82). In our study the cut-off point of the highest quartile was 19.4 g/day and this might be the reason, why we did not observe an increased colorectal cancer risk with high alcohol intakes. When we divided alcohol intake into categories (cut off points: 0, 0-15, 15-30, 30-45, 45-60, >60 g/day), we did not observe an increased risk for intakes of more than 30 g/day but we did observe a significant increased risk for intakes of more than 60g/day, which was statistically significant for model I (p=0.02) but not statistically significant for models II (p=0.08) and III (p=0.21) (Table 84).

Regarding the genetic findings of the current study, none of the four examined SNPs was associated with colorectal cancer (data not shown). In addition, our data did not

support the hypothesis that folate or any of the vitamins B2, B6, B12 interacts with the rs1801133 (*MTHFR* 677TT) variant or with any of the rs1801131 (*MTHFR* A1298G), rs1805087 (*MTR* A2756G) or rs1801339 (*MTRR* A66G) variants (data not shown).

Findings from the current study in relation to previous studies

Folate

A substantial body of observational studies investigating the association between colorectal cancer and dietary intakes, total intakes or plasma measurements of folate have been conducted. Ten of 14 cohort and 13 of 24 case-control studies that were identified from the literature review reported statistically significant or statistically non-significant inverse associations between folate and colorectal cancer with an average 30% decrease in risk (Table 18, Table 19). In addition, two recent meta-analyses (published in 2001 and 2005) reported a 20 to 25% reduction in colorectal cancer risk with high intakes of dietary folate (184;321). In some studies these inverse associations were attenuated after adjustment for confounding factors (e.g. fibre) or were observed only between intakes of dietary folate and colorectal cancer and in some other studies inverse associations were not replicated at all (30). Furthermore, results from two recent studies showed a positive effect of folate on colorectal cancer risk, which followed a bell-shaped pattern similar to the one that was observed in the current study (212;216) and a third study reported a not statistically significant positive association (196).

The inconsistency between different studies, with some studies reporting a positive association, other studies reporting a negative association and other no significant association might be due to failing to adequately control for particular confounding factors such as fibre. Differences in the median and range of folate intakes might also explain the inconsistent findings. In particular for our study population, folate intakes were lower than the ones reported in other studies (193;195;198), but they were similar to intakes of a recent Scottish study, with this study also reporting a bell-shaped relationship between folate and colorectal cancer (212). In addition, failure of observing an association might be due to low variability in intakes between the participants of a study. Indeed, the variability in intakes in our study was low with 75.5% of the control reporting intakes between 0 and 200 μg/day, and that might explain why we did not

observe a significant association between high folate intakes and colorectal cancer. Furthermore, it has been suggested that a total folate intake of more than 600 μg/day may be required in order to observe a preventive effect against colorectal cancer. However, when we focused our analysis on subjects with high dietary or total folate intake, we did not observe any significant associations. In particular, the new cut-off points for total (dietary and from supplements) folate intakes were: 0-200 μg/day (45 cases, 58 controls), 200-400 μg/day (1,629 cases, 2,096 controls), 400-600 μg/day (330 cases, 532 controls), >600 μg/day (57 cases, 90 controls). The OR (95% CI) of the 4th versus the 1st category was 1.26 (0.69, 2.29) with a p-value for trend of 0.14 (model III; data not shown). Finally, another possible explanation might be that folate acts in a dual way during colorectal carcinogenesis, reducing risk for healthy individuals but promoting progression of colorectal adenomas or neoplasms for individuals that have already developed colorectal cancer (321).

The main biological mechanism of folate is its involvement in the one-carbon metabolic pathway, which leads to the synthesis of certain nucleotides (purines and thymidilate) and provides the methyl groups for DNA methylation (321;322). It has been hypothesised, therefore that high folate intakes will protect against colorectal carcinogenesis, maintaining a healthy colorectal epithelium. However, on the other hand it has been suggested that folate may assist in the progression of existing preneoplastic or neoplastic lesions, by providing the highly proliferative cancerous cells with nucleotides. Therefore, there is a possibility of a dual role of folate on colorectal cancer depending on the status of the colorectal epithelium (321;322).

The evidence that folate protects against colorectal cancer has been convincing for several years. However, reports of positive associations between folate and colorectal cancer as well as the biological plausibility of an increased risk have challenged its chemoprotective role. Therefore, further investigation of the role of folate in prospective observational studies and examination of the results of two clinical trials investigating the folate effect on cancer (including colorectal) is recommended prior to the mandatory folic acid fortification in the UK.

Vitamin B6

Regarding vitamin B6, results from published cohort and case-control studies showed inverse associations between colorectal cancer and dietary or total (including supplements) vitamin B6 intake (Table 22, Table 23). Three cohort studies reported statistically significant and dose-dependent inverse associations with a 30 to 35% reduction in colorectal cancer risk. In particular, in the Swedish Mammography Cohort both colon and rectal cancer were inversely associated with vitamin B6, in the Japan Public Health Centre-based Prospective Study statistically significant inverse associations were reported only for males and in the Women's Health Study dietary but not total vitamin B6 intake was associated with a decreased risk of colorectal cancer. In contrast, the Iowa Women's Health Study reported a significant positive association for rectal cancer and no association for colon cancer. Regarding the case-control studies, of the 11 identified studies, one nested case-control (Nurses' Health study) and four case-control studies reported inverse associations with colorectal cancer (Table 23). However, even if most of the studies investigating the vitamin B6 effects reported statistically significant findings, since most studies of dietary factors of one-carbon metabolic pathway were focused on folate, non-significant findings for vitamin B6 could have been omitted from publications. Therefore the results of this literature review might be subject to publication bias. It has also been proposed that vitamin B6 effects might be modified by the intake of other nutrients, such as alcohol and folate (223) but our data showed no evidence of this. From the three published studies that investigated alcohol and vitamin B6 (223;225;323), only one found a clear interaction especially among women with high alcohol intake (>30g/day) (223). In addition, all three studies that investigated plasma or dietary folate and B6 (195;225;323) failed to show significant interactions. Finally, in our population, when we further adjusted for folate intakes the associations between colorectal cancer and vitamin B6 remained constant (high versus low quartile: OR (95% CI), p-value for trend: 0.80 (0.62, 1.03), 0.06) (data not shown).

Vitamin B6 plays a key role in the one-carbon metabolic pathway as a co-enzyme of the cystathionine β-synthase, which converts homocysteine into cystathionine (324). In addition, its role as a co-enzyme in the synthesis of 5,10MTHF might be critical for synthesis, repair and methylation of DNA and inhibition of single and double DNA

breaks (325-327). Further more, laboratory studies on mice suggest that high intake of pyridoxine (vitamin B6) has other anticarcinogenic effects by reducing cell proliferation, oxidative stress, nitric oxide production and angiogenesis (328;329) and a cultured human lymphocyte study reported a protective action against chromosomal damage (330). Finally, it has been proposed that vitamin B6's inhibition of DNA polymerases and steroid receptors may be useful and vitamin B6 might be a promising adjuvant in cancer chemotherapy (331).

High intakes of vitamin B6 have been found to be associated with a decreased colorectal cancer both in the current and previous studies. However, vitamin B6 intakes were attenuated and became marginally not statistically significant after further adjustment (model III). In addition, even if the majority of the published prospective and retrospective studies support an inverse association, the possibility of publication bias cannot be ruled out. Therefore, specific recommendations regarding intakes of vitamin B6 are not suggested. In contrast, further investigation of their associations with colorectal cancer in prospective observational studies is proposed.

Vitamins B2 and B12

Few studies have investigated the association between colorectal cancer and intakes of vitamin B2 or vitamin B12, even if they are important co-enzymes in the one-carbon metabolic pathway. Regarding vitamin B2, we identified only one case-control study that reported a significant inverse association between high intakes of vitamin B2 and colorectal cancer (Table 20, Table 21), findings that were not replicated in our study. Regarding vitamin B12, one cohort study reported a significant increased colorectal cancer risk with high intakes of dietary vitamin B12, but they suggested that this finding might be confounded by smoking (196). The majority of the case-control studies reported either non-significant inverse or null associations (Table 24, Table 25), whereas in our analysis we observed an inverse and dose-dependent association that remained constant in models II, III and after further adjusting for folate (high versus low quartile: OR (95% CI), p-value for trend: 0.80 (0.66, 0.97), 0.05) (data not shown). However, the main source of vitamin B12 was oily fish, therefore the observed inverse association might be due to a confounding effect from either ω3PUFAs or vitamin D, which main

source is also oily fish. Indeed, when we further controlled for either ω3PUFAs or vitamin D intakes the inverse association between vitamin B12 and colorectal cancer was diluted (high vs. low quartile: OR (95% CI), p-value for trend: 1.01 (0.80, 1.27), 0.62; 0.95 (0.75, 1.22), 0.94; respectively). However, vitamin B12 intakes were highly correlated with both ω3PUFAs (r=0.79) and vitamin D (r=0.85), and therefore it is very difficult to know whether the inverse association with colorectal cancer is driven by vitamin B12, ω3PUFAs or vitamin D. According though to the findings from previous studies, it is more likely that the inverse association between vitamin B12 and colorectal cancer observed in model II and III is confounded by either ω3PUFAs or vitamin D intakes.

Alcohol

High alcohol intake has been considered as an important risk factor for colorectal cancer. However, it has been suggested that this positive association is not dose-dependent. In particular, evidence suggest that alcohol intake of 30 g/day or lower are not associated with colorectal cancer, whereas alcohol intake of more than 30g/d is linked with male colorectal cancer and probably linked with female colorectal cancer (30). Results from the EPIC study (80) as well as from two pooled meta-analyses (81;82) support this finding suggesting alcohol consumption of more than 30 g/day is significantly associated with an increased colorectal cancer risk.

In our study, when alcohol intake was divided into quartiles, high alcohol consumption was associated with a significant and dose-dependent decreased colorectal cancer risk. However, the cut-off point of the highest quartile (19.20 g/day) was lower than the 30 g/day threshold. Therefore, we divided alcohol intake in categories (0, 0-15, 15-30, 30-45, 45-60, >60 g/day) and we observed an increased colorectal cancer risk for intakes of higher than 60 g/day. One possible reason why the threshold of an increased colorectal cancer risk in our study was 60 instead of 30 g/day might be that the study participants underreported their alcohol intakes.

The reference category used in the latter analysis included subjects reporting that they had never consumed any alcoholic beverage weekly. This might be a limitation since complete abstainers may not be a representative group of subjects and therefore not an

ideal reference group. However, subjects that had consumed less than one measure a week of any alcoholic beverage were also asked to circle 0. Therefore the reference category probably included both complete abstainers and occasional drinkers. When alcohol intake was divided in new categories using as reference group the low alcohol consumers (0-15g/day) an increased colorectal cancer risk for intakes higher than 60 g/ day was still observed (OR (95% CI), p-value: Model I 1.55 (1.03, 2.32), 0.03; Model II 1.37 (0.91, 2.07), 0.13; Model III 1.26 (0.80, 2.00)) (data not shown).

Associations with genetic variants, gene-nutrient interactions

Two previous meta-analyses (332;333) have reported inverse associations between the *MTHFR* 677TT genotype and colorectal cancer risk. Therefore lack of a statistically significant association in our study might be either a chance finding or due to limited power. A smaller number of observational studies have investigated the associations between colorectal cancer and the other genetic variants: rs1801131 (*MTHFR* A1298C), rs1805087 (*MTR* A2756G) and rs1801339 (*MTRR* A66G). Regarding the rs1801131 (*MTHFR* A1298C) variant, a decreased though not statistically significant association between the CC genotype and colorectal cancer is reported in the majority of the studies (178). However, since rs1801133 (*MTHFR* C677T) and rs1801131 (*MTHFR* A1298C) are in strong linkage disequilibrium and the pattern of association between the *MTHFR* 1298CC genotype and colorectal cancer is similar to the pattern of association *MTHFR* 677TT and colorectal cancer, it raises the possibility that the rs1801131-cancer relation is actually due to the rs1801133 variant. Studies about associations between rs1805087 (*MTR* A2756G) and colorectal cancer and between rs1801339 (*MTRR* A66G) and colorectal cancer have reported null or not statistically significant associations (178).

Regarding gene-nutrient interactions, it has been suggested that the decreased colorectal cancer risk for the *MTHFR* 677TT individuals is not apparent when folate or methionine intakes are low or when alcohol intakes are high (216). However, this hypothesis was not replicated by the current study and also results from observational studies examining these interactions are inconsistent suggesting that further investigation might be necessary (216). In addition, some observational studies have investigated interaction relationships between *MTHFR* 1298CC genotype and folate intakes. Similarly to the

MTHFR 677TT interactions, results are inconsistent and might be driven by the rs1801133 (*MTHFR* C677T) variant due to the strong linkage disequilibrium (216). Furthermore, at least three studies reported a lower risk of colorectal cancer (334;335) and adenomas (336) in subjects of the *MTHFR* 677TT genotype and reporting high vitamin B6 intake.

In addition to individual effects and specific gene-nutrient interactions, several previous prospective and retrospective studies have investigated combinations of dietary factors and/ or the genetic factors involved in one-carbon metabolism and their association with colorectal cancer risk. Results tend to support an inverse association between a "high methyl-donor" diet (high folate and in some cases high vitamin B6 and vitamin B12 intakes, high methionine intakes and low alcohol intakes) even in studies where individual effects were not significant (226).

Summary

To summarise, according to the findings of the current study, folate intakes were not associated with a decreased colorectal cancer, but instead a bell-shaped relationship was observed. Even if the majority of the published studies support a protective effect of folate, the possibility of a dual folate role (protecting against colorectal cancer onset but enhancing colorectal cancer progression) needs further investigation from observational studies. Vitamin B2 was not associated with colorectal cancer, but both vitamin B6 and vitamin B12 were found to decrease colorectal cancer risk. However, inverse associations with vitamin B6 were attenuated after applying the multivariable model and similarly inverse association with vitamin B12 were found to be confounded by ω3PUFAs or vitamin D intakes. Vitamin B6 can act as an important chemopreventive agent, but further investigation of its effect on colorectal cancer would need to be conducted. Similarly, even if vitamin B12 findings are interesting, they might be confounded, especially if we consider the published evidence regarding the effects of ω3PUFAs and/ or of vitamin D. Finally, when alcohol intake was divided into quartiles, high alcohol consumption was associated with a significant and dose-dependent decreased colorectal cancer risk. However, when alcohol intake was divided in

categories an increased colorectal cancer risk for intakes of higher than 60 g/day was observed.

9.3.1.5 Vitamin D and calcium

Introduction

A protective effect of vitamin D on colorectal cancer has been initially proposed in 1980 by Garland and Garland, who suggested that different incident and mortality rates of colorectal cancer, could be explained by the different sunlight exposure according to the geographic latitude (337). Since then, several ecological studies investigated the association between UVB exposure and colorectal cancer, with most of them confirming the initial observation (338). However, results from prospective or retrospective studies investigating the association between mainly dietary intakes of vitamin D and colorectal cancer are not as strong (338), whereas the epidemiological evidence regarding calcium intake and its effect on colorectal cancer is relatively stronger (339). In the current study we used estimates obtained from the FFQ, in order to investigate the associations between colorectal cancer, vitamin D and calcium. The foods that contributed to the intake of the vitamin D and calcium in our study included oily fish (fried, smoked or grilled), milk and cheese (Table 89). Median and range estimations of vitamin D and calcium in the Scottish population as they were estimated from the population-based controls that participated in the current study were: 3.9 mg/d (2.5 - 5.8mg/d) for vitamin D and 1.1 g/d (0.8 -1.4 g/d) for calcium (Table 87). In addition, nutrient data from supplements were extracted for vitamin D and calcium and they were added to their daily dietary intakes (after energy adjustment). Finally, the estimates of this FFQ for vitamin D and calcium intakes have been compared with 4 day weighed record estimates in the Scottish population and the Spearman rank correlations were: 0.38 and 0.49 for men, and 0.37 and 0.75 for women, respectively (270).

Main findings

Regarding vitamin D, significant inverse dose-dependent associations were observed between colorectal cancer and dietary vitamin D in both models II ($p=0.01$) and III ($p=0.03$) (Table 90). However, when we further adjusted for ω3PUFAs, since they share common food sources (oily fish), this inverse association between vitamin D and

colorectal cancer was attenuated (Table 91). Regarding calcium, high dietary intakes were associated with an increased colorectal cancer risk in the crude model (model I), whereas dietary and total calcium intakes were not associated with colorectal cancer risk in any of the other models (Table 90 and Table 91). After correcting for multiple testing using the FDR method by taking into account the tests that were conducted in hypothesis 4 (eight tests) or the tests that were conducted in all four hypotheses (93 tests in the individual compound analysis), associations between dietary intakes of calcium (p= 0.001; model I) and vitamin D (p=0.01; model II) remained significant (Table 90). Finally, analysis of the main food sources of vitamin D and calcium, suggested that there is some evidence in favour of a significant inverse association between colorectal cancer and intakes of fried and smoked oily fish (vitamin D sources), whereas there is some evidence of a positive association between colorectal cancer and full fat hard cheese (calcium source) (Table 92).

Regarding the genetic findings of the current study, none of the four SNPs examined was associated with colorectal cancer (data not shown). In addition, we investigated the associations between colorectal cancer, vitamin D and calcium after genotype stratification to test whether their associations are modified according to the particular genotype (data not shown). We observed that the inverse association between vitamin D and colorectal cancer was more profound for individuals of the rs10735810 CC genotype than for individuals of the CT or TT genotypes. Furthermore, calcium intake was inversely though not significantly associated with colorectal cancer for the rs10735810 CC individuals, whereas it was positively associated for the TT individuals (data not shown). Finally, there was some evidence that rs10735810, a SNP that affects VDR function, interacts with vitamin D (p for interaction 0.06) and calcium dietary intakes (p for interaction 0.13) (data not shown). However, given the multiple interactions examined, we can not rule out the possibility that the observed interaction between rs10735810, vitamin D and calcium might be due to chance.

Findings from the current study in relation to previous studies

Vitamin D

A recent clinical trial of vitamin D (10 μg/day) and calcium supplementation (1000 mg/day) for seven years in post-menopausal women did not show any association with colorectal cancer (234). However, a large proportion of women assigned to vitamin D/ calcium supplementation or of women assigned to placebo were also taking supplements on their own and the authors suggested that this may have limited their ability to affect the rates of colorectal cancer further. In addition, this finding might be due to insufficient time for vitamin D to affect colorectal carcinogenesis, since it has been proposed that vitamin D may require at least 10 years to act. Furthermore, this finding might be due to low dosage of vitamin D supplementation and therefore the contrast between the treatment participants and the control ones might not have been adequate. Evidence from observational studies measuring serum (plasma) vitamin D (25(OH)D) was strong and statistically significant, suggesting an average 40% reduction in colorectal cancer (Table 28). In addition, results from a meta-analysis combining seven nested case-control studies investigating the association between 25(OH)D in the blood and colorectal cancer showed a significant inverse association with a combined OR of 0.70 (95% CI 0.56, 0.87) (250). Regarding dietary and total vitamin D, numerous case-control and cohort studies have examined vitamin D intake in relation to risk of colorectal cancer (Table 26, Table 27) and findings from most of them have been discussed in detail in recent review articles (338;340;341). Whereas, some cohort (including the Nurses' Health Study, the Health Professionals' study and the Iowa Women's Health Study) and some case-control studies reported statistically significant and dose dependent inverse associations between vitamin D intakes and colorectal cancer, other studies reported no associations. In addition, results of two meta-analyses combining 11 cohort and nine case-control studies showed a weak statistically significant inverse association for the cohort studies and a weak statistically non-significant inverse association for the case-control studies (combined RR=0.91, 95% CI 0.84, 1.00; combined OR=0.90, 95% CI 0.80, 1.02; respectively) (250). These inconsistent and weak associations might be due to the fact that the studies included in the meta-analyses did not capture total vitamin D intake (dietary intake, supplementary

intake and skin production) coupled to the measurement error in dietary measures of vitamin D intake.

Regarding the current study, it is worth mentioning that only dietary and supplementary vitamin D intake was considered, since we did not have information regarding vitamin D skin production by UV sunlight. Therefore, the possibility of misclassification due to the lack of measuring sunshine-produced vitamin D can not be ruled out. It has been suggested though that a UVB irradiation threshold of 20mJ/cm^2 is required to induce the vitamin D3 skin production and apparently this threshold is not reached for countries above latitude 40^o during the winter months (342). Since Scotland's latitude is 55^o, the sun exposure, especially during the winter months, is relatively low and this will probably make diet a more important contributor. Finally, the inverse association that was observed between vitamin D and colorectal cancer in our study was attenuated after further adjustment for ω3PUFAs. However, these two nutrients were highly correlated with each other (r=0.82, p-value<0.00005) and therefore it is very difficult to know whether the inverse association with colorectal cancer is driven by vitamin D and/or by ω3PUFAs or whether the dilution of the association might be due to an over-control of the vitamin D intake.

Vitamin D has been suggested to affect colorectal cancer carcinogenesis mainly through the binding of 1α,25$(OH)_2D_3$ (vitamin's D most active form) on VDR (343). *In vitro* laboratory studies suggest that the main anti-neoplastic activities of vitamin D include inhibition of cell proliferation, induction of differentiation and apoptosis, inhibition of growth effects and modulation of the signalling pathway of particular cytokines. If vitamin D is proved to be truly linked with colorectal cancer, then it could be a very promising chemopreventive agent for colorectal cancer. However, adverse side-effects of natural vitamin D (in high doses), such as hypocalcaemia, should be overcome (227).

Calcium

Results from several animal studies have suggested that calcium has a protective effect against colorectal carcinogenesis. In addition, results from a recent Cochrane systematic review (including findings from two clinical trials) and a meta-analysis based on three randomised controlled trials, studying the effect of calcium supplementation on

colorectal adenoma incidence and recurrence respectively, suggested that daily intake of calcium (dietary or from supplements) may have a moderate protective effect on development or recurrence of colorectal adenomas (344;345). However as it has been already mentioned, the randomised clinical trial from the Women's Health Initiative found no effect of calcium plus vitamin D supplementation among postmenopausal women. Regarding observational studies, results from cohort and case-control studies are inconsistent (Table 29, Table 30). In particular for the prospective studies, some of the large cohort studies (including the Multiethnic cohort study, the Breast Cancer Detection Demonstration Project, Professionals Follow-Up Study, the Swedish Mammography Cohort Study and the Iowa Women's Health Study) support an inverse association between either dietary or total calcium intake with an average 30% reduction in colorectal cancer risk, whereas some others (including the Netherlands Cohort Study, the Nurses Health Study and the E3N-EPIC prospective study) failed to replicate these findings (Table 29).

A possible reason for this inconsistency regarding the associations between calcium intakes and colorectal cancer might be the fact that many studies did not account for calcium intake from supplements, which might be important contributors of total daily intakes. An alternative possible explanation of this difference might be the different levels of calcium intake. In particular, some investigators have suggested that calcium affects colorectal cancer at the low range of intake with some studies suggesting a cut-off at 600-800 mg/day where there is no further benefit (261). However, when we limited our analysis to subjects with a dietary calcium intake of ≤1000 mg, we did not find a significant association between calcium intake and colorectal cancer. The cut-off points for the new categories were: 0-600 mg/day (36 cases, 55 controls), 600-800 mg/day (194 cases, 249 controls), 800-900 mg/day (208 cases, 307 controls) and 900-1000 mg/day (295 cases, 402 controls). After applying model III for the 4^{th} versus the 1^{st} category the reported OR (95% CI) was 1.37 (0.81, 2.32) with a p-value for trend 0.98 (data not shown). In contrast, other investigators have suggested that a total calcium intake of more than 1,200 mg/day may be required in order to observe a preventive effect against colorectal cancer (346). When we focused our analysis on subjects with

high dietary or total calcium intake, inverse associations for intakes of more than 1500mg/day were observed. In particular the new cut-off points for total (dietary and from supplements) calcium intakes were: 0-1000 mg/day (708 cases, 978 controls), 1000-1250 mg/day (773 cases, 975 controls), 1250-1500 mg/day (414 cases, 539 controls), 1500-1750 mg/day (127 cases, 197 controls), >1750 mg/day (39 cases, 87 controls). The results after applying model III for the 4th versus the 1st category and for the 5th versus the 1st category were OR (95% CI): 0.82 (0.62, 1.09) and 0.63 (0.41, 0.97) respectively with a p-value for trend 0.14 (data not shown). Therefore, a possible explanation of the inconsistency between different studies might be that like in our study high intakes of dietary or supplementary calcium (of more than 1500mg/day) might be necessary, before a protective effect could take place.

Calcium has also been evaluated as a possible chemopreventive agent against colorectal cancer mainly due to its anti-inflammatory and anti-proliferative properties (343). Calcium mainly exerts its chemopreventive actions through activation of a calcium-sensing receptor. This leads to an increase in the levels of intracellular calcium, inducing a wide range of biological effects including the restrain and differentiation of neoplastic colon cells (347). Finally, it has also been proposed that calcium can bind on bile and fatty acids in the colonic lumen reducing the toxicity of these agents (339).

Associations with *VDR* variants, gene-nutrient interactions

Regarding previous studies on the genetic variants of the *VDR* in agreement with the findings of the current study, the combined analysis of five case-control studies investigating the effect of the *VDR* rs10735810 variant on colorectal cancer showed no significant associations (250). However the variant genotype of rs1544410 GG was found to be significantly associated with colorectal cancer in a meta-analysis of four studies (combined OR=1.18, 95% CI 1.04, 1.33) (250). We did not replicate this finding, possibly because rs1544410 was not in Hardy Weinberg equilibrium in our study. In addition, the few studies that have performed stratified and interaction analyses by vitamin D and/ or calcium status suggest that the effect of *VDR* variants might depend on the intake of these nutrients (250).

The F (C) allele of *Fok*I (rs10735810) has been found to result in a 3 amino acid shorter version of the VDR protein that is more efficient in binding vitamin D than the longer version coded by the f (T) allele. Therefore higher vitamin D or calcium intake might enhance its activity (348). Both vitamin D and calcium interact biologically with VDR and it has been suggested that they act together in their anticarcinogenic properties, with their effects being mainly at the earlier stages of carcinogenesis (adenomas) (349). Ingles *et al* (350) showed that the f (T) allele was inversely associated with large colorectal adenomas (>1cm in diameter; more likely to progress to adenocarcinomas) among individuals with low vitamin D and calcium intake and concluded that the association between *VDR* variants and colorectal adenoma risk are modified by vitamin D and calcium intake; findings which are in accordance with our results.

Summary

Findings from the current and previous studies suggest an inverse association between vitamin D intakes and colorectal cancer. Associations in the current study though were attenuated after adjusting for ω3PUFAs (common food source). These nutrients are highly correlated and it is therefore difficult to draw specific conclusions regarding which nutrient is truly associated with colorectal cancer and which not. Vitamin D might be a particularly useful chemopreventive agent against colorectal cancer (considering that its main side effects will be prevented) and therefore further investigation of the vitamin D effect on colorectal cancer by prospective and retrospective studies is very important. In addition, alternative analytical approaches (e.g. Mendelian randomisation) that overcome the problems of traditional epidemiological methods (such as confounding and reverse causation) might be useful in order to establish the relationship between vitamin D and colorectal cancer. Regarding calcium, high intakes (when divided into quartiles) were not found to be associated with colorectal cancer. However, calcium intakes of more than 1500mg/day were significantly associated with a decreased risk. In addition, results from prospective and retrospective studies are inconsistent and this inconsistency might be due to different levels of calcium intake. Therefore, based on the current findings as well as on the inconsistent results from previous studies, the

effect of calcium should be further investigated in observational studies, considering that high intakes of calcium could be required for a protective effect to be apparent.

9.3.1.6 Summary

Main findings of part 1 of the currents thesis

To summarise, the main findings of the first part of the current thesis support the overall evidence that lifestyle and in particular dietary exposures are linked with colorectal cancer either by increasing or decreasing risk.

The particular dietary factors that were found to be inversely associated with colorectal cancer after applying several multivariable logistic regression models and after controlling for multiple testing error were the following subgroups and individual compounds: flavonols, quercetin, catechin, ω3PUFAs, EPA, DHA, vitamin B6 and vitamin B12. In addition, high intakes of stearic acid were found to be positively associated with colorectal cancer and this association persisted even after further energy or total fatty acids adjustment. In contrast, high intakes of dietary and total folate were associated with a decreased colorectal cancer risk in the energy-adjusted model, but these inverse associations were attenuated and a bell shaped association was observed after further adjustment for several confounding factors including fibre. Regarding alcohol intake, when it was divided into quartiles, high alcohol consumption was associated with a significant and dose-dependent decreased colorectal cancer risk. However, when alcohol intake was divided in categories an increased colorectal cancer risk for intakes of higher than 60 g/day was observed. Furthermore, high intakes of vitamin D were also inversely associated with colorectal cancer after applying model II and III, but the effect was diluted after further adjusting for ω3PUFAs. Finally, it was observed that for calcium intakes to be inversely associated with colorectal cancer, a dosage of 1500mg/day or higher was necessary.

Finally, in the current study high BMI ($\geq$30kg/m^2) versus normal BMI (18.5-25 kg/m^2) was associated with a not statistically significant decreased colorectal cancer risk in both the matched and unmatched analysis (OR (95% CI), p-value: matched dataset 0.87 (0.71, 1.07), 0.14; unmatched dataset 0.92 (0.78, 1.08), 0.30). These results are not in accordance with the findings of the associations between colorectal cancer, physical

activity and dietary energy intake from the current study. In particular, since high levels of physical activity and low levels of dietary energy intake were associated with a decreased colorectal cancer risk, it would be expected that high BMI would be associated with an increased colorectal cancer risk. In addition, the inverse association between BMI and colorectal cancer is not consistent with many observational studies that have concluded that obesity is an important risk factor for colorectal cancer (71) (summarised on page 54). One possible reason for this inconsistent finding might be a weight underreporting from the cases or a weight misreporting due to their weight change after their cancer diagnosis. The validity of the LCQ regarding the weight and height report will be checked in healthy controls by comparing self reported measurements with measurements conducted from a trained research nurse.

General comments

Observational analytical studies examining the associations between the nutrients of our primary hypotheses (flavonoids, fatty acids, folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium) and colorectal cancer reported generally inconsistent results. Many possible explanations for these inconsistent findings have been suggested. In particular, the inconsistent findings might be due to different levels of intakes (resulting in different median and range of intakes) of the nutrients under investigation among the populations, which might be particularly important for nutrients that have an effect threshold (as for example it has been suggested for alcohol or calcium intake). However, it is more likely that most of the inconsistent findings are due to several methodological issues that could affect the accuracy of the reported results. Generally, case-control studies are more prone to report biased results mainly due to recall bias. However, other methodological problems including measurement errors, lack of controlling for all confounding factors and/ or residual confounding can affect equally results from both case-control and cohort studies.

One of the most important limitations of the majority of the published observational studies is their inability to detect small effect sizes due to small sample sizes and therefore limited power. Therefore, for a study to have 80% power to detect a difference of 20%, which are similar to the effect sizes observed in the current study, a sample size

of at least 2,500 cases and controls is required (α=0.05). And if we wish to increase the power to 90% then the study sample of the observational study should be up to at least 3,400 cases and controls. Furthermore, for a study to have 80% power to detect even smaller effects (e.g. OR=0.90) then a sample size as large as 11,324 may be required. However, a sample size of more than 11,000 individuals according to the above traditional power calculations is based on ideal study settings and probably is an underestimate of the true required sample size. In particular according to a recent publication, traditional power calculations fail to consider several key elements of the analysis complexity including for example errors in disease assessment and measurement errors of the explanatory variables (351).

Observational epidemiology has identified several important risk factors that have been verified to be causally linked with a disease. A few examples are the effects of smoking on lung cancer, lipids on coronary disease, high blood pressure on stroke and aspirin or NSAIDs use on colorectal cancer (352). However, there are many other examples that findings from observational studies were proven (mainly from randomised clinical trials) to be false, like the effects of anti-oxidant beta carotene on smoking related cancers, vitamin E and vitamin C on coronary heart disease. Observational epidemiology though is an important tool for medical research of disease causes, especially since it is not possible to conduct randomised clinical trials (which are considered as the gold standard) for all the potential risk factors and in some cases it is not possible to conduct a randomised clinical trial at all due to ethical reasons. Therefore, effort to improve the design of the case-control and cohort studies is essential. In addition, for researchers to be able to judge and draw conclusions about published studies, the reported results should be transparent and complete. One way to do that is by applying the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria, which is a guidance of how researchers should report findings from observational studies (353).

9.3.2 Main findings of part 2: Overall and stepwise regression analysis

9.3.2.1 Introduction

In this part of the chapter the main results of the overall and stepwise regression analysis will be presented and discussed. Regarding the overall analysis, univariable logistic regression models were fitted for the selected demographic, lifestyle, food and nutrient variables and OR, 95% CI and p-values for trend were calculated for each quartile of the continuous variables and each category of the categorical variables (Table 96). Regarding the stepwise regression analysis, forward and backward stepwise regression models were applied in the whole sample for three different sets of variables using the quartile form of the continuous variables (Table 97, Table 98, Table 99, Table 100, Table 101) and this procedure was repeated in sex stratified samples (data not shown). The explanatory variables that were included in the stepwise regression models consisted of selected demographic, lifestyle, food and nutrient variables.

9.3.2.2 Main results from overall analysis

The risk factors that were found to be significantly associated with colorectal cancer were: the demographic and lifestyle risk factors: family history of cancer ($p=1.1x10^{-51}$), NSAIDs intake ($p=7.3x10^{-7}$), dietary energy intake ($p=2.0x10^{-5}$), HRT intake (p=0.0003) and physical activity (p=0.02) (Table 96); the food group variables: vegetables ($p=2.4x10^{-8}$), eggs ($p=4.0x10^{-7}$), sweets ($p=7.9x10^{-7}$), fruit/ vegetable juice ($p=1.7x10^{-6}$), oily fish (p=0.001), coffee (p=0.001), fruit (p=0.009), savoury foods (p=0.009) and white fish (p=0.04) (Table 96); and the nutrient variables: *t*MUFAs ($p=6.7x10^{-6}$), ω3PUFAs ($p=1.3x10^{-5}$), SFAs (p=0.0001), *t*FAs (p=0.001) and MUFAs (p=0.01); quercetin (p=0.001), catechin (p=0.001) and phytoestrogen (p=0.04); cholesterol ($p=1.4x10^{-5}$), fibre ($p=3.3x10^{-5}$), protein (p=0.001) and starch (p=0.05); magnesium ($p=2.7x10^{-11}$), potassium ($p=9.1x10^{-8}$), manganese ($p=1.8x10^{-7}$), copper ($p=2.0x10^{-6}$), iron ($p=1.3x10^{-5}$), zinc ($p=4.6x10^{-5}$), phosphorus (p=0.0001) and selenium (p=0.009); niacin ($p=8.2x10^{-7}$), vitamin B6 ($p=7.1x10^{-6}$), carotenes ($p=2.6x10^{-5}$), vitamin C ($p=4.6x10^{-5}$), vitamin A (p=0.001), potential niacin (p=0.001), biotin (p=0.001), folate

(p=0.003), pantothenic acid (p=0.006), vitamin D (p=0.01), vitamin B1 (p=0.02) and vitamin B12 (p=0.02) (Table 96).

9.3.2.3 Main results from stepwise regression - Original sample

Whole, female and male samples

After applying forward and backward stepwise regression using three different sets of variables in the whole sample, the variables that were included: 1) in all models were family history (6/6 of models), dietary energy (6/6 of models), NSAIDs (6/6 of models), white fish (4/4 of models) sweets (4/4 models), coffee (4/4 models), fruit/ vegetable juice (4/4 models) and magnesium (4/4 of models) and 2) in more than 75% of models were: physical activity (5/6 of models), eggs (3/4 of models), oily fish (3/4 of models), vegetables (3/4 of models), ω3PUFAs (3/4 of models), quercetin (3/4 of models), cholesterol (3/4 of models), fibre (3/4 of models) and copper (3/4 of models) (Table 102).

After sex stratification, the following variables were included: 1) in all female and male derived models: family history (12/12 of models), NSAIDs (12/12 of models), sweets (8/8 of models) and fruit/ vegetable juice (8/8 of models) and 2) in more than 75% of female and male models: eggs (6/8 of models), white fish (6/8 of models) and *t*MUFAs (6/8 of models) (Table 102). However, few risk factors were included only in female or male derived models. In particular, the following variables were included only in at least 75% of the models derived from the female sample: *t*FAs (4/4 of the female models), vegetables (3/4 of the female models), ω3PUFAs (3/4 of the female models) and HRT (4/6 of the female models) (Table 102). In addition, the following variables were included only in at least 75% of the models derived from the male sample: dietary energy intake (6/6 of the male models), physical activity (5/6 of the male models), quercetin (3/4 of the male models), flavanones (3/4 of the male models) and manganese (3/4 of the male models) (Table 102).

To summarise, the variables that were included in all models derived from the whole, female and male analysis of the original sample for all three sets of variables were family history, NSAIDs, sweets and fruit/ vegetable juice and the variables that were included in at least 75% of the models were: eggs and white fish. In addition, the

variables vegetables and ω3PUFAs were selected to be included in the vast majority of the models derived from the whole and female samples and similarly, dietary energy and physical activity were selected to be included in the vast majority of the models derived from the whole and male samples (Table 102).

The variables with the strongest and most significant associations were among the ones that were included in the majority of the models. In particular, the lowest p-values were observed for associations between colorectal cancer and family history (p-value range: $3.6x10^{-50}$ to $1.8x10^{-24}$), NSAIDs (p-value range: $6.1x10^{-6}$ to 0.009), dietary energy intake (p-value range: $4.6x10^{-7}$ to 0.002), sweets (p-value range: $4.4x10^{-8}$ to 0.005), fruit/ vegetable juice (p-value range: $1.3x10^{-6}$ to 0.01), eggs (p-value range: $8.3x10^{-8}$ to 0.08) and white fish (p-value range: $5.4x10^{-5}$ to 0.02) (Table 97, Table 98, Table 99, Table 100, Table 101). In addition, regarding the direction of the associations, the variables family history, dietary energy, sweets, fruit/ vegetable juice, eggs and white fish were associated with an increased colorectal cancer risk, whereas the variable NSAIDs was associated with a decreased risk. Finally, regarding the size of the associations, family history was observed to have the strongest associations with colorectal cancer (OR range: 14.68 to 29.53), followed by NSAIDs intake (OR range: 0.68 to 0.79). For the remaining variables (dietary energy, sweets, fruit/ vegetable juice, eggs and white fish) the observed association were moderate or weak, with ORs ranging from 1.09 to 1.26 (Table 97, Table 98, Table 99, Table 100, Table 101). However, these observed ORs might not be accurate since stepwise regression either forward or backward, is not an appropriate method to draw conclusions regarding effect sizes.

9.3.2.4 Main results from stepwise regression - Bootstrap samples

The bootstrap method was applied to investigate the stability of the models and it was applied for forward and backward stepwise regression of all three sets of variables (whole sample). One hundred bootstrap samples were randomly drawn from the original sample. Then, each bootstrap sample was used to apply forward and backward stepwise regression for each set of variables (set 1, 2 and 3).

The variables that were selected to be included in the final models using forward stepwise regression were highly dependent on the subjects that were included in each

bootstrap sample, since all 100 models were chosen once (for all sets of variables) and the same was observed for the 100 models derived after applying backward stepwise regression.

Our findings suggest that the number of noise (false positive) variables that were selected to be included in the models increased as the number of candidate variables increased. In particular, the agreement between the models derived from forward and backward stepwise regression within the same bootstrap sample decreased as the number of the potential risk factors (number of variables for each set of variables) increased. The mean percentage of agreement for the analysis of set 1 (30 variables), set 2 (52 variables) and set 3 (82 variables) was 96.97%, 84.36%, 83.12%, respectively. The number of variables that were selected to be included in the models of the 100 bootstrap samples was smaller for the set 1 analysis (11-20 variables), than for the set 2 and set 3 analyses (10-31 and 15-39 variables respectively) (data not shown). Finally, for all sets of variables, more variables were selected to be included in models derived from backward stepwise regression than in models derived from forward stepwise regression (mean number of selected variables: 22.54, 20.02; respectively).

Regarding the variables that were selected to be included in the majority (more than 90%) of the models derived from the bootstrap samples were: 1) family history (600/600 of models), NSAIDs (596/600 of models) and dietary energy (587/600 of models), for variables that were included in all three sets of variables (3 sets of variables*2 types of stepwise regression*100 bootstrap samples = 600 models); and 2) sweets (397/400 of models), fruit/ vegetable juice (395/400 of models), eggs (388/400 of models), and white fish (381/400), for variables that were included in set 1 and 3 (2 sets of variables*2 types of stepwise regression*100 bootstrap samples = 400 models) (data not shown).

Therefore, most of the variables that were selected to be included in the majority (more than 90%) of the models derived from of the bootstrap samples are similar to the ones that were selected to be included in the majority (more than 90%) of the models derived from the original sample and these were: family history, NSAIDs, dietary energy intake, sweets, fruit/ vegetable juice, eggs and white fish. However, the variables coffee and

magnesium that were included in all 4 models derived from the original sample were included in 88.8% (355/400) and 77.8% (311/400) of the models derived from the bootstrap samples.

9.3.2.5 Comment on main findings of overall and stepwise regression analysis

Demographic and lifestyle factors

After applying forward and backward stepwise regression, some of the explanatory risk factors that were found to be associated with colorectal cancer in the majority of the selected models (>90% of the models derived from the original and bootstrap samples) were risk factors that have been found to affect colorectal cancer in many published observational studies. In particular, these factors included family history and dietary energy intake, which were associated with an increased colorectal cancer risk and NSAIDs, which was associated with a decreased colorectal cancer risk.

Family history has been considered as one of the main risk factors of colorectal cancer and for individuals that are in moderate or high family history risk colorectal cancer screening is offered. According to the findings of a recent meta-analysis (2006), which was summarised in the Introduction section (on page 48), the pooled colorectal cancer relative risk estimate when at least one first degree relative was affected was 2.24 (95% CI 2.06, 2.43) and it rose to 3.97 (95% CI 2.60, 6.06) when there were at least two affected relatives (47). In addition, the effect of NSAIDs on colorectal cancer has been investigated in numerous randomised clinical trials and observational studies (summarised in Introduction, on page 57), with the majority of the results suggesting that regular use of NSAIDs is associated with a reduced risk of colorectal cancer. On the other hand, even though the effect of dietary energy intake on colorectal cancer has been investigated in several observational studies (summarised in Introduction, on page 53) findings are generally inconsistent, with the case-control studies suggesting a significant inverse association, whereas cohort studies showing weaker or null associations (61). It is worth mentioning that findings of the current study suggest that dietary energy intakes is mainly associated with male colorectal cancer rather than with female colorectal cancer. An attractive hypothesis of this sex difference would be that high intakes of

dietary energy only affect male colorectal cancer. Indeed, sex is a factor that has been hypothesised to be an important effect modulator for several risk factors. However, many of the claimed sex differences have been proven to be spurious and failed to get replicated (354). Therefore, an alternative explanation of this finding might be that men and women misreported their dietary energy intakes in different ways. In particular, findings from previous studies support the hypothesis that under-reporting of dietary energy intake is unevenly distributed according to sex with women being more likely to underreport their dietary energy intakes (355-357).

Food groups

In addition to the widely studied risk factors a few less studied ones were found to be associated with colorectal cancer in the majority of the resultant models (>90% of the models derived from the original and bootstrap samples), which included the food groups sweets, fruit/ vegetable juice, eggs and white fish (with high intakes of all these food groups being associated with an increased colorectal cancer risk). In addition, coffee was selected to be included in >90% of the models derived from the original sample, but this finding was not replicated after applying the bootstrap sampling method, where coffee was selected to be included in 88.8% of the resultant models derived from the bootstrap samples. In the following paragraphs evidence from observational studies regarding the associations between colorectal cancer and these food groups (sweets, fruit/vegetable juice, eggs, white fish and coffee) will be briefly summarised.

Sweets

Sweets is a summary variable of high-fat and high-sugar foods, including pudding and deserts, chocolates, sweets, nuts and crisps, biscuits and cakes. This summary variable represents an unhealthy dietary pattern and it is moderately correlated with dietary energy intake ($r=0.61$, p-value$<10^{-5}$). Several observational studies have investigated the associations between colorectal cancer and dietary (food) patterns, which involves the joint analyses of foods that are consumed together by forming clusters of individuals with similar dietary habits (cluster analysis) (358). The two patterns that appear in the majority of the studies are: 1) a pattern of high intakes in fruit, vegetables and other

healthy foods ("healthy" pattern) and 2) a pattern of high intakes in meat, high fat and high sugar foods ("western" pattern) (358). In most of the studies the "healthy" dietary pattern was found to be associated with a decreased colorectal cancer risk (358-362), whereas the "western" dietary pattern has been found to be associated with an increased risk (359;363;364).

Fruit/ vegetable juice

The finding of the positive association between fruit/ vegetable juice and colorectal cancer is difficult to explain. Generally fruit and vegetable juices have different properties than the whole fruit or vegetable they come from, since juices contain limited amount of fibre and the majority of them contain sugars, preservatives and other additives (30). However, in many studies juice intakes are combined with fruit and vegetable intakes and their association with colorectal cancer is rarely investigated independently (365). Fruit and vegetable juices might affect colorectal cancer due to their high sugar content, however association between sugar intakes (as nutrient) and colorectal cancer are also inconsistent (30).

Eggs

Eggs are a food group that contains mainly protein, fat (saturated and mono-unsaturated fat) and cholesterol and are good sources of vitamin D, vitamin A, vitamin B2 and iodine. High consumption of them has been hypothesised to be associated with an increased colorectal cancer risk mainly due to their high content in fat and cholesterol. However, the results from case-control and cohort studies have been inconsistent. In the first AICR/WCRF report (1997) after reviewing 16 case-control studies, eggs were classified as a possible risk factor of colorectal cancer (58). However, in the second WCRF/AICR report (2007), the association between eggs and colorectal cancer was not investigated (30). In a recent population based case-control study (Shangai, China) an increased risk of colorectal cancer was reported for the ones of the highest intake of eggs versus the ones of the lowest (OR (95% CI): 1.4 (1.0, 1.9) for men and 1.3 (0.9, 1.9) for women) (366). However, results from a recent prospective study failed to replicate this inverse association (157). Finally, a review that summarised findings regarding the associations between colorectal cancer and various food groups, concluded that there is

some though inconsistent evidence that high consumption of eggs is associated with an increased colorectal cancer risk (367).

White fish

The finding of the positive association between high intakes of white fish and colorectal cancer is also difficult to explain. The majority of observational studies have investigated the associations between total fish intake (white fish, oily fish and shellfish) (368). The AICR/WCRF second report (2007) summarised the findings of 55 case-control studies and 19 cohort studies and concluded that even if there is some evidence supporting an inverse association between fish consumption and colorectal cancer the results are inconsistent and findings might be residually confounded by other food groups (e.g. meat) (30). In addition a meta-analysis of prospective cohort studies published in 2007 reported high fish consumption was associated with a borderline significant decreased colorectal cancer (312). These observed inverse associations between fish and colorectal cancer might be mainly due to high intakes of oily fish, which are rich sources of ω3PUFAs, vitamin D and vitamin A. However, it is unlikely that high intakes of white fish increase colorectal cancer risk. A possible explanation of the current study's findings is that 64.3% of the white fish intakes were from fried, cooked in butter or smoked white fish, whereas only 24.3% were from grilled or poached white fish. Fried and cooked in butter foods generally have a high content in fat (both saturated and trans fat) and in heterocyclic amines, which are formed during the frying process. And therefore fried fish might be positively associated with colorectal cancer due to these compounds (368). In addition smoked fish is rich in *N*-nitroso compounds, which also have been hypothesised to be positively associated with colorectal cancer (368;369). Therefore, the observed increased risk might be associated with the cooking preparation rather than the intake of the white fish itself.

Coffee

Finally, coffee may be associated with a decreased colorectal cancer risk either because it contains particular anticarcinogenic substances, such as phenolic compounds, or because it increases the motility of the large bowel (370). Some case-control and a few cohort studies have investigated the association between coffee consumption and

colorectal cancer. Findings from the majority of the case-control studies, as they were summarised in a review and a meta-analysis, suggest that coffee may be inversely associated with colorectal cancer risk, with those that consume four or more cups per day to have a 24% lower colorectal cancer risk (371;372). However, findings from cohort studies are less consistent, with the majority of them reporting no significant associations (370;373).

Nutrients

In marked contrast resultant models after using the set of variables that included nutrients (set 2 and 3) were not as stable as the derived models after using the sets of variables that included food groups. Only magnesium was selected to be included in the majority of the resultant models (>90% of the models derived from the original sample), but this finding was not replicated after applying the bootstrap sampling method, where magnesium was selected to be included in 77.8% of the resultant models derived from the bootstrap samples. One possible explanation of the limited number of nutrients that were selected to be included in the resultant models might be that nutrients are usually highly correlated with each other. Therefore multi-collinearity issues, when attending to fit highly correlated variables in the same model, might lead to unstable resultant models. Regarding the observed inverse association between magnesium and colorectal cancer, it has been supported by findings from a few other observational studies (374-376), whereas some other reported null associations (377-379). One possible reason for the different findings among these studies might be the different levels of magnesium intakes between the different populations.

Regarding the nutrients that were investigated in the first part of the thesis (flavonoids, fatty acids, folate, vitamin B2, vitamin B6, vitamin B12, alcohol, vitamin D and calcium) the ones that were found to be associated in some of the selected models were the nutrients: *t*MUFAs (found in 3/4 of the models derived from the original sample and in 73.0% of the models derived from the bootstrap samples), *t*FAs (found in 3/4 of the models derived from the original sample and in 52.8% of the models derived from the bootstrap samples), quercetin (found in 3/4 of the models derived from the original sample and in 47.5% of the models derived from the bootstrap samples) and ω3PUFAs

(found in 3/4 of the models derived from the original sample and in 47.3% of the models derived from the bootstrap samples). After sex stratification, *t*FAs and ω3PUFAs were found to be inversely associated with female but not male colorectal cancer and similarly quercetin was found to be inversely associated with male but not female colorectal cancer. However, similarly to the explanation provided for the finding that high dietary energy intakes were found to be associated with male and not female colorectal cancer, these sex specific differences for *t*FAs, ω3PUFAs and quercetin might be due to measurement errors with men and women misreporting the intakes of these particular nutrients.

9.3.2.6 Summary

In the overall analysis several risk factors were found to be significantly associated with colorectal cancer including demographic and lifestyle factors (family history of cancer, NSAIDs intake, dietary energy intake, HRT intake and physical activity), food group variables (vegetables, eggs, sweets, fruit/ vegetable juice, oily fish, coffee, fruit, savoury foods and white fish) and nutrient variables (*t*MUFAs, ω3PUFAs, SFAs, *t*FAs, MUFAs, quercetin, catechin, phytoestrogen, cholesterol, fibre, protein, starch, magnesium, potassium, manganese, copper, iron, zinc, phosphorus, selenium, niacin, vitamin B6, carotenes, vitamin C, vitamin A, potential niacin, biotin, folate, pantothenic acid, vitamin D, vitamin B1 and vitamin B12).

Regarding forward and backward stepwise regression models, the variables that were selected to be included in 100% of the models derived from the whole, female and male analysis of all three sets were family history, NSAIDs, sweets and fruit/ vegetable juice. In contrast, the variables *t*FAs, vegetables and ω3PUFAs were selected to be included in models derived from the female sample, but not in models derived from the male samples. Similarly, the variables dietary energy intake, physical activity, quercetin, flavanones and manganese were selected to be included in models derived from the male sample, but not in models derived from the female sample

Finally, the bootstrap method was applied to investigate the stability of the models of the whole sample and it was applied for forward and backward stepwise regression of all three sets of variables. The variables that were selected to be included in models for the

majority of the bootstrap samples (more than 90%) were: 1) family history, NSAIDs and dietary energy, if we consider all three sets of variables; 2) family history, NSAIDs, dietary energy, eggs, sweets, fruit/ vegetable juice and white fish, if we consider set 1 and set 3; and 3) family history, NSAIDs and dietary energy, if we consider set 2 and 3.

9.4 Conclusions and recommendations

In this last part of the chapter, the main conclusions and the hypotheses that were generated will be outlined. In addition, recommendations for future studies according to the findings of the present study will be presented and discussed.

9.4.1 Conclusions

Analysis of the current thesis was divided in two parts. The first part was focused on the analysis of specific hypotheses using logistic regression models adjusted for several confounding factors, whereas the second part consisted of the overall and stepwise regression analysis of a number of demographic, lifestyle and dietary risk factors.

9.4.1.1 Main conclusions of first part of the thesis

The main conclusions derived from the analysis of the first part of the thesis (analysis of hypotheses 1-4) are described below.

1. The flavonoid subgroups flavonols and procyanidins and the flavonoid individual compounds quercetin and catechin were inversely and dose dependently associated with colorectal cancer risk after applying the energy-adjusted model (model II). After applying the full multivariable conditional logistic regression model (model III) the inverse association with intakes of quercetin and catechin remained statistically significant, whereas the inverse associations with intakes of flavonols and procyanidins was marginally not statistically significant (at p=0.05 level). In addition, the associations with flavonols and catechin remained significant and became stronger after mutually adjusting between flavonoid categories (model V of flavonoid analysis). Finally, the associations between colorectal cancer and the intakes of quercetin and catechin (model II) and the intakes of flavonols and catechin (model V) remained statistically significant after correcting the p-values for multiple testing using either the Bonferroni or the FDR method.

2. Crude intakes of total FAs, of the subgroups SFAs, MUFAs, PUFAs, ω6PUFAs, *t*FAs, *t*MUFAs, and of the individual fatty acids palmitic, stearic, oleic, linoleic, γ-linolenic and arachidonic were associated with an increased colorectal cancer (model I). After applying the energy-adjusted model (model II), the fatty acid subgroup ω3PUFAs and the fatty acid compounds EPA and DHA were inversely associated with colorectal cancer whereas total FAs, the fatty acid subgroups SFAs, MUFAs, *t*FAs, *t*MUFAs, and the individual fatty acids palmitic, stearic and oleic were positively associated with colorectal cancer. Furthermore, the associations that remained statistically significant after applying the full multivariable conditional logistic regression model (model III), after further energy adjustment (model IV of fatty acid analysis) and after total fatty acid intake adjustment (model V of fatty acid analysis) were the inverse associations with high intakes of ω3PUFAs, EPA and DHA and the positive association with high intakes of stearic acid. Finally, all the aforementioned associations except for the associations with linoleic and γ-linolenic acids (model I) remained statistically significant after correcting the p-values for multiple testing using either the Bonferroni or the FDR method.

3. High intakes of folate and of vitamin B6 were associated with a decreased colorectal cancer risk in the energy-adjusted model (model II), and these associations remained statistically significant after correcting the p-values for multiple testing using either the Bonferroni or the FDR method. In the full multivariable model (model III) though, the inverse association between folate and colorectal cancer was attenuated and a bell shaped association with an increased colorectal cancer risk for medium folate intakes was observed. In addition, the association between vitamin B6 and colorectal cancer was slightly attenuated and became marginally not statistically significant (at the p=0.05 level). Regarding vitamin B12, high intakes were associated with a decreased colorectal cancer risk after applying both model II and III. However, the associations were not statistically significant after correcting the p-values for multiple testing and they were diluted after further adjusting for ω3PUFAs.

4. High intakes of calcium were associated with an increased colorectal cancer after applying the unadjusted crude model (model I), but no statistically significant

associations were observed after further adjustment. However, higher intakes of calcium of more than 1500mg/day were associated with a statistically significant decreased colorectal cancer risk (after applying model III). High intakes of vitamin D were inversely associated with colorectal cancer in the energy-adjusted model (model II) and the full multivariable logistic regression model (model III). This inverse association though was diluted after further adjusting for ω3PUFAs (model IV of vitamin D analysis). Finally, the associations between colorectal cancer and intakes of calcium (model I) and vitamin D (model II) remained statistically significant after correcting the p-values for multiple testing using either the Bonferroni or the FDR method.

5. Finally, analysis of the main food sources of the aforementioned nutrients generally confirmed these findings, even if in most cases the associations between colorectal cancer and food group or item intakes were less clear. Briefly, the food groups or items that were investigated included: the food items regular tea, onions, apples and red wine for the flavonoids, the food groups meat and meat products, confectionery and savoury snacks, fish and fish products for the fatty acids, the food items baked or boiled potatoes, bran flakes, bananas, fried oily fish and liver or liver products for folate, vitamin B6 and vitamin B12 and fried oily fish, smoked oily fish, semi-skimmed milk and full fat cheese for vitamin D and calcium.

9.4.1.2 Main conclusions of second part of the thesis

The main conclusions derived from the analysis of the second part of the thesis (overall and stepwise regression analyses) are described below.

1. The risk factors that were found to be statistically significantly associated with colorectal cancer in the overall analysis after applying univariable logistic regression model (residually energy-adjusted) were:

a. The demographic and lifestyle factors: family history of cancer, NSAIDs intake, dietary energy intake, HRT intake and physical activity;

b. The food group variables: vegetables, eggs, sweets, fruit/ vegetable juice, oily fish, coffee, fruit, savoury foods and white fish;

c. The nutrient variables: *t*MUFAs, ω3PUFAs, SFAs, *t*FAs, MUFAs, quercetin, catechin, phytoestrogen, cholesterol, fibre, protein, starch, magnesium, potassium,

manganese, copper, iron, zinc, phosphorus, selenium, niacin, vitamin B6, carotenes, vitamin C, vitamin A, potential niacin, biotin, folate, pantothenic acid, vitamin D, vitamin B1 and vitamin B12.

2. Regarding stepwise regression analysis, the variables family history, NSAIDs, sweets and fruit/ vegetable juice were selected to be included in all models derived from the whole, female and male analysis of all three sets of variables after applying forward and backward stepwise regression. In contrast, the variables *t*FAs, vegetables and ω3PUFAs, were selected to be included in models derived from the female sample, and similarly the variables dietary energy intake, physical activity, quercetin, flavanones, and manganese were selected to be included in models derived from the male sample.

3. The main conclusions of the bootstrap sampling analysis, which was applied in order to check the stability of the derived models are:

a. All 100 models derived after forward stepwise regression were chosen once (for all sets of variables), and the same was observed for the 100 models derived after applying backward stepwise regression.

b. The agreement between the models derived from forward and backward stepwise regression within the same bootstrap sample was high for the analysis of the set 1 variables, whereas it was lower for the analysis of the set 2 and set 3 variables.

c. The number of variables that were selected to be included in the models of the 100 bootstrap samples was smaller for the set 1 analysis, than for the set 2 and set 3 analyses.

d. More variables were selected to be included in models derived from backward stepwise regression than in models derived from forward stepwise regression.

e. The variables that were selected to be included in models for the majority of the bootstrap samples (more than 90%) were: i) family history, NSAIDs and dietary energy, if we consider all three sets of variables; ii) family history, NSAIDs, dietary energy, eggs, sweets, fruit/ vegetable juice and white fish, if we consider set 1 and set 3; and iii) family history, NSAIDs and dietary energy, if we consider set 2 and 3.

9.4.2 Recommendations

The recommendations that are derived from the findings of the current thesis can be divided in two parts: 1) recommendations regarding the specific findings of the current study and 2) general recommendations regarding methodological and analytical issues.

9.4.2.1 Recommendations regarding findings of the current thesis

1. The findings of the current study suggest that high intakes of the subgroups flavonols and procyanidins and of the individual compounds quercetin and catechin might be inversely and dose dependently associated with colorectal cancer risk. However, specific recommendation regarding the intakes of these particular flavonoids is not suggested, mainly because the observed associations were not statistically significant in all applied models and also because there are inconsistent findings from previous studies. On the other hand, the subgroups flavones, flavan3ols, flavanones and phytoestrogens were not associated with colorectal cancer in any of the applied models. However, interpretation of the findings for these compounds is problematic due to: a) limited ability of the FFQ to rank individuals according to flavones and flavanones intakes (based on the validation study results), b) problematic distribution of flavan3ols intakes (except for catechin and epicatechin intakes) and c) low levels of dietary intake of phytoestrogens in Scotland leading to insufficient variation. Therefore, further investigation of the associations between colorectal cancer and intakes of flavonoid subgroups and individual compounds in future observational studies is recommended.

2. High intakes of SFAs, MUFAs, *t*FAs and *t*MUFAs were found to be associated with an increased colorectal cancer in the current study. However, these associations were attenuated after further adjustment for various confounding factors. These fatty acids are mainly found in red and processed meat and they also contribute highly to the dietary energy intake, which has been found to increase colorectal cancer risk in the current and other observational studies. Therefore, they still should be considered as important colorectal cancer risk factors even if they were not found to be statistically significantly associated with colorectal cancer in all applied models. Health promotion policies should consider including recommendations for low intakes of these types of fat or their food sources. One such example is the recommendations published from

AICR/WCRF report (2007), where it has been suggested that intakes of red meat should be limited to less than 300g/week and intakes of processed meats should be completely avoided.

3. In contrast high intakes of ω3PUFAs were found to be inversely and dose dependently associated with colorectal cancer risk in all applied models (except for the crude one). It is suggested therefore that ω3PUFAs operate differently than the other types of fat, decreasing colorectal cancer risk. However, ω3PUFAs share common sources (main food source: oily fish) with other nutrients that may affect colorectal carcinogenesis (vitamin B12, vitamin D) and therefore these inverse associations might be confounded. Therefore, specific recommendations regarding intakes ω3PUFAs are not suggested. In contrast further investigation of their associations with colorectal cancer in prospective observational studies is proposed.

4. The findings of the current study suggest that high intakes of folate are not associated with an increased or decreased colorectal cancer risk. A bell shaped relationship was observed instead with those of medium folate intakes being at higher risk. Mandatory folic acid fortification has been introduced in several countries (including USA and Canada) and has been decided but suspended in the UK. Considering the findings of the current study as well as the possibility of folate enhancing colorectal cancer risk, further investigation of the role of folate in prospective observational studies and examination of the results of two clinical trials investigating the folate effect on cancer (including colorectal) is recommended prior to the mandatory folic acid fortification in the UK.

5. High intakes of vitamin B6 and vitamin B12, which act as coenzymes in the one-carbon metabolic pathway have been found to be associated with a decreased colorectal cancer risk. However, vitamin B6 intakes were attenuated and became marginally not statistically significant after further adjustment (model III). Similarly, association between high intakes of vitamin B12 and colorectal cancer was found to be confounded by ω3PUFAs (common food source). Therefore, specific recommendations regarding intakes of vitamin B6 or vitamin B12 are not suggested. In contrast, further

investigation of their associations with colorectal cancer in prospective observational studies is proposed.

6. Folate, vitamin B2, vitamin B6 and vitamin B12 are all involved in the one-carbon metabolic pathway. In addition, the enzymes MTHFR, MTR and MTRR are also involved in this pathway and are coded from polymorphic genes. All these factors have been proposed to be independently linked to colorectal cancer risk, however results from the current and other observational studies failed to replicate these associations. Combined analysis of these factors allowing for possible genetic and environmental effects on intermediate phenotypes, together with gene-gene and gene-environment interactions is therefore recommended, in order to further investigate associations between these risk factors and colorectal cancer. Both conventional (such as stepwise regression) and more novel analytical methods are proposed to be applied. An example of a novel analytical model for investigating both the independent associations as well as various combinations (nutrient-nutrient, gene-gene and gene-nutrient interactions) of risk factors of a particular pathway, is an approximate method known as Variational Bayes (380-382). One of the main advantages of the Variational Bayes algorithm is that it allows effects unsupported by the data to be "switched off" (automatic relevance determination) and can then prune the developed models to the simplest form that is supported by the data.

7. Whereas calcium intakes (when divided into quartiles) were not found to be associated with colorectal cancer, calcium intakes of more than 1500mg/day were significantly associated with a decreased risk. In addition, results from prospective and retrospective studies are inconsistent and this inconsistency might be due to different levels of calcium intake. A current systematic review of two clinical trials investigating the effect of calcium supplementation on colorectal polyps reported a moderate reduction in risk of colorectal polyps. However, it concluded that there is not enough evidence to recommend general use of calcium supplements to prevent colorectal cancer (344). Based on the current findings as well as on the inconsistent results of previous studies, the effect of calcium should be further investigated in observational studies,

considering that high intakes of calcium could be required for a protective effect to be apparent.

8. Association between vitamin D intakes and colorectal cancer were statistically significant, however they were attenuated after further adjusting for ω3PUFAs (common food source). However, due to the fact that these nutrients are highly correlated it is difficult to draw specific conclusions regarding which nutrient is truly associated with colorectal cancer and which not. Vitamin D might be a particularly useful chemopreventive agent against colorectal cancer (considering that its main side effects will be prevented) and therefore further investigation of the vitamin D effect on colorectal cancer by prospective and retrospective studies is very important. In addition, alternative analytical approaches that overcome the problems of traditional epidemiological methods (such as confounding and reverse causation) might be used in order to establish the relationship between vitamin D and colorectal cancer. One such method is the Mendelian randomisation approach, where a genetic variant is treated as an instrument which is assumed to be associated with the disease only through its association with the intermediate phenotype (383;384). Finally, given the fact that prevalence of vitamin D deficiency is high in Scotland (due to high latitude and low sunshine exposure), if vitamin D will be proven to be significantly linked to colorectal cancer, health promotion policies should consider including recommendations for an increase in vitamin D intake by the general public (especially during the winter months).

9. Results from the overall and stepwise regression analysis supported previous findings of an increased colorectal cancer risk due to a high or moderate family history risk. Therefore, colorectal cancer screening is recommended for individuals with a high family history risk. In the current thesis, individuals with moderate and high family history risk were compared to individuals with low family history risk. However, investigation of the association between colorectal cancer and a more detailed family history score is recommended. An example of a comprehensive family history score for a particular individual is one that takes into consideration the actual number of first, second and other-degree affected relatives assigning a specific number of points. This or

similar family history scoring systems will probably make risk assessment and development of screening programs easier (385).

10. High intakes of dietary energy were found to be positively associated with increased colorectal cancer risk in the overall analysis and in addition dietary energy was selected to be included in the majority of the stepwise regression models. Increased dietary energy intake, when combined to limited physical activity, is one of the main risk factors of obesity, which is considered as one of the established colorectal cancer risk factors (even if high BMI was not found to be associated with colorectal cancer in the current study). Taking all these into consideration, health promotion policies should possibly include recommendations for limiting dietary energy intakes in order to prevent colorectal carcinogenesis and other chronic diseases.

11. Regular intake of NSAIDs was found to be inversely associated with colorectal cancer risk in the overall analysis and in the majority of the stepwise regression models. This finding is supported by findings of a significant amount of observational studies and randomised clinical trials. NSAIDs can be considered and recommended as chemopreventive agents against colorectal carcinogenesis. However, their gastrointestinal side effects mainly due to reduction of the prostaglandins that protect the gastric epithelium should be overcome and also an assessment regarding of their other effects should be evaluated.

12. The overall and stepwise regression analyses generated a few new hypotheses suggesting that low intakes of fruit/ vegetable juice, eggs, white fish and sweets (a combined variable of high-fat and high-sugar foods) and high intakes of coffee and magnesium were associated with a decreased colorectal cancer. Further investigation of the associations between the aforementioned risk factors and colorectal cancer in future prospective and retrospective studies is recommended.

13. Finally, in the current study, the associations between particular nutrients and food groups/ items were examined. A small amount of studies suggest that dietary pattern analysis should be also conducted investigating the associations between clusters of particular foods and colorectal cancer. Therefore, application of dietary pattern analysis on the data of the current thesis is suggested.

9.4.2.2 General recommendations regarding methodological and analytical issues

1. According to the findings of the current thesis as well as of other observational studies, it is clear that establishing causal relationships between environmental exposures and common diseases using conventional methods of observational epidemiology is usually problematic. Particular examples include the collinearity issues between nutrients that have common dietary sources, not allowing to identify the nutrient that is truly associated with a disease (as for example with ω3PUFAs, vitamin B12 and vitamin D), or limited power of observational studies to detect gene-environment interactions. Therefore, the application of novel analytical methods, such as the already mentioned Mendelian randomisation method or the Variational Bayes method, might be a way to overcome these limitations. Funding from CR-UK (36-month CR-UK Population and Behavioural Science Training Fellowship) and CSO (27-month CSO research grant) has been already secured for exploring these novel methodologies (Mendelian randomisation and Variational Bayes) using the current dataset (SOCCS study).

2. Additionally, one of the most problematic areas of observational epidemiology is the limited power to detect weak and sometimes even moderate associations. Traditional power calculations tend to underestimate sample size requirements and therefore it has been suggested that the majority of observational studies is under-powered. Considerable effort should be made to improve measurement procedures in order to increase the accuracy and precision of a study, to increase the sample size of individual studies and to set specific protocols of collaboration and data sharing.

3. Energy adjustment is one of the main issues of nutritional epidemiology, particularly when investigating associations with nutrients that highly contribute to the total dietary energy intake. We elected to use the residual energy adjustment method, as this method is considered to be analogous to a study in which total dietary energy intake remains constant, whereas the amount of nutrients (composition of diet) varies between groups. However, to be able to apply this method the nutrient under investigation should be normally distributed (with or without transformation). For a few nutrients, which

distributions were not normal even after data transformation, we elected to apply the standard method of energy adjustment, which is the method that is more closely related to the residual energy adjustment. In addition, it has been suggested that application of the residual energy adjustment results to over-correcting and attenuating any statistically significant associations. However, when we compared four different energy adjustment methods for the investigation of the associations between specific fatty acids (subgroups or individual compounds) and colorectal cancer, we did not observe any significant differences between the different methods. According to this finding, the application of residual energy adjustments is recommended in all cases, except for when the nutrient under investigation is not normally distributed, where an alternative method should be used like the standard method.

4. Matching for particular risk factors is a way to control for the confounding effect of these risk factors. In addition, it generally increases the precision and power of the study. However, important limitations include that cases with no controls fulfilling the matching criteria need to be excluded from the analysis and that recruitment of controls that are finely matched to the cases is a time consuming and expensive procedure. In our study, the matched and unmatched datasets were similarly powered to detect moderate and strong associations, even if the matched dataset included fewer cases and controls. In addition, we compared the associations between specific fatty acids (subgroups or individual compounds) and colorectal cancer after applying logistic regression models on the matched and unmatched datasets and we did not observe any significant differences. Even if the increase in precision and power is significant when a matching protocol is employed, future case-control studies should decide whether the effort and costs of a matched design are necessary by considering the type of their research questions and how important matching will be in order to address them.

5. According to the findings from the overall and stepwise regression analyses high intakes of fruit/ vegetable juice were associated with an increased colorectal cancer risk. Just a few studies though have reported separate associations between colorectal cancer and intakes of fruit/ vegetable juice and raw fruit or vegetables. Fruit/ vegetable juice consists of many other ingredients (including sugars, preservatives, etc.) apart from the

nutrients that are found in the fruit and vegetables they come from. Therefore, it is recommended that fruit/ vegetable juice should be studied separately from raw fruit or vegetables.

6. Similarly, in many studies white and oily fish intakes are grouped together when investigating colorectal cancer risk. However, according to the findings of the current thesis high intakes of white fish were associated with an increased colorectal cancer risk, whereas high intakes of oily fish were associated with a decreased colorectal cancer risk. It is therefore recommended that white and oily fish should be studied separately. In addition, weight should be given for selecting information regarding the ways of both food preparation and cooking methods, since they might be equally important for colorectal carcinogenesis as the foods and nutrients themselves.

7. As it has been shown from the stepwise regression and bootstrap sampling results, both forward and backward stepwise regression does not produce very stable models. In addition, the agreement between the models derived from forward and backward stepwise regression within the same bootstrap sample decreased as the number of the potential risk factors increased. This finding suggests that the number of noise variables that were selected to be included in the models increased as the number of candidate variables increased. Therefore, it might be necessary that the number of the candidate variables needs to be kept relatively small for the production of more reliable models.

8. Furthermore, high correlation between the candidate variables can affect the reliability of the selected models. According to our findings, when stepwise regression was applied on sets of highly correlated variables (nutrients), then the resultant models were less stable than when stepwise regression was applied on sets of less correlated variables (food groups). Multicollinearity issues are particularly important when applying backward stepwise regression, since the first step of the backward procedure is to include all the risk factors in the model. However, inclusion of highly correlated variables in the same model will probably result to spurious findings. Therefore, it is recommended that when applying stepwise regression models, and backward stepwise

regression in particular, to avoid including variables that are highly correlated with each other.

9. Finally, findings from the bootstrap sampling method indicated that the stability of the stepwise regression models, either forward or backward is generally low. Therefore, results derived after applying these methods should be treated with caution. In addition, efforts for replication of any positive findings should be made, either by applying the selected model to an independent dataset or by examining the stability of the model with the bootstrap sampling method. In the current study, the stability of the selected models was tested in 100 bootstrap samples due to time and computer power issues, however ideally 1,000 to 10,000 samples should be used for adequately examining the validity of the stepwise regression procedure.

Reference List

(1) Ponz de LM, Di GC. Pathology of colorectal cancer. Dig Liver Dis 2001; 33(4):372-388.
(2) Cruz-Bustillo CD. Molecular genetics of colorectal cancer. Rev Esp Enferm Dig 2004; 96(1):48-59.
(3) Mitchell RJ, Farrington SM, Dunlop MG, Campbell H. Mismatch repair genes hMLH1 and hMSH2 and colorectal cancer: a HuGE review. Am J Epidemiol 2002; 156(10):885-902.
(4) Dunlop MG, Farrington SM, Nicholl I et al. Population carrier frequency of hMSH2 and hMLH1 mutations. Br J Cancer 2000; 83(12):1643-1645.
(5) Aarnio M, Sankila R, Pukkala E et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999; 81(2):214-218.
(6) Millar AL, Pal T, Madlensky L et al. Mismatch repair gene defects contribute to the genetic basis of double primary cancers of the colorectum and endometrium. Hum Mol Genet 1999; 8(5):823-829.
(7) Diergaarde B, van Geloof WL, van Muijen GN, Kok FJ, Kampman E. Dietary factors and the occurrence of truncating APC mutations in sporadic colon carcinomas: a Dutch population-based study. Carcinogenesis 2003; 24(2):283-290.
(8) Senda T, Iizuka-Kogo A, Onouchi T, Shimomura A. Adenomatous polyposis coli (APC) plays multiple roles in the intestinal and colorectal epithelia. Med Mol Morphol 2007; 40(2):68-81.
(9) Segditsas S, Tomlinson I. Colorectal cancer and genetic alterations in the Wnt pathway. Oncogene 2006; 25(57):7531-7537.
(10) Olschwang S, Serova-Sinilnikova OM, Lenoir GM, Thomas G. PTEN germ-line mutations in juvenile polyposis coli. Nat Genet 1998; 18(1):12-14.
(11) Howe JR, Bair JL, Sayed MG et al. Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. Nat Genet 2001; 28(2):184-187.
(12) Gustafson S, Zbuk KM, Scacheri C, Eng C. Cowden syndrome. Semin Oncol 2007; 34(5):428-434.
(13) McGarrity TJ, Kulin HE, Zaino RJ. Peutz-Jeghers syndrome. Am J Gastroenterol 2000; 95(3):596-604.
(14) Xu Y, Pasche B. TGF-beta signaling alterations and susceptibility to colorectal cancer. Hum Mol Genet 2007; 16 Spec No 1:R14-R20.
(15) Laken SJ, Petersen GM, Gruber SB et al. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. Nat Genet 1997; 17(1):79-83.
(16) White S, Bubb VJ, Wyllie AH. Germline APC mutation (Gln1317) in a cancer-prone family that does not result in familial adenomatous polyposis. Genes Chromosomes Cancer 1996; 15(2):122-128.
(17) Chen SP, Tsai ST, Jao SW et al. Single nucleotide polymorphisms of the APC gene and colorectal cancer risk: a case-control study in Taiwan. BMC Cancer 2006; 6:83.
(18) Al-Tassan N, Chmiel NH, Maynard J et al. Inherited variants of MYH associated with somatic G:C-->T:A mutations in colorectal tumors. Nat Genet 2002; 30(2):227-232.
(19) Sieber OM, Lipton L, Crabtree M et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. N Engl J Med 2003; 348(9):791-799.
(20) Kury S, Buecher B, Robiou-du-Pont S et al. The Thorough Screening of the MUTYH Gene in a Large French Cohort of Sporadic Colorectal Cancers. Genet Test 2007.
(21) Shaheen NJ, Silverman LM, Keku T et al. Association between hemochromatosis (HFE) gene mutation carrier status and the risk of colon cancer. J Natl Cancer Inst 2003; 95(2):154-159.
(22) David SS, O'Shea VL, Kundu S. Base-excision repair of oxidative DNA damage. Nature 2007; 447(7147):941-950.
(23) Cheadle JP, Sampson JR. MUTYH-associated polyposis--from defect in base excision repair to clinical genetic testing. DNA Repair (Amst) 2007; 6(3):274-279.
(24) Ilyas M, Straub J, Tomlinson IP, Bodmer WF. Genetic pathways in colorectal and other cancers. Eur J Cancer 1999; 35(14):1986-2002.
(25) Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet 2005; 365(9454):153-165.

(26) Harvey NT, Ruszkiewicz A. Serrated neoplasia of the colorectum. World J Gastroenterol 2007; 13(28):3792-3798.
(27) Speake D, Biyani D, Frizelle FA, Watson AJ. Flat adenomas. ANZ J Surg 2007; 77(1-2):4-8.
(28) Martin ES, Tonon G, Sinha R et al. Common and distinct genomic events in sporadic colorectal cancer and diverse cancer types. Cancer Res 2007; 67(22):10736-10743.
(29) Souglakos J. Genetic alterations in sporadic and hereditary colorectal cancer: implementations for screening and follow-up. Dig Dis 2007; 25(1):9-19.
(30) Food, Nutrition, Physical Activity and the Prevention of Cancer. 2007. American Institute for Cancer Research, World Cancer Research Fund.
(31) Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 2007; 18(3):581-592.
(32) Berrino F, De AR, Sant M et al. Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995-99: results of the EUROCARE-4 study. Lancet Oncol 2007; 8(9):773-783.
(33) Gatta G, Faivre J, Capocaccia R, Ponz de LM. Survival of colorectal cancer patients in Europe during the period 1978-1989. Eur J Cancer 1998; 34(14):2176-2183.
(34) Gatta G, Capocaccia R, Sant M et al. Understanding variations in survival for colorectal cancer in Europe: a EUROCARE high resolution study. Gut 2000; 47(4):533-538.
(35) Sant M, Aareleid T, Berrino F et al. EUROCARE-3: survival of cancer patients diagnosed 1990-94--results and commentary. Ann Oncol 2003; 14 Suppl 5:v61-118.
(36) Sant M, Capocaccia R, Coleman MP et al. Cancer survival increases in Europe, but international differences remain wide. Eur J Cancer 2001; 37(13):1659-1667.
(37) Winawer S, Fletcher R, Rex D et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. Gastroenterology 2003; 124(2):544-560.
(38) Rex DK, Kahi CJ, Levin B et al. Guidelines for colonoscopy surveillance after cancer resection: a consensus update by the American Cancer Society and the US Multi-Society Task Force on Colorectal Cancer. Gastroenterology 2006; 130(6):1865-1871.
(39) Bouvier AM, Latournerie M, Jooste V, Lepage C, Cottet V, Faivre J. The lifelong risk of metachronous colorectal cancer justifies long-term colonoscopic follow-up. Eur J Cancer 2008; 44(4):522-527.
(40) Enblad P, Adami HO, Glimelius B, Krusemo U, Pahlman L. The risk of subsequent primary malignant diseases after cancers of the colon and rectum. A nationwide cohort study. Cancer 1990; 65(9):2091-2100.
(41) Hoar SK, Wilson J, Blot WJ, McLaughlin JK, Winn DM, Kantor AF. Second cancer following cancer of the digestive system in Connecticut, 1935-82. Natl Cancer Inst Monogr 1985; 68:49-82.
(42) Slattery ML, Mori M, Gao R, Kerber RA. Impact of family history of colon cancer on development of multiple primaries after diagnosis of colon cancer. Dis Colon Rectum 1995; 38(10):1053-1058.
(43) Teppo L, Pukkala E, Saxen E. Multiple cancer--an epidemiologic exercise in Finland. J Natl Cancer Inst 1985; 75(2):207-217.
(44) Mitchell PJ, Haboubi NY. The malignant adenoma: when to operate and when to watch. Surg Endosc 2008; 22(7):1563-1569.
(45) Rapuri S, Spencer J, Eckels D. Importance of postpolypectomy surveillance and postpolypectomy compliance to follow-up screening--review of literature. Int J Colorectal Dis 2008; 23(5):453-459.
(46) Neugut AI, Jacobson JS, De V, I. Epidemiology of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 1993; 2(2):159-176.
(47) Butterworth AS, Higgins JP, Pharoah P. Relative and absolute risk of colorectal cancer for individuals with a family history: a meta-analysis. Eur J Cancer 2006; 42(2):216-227.
(48) Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. World J Gastroenterol 2008; 14(3):378-389.
(49) Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut 2001; 48(4):526-535.
(50) von Roon AC, Reese G, Teare J, Constantinides V, Darzi AW, Tekkis PP. The risk of cancer in patients with Crohn's disease. Dis Colon Rectum 2007; 50(6):839-855.

(51) Ryan-Harshman M, Aldoori W. Diet and colorectal cancer: Review of the evidence. Can Fam Physician 2007; 53(11):1913-1920.
(52) Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int J Cancer 2006; 119(11):2657-2664.
(53) Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. PLoS Med 2007; 4(12):e325.
(54) Burkitt DP. Related disease--related cause? Lancet 1969; 2(7632):1229-1231.
(55) Park Y, Hunter DJ, Spiegelman D et al. Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. JAMA 2005; 294(22):2849-2857.
(56) Nomura AM, Hankin JH, Henderson BE et al. Dietary fiber and colorectal cancer risk: the multiethnic cohort study. Cancer Causes Control 2007; 18(7):753-764.
(57) Bingham SA, Norat T, Moskal A et al. Is the association with fiber from foods in colorectal cancer confounded by folate intake? Cancer Epidemiol Biomarkers Prev 2005; 14(6):1552-1556.
(58) Food, Nutrition and the Prevention of Cancer: a global prospective. 1997. American Institute for Cancer Research, World Cancer Research Fund.
(59) Park Y, Subar AF, Kipnis V et al. Fruit and vegetable intakes and risk of colorectal cancer in the NIH-AARP diet and health study. Am J Epidemiol 2007; 166(2):170-180.
(60) Beresford SA, Johnson KC, Ritenbaugh C et al. Low-fat dietary pattern and risk of colorectal cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 2006; 295(6):643-654.
(61) Satia-Abouta J, Galanko JA, Potter JD, Ammerman A, Martin CF, Sandler RS. Associations of total energy and macronutrients with colon cancer risk in African Americans and Whites: results from the North Carolina colon cancer study. Am J Epidemiol 2003; 158(10):951-962.
(62) Franceschi S, La VC, Russo A et al. Macronutrient intake and risk of colorectal cancer in Italy. Int J Cancer 1998; 76(3):321-324.
(63) Gaard M, Tretli S, Loken EB. Dietary factors and risk of colon cancer: a prospective study of 50,535 young Norwegian men and women. Eur J Cancer Prev 1996; 5(6):445-454.
(64) Giovannucci E, Goldin B. The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. Am J Clin Nutr 1997; 66(6 Suppl):1564S-1571S.
(65) Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. Nutr Cancer 1997; 28(3):276-281.
(66) Levi F, Pasche C, Lucchini F, La VC. Macronutrients and colorectal cancer: a Swiss case-control study. Ann Oncol 2002; 13(3):369-373.
(67) Slattery ML, Caan BJ, Potter JD et al. Dietary energy sources and colon cancer risk. Am J Epidemiol 1997; 145(3):199-210.
(68) Stemmermann GN, Nomura AM, Heilbrun LK. Cancer risk in relation to fat and energy intake among Hawaii Japanese: a prospective study. Princess Takamatsu Symp 1985; 16:265-274.
(69) West DW, Slattery ML, Robison LM et al. Dietary intake and colon cancer: sex- and anatomic site-specific associations. Am J Epidemiol 1989; 130(5):883-894.
(70) Slattery ML, Potter J, Caan B et al. Energy balance and colon cancer--beyond physical activity. Cancer Res 1997; 57(1):75-80.
(71) Pendyala S, Holt P. Obesity and colorectal cancer risk. Gastroenterology 2008; 134(3):896.
(72) 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 2006; 98(13):920-931.
(73) Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr 2007; 86(3):556-565.
(74) 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 2007; 16(12):2533-2547.
(75) Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008; 371(9612):569-578.

(76) Howard RA, Freedman DM, Park Y, Hollenbeck A, Schatzkin A, Leitzmann MF. Physical activity, sedentary behavior, and the risk of colon and rectal cancer in the NIH-AARP Diet and Health Study. Cancer Causes Control 2008.
(77) Friedenreich C, Norat T, Steindorf K et al. Physical activity and risk of colon and rectal cancers: the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev 2006; 15(12):2398-2407.
(78) Samad AK, Taylor RS, Marshall T, Chapman MA. A meta-analysis of the association of physical activity with reduced risk of colorectal cancer. Colorectal Dis 2005; 7(3):204-213.
(79) Toriola AT, Kurl S, Laukanen JA, Mazengo C, Kauhanen J. Alcohol consumption and risk of colorectal cancer: the Findrink study. Eur J Epidemiol 2008; 23(6):395-401.
(80) Ferrari P, Jenab M, Norat T et al. Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC). Int J Cancer 2007; 121(9):2065-2072.
(81) Moskal A, Norat T, Ferrari P, Riboli E. Alcohol intake and colorectal cancer risk: a dose-response meta-analysis of published cohort studies. Int J Cancer 2007; 120(3):664-671.
(82) Cho E, Smith-Warner SA, Ritz J et al. Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. Ann Intern Med 2004; 140(8):603-613.
(83) Bongaerts BW, van den Brandt PA, Goldbohm RA, de Goeij AF, Weijenberg MP. Alcohol consumption, type of alcoholic beverage and risk of colorectal cancer at specific subsites. Int J Cancer 2008.
(84) Longnecker MP, Orza MJ, Adams ME, Vioque J, Chalmers TC. A meta-analysis of alcoholic beverage consumption in relation to risk of colorectal cancer. Cancer Causes Control 1990; 1(1):59-68.
(85) Botteri E, Iodice S, Raimondi S, Maisonneuve P, Lowenfels AB. Cigarette smoking and adenomatous polyps: a meta-analysis. Gastroenterology 2008; 134(2):388-395.
(86) Giovannucci E. An updated review of the epidemiological evidence that cigarette smoking increases risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2001; 10(7):725-731.
(87) Paskett ED, Reeves KW, Rohan TE et al. Association between cigarette smoking and colorectal cancer in the Women's Health Initiative. J Natl Cancer Inst 2007; 99(22):1729-1735.
(88) Doll R, Peto R, Boreham J, Sutherland I. Mortality from cancer in relation to smoking: 50 years observations on British doctors. Br J Cancer 2005; 92(3):426-429.
(89) Ho JW, Lam TH, Tse CW et al. Smoking, drinking and colorectal cancer in Hong Kong Chinese: a case-control study. Int J Cancer 2004; 109(4):587-597.
(90) Slattery ML, Edwards S, Curtin K, Schaffer D, Neuhausen S. Associations between smoking, passive smoking, GSTM-1, NAT2, and rectal cancer. Cancer Epidemiol Biomarkers Prev 2003; 12(9):882-889.
(91) Luchtenborg M, Weijenberg MP, Kampman E et al. Cigarette smoking and colorectal cancer: APC mutations, hMLH1 expression, and GSTM1 and GSTT1 polymorphisms. Am J Epidemiol 2005; 161(9):806-815.
(92) Ghadirian P, Maisonneuve P, Perret C, Lacroix A, Boyle P. Epidemiology of sociodemographic characteristics, lifestyle, medical history, and colon cancer: a case-control study among French Canadians in Montreal. Cancer Detect Prev 1998; 22(5):396-404.
(93) Diergaarde B, Vrieling A, van Kraats AA, van Muijen GN, Kok FJ, Kampman E. Cigarette smoking and genetic alterations in sporadic colon carcinomas. Carcinogenesis 2003; 24(3):565-571.
(94) Baron JA, Cole BF, Sandler RS et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med 2003; 348(10):891-899.
(95) Benamouzig R, Deyra J, Martin A et al. Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. Gastroenterology 2003; 125(2):328-336.
(96) Sandler RS, Halabi S, Baron JA et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 2003; 348(10):883-890.
(97) Bertagnolli MM, Eagle CJ, Zauber AG et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 2006; 355(9):873-884.
(98) Arber N, Eagle CJ, Spicak J et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 2006; 355(9):885-895.

(99) Baron JA, Sandler RS, Bresalier RS et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology 2006; 131(6):1674-1682.
(100) Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Wu K, Fuchs CS. Aspirin dose and duration of use and risk of colorectal cancer in men. Gastroenterology 2008; 134(1):21-28.
(101) Gann PH, Manson JE, Glynn RJ, Buring JE, Hennekens CH. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst 1993; 85(15):1220-1224.
(102) Cook NR, Lee IM, Gaziano JM et al. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005; 294(1):47-55.
(103) Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet 2007; 369(9573):1603-1613.
(104) Peto R, Gray R, Collins R et al. Randomised trial of prophylactic daily aspirin in British male doctors. Br Med J (Clin Res Ed) 1988; 296(6618):313-316.
(105) Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry 1991; 54(12):1044-1054.
(106) Dube C, Rostom A, Lewin G et al. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med 2007; 146(5):365-375.
(107) Grodstein F, Newcomb PA, Stampfer MJ. Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis. Am J Med 1999; 106(5):574-582.
(108) McMichael AJ, Potter JD. Reproduction, endogenous and exogenous sex hormones, and colon cancer: a review and hypothesis. J Natl Cancer Inst 1980; 65(6):1201-1207.
(109) Calle EE, Miracle-McMahill HL, Thun MJ, Heath CW, Jr. Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. J Natl Cancer Inst 1995; 87(7):517-523.
(110) Farquhar CM, Marjoribanks J, Lethaby A, Lamberts Q, Suckling JA. Long term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev 2005;(3):CD004143.
(111) Hodis HN, Mack WJ, Lobo RA et al. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 2001; 135(11):939-953.
(112) Grady D, Applegate W, Bush T, Furberg C, Riggs B, Hulley SB. Heart and Estrogen/progestin Replacement Study (HERS): design, methods, and baseline characteristics. Control Clin Trials 1998; 19(4):314-335.
(113) Espeland MA, Bush TL, Mebane-Sims I et al. Rationale, design, and conduct of the PEPI Trial. Postmenopausal Estrogen/Progestin Interventions. Control Clin Trials 1995; 16(4 Suppl):3S-19S.
(114) Chlebowski RT, Wactawski-Wende J, Ritenbaugh C et al. Estrogen plus progestin and colorectal cancer in postmenopausal women. N Engl J Med 2004; 350(10):991-1004.
(115) Heiss G, Wallace R, Anderson GL et al. Health risks and benefits 3 years after stopping randomized treatment with estrogen and progestin. JAMA 2008; 299(9):1036-1045.
(116) Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13(10):572-584.
(117) Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 2005; 81(1 Suppl):317S-325S.
(118) Knekt P, Kumpulainen J, Jarvinen R et al. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 2002; 76(3):560-568.
(119) Neuhouser ML. Dietary flavonoids and cancer risk: evidence from human population studies. Nutr Cancer 2004; 50(1):1-7.
(120) Peterson J, Lagiou P, Samoli E et al. Flavonoid intake and breast cancer risk: a case--control study in Greece. Br J Cancer 2003; 89(7):1255-1259.
(121) Yao LH, Jiang YM, Shi J et al. Flavonoids in food and their health benefits. Plant Foods Hum Nutr 2004; 59(3):113-122.
(122) Liggins J, Grimwood R, Bingham SA. Extraction and quantification of lignan phytoestrogens in food and human samples. Anal Biochem 2000; 287(1):102-109.
(123) Moon YJ, Wang X, Morris ME. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. Toxicol In Vitro 2006; 20(2):187-210.

(124) Volate SR, Davenport DM, Muga SJ, Wargovich MJ. Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin). Carcinogenesis 2005; 26(8):1450-1456.
(125) Arts IC, Jacobs DR, Jr., Gross M, Harnack LJ, Folsom AR. Dietary catechins and cancer incidence among postmenopausal women: the Iowa Women's Health Study (United States). Cancer Causes Control 2002; 13(4):373-382.
(126) De Stefani E, Boffetta P, Deneo-Pellegrini H, et al. Dietary antioxidants and lung cancer risk: a case control study in Uruguay. Nutr Cancer 1999; 34:100-110.
(127) Garcia-Closas R, Agudo A, Gonzalez CA, Riboli E. Intake of specific carotenoids and flavonoids and the risk of lung cancer in women in Barcelona, Spain. Nutr Cancer 1998; 32(3):154-158.
(128) Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P. Flavonol and flavone intake and the risk of cancer in male smokers (Finland). Cancer Causes Control 2001; 12(9):789-796.
(129) Knekt P, Jarvinen R, Seppanen R et al. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 1997; 146(3):223-230.
(130) Le Marchand L, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN. Intake of flavonoids and lung cancer. J Natl Cancer Inst 2000; 92(2):154-160.
(131) Cutler GJ, Nettleton JA, Ross JA et al. Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. Int J Cancer 2008; 123(3):664-671.
(132) Goldbohm RA, Hertog MGL, Brants HAM, van Poppel G, van der Brandt PA. Intake of flavonoids and cancer risk: a prospective cohort study. In: Armado R, Andersson H, Bardσcz S, Serra F, editors. Polyphenols in foods. Luxembourg: Office for Official Publications of the European Community, 1998: 159-166.
(133) Lin J, Zhang SM, Wu K, Willett WC, Fuchs CS, Giovannucci E. Flavonoid Intake and Colorectal Cancer Risk in Men and Women. Am J Epidemiol 2006; 164:644-651.
(134) Mursu J, Nurmi T, Tuomainen TP, Salonen JT, Pukkala E, Voutilainen S. Intake of flavonoids and risk of cancer in Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. Int J Cancer 2008; 123(3):660-663.
(135) Rossi M, Negri E, Talamini R et al. Flavonoids and colorectal cancer in Italy. Cancer Epidemiol Biomarkers Prev 2006; 15(8):1555-1558.
(136) Theodoratou E, Kyle J, Cetnarskyj R et al. Dietary flavonoids and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2007; 16(4):684-693.
(137) Yuan JM, Gao YT, Yang CS, Yu MC. Urinary biomarkers of tea polyphenols and risk of colorectal cancer in the Shanghai Cohort Study. Int J Cancer 2007; 120(6):1344-1350.
(138) Cotterchio M, Boucher BA, Manno M, Gallinger S, Okey A, Harper P. Dietary phytoestrogen intake is associated with reduced colorectal cancer risk. J Nutr 2006; 136(12):3046-3053.
(139) Oba S, Nagata C, Shimizu N et al. Soy product consumption and the risk of colon cancer: a prospective study in Takayama, Japan. Nutr Cancer 2007; 57(2):151-157.
(140) Berg JM, Tymoczko JL, Stryer L. The Molecular Design of Life. In: Freeman WH, editor. Biochemistry. 2002.
(141) Lin J, Zhang SM, Cook NR, Lee IM, Buring JE. Dietary fat and fatty acids and risk of colorectal cancer in women. Am J Epidemiol 2004; 160(10):1011-1022.
(142) Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. Specific fatty acids and human colorectal cancer: an overview. Cancer Detect Prev 2003; 27(1):55-66.
(143) Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004; 79(6):935-945.
(144) MacLean CH, Newberry SJ, Mojica WA et al. Effects of omega-3 fatty acids on cancer risk: a systematic review. JAMA 2006; 295(4):403-415.
(145) Wynder EL, Kajitani T, Ishikawa S, Dodo H, Takano A. Environmental factors of cancer of the colon and rectum. II. Japanese epidemiological data. Cancer 1969; 23(5):1210-1220.
(146) Reddy BS. Omega-3 fatty acids in colorectal cancer prevention. Int J Cancer 2004; 112(1):1-7.
(147) Bostick RM, Potter JD, Kushi LH et al. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control 1994; 5(1):38-52.
(148) Brink M, Weijenberg MP, de Goeij AF et al. Fat and K-ras mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis 2004; 25(9):1619-1628.

(149) Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 1994; 54(9):2390-2397.
(150) Le Marchand L, Wilkens LR, Hankin JH, Kolonel LN, Lyu LC. A case-control study of diet and colorectal cancer in a multiethnic population in Hawaii (United States): lipids and foods of animal origin. Cancer Causes Control 1997; 8(4):637-648.
(151) Trichopoulou A, Tzonou A, Hsieh CC, Toupadaki N, Manousos O, Trichopoulos D. High protein, saturated fat and cholesterol diet, and low levels of serum lipids in colorectal cancer. Int J Cancer 1992; 51(3):386-389.
(152) Weijenberg MP, Luchtenborg M, de Goeij AF et al. Dietary fat and risk of colon and rectal cancer with aberrant MLH1 expression, APC or KRAS genes. Cancer Causes Control 2007; 18(8):865-879.
(153) Ghadirian P, Lacroix A, Maisonneuve P et al. Nutritional factors and colon carcinoma: a case-control study involving French Canadians in Montreal, Quebec, Canada. Cancer 1997; 80(5):858-864.
(154) De SE, Mendilaharsu M, eo-Pellegrini H, Ronco A. Influence of dietary levels of fat, cholesterol, and calcium on colorectal cancer. Nutr Cancer 1997; 29(1):83-89.
(155) Goldbohm RA, van den Brandt PA, van ', V et al. A prospective cohort study on the relation between meat consumption and the risk of colon cancer. Cancer Res 1994; 54(3):718-723.
(156) Flood A, Velie EM, Sinha R et al. Meat, fat, and their subtypes as risk factors for colorectal cancer in a prospective cohort of women. Am J Epidemiol 2003; 158(1):59-68.
(157) Jarvinen R, Knekt P, Hakulinen T, Rissanen H, Heliovaara M. Dietary fat, cholesterol and colorectal cancer in a prospective study. Br J Cancer 2001; 85(3):357-361.
(158) Kimura Y, Kono S, Toyomura K et al. Meat, fish and fat intake in relation to subsite-specific risk of colorectal cancer: The Fukuoka Colorectal Cancer Study. Cancer Sci 2007; 98(4):590-597.
(159) Kojima M, Wakai K, Tokudome S et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. Am J Epidemiol 2005; 161(5):462-471.
(160) Kuriki K, Wakai K, Hirose K et al. Risk of colorectal cancer is linked to erythrocyte compositions of fatty acids as biomarkers for dietary intakes of fish, fat, and fatty acids. Cancer Epidemiol Biomarkers Prev 2006; 15(10):1791-1798.
(161) Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. Assessment of risk associated with specific fatty acids and colorectal cancer among French-Canadians in Montreal: a case-control study. Int J Epidemiol 2003; 32(2):200-209.
(162) Pietinen P, Malila N, Virtanen M et al. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999; 10(5):387-396.
(163) Slattery ML, Potter JD, Duncan DM, Berry TD. Dietary fats and colon cancer: assessment of risk associated with specific fatty acids. Int J Cancer 1997; 73(5):670-677.
(164) Theodoratou E, McNeill G, Cetnarskyj R et al. Dietary fatty acids and colorectal cancer: a case-control study. Am J Epidemiol 2007; 166(2):181-195.
(165) Zaridze D, Filipchenko V, Kustov V, Serdyuk V, Duffy S. Diet and colorectal cancer: results of two case-control studies in Russia. Eur J Cancer 1992; 29A(1):112-115.
(166) Wakai K, Hirose K, Matsuo K et al. Dietary risk factors for colon and rectal cancers: a comparative case-control study. J Epidemiol 2006; 16(3):125-135.
(167) Senesse P, Meance S, Cottet V, Faivre J, Boutron-Ruault MC. High dietary iron and copper and risk of colorectal cancer: a case-control study in Burgundy, France. Nutr Cancer 2004; 49(1):66-71.
(168) Chyou PH, Nomura AM, Stemmermann GN. A prospective study of colon and rectal cancer among Hawaii Japanese men. Ann Epidemiol 1996; 6(4):276-282.
(169) Hall MN, Campos H, Li H et al. Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2007; 16(2):314-321.
(170) Hall MN, Chavarro JE, Lee IM, Willett WC, Ma J. A 22-year Prospective Study of Fish, n-3 Fatty Acid Intake, and Colorectal Cancer Risk in Men. Cancer Epidemiol Biomarkers Prev 2008; 17(5):1136-1143.
(171) Kobayashi M, Tsubono Y, Otani T, Hanaoka T, Sobue T, Tsugane S. Fish, long-chain n-3 polyunsaturated fatty acids, and risk of colorectal cancer in middle-aged Japanese: the JPHC study. Nutr Cancer 2004; 49(1):32-40.

(172) Tavani A, Pelucchi C, Parpinel M et al. n-3 polyunsaturated fatty acid intake and cancer risk in Italy and Switzerland. Int J Cancer 2003; 105(1):113-116.
(173) Terry P, Bergkvist L, Holmberg L, Wolk A. No association between fat and fatty acids intake and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2001; 10(8):913-914.
(174) Koh WP, Yuan JM, van den BD, Lee HP, Yu MC. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: the Singapore Chinese Health Study. Br J Cancer 2004; 90(9):1760-1764.
(175) Slattery ML, Benson J, Ma KN, Schaffer D, Potter JD. Trans-fatty acids and colon cancer. Nutr Cancer 2001; 39(2):170-175.
(176) Limburg PJ, Liu-Mares W, Vierkant RA et al. Prospective evaluation of trans-fatty acid intake and colorectal cancer risk in the Iowa Women's Health Study. Int J Cancer 2008.
(177) Kim YI. Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? Cancer Epidemiol Biomarkers Prev 2004; 13(4):511-519.
(178) Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004; 159(5):423-443.
(179) Choi SW, Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr 2002; 132(8 Suppl):2413S-2418S.
(180) Mason JB, Dickstein A, Jacques PF et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev 2007; 16(7):1325-1329.
(181) De Wals P, Tairou F, Van Allen MI et al. Reduction in neural-tube defects after folic acid fortification in Canada. N Engl J Med 2007; 357(2):135-142.
(182) Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? Am J Clin Nutr 2008; 87(3):517-533.
(183) Hubner RA, Houlston RD, Muir KR. Should folic acid fortification be mandatory? No. BMJ 2007; 334(7606):1253.
(184) Sanjoaquin MA, Allen N, Couto E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer 2005; 113(5):825-828.
(185) Benito E, Stiggelbout A, Bosch FX et al. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. Int J Cancer 1991; 49(2):161-167.
(186) Boutron-Ruault MC, Senesse P, Faivre J, Couillault C, Belghiti C. Folate and alcohol intakes: related or independent roles in the adenoma-carcinoma sequence? Nutr Cancer 1996; 26(3):337-346.
(187) Brink M, Weijenberg MP, de Goeij AF et al. Dietary folate intake and k-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study. Int J Cancer 2005; 114(5):824-830.
(188) Coogan PF, Rosenberg L. The use of folic acid antagonists and the risk of colorectal cancer. Pharmacoepidemiol Drug Saf 2007; 16(10):1111-1119.
(189) de Vogel S, van EM, Luchtenborg M et al. Dietary folate and APC mutations in sporadic colorectal cancer. J Nutr 2006; 136(12):3015-3021.
(190) Ferraroni M, La VC, D'Avanzo B, Negri E, Franceschi S, Decarli A. Selected micronutrient intake and the risk of colorectal cancer. Br J Cancer 1994; 70(6):1150-1155.
(191) Flood A, Caprario L, Chaterjee N, Lacey JV, Jr., Schairer C, Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. Cancer Causes Control 2002; 13(6):551-561.
(192) Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate intake and carcinogenesis of the colon and rectum. Int J Epidemiol 1991; 20(2):368-374.
(193) Giovannucci E, Stampfer MJ, Colditz GA et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 1998; 129(7):517-524.
(194) Glynn SA, Albanes D, Pietinen P et al. Colorectal cancer and folate status: a nested case-control study among male smokers. Cancer Epidemiol Biomarkers Prev 1996; 5(7):487-494.
(195) Harnack L, Jacobs DR, Jr., Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. Nutr Cancer 2002; 43(2):152-158.
(196) Ishihara J, Otani T, Inoue M, Iwasaki M, Sasazuki S, Tsugane S. Low Intake of Vitamin B-6 Is Associated with Increased Risk of Colorectal Cancer in Japanese Men. J Nutr 2007; 137(7):1808-1814.

(197) Jiang Q, Chen K, Ma X et al. Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer. Cancer Detect Prev 2005; 29(2):146-154.
(198) Kato I, Dnistrian AM, Schwartz M et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 1999; 79(11-12):1917-1922.
(199) Konings EJ, Goldbohm RA, Brants HA, Saris WH, van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. Cancer 2002; 95(7):1421-1433.
(200) Kune G, Watson L. Colorectal cancer protective effects and the dietary micronutrients folate, methionine, vitamins B6, B12, C, E, selenium, and lycopene. Nutr Cancer 2006; 56(1):11-21.
(201) La VC, Negri E, Pelucchi C, Franceschi S. Dietary folate and colorectal cancer. Int J Cancer 2002; 102(5):545-547.
(202) Larsson SC, Giovannucci E, Wolk A. A prospective study of dietary folate intake and risk of colorectal cancer: modification by caffeine intake and cigarette smoking. Cancer Epidemiol Biomarkers Prev 2005; 14(3):740-743.
(203) Le Marchand L., Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control 2002; 13(3):239-248.
(204) Levi F, Pasche C, Lucchini F, La VC. Selected micronutrients and colorectal cancer. a case-control study from the canton of Vaud, Switzerland. Eur J Cancer 2000; 36(16):2115-2119.
(205) Meyer F, White E. Alcohol and nutrients in relation to colon cancer in middle-aged adults. Am J Epidemiol 1993; 138(4):225-236.
(206) Murtaugh MA, Curtin K, Sweeney C et al. Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer. Cancer Causes Control 2007; 18(2):153-163.
(207) Otani T, Iwasaki M, Hanaoka T et al. Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. Nutr Cancer 2005; 53(1):42-50.
(208) Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S. Plasma folate and risk of colorectal cancer in a nested case-control study: the Japan Public Health Center-based prospective study. Cancer Causes Control 2008; 19(1):67-74.
(209) Pufulete M, Al-Ghnaniem R, Leather AJ et al. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology 2003; 124(5):1240-1248.
(210) Rossi E, Hung J, Beilby JP, Knuiman MW, Divitini ML, Bartholomew H. Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia. Ann Epidemiol 2006; 16(3):206-212.
(211) Satia-Abouta J, Galanko JA, Martin CF, Potter JD, Ammerman A, Sandler RS. Associations of micronutrients with colon cancer risk in African Americans and whites: results from the North Carolina Colon Cancer Study. Cancer Epidemiol Biomarkers Prev 2003; 12(8):747-754.
(212) Sharp L, Little J, Brockton NT et al. Polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene, intakes of folate and related B vitamins and colorectal cancer: a case-control study in a population with relatively low folate intake. Br J Nutr 2008; 99(2):379-389.
(213) Slattery ML, Potter JD, Coates A et al. Plant foods and colon cancer: an assessment of specific foods and their related nutrients (United States). Cancer Causes Control 1997; 8(4):575-590.
(214) Su LJ, Arab L. Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. Ann Epidemiol 2001; 11(1):65-72.
(215) Terry P, Jain M, Miller AB, Howe GR, Rohan TE. Dietary intake of folic acid and colorectal cancer risk in a cohort of women. Int J Cancer 2002; 97(6):864-867.
(216) Van Guelpen B, Hultdin J, Johansson I et al. Low folate levels may protect against colorectal cancer. Gut 2006; 55(10):1461-1466.
(217) Wei EK, Giovannucci E, Wu K et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004; 108(3):433-442.
(218) Zhang SM, Moore SC, Lin J et al. Folate, vitamin B6, multivitamin supplements, and colorectal cancer risk in women. Am J Epidemiol 2006; 163(2):108-115.
(219) White E, Shannon JS, Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. Cancer Epidemiol Biomarkers Prev 1997; 6(10):769-774.

(220) Sellers TA, Bazyk AE, Bostick RM et al. Diet and risk of colon cancer in a large prospective study of older women: an analysis stratified on family history (Iowa, United States). Cancer Causes Control 1998; 9(4):357-367.
(221) La Vecchia C., Braga C, Negri E et al. Intake of selected micronutrients and risk of colorectal cancer. Int J Cancer 1997; 73(4):525-530.
(222) Shin A, Li H, Shu XO, Yang G, Gao YT, Zheng W. Dietary intake of calcium, fiber and other micronutrients in relation to colorectal cancer risk: Results from the Shanghai Women's Health Study. Int J Cancer 2006; 119(12):2938-2942.
(223) Larsson SC, Giovannucci E, Wolk A. Vitamin B6 intake, alcohol consumption, and colorectal cancer: a longitudinal population-based cohort of women. Gastroenterology 2005; 128(7):1830-1837.
(224) Theodoratou E, Farrington SM, Tenesa A et al. Dietary vitamin B6 intake and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2008; 17(1):171-182.
(225) Wei EK, Giovannucci E, Selhub J, Fuchs CS, Hankinson SE, Ma J. Plasma vitamin B6 and the risk of colorectal cancer and adenoma in women. J Natl Cancer Inst 2005; 97(9):684-692.
(226) Dahlin AM, Van GB, Hultdin J, Johansson I, Hallmans G, Palmqvist R. Plasma vitamin B12 concentrations and the risk of colorectal cancer: a nested case-referent study. Int J Cancer 2008; 122(9):2057-2061.
(227) Jimenez-Lara AM. Colorectal cancer: potential therapeutic benefits of Vitamin D. Int J Biochem Cell Biol 2007; 39(4):672-677.
(228) Hypponen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007; 85(3):860-868.
(229) Burleigh E, Potter J. Vitamin D deficiency in outpatients:--a Scottish perspective. Scott Med J 2006; 51(2):27-31.
(230) Larsson SC, Bergkvist L, Rutegard J, Giovannucci E, Wolk A. Calcium and dairy food intakes are inversely associated with colorectal cancer risk in the Cohort of Swedish Men. Am J Clin Nutr 2006; 83(3):667-673.
(231) Park SY, Murphy SP, Wilkens LR, Nomura AM, Henderson BE, Kolonel LN. Calcium and vitamin D intake and risk of colorectal cancer: the Multiethnic Cohort Study. Am J Epidemiol 2007; 165(7):784-793.
(232) Gross MD. Vitamin D and calcium in the prevention of prostate and colon cancer: new approaches for the identification of needs. J Nutr 2005; 135(2):326-331.
(233) Slattery ML, Samowitz W, Hoffman M, Ma KN, Levin TR, Neuhausen S. Aspirin, NSAIDs, and colorectal cancer: possible involvement in an insulin-related pathway. Cancer Epidemiol Biomarkers Prev 2004; 13(4):538-545.
(234) Wactawski-Wende J, Kotchen JM, Anderson GL et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 2006; 354(7):684-696.
(235) Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH, Folsom AR. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. Am J Epidemiol 1993; 137(12):1302-1317.
(236) Jarvinen R, Knekt P, Hakulinen T, Aromaa A. Prospective study on milk products, calcium and cancers of the colon and rectum. Eur J Clin Nutr 2001; 55(11):1000-1007.
(237) Kearney J, Giovannucci E, Rimm EB et al. Calcium, vitamin D, and dairy foods and the occurrence of colon cancer in men. Am J Epidemiol 1996; 143(9):907-917.
(238) Kesse E, Boutron-Ruault MC, Norat T, Riboli E, Clavel-Chapelon F. Dietary calcium, phosphorus, vitamin D, dairy products and the risk of colorectal adenoma and cancer among French women of the E3N-EPIC prospective study. Int J Cancer 2005; 117(1):137-144.
(239) Lin J, Zhang SM, Cook NR, Manson JE, Lee IM, Buring JE. Intakes of calcium and vitamin D and risk of colorectal cancer in women. Am J Epidemiol 2005; 161(8):755-764.
(240) Martinez ME, Giovannucci EL, Colditz GA et al. Calcium, vitamin D, and the occurrence of colorectal cancer among women. J Natl Cancer Inst 1996; 88(19):1375-1382.
(241) McCullough ML, Robertson AS, Rodriguez C et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). Cancer Causes Control 2003; 14(1):1-12.
(242) Terry P, Baron JA, Bergkvist L, Holmberg L, Wolk A. Dietary calcium and vitamin D intake and risk of colorectal cancer: a prospective cohort study in women. Nutr Cancer 2002; 43(1):39-46.

(243) Zheng W, Anderson KE, Kushi LH et al. A prospective cohort study of intake of calcium, vitamin D, and other micronutrients in relation to incidence of rectal cancer among postmenopausal women. Cancer Epidemiol Biomarkers Prev 1998; 7(3):221-225.
(244) Boutron MC, Faivre J, Marteau P, Couillault C, Senesse P, Quipourt V. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case--control study. Br J Cancer 1996; 74(1):145-151.
(245) Kampman E, Slattery ML, Caan B, Potter JD. Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). Cancer Causes Control 2000; 11(5):459-466.
(246) Marcus PM, Newcomb PA. The association of calcium and vitamin D, and colon and rectal cancer in Wisconsin women. Int J Epidemiol 1998; 27(5):788-793.
(247) Peters RK, Pike MC, Garabrant D, Mack TM. Diet and colon cancer in Los Angeles County, California. Cancer Causes Control 1992; 3(5):457-473.
(248) Pritchard RS, Baron JA, Gerhardsson d, V. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. Cancer Epidemiol Biomarkers Prev 1996; 5(11):897-900.
(249) Slattery ML, Neuhausen SL, Hoffman M et al. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer. Int J Cancer 2004; 111(5):750-756.
(250) Theodoratou E, Farrington SM, Tenesa A et al. Modification of the inverse association between dietary vitamin D intake and colorectal cancer risk by a FokI variant supports a chemoprotective action of Vitamin D intake mediated through VDR binding. Int J Cancer 2008; 123(9):2170-2179.
(251) Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. Am J Epidemiol 1995; 142(6):608-611.
(252) Feskanich D, Ma J, Fuchs CS et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 2004; 13(9):1502-1508.
(253) Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S. Plasma vitamin D and risk of colorectal cancer: the Japan Public Health Center-Based Prospective Study. Br J Cancer 2007; 97(3):446-451.
(254) Tangrea J, Helzlsouer K, Pietinen P et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes Control 1997; 8(4):615-625.
(255) Wu K, Feskanich D, Fuchs CS, Willett WC, Hollis BW, Giovannucci EL. A nested case control study of plasma 25-hydroxyvitamin D concentrations and risk of colorectal cancer. J Natl Cancer Inst 2007; 99(14):1120-1129.
(256) Gorham ED, Garland CF, Garland FC et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. Am J Prev Med 2007; 32(3):210-216.
(257) Cho E, Smith-Warner SA, Spiegelman D et al. Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. J Natl Cancer Inst 2004; 96(13):1015-1022.
(258) Flood A, Peters U, Chatterjee N, Lacey JV, Jr., Schairer C, Schatzkin A. Calcium from diet and supplements is associated with reduced risk of colorectal cancer in a prospective cohort of women. Cancer Epidemiol Biomarkers Prev 2005; 14(1):126-132.
(259) Kampman E, Goldbohm RA, van den Brandt PA, van ', V. Fermented dairy products, calcium, and colorectal cancer in The Netherlands Cohort Study. Cancer Res 1994; 54(12):3186-3190.
(260) Stemmermann GN, Nomura A, Chyou PH. The influence of dairy and nondairy calcium on subsite large-bowel cancer risk. Dis Colon Rectum 1990; 33(3):190-194.
(261) Wu K, Willett WC, Fuchs CS, Colditz GA, Giovannucci EL. Calcium intake and risk of colon cancer in women and men. J Natl Cancer Inst 2002; 94(6):437-446.
(262) Arbman G, Axelson O, Ericsson-Begodzki AB, Fredriksson M, Nilsson E, Sjodahl R. Cereal fiber, calcium, and colorectal cancer. Cancer 1992; 69(8):2042-2048.
(263) Freudenheim JL, Graham S, Marshall JR, Haughey BP, Wilkinson G. A case-control study of diet and rectal cancer in western New York. Am J Epidemiol 1990; 131(4):612-624.
(264) Kampman E, van ', V, Hiddink GJ, van Aken-Schneijder P, Kok FJ, Hermus RJ. Fermented dairy products, dietary calcium and colon cancer: a case-control study in The Netherlands. Int J Cancer 1994; 59(2):170-176.
(265) Ma J, Giovannucci E, Pollak M et al. Milk intake, circulating levels of insulin-like growth factor-I, and risk of colorectal cancer in men. J Natl Cancer Inst 2001; 93(17):1330-1336.

(266) Negri E, La VC, D'Avanzo B, Franceschi S. Calcium, dairy products, and colorectal cancer. Nutr Cancer 1990; 13(4):255-262.
(267) Slattery ML, Potter JD, Sorenson AW. Age and risk factors for colon cancer (United States and Australia): are there implications for understanding differences in case-control and cohort studies? Cancer Causes Control 1994; 5(6):557-563.
(268) Garton MJ, Abdalla MI, Reid DM, Russell IT. Estimating the point accuracy of population registers using capture-recapture methods in Scotland. J Epidemiol Community Health 1996; 50(1):99-103.
(269) Bolton-Smith C, Smith WC, Woodward M, Tunstall-Pedoe H. Nutrient intakes of different social-class groups: results from the Scottish Heart Health Study (SHHS). Br J Nutr 1991; 65(3):321-335.
(270) Masson LF, McNeill G, Tomany JO et al. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. Public Health Nutr 2003; 6(3):313-321.
(271) Kyle J, Masson LF, Grubb DA, Duthie GG, McNeill G. Estimating dietary flavonoid intake: comparison of a semi-quantitative food frequency questionnaire with 4-day weighed diet records in a Scottissh population. Proc.Nutr.Soc. 61[3], 69A. 2002.
Ref Type: Abstract
(272) Heald CL, Bolton-Smith C, Ritchie MR, Morton MS, Alexander FE. Phyto-oestrogen intake in Scottish men: use of serum to validate a self-administered food-frequency questionnaire in older men. Eur J Clin Nutr 2006; 60(1):129-135.
(273) Ainsworth BE, Haskell WL, Whitt MC et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000; 32(9 Suppl):S498-S504.
(274) Kyle JAM, Duthie GG. Flavonoids in Foods. In: Taylor & Francis, editor. Flavonoids: Chemistry, Biochemistry and Applications. 2006.
(275) Ritchie MR. Phyto-oestrogen database. http://medicine.st-andrews.ac.uk/research/docs/ritchie . 2004.
Ref Type: Electronic Citation
(276) Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986; 124(1):17-27.
(277) Moorman PG, Newman B, Millikan RC, Tse CK, Sandler DP. Participation rates in a case-control study: the impact of age, race, and race of interviewer. Ann Epidemiol 1999; 9(3):188-195.
(278) Slattery ML, Edwards SL, Caan BJ, Kerber RA, Potter JD. Response rates among control subjects in case-control studies. Ann Epidemiol 1995; 5(3):245-249.
(279) Stang A, Ahrens W, Jockel KH. Control response proportions in population-based case-control studies in Germany. Epidemiology 1999; 10(2):181-183.
(280) Morton LM, Cahill J, Hartge P. Reporting participation in epidemiologic studies: a survey of practice. Am J Epidemiol 2006; 163(3):197-203.
(281) Rogers A, Murtaugh MA, Edwards S, Slattery ML. Contacting controls: are we working harder for similar response rates, and does it make a difference? Am J Epidemiol 2004; 160(1):85-90.
(282) Franceschi S, Favero A, La VC et al. Food groups and risk of colorectal cancer in Italy. Int J Cancer 1997; 72(1):56-61.
(283) Willett W. Nutritional Epidemiology. Second Edition ed. USA: Oxford University Press, 1998.
(284) Moon TE, Micozzi MS. Nutrition and Cancer Prevention: Investigating the Role of Micronutrients. CRC Press, 1989.
(285) Berdanier CD. Handbook of nutrition and food. CRC Press, 2002.
(286) Kirkwood BR, Sterne JA. Essential medical statistics. Blackwell Publishing, 2003.
(287) Pols MA, Peeters PH, Ocke MC, Slimani N, Bueno-de-Mesquita HB, Collette HJ. Estimation of reproducibility and relative validity of the questions included in the EPIC Physical Activity Questionnaire. Int J Epidemiol 1997; 26 Suppl 1:S181-S189.
(288) Wareham NJ, Jakes RW, Rennie KL et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003; 6(4):407-413.
(289) Altman DG. Practical Statistics for Medical Research. London, UK: Chapman & Hall, 1991.
(290) False Discovery Rate Methods for Multiple Comparisons: Utility for Sociology. ASA Annual Meeting; 2004.

(291) Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) 1995; 57(1):289-300.
(292) Katz MH. Multivariable Analysis: A Practical Guide for Clinicians. Second Edition ed. Published by Cambridge University Press, 2006.
(293) Austin PC, Tu JV. Automated variable selection methods for logistic regression produced unstable models for predicting acute myocardial infarction mortality. J Clin Epidemiol 2004; 57(11):1138-1146.
(294) Austin PC, Tu JV. Bootstrap Methods for Developing Predictive Models. American Statistician 2004; 58:131-137.
(295) HILL AB. The environment and disease: Association or causation? Proc R Soc Med 1965; 58:295-300.
(296) Potischman N, Weed DL. Causal criteria in nutritional epidemiology. Am J Clin Nutr 1999; 69(6):1309S-1314S.
(297) Faria A, Calhau C, de Freitas V, Mateus N. Procyanidins as antioxidants and tumor cell growth modulators. J Agric Food Chem 2006; 54(6):2392-2397.
(298) Hoensch HP, Kirch W. Potential role of flavonoids in the prevention of intestinal neoplasia: a review of their mode of action and their clinical perspectives. Int J Gastrointest Cancer 2005; 35(3):187-195.
(299) Hoensch HP, Kirch W. Potential role of flavonoids in the prevention of intestinal neoplasia: a review of their mode of action and their clinical perspectives. Int J Gastrointest Cancer 2005; 35(3):187-195.
(300) Luceri C, Caderni G, Sanna A, Dolara P. Red wine and black tea polyphenols modulate the expression of cycloxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced f344 rat colon tumors. J Nutr 2002; 132(6):1376-1379.
(301) Veeriah S, Kautenburger T, Habermann N et al. Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. Molecular Carcinogenesis 2006; 45(3):164-174.
(302) Handayani R, Rice L, Cui Y et al. Soy Isoflavones Alter Expression of Genes Associated with Cancer Progression, Including Interleukin-8, in Androgen-Independent PC-3 Human Prostate Cancer Cells. J Nutr 2006; 136(1):75-82.
(303) Peng G, Dixon DA, Muga SJ, Smith TJ, Wargovich MJ. Green tea polyphenol (-)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. Mol Carcinog 2006; 45(5):309-319.
(304) Park CH, Chang JY, Hahm ER, Park S, Kim HK, Yang CH. Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells. Biochem Biophys Res Commun 2005; 328(1):227-234.
(305) Kim WK, Bang MH, Kim ES et al. Quercetin decreases the expression of ErbB2 and ErbB3 proteins in HT-29 human colon cancer cells. J Nutr Biochem 2005; 16(3):155-162.
(306) Mouat MF, Kolli K, Orlando R, Hargrove JL, Grider A. The effects of quercetin on SW480 human colon carcinoma cells: a proteomic study. Nutr J 2005; 4:11.
(307) Escrich E, Moral R, Grau L, Costa I, Solanas M. Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. Mol Nutr Food Res 2007; 51(10):1279-1292.
(308) Hooper L, Thompson RL, Harrison RA et al. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ 2006; 332(7544):752-760.
(309) He K, Song Y. Risks and benefits of omega 3 fats: A few thoughts on systematic review. BMJ 2006; 332(7546):915-91a.
(310) Burr ML, shfield-Watt PA, Dunstan FD et al. Lack of benefit of dietary advice to men with angina: results of a controlled trial. Eur J Clin Nutr 2003; 57(2):193-200.
(311) GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999; 354(9177):447-455.
(312) Geelen A, Schouten JM, Kamphuis C et al. Fish consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. Am J Epidemiol 2007; 166(10):1116-1125.
(313) Grimble RF. Nutritional modulation of immune function. Proc Nutr Soc 2001; 60(3):389-397.

(314) Grant WB. Fish consumption, cancer, and Alzheimer disease. Am J Clin Nutr 2000; 71(2):599-600.
(315) Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther 1999; 83(3):217-244.
(316) Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004; 79(6):935-945.
(317) Stender S, Dyerberg J. Influence of trans fatty acids on health. Ann Nutr Metab 2004; 48(2):61-66.
(318) Vinikoor LC, Schroeder JC, Millikan RC et al. Consumption of trans-fatty acid and its association with colorectal adenomas. Am J Epidemiol 2008; 168(3):289-297.
(319) Kuhnt K, Wagner A, Kraft J, Basu S, Jahreis G. Dietary supplementation with 11trans- and 12trans-18:1 and oxidative stress in humans. Am J Clin Nutr 2006; 84(5):981-988.
(320) Mozaffarian D, Pischon T, Hankinson SE et al. Dietary intake of trans fatty acids and systemic inflammation in women. Am J Clin Nutr 2004; 79(4):606-612.
(321) Kim YI. Folate and colorectal cancer: an evidence-based critical review. Mol Nutr Food Res 2007; 51(3):267-292.
(322) Sanderson P, Stone E, Kim YI et al. Folate and colo-rectal cancer risk. Br J Nutr 2007; 98(6):1299-1304.
(323) Slattery ML, Schaffer D, Edwards SL, Ma KN, Potter JD. Are dietary factors involved in DNA methylation associated with colon cancer? Nutr Cancer 1997; 28(1):52-62.
(324) Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004; 159(5):423-443.
(325) Blount BC, Mack MM, Wehr CM et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997; 94(7):3290-3295.
(326) Larsson SC, Giovannucci E, Wolk A. Vitamin B6 intake, alcohol consumption, and colorectal cancer: a longitudinal population-based cohort of women. Gastroenterology 2005; 128(7):1830-1837.
(327) Blount BC, Mack MM, Wehr CM et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997; 94(7):3290-3295.
(328) Komatsu SI, Watanabe H, Oka T, Tsuge H, Nii H, Kato N. Vitamin B-6-supplemented diets compared with a low vitamin B-6 diet suppress azoxymethane-induced colon tumorigenesis in mice by reducing cell proliferation. J Nutr 2001; 131(8):2204-2207.
(329) Komatsu S, Yanaka N, Matsubara K, Kato N. Antitumor effect of vitamin B6 and its mechanisms. Biochim Biophys Acta 2003; 1647(1-2):127-130.
(330) Takeuchi PL, Antunes LM, Takahashi CS. Evaluation of the clastogenicity and anticlastogenicity of vitamin B(6) in human lymphocyte cultures. Toxicol In Vitro 2007.
(331) Bolander FF. Vitamins: not just for enzymes. Curr Opin Investig Drugs 2006; 7(10):912-915.
(332) Huang Y, Han S, Li Y, Mao Y, Xie Y. Different roles of MTHFR C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. J Hum Genet 2007; 52(1):73-85.
(333) Hubner RA, Houlston RS. MTHFR C677T and colorectal cancer risk: A meta-analysis of 25 populations. Int J Cancer 2007; 120(5):1027-1035.
(334) Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999; 8(6):513-518.
(335) Le Marchand L., Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control 2002; 13(3):239-248.
(336) Ulrich CM, Kampman E, Bigler J et al. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol Biomarkers Prev 1999; 8(8):659-668.
(337) Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol 1980; 9(3):227-231.

(338) Giovannucci E. Epidemiological evidence for vitamin D and colorectal cancer. J Bone Miner Res 2007; 22 Suppl 2:V81-V85.
(339) Holt PR. New insights into calcium, dairy and colon cancer. World J Gastroenterol 2008; 14(28):4429-4433.
(340) Gorham ED, Garland CF, Garland FC et al. Vitamin D and prevention of colorectal cancer. J Steroid Biochem Mol Biol 2005; 97(1-2):179-194.
(341) Grant WB, Garland CF. A critical review of studies on vitamin D in relation to colorectal cancer. Nutr Cancer 2004; 48(2):115-123.
(342) Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 2005; 135(2):317-322.
(343) Das D, Arber N, Jankowski JA. Chemoprevention of colorectal cancer. Digestion 2007; 76(1):51-67.
(344) Weingarten MA, Zalmanovici A, Yaphe J. Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. Cochrane Database Syst Rev 2008;(1):CD003548.
(345) Shaukat A, Scouras N, Schunemann HJ. Role of supplemental calcium in the recurrence of colorectal adenomas: a metaanalysis of randomized controlled trials. Am J Gastroenterol 2005; 100(2):390-394.
(346) Wallace K, Baron JA, Cole BF et al. Effect of calcium supplementation on the risk of large bowel polyps. J Natl Cancer Inst 2004; 96(12):921-925.
(347) Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer 2003; 3(8):601-614.
(348) Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004; 338(2):143-156.
(349) Grau MV, Baron JA, Sandler RS et al. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. J Natl Cancer Inst 2003; 95(23):1765-1771.
(350) Ingles SA, Wang J, Coetzee GA, Lee ER, Frankl HD, Haile RW. Vitamin D receptor polymorphisms and risk of colorectal adenomas (United States). Cancer Causes Control 2001; 12(7):607-614.
(351) Burton PR, Hansell AL, Fortier I et al. Size matters: just how big is BIG?: Quantifying realistic sample size requirements for human genome epidemiology. Int J Epidemiol 2008.
(352) Rothwell PM, Bhatia M. Reporting of observational studies. BMJ 2007; 335(7624):783-784.
(353) von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ 2007; 335(7624):806-808.
(354) Patsopoulos NA, Tatsioni A, Ioannidis JP. Claims of sex differences: an empirical assessment in genetic associations. JAMA 2007; 298(8):880-893.
(355) Mendez MA, Wynter S, Wilks R, Forrester T. Under- and overreporting of energy is related to obesity, lifestyle factors and food group intakes in Jamaican adults. Public Health Nutr 2004; 7(1):9-19.
(356) Tomoyasu NJ, Toth MJ, Poehlman ET. Misreporting of total energy intake in older men and women. J Am Geriatr Soc 1999; 47(6):710-715.
(357) Bazelmans C, Matthys C, De HS et al. Predictors of misreporting in an elderly population: the 'Quality of life after 65' study. Public Health Nutr 2007; 10(2):185-191.
(358) Wirfalt E, Midthune D, Reedy J et al. Associations between food patterns defined by cluster analysis and colorectal cancer incidence in the NIH-AARP diet and health study. Eur J Clin Nutr 2008.
(359) Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. Eating patterns and risk of colon cancer. Am J Epidemiol 1998; 148(1):4-16.
(360) Terry P, Hu FB, Hansen H, Wolk A. Prospective study of major dietary patterns and colorectal cancer risk in women. Am J Epidemiol 2001; 154(12):1143-1149.
(361) Harnack L, Nicodemus K, Jacobs DR, Jr., Folsom AR. An evaluation of the Dietary Guidelines for Americans in relation to cancer occurrence. Am J Clin Nutr 2002; 76(4):889-896.
(362) Mizoue T, Yamaji T, Tabata S et al. Dietary patterns and colorectal adenomas in Japanese men: the Self-Defense Forces Health Study. Am J Epidemiol 2005; 161(4):338-345.
(363) Fung T, Hu FB, Fuchs C et al. Major dietary patterns and the risk of colorectal cancer in women. Arch Intern Med 2003; 163(3):309-314.

(364) 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 2004; 80(4):1003-1011.
(365) Smith-Warner SA, Elmer PJ, Fosdick L et al. Fruits, vegetables, and adenomatous polyps: the Minnesota Cancer Prevention Research Unit case-control study. Am J Epidemiol 2002; 155(12):1104-1113.
(366) Chiu BC, Ji BT, Dai Q et al. Dietary factors and risk of colon cancer in Shanghai, China. Cancer Epidemiol Biomarkers Prev 2003; 12(3):201-208.
(367) Marques-Vidal P, Ravasco P, Ermelinda CM. Foodstuffs and colorectal cancer risk: a review. Clin Nutr 2006; 25(1):14-36.
(368) Engeset D, Andersen V, Hjartaker A, Lund E. Consumption of fish and risk of colon cancer in the Norwegian Women and Cancer (NOWAC) study. Br J Nutr 2007; 98(3):576-582.
(369) Knekt P, Jarvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Int J Cancer 1999; 80(6):852-856.
(370) Larsson SC, Bergkvist L, Giovannucci E, Wolk A. Coffee consumption and incidence of colorectal cancer in two prospective cohort studies of Swedish women and men. Am J Epidemiol 2006; 163(7):638-644.
(371) Tavani A, La VC. Coffee, decaffeinated coffee, tea and cancer of the colon and rectum: a review of epidemiological studies, 1990-2003. Cancer Causes Control 2004; 15(8):743-757.
(372) Giovannucci E. Meta-analysis of coffee consumption and risk of colorectal cancer. Am J Epidemiol 1998; 147(11):1043-1052.
(373) Michels KB, Willett WC, Fuchs CS, Giovannucci E. Coffee, tea, and caffeine consumption and incidence of colon and rectal cancer. J Natl Cancer Inst 2005; 97(4):282-292.
(374) Folsom AR, Hong CP. Magnesium intake and reduced risk of colon cancer in a prospective study of women. Am J Epidemiol 2006; 163(3):232-235.
(375) van den Brandt PA, Smits KM, Goldbohm RA, Weijenberg MP. Magnesium intake and colorectal cancer risk in the Netherlands Cohort Study. Br J Cancer 2007; 96(3):510-513.
(376) Larsson SC, Bergkvist L, Wolk A. Magnesium intake in relation to risk of colorectal cancer in women. JAMA 2005; 293(1):86-89.
(377) Lin J, Cook NR, Lee IM, Manson JE, Buring JE, Zhang SM. Total magnesium intake and colorectal cancer incidence in women. Cancer Epidemiol Biomarkers Prev 2006; 15(10):2006-2009.
(378) Yang CY, Chiu HF. Calcium and magnesium in drinking water and risk of death from rectal cancer. Int J Cancer 1998; 77(4):528-532.
(379) Yang CY, Chiu HF, Chiu JF, Tsai SS, Cheng MF. Calcium and magnesium in drinking water and risk of death from colon cancer. Jpn J Cancer Res 1997; 88(10):928-933.
(380) Beal MJ. Variational Algorithms for Approximate Bayesian Inference. 2003.
(381) Jordan MI, Ghahramani Z, Jaakkola TS, Saul LK. An introduction to variational methods for graphical models. Machine Learning 1999; 37:183-233.
(382) Winn J. Variational Message Passing and its Applications. 2004.
(383) Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Stat Med 2007.
(384) Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. Stat Methods Med Res 2007; 16(4):309-330.
(385) Church JM. A scoring system for the strength of a family history of colorectal cancer. Dis Colon Rectum 2005; 48(5):889-896.