# Nutrition and colorectal cancer - The role of BMI, sex, biomarkers and dietary index

Vulcan, Alexandra

2018

*Document Version:*
Publisher's PDF, also known as Version of record

# Nutrition and colorectal cancer

## The role of BMI, sex, biomarkers and dietary index

ALEXANDRA VULCAN
DEPARTMENT OF CLINICAL SCIENCES | FACULTY OF MEDICINE | LUND UNIVERSITY

Alexandra Vulcan is a dietitian specialised in gastroenterology at Skåne University Hospital in Malmö. Her thesis focuses on associations between nutrition and colorectal cancer in a population-based cohort from Malmö, the Malmö Diet and Cancer cohort. This research is useful in order to more fully comprehend that the associations between food and colorectal cancer may depend on preconditions of the individual.

Printed by Media-Tryck, Lund 2018 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2018:72
ISBN 978-91-7619-638-0
ISSN 1652-8220

# Nutrition and colorectal cancer

## The role of BMI, sex, biomarkers and dietary index

Alexandra Vulcan

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty Medicine, Lund University, Sweden.
To be defended at Jubileumsaulan, Jan Waldenströms gata 5,
Skåne University Hospital, Malmö
Friday15th of June at 13.00

*Faculty opponent*
Rolf Hultcrantz

Department of Medicine, Karolinska Institutet, Stockholm, Sweden

| Organization | Document name |
|---|---|
| LUND UNIVERSITY, Faculty of Medicine, Department of Clinical Sciences | DOCTORAL DISSERTATION |
| | Date of issue<br>June 15, 2018 |
| Author(s)<br>Alexandra Vulcan | Sponsoring organisation |


Title and subtitle

Nutrition and colorectal cancer – The role of BMI, sex, biomarkers and dietary index


Abstract

Colorectal cancer (CRC) is one of the most common forms of cancer. The cause of CRC is multifactorial, and lifestyle factors are thought to be a major contributor to the development of CRC. It can be hypothesised that the association between food intake and the risk of developing CRC depends not only on the food consumed, but what it is consumed in combination with. The associations between food and CRC may also depend on other lifestyle-related factors, such as blood glucose and insulin levels, insulin resistance, and body composition, or sex and clinicopathological characteristics, such as tumour location and tumour-stage. It is not confirmed that the association between a nutrient and CRC is equal to the associations between the different food groups that a nutrient comes from or that the food sources of the nutrients all creates the same associations.

The aim of this thesis is therefore to examine associations between dietary intakes and CRC in the Malmö Diet and Cancer cohort, and whether the associations are modified by different preconditions.

In the Malmö Diet and Cancer study (MDC) we examined food intake and levels of blood glucose, plasma insulin and insulin resistance by hazard regression regarding incident CRC. In MDC, baseline examinations were performed between 1991 and 1996, where information on body composition, and socioeconomic- and lifestyle factors was obtained, together with collection of blood samples. Food intake was recorded by a modified diet history method. Information on incident cases of CRC was identified via the Swedish cancer registry.

We found that high fibre intake was associated with lower risk for CRC, especially with lower risk for colon cancer in women, and that high intake of fruits and berries was associated with lower risk of CRC in women. Regarding meat intake, we found that high intake of pork, as well as intake of processed meat, was associated with risk of CRC. In addition, we found that high fasting blood glucose was associated with higher risk of CRC, especially in colon cancer in men.

Finally, we found that high adherence to a predefined CRC-specific diet quality index, based on World Cancer Research Found's conclusions regarding diet, was inversely associated with risk of CRC, and gave a stronger association with CRC than when analysing the components of the index individually.

In conclusion, this thesis found different preconditions for associations between food intake and CRC, dependent on sex, meat subtype, fibre source, and the location of the tumour, but not for presence of overweight and diabetes in this population of inhabitants in Malmö.



Key words

Colorectal cancer, nutrition, fibre, meat, dietary index, CDQI, glucose, insulin, insulin resistance, MDC

Classification system and/or index terms (if any)

| Supplementary bibliographical information | | Language<br>English |
|---|---|---|
| ISSN and key title<br>1652-8220 Nutrition and colorectal cancer | | ISBN<br>978-91-7619-638-0 |
| Recipient's notes | Number of pages<br>136 | Price |
| | Security classification | |

# Nutrition and colorectal cancer

## The role of BMI, sex, biomarkers and dietary index

Alexandra Vulcan


LUND
UNIVERSITY

Coverphoto by Alexandra Vulcan, with graphic editing by Christian Hjerp

Illustrations by Alexandra Vulcan



Lund University, Faculty of Medicine, Doctoral Dissertation Series 2018:72

ISBN 978-91-7619-638-0
ISSN 1652-8220
Printed in Sweden by Media-Tryck, Lund University
Lund 2018



MADE IN SWEDEN

*To Anders, my love*

# Table of Contents

List of papers ..... 9
Abbreviations ..... 11
Introduction ..... 13
Colon and rectum ..... 14
Colorectal cancer epidemiology ..... 14
Colorectal carcinogenesis ..... 14
Chromosomal instability ..... 15
Microsatellite instability ..... 15
CpG island methylator phenotype ..... 16
Tumour-classification ..... 16
Clinical aspects ..... 16
Colorectal cancer – A preventable cancer? ..... 17
Age ..... 17
Socioeconomic factors ..... 18
Sex and hormonal factors ..... 18
Hereditary factors ..... 18
Adult height ..... 19
Inflammation ..... 19
Lifestyle-related diseases ..... 20
Physical activity ..... 20
Smoking ..... 21
Alcohol consumption ..... 21
Diet ..... 21
Dietary fibre ..... 21
Dairy products ..... 22
Meat ..... 23
Fish ..... 23
Dietary pattern ..... 23
Aims ..... 25
Material and Methods ..... 27
Ethics ..... 27

Study population ..... 27
Method of Malmö Diet and Cancer study ..... 28
Data collection ..... 28
Dietary data ..... 29
Handling of dietary data ..... 29
Age, sex, BMI, and lifestyle and socioeconomic variables ..... 30
Plasma insulin, blood glucose and insulin resistance ..... 31
Cancer cases ..... 31
Tumour characteristics ..... 31
Diabetes cases ..... 32
Statistical methods ..... 32
Main results ..... 37
General discussion ..... 41
Main findings and interpretation ..... 41
Fibre intake and colorectal cancer ..... 41
Meat intake and colorectal cancer ..... 43
Intake of dairy products and colorectal cancer ..... 44
The Colorectal cancer Dietary Quality Index ..... 44
Overweight, meat intake and colorectal cancer ..... 45
Hyperglycaemia, hyperinsulinemia, and insulin resistance and colorectal cancer ..... 45
Methodological considerations ..... 46
Misclassification and measurement error ..... 47
Energy adjustment ..... 48
Confounding and correlated intakes ..... 49
Residual confounding ..... 49
Generalisability ..... 50
Power ..... 50
Conclusions ..... 51
Clinical and future perspectives ..... 53
Populärvetenskaplig sammanfattning (Summary in Swedish) ..... 55
Acknowledgement ..... 57
References ..... 59
Original papers 1-4 ..... 73

# List of papers

This thesis is based on the following papers, which will be referred to in the text by their arabic numerals

1. **Vulcan A**, Brändstedt J, Manjer J, Jirström K, Ohlsson B, Ericson U (2015) Fibre intake and incident colorectal cancer depending on fibre source, sex, tumour location and Tumour, Node, Metastasis stage. The British journal of nutrition 114 (6):959-969. doi:10.1017/S0007114515002743.

2. **Vulcan A**, Manjer J, Ohlsson B (2017) High blood glucose levels are associated with higher risk of colon cancer in men: A Cohort study. BMC Cancer 17(1):842. doi:10.1186/s12885-017-3874-4.

3. **Vulcan A**, Manjer J, Ericson U, Ohlsson B (2017) Intake of different types of red meat, poultry, and fish and incident colorectal cancer in women and men: results from the Malmo Diet and Cancer Study. Food & nutrition research 61 (1):1341810. doi:10.1080/16546628.2017.1341810.

4. **Vulcan A**, Manjer J, Ericson U, Ohlsson B (2017) A colorectal diet quality index is inversely associated with colorectal cancer in the Malmö Diet and Cancer study. (Under submission)

# Abbreviations

| | |
|---|---|
| APC | Adenomatous Polyposis Coli |
| AICR | American Institute for Cancer Research |
| BMI | Body Mass Index |
| BRAF | v-Raf murine sarcoma viral oncogene homolog B1 |
| CpG | Guanine-containing dinucleotide |
| CRC | Colorectal cancer |
| DI | Dietary Inflammatory Index |
| DNA | Deoxyribonucleic acid |
| E% | Percentage of energy |
| EMT | Epithelial-mesenchymal transition |
| FAP | Familial Adenomatous Polyposis |
| HEI | Healthy Eating Index |
| HOMA-IR | Homeostasis model Assessment for Insulin resistance |
| HNPCC | Hereditary Non-Polyposis Colorectal Cancer |
| IBD | Inflammatory bowel disease |
| IGF | Insulin-like Growth-Factor |
| KRAS | v-Ki-ras2 Kirsten ray sarcoma viral oncogene homolog |
| LOH | Loss of heterozygosity |
| MAP-kinase | Mitogen-activated protein kinase |
| MDC | Malmö Diet and Cancer Study |
| MDC-CC | Malmö Diet and Cancer Study's Cardiovascular Cohort |
| MDS | Mediterranean Diet Score |
| MHT | Menopausal hormonal replacement therapy |

| | |
|---|---|
| NSAID | Non-Steroidal Anti-Inflammatory Drugs |
| ROS | Reactive Oxygen Species |
| SCFA | Short-Chain Fatty Acids |
| TNM | Tumour, Node, Metastasis |
| TP | Tumour suppressor gene |
| WCRF | World Cancer Research Fund International |
| Wnt | Wingless-type MM_TV integration site family members |

# Introduction

Colorectal cancer (CRC), a form of cancer that was quite rare in the middle of the 20th century, is now one of the most common forms of cancer in the world, and the number of cases diagnosed per year is continuing to rise globally as well as in Sweden (1). As CRC develops over a long timespan, early detection and accurate prevention are crucial in order to counteract further increases.

The cause of CRC is multifactorial, and lifestyle is thought to be a major contributor to the development of CRC. There are numerous studies examining the associations between diet and CRC. Associations with CRC have mostly been found for intake of fibre, foods containing wholegrains, red meat, processed meat, dairy products, and calcium supplements, factors which increase or decrease the risk of CRC (2). It is important to examine associations with foods and nutrients in different populations, as risk factors have been seen to differ in between populations.

It can be hypothesised that associations between intake of specific foods and risk of developing CRC depends on intakes of other foods. In addition, effects of food components may depend on the food source, as nutrients within the foods may interact. Other lifestyle-related factors, such as body composition, blood glucose, insulin levels and insulin resistance, or sex and clinicopathological characteristics, such as tumour location and tumour-stage, may also influence the risk from food components.

If the associations between foods and CRC differ by sex, tumour location, weight status or diabetes is not yet confirmed, and associations between dietary intake and different stages of CRC has not previously been examined.

This thesis therefore aimed to examine associations between 1) different fibre sources and CRC; 2) different meat types and CRC; 3) combinations of food intakes and CRC; 4) blood glucose, plasma insulin, and insulin resistance and CRC, as well as 5) whether associations with CRC differ depending on sex, tumour location, and weight status.

# Colon and rectum

Cecum, appendix, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum all together form the large intestine. The main function of the large intestine is to store and concentrate faecal material before defecation. The colon and rectum absorb fluids and salt left in the faeces. It is also where the immune system interfaces with diverse arrays of antigens in food and gut microbes, and where a production of short-chain fatty acids (SCFA), vitamin K and biotin by the microbiota is taking place (3).
The mucosal layer in the large intestine acts as a protectant against microbial infections and invasions, and produces bicarbonates to neutralize the acidity of the faecal content. Some tissue in the large intestine are of lymphoid tissue, especially in the appendix, and are involved in the immune system, and also help to produce antibodies, as well as cross-reactive antibodies (3).

# Colorectal cancer epidemiology

In the Western world CRC is one of the most common forms of cancer (4, 5). In Sweden, it is the fourth most common form of cancer. It is roughly 7.000 persons per year who develops the disease, and the incidence is increasing, and has been doing so for at least half a century (6).
Colon cancer is more common than rectal cancer, and the ratio is roughly two to one. The incidence of CRC differs throughout the world, and it is more common in developed countries. The highest incidence is found in Australia and New Zeeland, whereas the lowest is found in Western Africa (7).
The incidence rate for CRC is similar for women and men worldwide. In Sweden, the incidence rate for women is 26.5 per 100 000 individuals, and for men 32.3 per 100 000 individuals (1).

# Colorectal carcinogenesis

CRC is a slowly growing cancer, which develops during 10 to 15 years. It is more common that CRC arises in the distal colon, than in the proximal (8), and adenocarcinoma is the most common type of CRC. CRC develops from a polyp in most cases, and the polyp turns into an adenomatous polyp which is a precursor to adenocarcinoma. Adenocarcinoma can also occur in the sessile polyp, or hyperplastic polyp, which at first was considered to be benign, but the sessile

lesions has been seen to transform into serrated sessile polyps, which in turn can advance into cancer (9). One of the earliest signs of carcinogenesis in the colon is hypothesised to be the aberrant crypt foci, which are clusters of abnormal tube-like glands developed in the lining of the colon (10).
The molecular events that lead to CRC are heterogeneous, and there are three pathways known; the chromosomal, the guanine-containing dinucleotide (CpG) island methylator phenotype and the microsatellite pathway (11). The pathways can both individually and in combination with each other cause CRC. The development of most CRC is caused by chromosomal instability, and secondly by microsatellite instability. Microsatellite instability and CpG island methylator phenotype is more common in proximal colon cancer, whereas it is more common with chromosomal instability in distal colon cancer (12).

## Chromosomal instability

In chromosomal instability it is mutations in the adenomatous polyposis coli (APC) gene and deoxyribonucleic acid (DNA) hypomethylation that activates Wnt-signalling pathways by increase of β-catenin, which in turn causes the polyps to change into adenoma (13). Activation of the oncogene v-Ki-ras2 Kristen rat sarcoma viral oncogene homolog (KRAS) and genetic alterations to genes on chromosome 18q, with loss of heterozygosity (LOH), results in adenoma growth. KRAS mutations lead to a permanently active state that allows the cell to evade apoptosis. Inactivation of tumour suppressor gene (TP) 53, which plays a major part in the cell cycle and apoptosis, then leads to formation of carcinoma. Chromosomal instability is clinically characterised by distal location, high differentiation grade and intermediate prognosis (12).

## Microsatellite instability

Microsatellite instability is caused by defected DNA repair by inactivation of mismatch repair caretaker genes, and is the start of the pathway towards CRC (11). A decrease in activated genes involved in the mismatch repair, leads to forming of adenoma. Oncogenes, e.g. mutated v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) gene, later triggers the adenoma into carcinoma. Microsatellite instability is clinically characterised by proximal location, poor differentiation, increased numbers of tumour infiltrating lymphocytes, and/or mucinous histology, and is quite rare in metastatic CRC (14).

### CpG island methylator phenotype

In the CpG island methylator phenotype pathway, there is a larger part of the genes that are hypermethylated, and transcriptional silencing of promotors important in development of carcinoma occur, which lead to development of adenoma, and further alterations leads to carcinoma, and then to metastatic disease (15). CpG island methylator phenotype is clinically characterised by proximal location, poor prognosis, poor differentiation, female sex and older age (16).

## Tumour-classification

A simple classification of CRC is to classify it as colon and rectal cancer or distal and proximal CRC. Although, the most widely used clinical classification system is the Tumour (T)- Node (N)-, Metastasis (M)-classification (17). It classifies the tumour by size, if it is integrated in the surrounding tissue, and by its spread. The most important prognostic factors are depth of the invasion and the presence or absence of lymph node metastasis. To decide which molecular pathway is involved is also an important factor in later treatment (18).

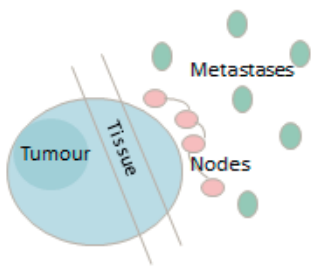


**Figure 1. TNM-classification**
Illustration of classification by tumour size, integration and spread, where the size of the tumour, its integration in adjacent tissue, and presence of lymph node metastases and metastases classifies the tumour in the Tumour (T)-Node (N)-, Metastasis (M)-classification system (17).

## Clinical aspects

CRC is diagnosed by rectoscopy and colonoscopy with biopsy sampling and histopathologic examination (19). Computed tomography (CT) scan of the abdomen and thorax is used for assessment of liver and lung metastasis, and a pelvis magnetic resonance tomography (MRT) is used in rectal cancer for

assessment of pelvis metastasis (19). The symptoms are diffuse at first in most cases, although approximately 20% are discovered because of acute colonic obstruction. Right-sided colon cancers are most likely to be discovered by fatigue or iron-deficiency anaemia, and proximal adenocarcinomas by occult bleeding, changes in bowel habits, or left lower quadrant discomfort in form of cramping (20). Symptoms later in the development of the disease are fatigue, low energy intake and weight loss. If metastasises occur, the most common sites are the liver and the thorax (21).
The 5-year survival rate for CRC is about 65% in Sweden (22). For cancers which are early discovered, the 5-year survival rate rises markedly (23). To earlier discover CRC, and increase the survival rate, some countries have introduced screening programs with faecal occult blood test and colonoscopy to earlier remove precancerous polyps (8). These programs have been found to lower the incidence of CRC (24). In the Gotland and Stockholm regions in Sweden, screening is conducted with faecal occult blood test, and with a following colonoscopy if tested positive (25). There is also a screening study, Screening of Swedish colons, calculated to end in 2019, investigating which screening method is the most suitable to early discover CRC (26).

# Colorectal cancer – A preventable cancer?

The aetiology behind CRC is diverse. There are both risk factors that are modifiable and those that are not, such as age, sex, hereditary factors and adult height (2). CRC most commonly is the cancer type where the highest number of non-synonymous mutations takes place, which indicate that CRC are most sensitive to environmental factors (27). It has therefore been discussed that lifestyle and lifestyle-related diseases, such as the metabolic syndrome, are attributed to 40-70% of the development of CRC (28-30), giving the opportunity to prevent the development of most CRCs.

## Age

More than 65% of all CRC occur after the age of 65 years (22), and age-specific incidence rates increase abruptly after the age of 50 years (4). It is predicted that the proportion of CRC occurring in the elderly will increase to 70% of all CRCs at the year of 2030 (31).

## Socioeconomic factors

Lower educational level (32), as a marker for lower socioeconomic status, together with lower socioeconomic status, has been found to be associated with CRC (33). It has been discussed if this association depends on higher incidence of smoking, exposure to work-related carcinogens, higher incidence of obesity, a more sedentary lifestyle and less healthy dietary habits in individuals with lower socioeconomic status (33).

## Sex and hormonal factors

There is a possible difference in the way how CRC develops between the sexes, and different factors may determine the sensitivity for carcinogens and development of neoplasia between the sexes. Distal CRC is more common in men, and proximal CRC is more common in women (1). It has been discussed if proximal and distal CRC have very different origins (12). The difference in transit time between the sexes (34) could alter the contact between carcinogenic or anti-carcinogenic compounds and the colonic mucosa.

There are also sex-differences in glucose metabolism, and as glucose metabolism in the cancer cell is an important factor for development of CRC, this may explain differences in cancer development between the sexes. Sex-differences in DNA methylation has been found with an association between altered expressions of DNA and insulin secretion in the human pancreatic islet in women, giving women a higher glucose-induced insulin secretion (35).

The sex hormones also play a part in cancer development, where oestrogen strengthens the tight junctions and facilitates the transport of glucose to the brain and promotes neural aerobic glycolysis (36). On the other hand, 5-DHEA, an adrenal steroid hormone, modulates glucose uptake (37).

Menopausal hormonal replacement therapy (MHT) has been found to be inversely associated with the risk of CRC (38). It has been speculated that MHT is preventing DNA methylation-induced silencing of oestrogen receptor expression, or that the mismatch repair gene might be oestrogen-responsive (39).

## Hereditary factors

With a family history of CRC, there is a greater risk that CRC arises in the individual (40). About 20% of all sporadic CRCs are of familiar origin. Familial adenomatous polyposis (FAP) and Lynch syndrome, also called Hereditary non-polyposis colorectal cancer, are the two most common forms of hereditary CRC. The Lynch syndrome represents approximately 3% of all CRCs (41), and is caused

by microsatellite instability after germline mutation of the mismatch repair genes (42). Lynch syndrome most likely develops between the age of 20 and 30 years. FAP often occurs before the age of 45 years, and is caused by mutations in germline and APC genes, causing chromosomal instability (15). FAP represents 1-2% of all CRCs (43), and if colectomy is not performed, 100% of persons with FAP will develop CRC (15).

## Adult height

The World Cancer Research Fund international (WCRF), together with American Institute for Cancer Research (AICR), has concluded that there is strong evidence for that tall adult height is associated with increased risk of CRC (2). This has been shown in a meta-analysis, where they also reported a 60%-risk increase per 10 cm increase in genetically predicted height (44). It has been speculated that a larger cell mass, with a greater risk of developing CRC, might be the cause (44), together with early nutrition and its hormone-related effects (45).

## Inflammation

Chronic inflammation affects development of cancer and later occurrence of metastasis through different mechanisms (46). Inflammation is enabling the transformation into cancer via the Epithelial-Mesenchymal Transition (EMT). In EMT, epithelial cells become mesenchymal cells and thereby can differentiate, and in later stage start the initiation of metastases. The healing process of the cell, which occurs during the inflammatory response, creates an increase in angiogenesis, which facilitates tumour progression and later invasion. Inflammation also causes genetic instability where reactive oxygen species (ROS) affects genomic instability by creating mutations and interleukin-10 and activation-induced cytidine deaminase (AID) initiates mutations in genes such as p53. Finally, inflammation initiates proliferation and apoptosis when overexpression of genes, such as the peroxisome proliferator-activating receptor (P-PAR) gene, p 53 and AID occurs.
A 3-fold risk increase to develop CRC has been seen in chronic Inflammatory Bowel Disease (IBD) (40), and the risk correlates with duration of the disease (47), presence of Primary Sclerosing Cholangitis (PSC) (48) and severity of inflammation (49). The chronic inflammation in IBD has been found to lead to shortening of telomeres, DNA damage and DNA senescence (50).
Regular use of Non-Steroidal Anti-Inflammatory Drugs (NSAID) has been shown to be associated with decreased risk of CRC (51), although, this is not the case when there is a BRAF mutation-caused CRC (52). Aspirin is an inhibitor of

cyclooxygenase-2, which is an important mediator of the inflammatory response, and a factor in CRC development (53).

## Lifestyle-related diseases

Lifestyle-related diseases, such as obesity (40), the metabolic syndrome (54), and type 2 diabetes (55), are associated with increased incidence of CRC.
Overweight and obesity are established risk factors for CRC, and the risk for CRC has been seen to increase with 30% per every 5-unit increase of Body Mass Index (BMI) (56). Factors explaining possible influence by obesity in CRC development is thought to be the presence of low-grade inflammation and changes in the gut microbiota (57), together with hormones such as oestrogen (58), Insulin-like Growth-Factors (IGF) and leptin (59).
Hyperinsulinemia, that has been associated with both obesity and type 2 diabetes (60, 61), as well as with CRC (62, 63), has been proposed to be a factor in the relation between dietary factors, overweight and development of CRC. It has also been discussed if the hyperglycaemia and the insulin resistance, together with the hyperinsulinemia, that defines type 2 diabetes, is an interstage to CRC, or if the aetiology between the two diseases is similar. Hyperinsulinemia is a factor in the carcinogenic process through influence on growth of cancer cells, stimulation of proliferation, decrease of apoptosis, and promotion of intestinal carcinogenesis (64). Hyperglycaemia, on the other hand, gives energy to malignant cells to facilitate their non-insulin dependent proliferation (65). In hyperglycaemia there is a chronic inflammation present, which may lead to imbalance between production of ROS, which leads to oxidative stress and DNA damage (66). Insulin resistance and metabolic pathways leads to overstimulation of mitogenic pathways and stimulation of cell proliferation leading to carcinogenic disease (64).

## Physical activity

Physical activity is inversely associated with risk of CRC (2, 67). There are several plausible mechanisms explaining why physical activity may protect against CRC. Physical activity may lead to higher insulin sensitivity and reduced plasma insulin, which in turn might reduce the risk of developing CRC (68). Physical activity also stimulates peristalsis and decreases colon transit time, thus decreasing the time for the carcinogens to be in contact with the mucosa (69). In addition, effect on body fatness (70) and effect on hormone levels (71) may be of importance.

### Smoking

Smoking increases the risk of developing CRC, and the risk increases with increasing pack-years (40). It has been seen that the association is somewhat stronger for rectal cancer, than for colon cancer (72). Smoking causes irreversible genetic damage to the colon mucosa, which in turn may cause formation, and increase in the growth rate, of adenomatous polyps (73).

### Alcohol consumption

Associations have been found between higher intake of alcohol and increased risk of CRC (74). A 7%-risk increase have been found per 10 g increase of daily alcohol intake (74). Alcohol is believed to be carcinogenic to humans, and a risk factor in many different cancers (75). Alcohol consumption may also be associated with lower intake of essential nutrients (76, 77) and thereby indirectly with higher susceptibility to CRC. Alcohol increases production of ROS and facilitates the uptake of carcinogens (78). Alcohol has been associated with certain types of CRC, where hypermethylation of IGF-2 takes place (79).

## Diet

Diet seems to be an important factor in the development of CRC, especially in CRC caused by microsatellite instability, as it is a type of cancer most sensitive to environmental factors because of its high number of non-synonymous mutations (27). High intakes of dietary fibre, wholegrain, red and processed meat, dairy products, and calcium supplements have all been associated with either higher or lower risk of CRC (2).

### Dietary fibre

Intakes of dietary fibre and wholegrain have been associated with decreased risk of CRC (2), and WCRF and AICR conclude that there is strong evidence that intake of wholegrain and foods containing fibre decrease the risk of CRC, and suggestive evidence for that low intake of fruit probably increases the risk of CRC (2). The evidence for the associations between the different food sources of fibre and CRC has not been deemed as strong as the association for fibre, except for the association between wholegrain and CRC (2). However, high intake of fruit and

vegetables has been seen to associate with lower risk of CRC (80), and similar, but non-significant results, have been seen in a meta-analysis (81).
The microbiota uses dietary fibre to produce SCFAs, such as butyrate. The microbial production of SCFAs seems to be the most probable cause up to date, as to why fibre intake is inversely associated with CRC. SCFAs constitute the predominant energy source for colonocytes and increase the strengthening of tight junction assembly and are mediators between the microbiota and host immune system for maintenance of gut homeostasis. The SCFAs also conditions gut epithelial cells to mount protective immunity through mitogen-activated protein (MAP) kinase signalling, inhibit pro-inflammatory cytokines, such as nuclear factor kappa-light-chain-enhancer of activated B cells and tumour necrosis factor-α, and inactivate mutagens (82).
Other potential mechanisms may be decreased colon transit time and dilution of colonic content with subsequent increased stool output (83), which could decrease the contact between mutagenic compounds and the colonic mucosa. In addition, fibre intake could decrease secondary bile acid production. Bioactive components in fibre-rich foods, such as cytoprotectants (vitamins, minerals, polyphenol flavonoids, and anthocyanins), have antiproliferative effects, and may also be important factors in the association seen between fibre intake and CRC. High intake of vitamin C has been found to be associated with CRC, although the evidence for a causal relation is deemed to be limited (2). The proposed mechanisms behind the association is the antioxidant qualities of vitamin C, leading to reduction of ROS and nitrate levels, inhibition of lipid peroxidation, as well as inhibition of production of carcinogens (84).

## Dairy products

The WCRF and AICR have concluded that there is strong evidence for that intake of dairy products is associated with decreased risk of CRC (2). Dairy products are foods rich in calcium and vitamin D, whose anti-carcinogenic properties are some of the suggested mechanisms for the association between dairy products and CRC. There is limited evidence for that intake of vitamin D decreases the risk of CRC, and strong evidence for that intake of calcium supplements decreases the risk of CRC (2, 85). Calcium may bind free fatty acids and bile acids, promote cell differentiation, decrease cell proliferation, prevent KRAS mutations and inhibit heme iron's effects on carcinogenesis (86, 87). Other suggested mechanisms in the association between dairy products and CRC are their content of lactic acid bacteria, lactoferrin, folate and butyrate of which may have cancer protective properties (88).

### Meat

High intake of processed meat is associated with increased risk of CRC (2), and even low intakes seem to increase the risk. A risk increase of 17% per 50 g daily intake of processed meat has been found (89). Although the support for that red meat intake increases the risk of CRC is not as convincing as that for processed meat, the WCRF and AICR have concluded that there is probable evidence for that high intake of red meat increases the risk of CRC (2). The National Food agency in Sweden recommends an intake of red meat not higher than 500 g/week (90), and the average intake of meat in Sweden is just below of 500 g/week (91).
The mechanisms behind the associations between meat and CRC are not known, but it has been speculated that for example heme iron may damage the epithelial cells in the colon (92) and amplify the production of ROS (93). There is limited evidence that foods containing heme iron are associated with risk of CRC (2). Fat content and fat quality of the meat may be of importance for CRC development (94, 95). Moreover, the cooking- and preservation methods might induce cancerous compounds such as heterocyclic amines, polycyclic aromatic hydrocarbons and N-nitroso compounds (96, 97). As processed meat often contains high amounts of salt (98), salt intake has also been discussed as a factor in CRC development (99).

### Fish

Even though the evidence that high intakes of fish and lower risk of CRC are considered to be limited (2), such associations have been suggested (100). Fish contains some of the nutrients that might be inversely associated with risk of CRC, e.g. vitamin D (2). Vitamin D promotes cell differentiation and decreases cell proliferation and apoptosis (101). It may also promote the innate and adaptive immune system, together with reduction of inflammation and inhibition of angiogenesis. Fatty fish is also high in omega-3 fatty acids, which *in vitro* has been seen to have anti-inflammatory properties, probably due to inhibition of cyclo-oxygenase-2 and the omega-6 polyunsaturated fatty acid production of eicosanoids (102).

## Dietary pattern

As foods are not consumed as a single unit, but combined with other foods, it is important to not only study them one by one, but also to study the combinations of foods. There are also complex interactions that occur between foods, and the

combined effect foods exert on a person might be stronger than that of individual foods.

Pattern analysis describes the way that foods are combined and there are two main ways of conducting pattern analysis: the a posteriori (data-driven) or the a priori (hypothesis-driven) constructed index. The data-driven pattern analysis is made either from cluster analysis, factor analysis or reduced rank regression. In the hypothesis-driven pattern analysis, the pattern is based on the interpretation of evidence of associations between diet and health (103).

Although pattern analysis gives a wider understanding, it also has to be combined with the analysis of the individual component of the pattern to complete the understanding on how the nutrients or foods included in the pattern may interact in influencing the disease (104). An overall dietary pattern may not enhance the understanding of the mechanism of the disease, but the analysis of an individual food may well do so.

The association between CRC and the interaction of foods has been examined with several dietary patterns and indexes, and CRC has been found to be associated with several dietary indexes. The Healthy Eating Index (HEI), the Mediterranean Diet Score (MDS), and the Dietary Inflammatory Index (DII) are hypothesis-driven constructed indexes with nine or more items, which has been associated with risk of CRC (105). The HEI and the MDS have both been found to be associated with decreased risk for CRC, and the DII with increased risk of CRC (106-112). A high MDS score was seen to lower the risk with 11-28% compared with a low score, and high HEI score lowered the risk with 20-30% compared with low score, high DII score increased the risk for CRC with up to 40% compared with low score.

The association has also been examined with a data-driven pattern analysis. In a study of the EPIC cohort, the patterns that were inversely associated with CRC were either characterised by a high variety of vitamins and minerals or by vitamin $B_{12}$, riboflavin, calcium, cholesterol, total protein and phosphorous (113). Other data-driven pattern analyses have shown that a prudent dietary pattern was inversely associated with risk of CRC and a westernized dietary pattern was associated with higher risk of CRC, both in a Japanese population (114) and in a North American population (115). An inverse association was also seen when comparing a traditional Korean dietary food pattern, rich in fruit and dairy, and a Westernized dietary pattern, abundant in meat, where the traditional pattern was inversely associated with risk of CRC, and the Westernized dietary pattern was positively associated with risk of CRC (116).

To the best of our knowledge, no previous study has examined an index based on the conclusions regarding diet by the WCRF in the context of CRC.

# Aims

## Overall aim

The aim of this thesis is to examine associations between dietary intakes and CRC in the MDC cohort, and whether the associations are modified by different preconditions.

## Specific aims

**Paper 1** To examine the association between fibre intake, and its food sources, and incident CRC. The secondary aim was to examine if the association differs depending on sex, tumour location and TNM-classification.

**Paper 2** To examine the association between blood glucose levels, insulin levels, insulin resistance and incident CRC. The secondary aim was to investigate whether tumour location or sex may modify the before-mentioned associations.

**Paper 3** To examine if intake of red meat, divided into beef and pork, unprocessed and processed meat, fish and poultry is associated with incident CRC. The secondary aim was to investigate whether sex, tumour location or overweight may modify the association between meat intakes and CRC.

**Paper 4** To examine the association between a constructed dietary index, the Colorectal Dietary Quality Index (CDQI), based on conclusions from WCRF, and risk of incident CRC, and weather the association differs depending on tumour site. The secondary aim was to extend our earlier studies on associations between fibre and processed meat intake and risk of CRC, with an additional 4-years follow-up and 195 new cases of incident CRC, and to examine if there is an association between intake of dairy products and CRC.

# Material and Methods

## Ethics

The study was approved by the Regional Ethical Review Board in Lund (50-91, 2013/803).

## Study population

### The Malmö Diet and Cancer study

All papers in the thesis are based on the Malmö Diet and Cancer (MDC) cohort, which is a population-based cohort with a long follow-up time. The main objective of MDC was to evaluate the effect of diet on different types of cancer, for example CRC. In MDC, all inhabitants of Malmö, Sweden, born between 1926 and 1945 were invited to participate from March 1991 to May 1995. Although, after May 1995, the invite was extended to include men born between 1923 and 1945, and women born between 1923 and 1950 (117). Exclusion criteria were mental disability and inadequate Swedish language (n = 1975). Altogether, 28,098 participants completed the baseline examinations after having given their informed consent, which represented 41% of the eligible individuals. Of those having completed the baseline examinations, 167 individuals had been diagnosed with CRC before or at baseline examinations, and were therefore excluded from all the four studies. **Paper 2** is based on a smaller part of the MDC cohort, the cardiovascular cohort (MDC-CC). MDC-CC comprises of 6,103 individuals randomly selected from MDC, whereof 5,540 returned for collection of blood samples. The MDC-CC participants were examined between October 1991 and February 1994 (118). In **Paper 2**, individuals were excluded if they had prevalent diabetes (n = 219), prevalent CRC (n = 14) or had not left a blood glucose and plasma insulin sample (n = 397), leaving 4,910 individuals (1,992 men).

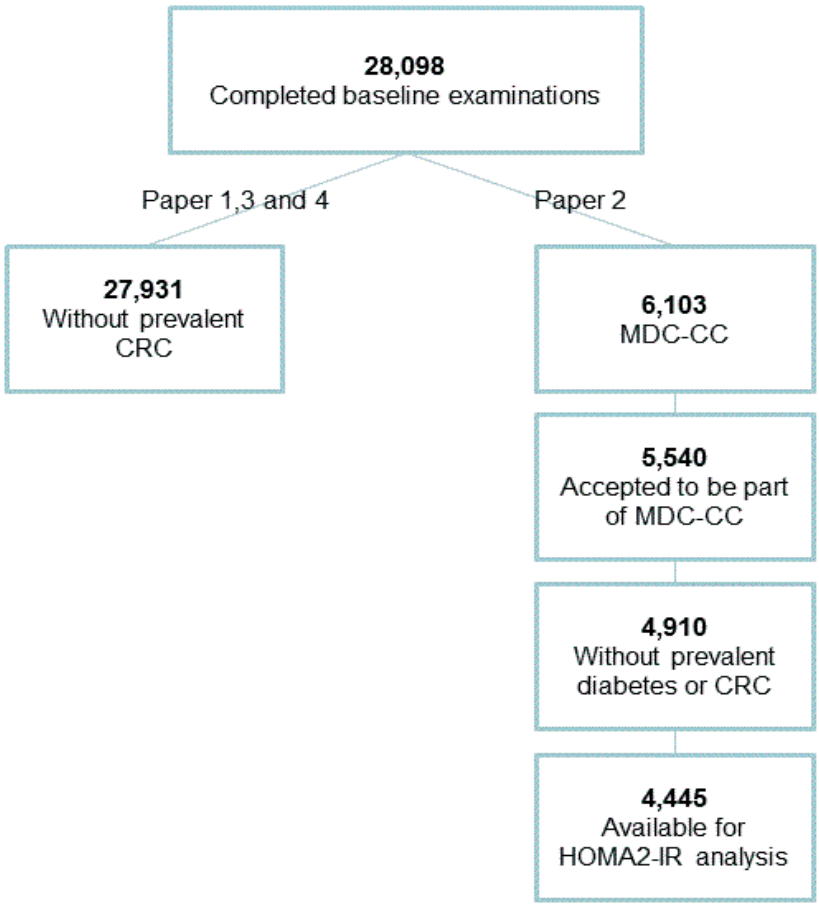


**Figure 2. Participant flow**
Paper 1, 3 and 4 is based on the Malmö Diet and Cancer (MDC) cohort and Paper 2 on the Malmö diet and Cancer cardiovascular cohort (MCD-CC).

# Method of Malmö Diet and Cancer study

## Data collection

At baseline, the participants visited the screening centre twice. On their first visit, they were instructed on how to fill out questionnaires on socioeconomic-, lifestyle-, and dietary factors, and how to register their cooked meals and cold beverages including alcoholic beverages in a seven day long food record. Weight, height, and waist circumference were measured and blood samples were collected by trained

nurses. Body composition was estimated with a single-frequency bio-impedance methodology (BIA 103, RJL systems, Detroit, MI, USA,). Body fat percentage was calculated using an algorithm provided by the manufacturer. Ten days after the first visit it was time for a second visit, where their questionnaires were controlled and a completing diet history interview was conducted by nutritionists to rule out overlap between the food frequency questionnaire and food record.

## Dietary data

Dietary data was collected through a modified diet history method with a 7-day menu book for registration of meals that varied from day to day, most likely lunch and dinner, cold beverages and nutrient supplement. In addition, a 168-item questionnaire was distributed to the participants for estimation of consumption frequencies and portion sizes of foods not covered in the menu book. A diet history interview completed the dietary assessment at the second visit. The interview contained questions about cooking methods and portion sizes for foods registered in the menu book. The interview was shortened from 60 minutes to 45 minutes due to economical reasons in 1994. The change in interview time did not affect the ranking of individuals (119). The data was then coded using the Swedish Food Data Base (120).

## Handling of dietary data

We used the following variables for daily nutrient intake in **Paper 1, 3 and 4**: total energy (MJ), non-alcoholic energy (MJ), carbohydrates (percentage of energy (En%)), fat (En%), protein (En%), fibre (g), calcium (mg) and folate (mg).
In **Paper 1,** we also used the variables fibre (g/MJ) and vitamin D (μg) for daily nutrient intake, in **Paper 3** saturated fat (g), iron (mg), and zinc (mg), and in **Paper 4** vitamin D (μg), iron (mg), and zinc (mg).
In **Paper 1,** the following daily intakes of foods were examined: vegetables (g/MJ), fruits and berries (g/MJ), fibre-rich cereal products (portions of fibre-rich bread and breakfast cereals/MJ) and red meat (g).
In **Paper 3,** the following daily intakes of foods were used: red meat (g), unprocessed red meat (g) processed red meat (sausages and cured meat) (g), beef (g), pork (g), poultry (g), fish (g), dairy products (portions of milk, yoghurt, sour milk, cream, cheese, and ice cream), fruit and berries (g), and sugar-sweetened beverages (g). Red meat was defined as pork, beef, lamb and game. Total red meat included both processed and unprocessed red meat. Intakes of pork and beef were mainly based on non-processed meat, as distinction between pork and beef was not

possible for all processed meats based on items included in the food questionnaire. The fish variable consisted of both processed and unprocessed fish.
In **Paper 4,** the following daily intakes of foods were used in the study: processed meat (g), dairy products (portions of milk, yogurt, cream, cheese, and ice-cream), and vegetables (g).
Portion sizes of dairy products and fiber-rich cereal products were used instead of grams, to analyze products with different water content and usually consumed in different weights. Standard portion sizes from the National Food Agency in Sweden were used: milk and yoghurt (200 g/portion), cheese (20 g/portion), cream (25 g/portion), ice cream (75 g/portion), fibre-rich soft bread (50g/portion), fibre-rich crisp bread (30g/portion), and fibre-rich breakfast cereals (25g/portion) (121).
Energy-adjusted food variables were created by either dividing dietary-intakes by non-alcohol energy intake (**Paper 1,4**) or by regressing the food intakes on non-alcohol energy intake (**Paper 3**) (122).
The diet analyses were adjusted for the variables called "method version" and "season". Method version was used because of the altered coding routines of dietary data introduced in September 1994 in order to shorten the interview time (from 1 h to 45 min). This resulted in two somewhat different method versions, before and after September 1994, but did not have any major influence on ranking of individuals (119). The variable season was divided into spring, summer, autumn, and winter depending on when in the year the baseline examination was executed. Information on dietary change in the past was available, based on the question "Have you substantially changed your eating habits because of illness or some other reasons?" Dietary change in the past was reported by 24.3%. The relative validity of the MDC method was evaluated in the Malmö Food study 1984-1985 in a sample of Malmö residents, 105 women and 101 men, 50-69 years old. An 18-day weighted food record was used as the reference method, comprised of three days every second month over a one-year period (123, 124).

## Age, sex, BMI, and lifestyle and socioeconomic variables

Age and sex was obtained via the personal identification number. BMI was calculated from measured weight and height. From the questionnaire, data on level of education, physical activity, alcohol intake, smoking and use of NSAID or MHT were collected. Level of education was divided into four different categories: ≤ 8 years; 9–10 years; 11–13 years of education; and university degree. The participants were asked to estimate their physical activity in how many minutes per week they spent on 17 different activities. The duration was multiplied with an activity-specific intensity coefficient and an overall leisure-time physical activity score was created (125). Alcohol intake, based on both the questionnaire and the dietary history method, was divided into four categories: zero; < 15 g/d for

women and < 20 g/d for men; 15–30 g/d for women and 20–40 g/d for men; and > 30 g/d for women and > 40 g/d for men. Smokers were divided into three categories: current smokers, including irregular smokers; ex-smokers; and non-smokers. Current use of MHT and regular use of NSAID were divided into non-users and users.

## Plasma insulin, blood glucose and insulin resistance

In the MDC-CC, all the blood and plasma samples were collected by a trained nurse in the morning after 12 hours of fasting, and plasma was separated and immediately frozen at -20˚C until analysed (118). The blood samples were on average collected eight months after the first visit. Analyses were performed according to clinical routines at the Department of Clinical Chemistry, Malmö Sweden. Blood glucose was analysed using a routine hexokinase method. Insulin levels were measured in mIU/ml by a radioimmunoassay, where the lowest limit for detection was 3 mIU/ml.
Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated with the use of a HOMA-IR calculator (126). In the analysis of HOMA-IR, extreme values of blood glucose (< 3.5 or > 25 mmol/l) were excluded, as were extreme values of plasma insulin (< 3 or > 57.5 mIU/ml), thus leaving 4,451 individuals for analysis of HOMA-IR.

## Cancer cases

Cancer cases were identified from the Swedish Cancer Registry. Information on date of death was collected from the Swedish Cause-of-death Registry. In **Paper 1-3,** cancer cases were identified until 31 December 2010, and in **Paper 4**, until 31 December 2014. Until last follow-up, 4.0% of the men, and 2.8% of the women had developed CRC, of which 590 were colon cancers (322 women), 317 were rectal cancers (152 women), and 16 were synchronous colon- and rectal cancers (6 women), during 502,136 person-years of follow-up.

## Tumour characteristics

Classification of the CRC cases used in **Paper 1** was possible in 635 cases of CRC, whereof 363 were colonic cancers and 272 rectal cancers. The classification was performed by examining clinical- and/or pathology records. In addition, the histopathological examination was re-evaluated by a senior pathologist. Colorectal tumours were classified according to the TNM-system. The method used to

identify the tumour characteristics of CRC has been described elsewhere and the clinicopathological characteristics did not differ between CRC tumours in the MDC and those in the EPIC cohort (127). The cases were identified until end of 2008. Of the cancer cases where cancer-staging was possible: 113 were tumour (T)-stage 1 and 2; 405 were T-stage 3 and 4; 292 were node-negative (N0) disease; 193 were lymph node positive (N1 and N2) disease; 451 were non-metastasis (M0) disease; and 116 were metastatic (M1) disease.

### Diabetes cases

Prevalent diabetes diagnosis was determined from self-reported diagnosis, self-reported medication for diabetes or information from medical data registries with a date of diagnosis before inclusion in the MDC. Incident diabetes diagnosis was obtained either from the Regional Diabetes 2000 Register of Scania, the Malmö HbA1C Register or the Swedish National Diabetes Register. In the MDC, 1,183 prevalent cases of diabetes and 3,245 incident cases of diabetes were identified until end of follow-up, 31 December 2010. In all those with diabetes, 185 cases of incident CRC were found.

## Statistical methods

All statistical analyses were performed in the SPSS version 21-23 (SPSS Inc. Chicago, Il, USA). All statistical analyses were two-sided and significance was assumed at p-value $< 0.05$.

The general linear model was used when examining baseline continuous characteristics in the cases and non-cases, and adjusted for age, sex (when appropriate), and also for season and method version for the food variables. The $chi_2$-test was used when examining baseline categorical characteristics in the cases and non-cases.

Food variables were log-transformed (e-log) to normalize the distribution before analysis. A very small amount (0.0001) was added to the food variables before transformation to handle zero intakes (128).

Cox proportional hazard analysis was used for estimating hazard ratios (HR) for CRC in quintiles of food intake, and quartiles of insulin, glucose, and HOMA-IR, or Index groups. Time in the study was used as underlying time variable, defined as time between baseline to either diagnosis, death, migration or end of follow-up by 31 December 2010 (**Paper 1-3**) or 31 December 2014 (**Paper 4**). In the analysis of TNM-classification, end of follow-up was until 31 December 2008 (**Paper 1**).

Agreement for proportionality was tested with Kaplan-Meier curve or for interactions between the underlying time variable and the examined covariates. Spearman's test for correlations was used to examine correlations between variables.

## Paper 1

HR was estimated for incident CRC, colon- and rectal cancer, and TNM-stages, depending on energy-adjusted quintiles of fibre, vegetables, fruit and berries, and fibre-rich cereal products. Adjustments were made for age, sex (when applicable), season, method version, total energy intake, level of education, smoking, alcohol intake, physical activity, BMI, and when applicable, for current use of MHT. Additional adjustments were made for regular use of NSAID, intakes of folate, red meat, vitamin D or calcium. Test for interactions between sex and fibre or food component with regard to CRC incidence was performed by adding a multiplicative variable [sex × diet quintile (treated as continuous variables)] to the full model. In sensitivity analyses, individuals with a reported dietary change in the past or prevalent cancer (except cervix cancer in situ) were excluded.

## Paper 2

HR was estimated for incident CRC, colon cancer, and rectal cancer, depending on quartiles of blood glucose, plasma insulin and HOMA-IR. The proportionality assumption was tested for all the adjustment factors with a Kaplan-Maier curve before analysis. Two models were presented: one unadjusted model, and a full model. The full model was adjusted for the background variables indicating a difference between the cases and the non-cases ($p < 0.2$), i.e. age, sex (when appropriate), BMI, and smoking status.

A test for interaction between sex and blood glucose; plasma insulin levels; and HOMA-IR, respectively, with regard to CRC incidence was performed by adding a multiplicative variable (i.e. [sex × glucose quartiles (treated as continuous variables)] to the full model. If a significant interaction was found, subgroup analyses based on sex was performed.

In the sensitivity analysis, we excluded individuals with incident diabetes, and apart from that we performed one additional model, the full model glucose/insulin. The full model insulin was only used when estimating HR of incident CRC for quartiles of blood glucose levels and was adjusted for age, sex (when applicable), BMI, smoking status and plasma insulin. The full model glucose was only used when estimating HR of incident CRC for quartiles of plasma insulin levels and was adjusted for age, sex (when applicable), BMI, smoking status and blood

glucose. In addition, we excluded individuals with CRC diagnosis within two years of inclusion.

## Paper 3

HR was estimated for incident CRC, colon cancer, and rectal cancer, depending on quintiles of energy-adjusted food intakes (red meat, beef, pork, unprocessed red meat, processed red meat, poultry, and fish). The energy-adjustment was made with the residual method (122). The proportionality assumption was tested for all the adjustment factors with a Kaplan-Maier curve before analysis. Adjustments for age, sex (when appropriate), season, total energy intake, level of education, smoking status, alcohol intake, physical activity, BMI, NSAID use, and when appropriate, for current use of MHT. Additional models were constructed with further adjustments for diabetes (prevalent and incident) or potential dietary confounders found in previous studies (intake of: fibre; protein; saturated fat; calcium; folate; iron; zinc; fruits and vegetables; milk products; and sugar-sweetened beverages) (2).

Spearman's correlation matrix was used to examine the correlation between energy-adjusted food intakes (total red meat, beef, pork, unprocessed red meat, processed red meat, poultry, and fish). For intakes where a correlation over 0.40 was found, additional models were constructed with mutual adjustments in the full models.

Test for interaction between sex or BMI status ($< 25$ and $\geq 25$) and dietary intakes with regard to CRC incidence was performed by adding a multiplicative variable (e.g. [sex × diet quintile (treated as continuous variables)] to the full model.

In sensitivity analysis, we excluded individuals with a reported dietary change in the past, all forms of prevalent cancer except cervix cancer in situ or incident and prevalent diabetes.

## Paper 4

An a priori defined dietary index for CRC, the Colorectal cancer Dietary Quality Index (CDQI), was constructed by classifying the individuals according to intake quintiles of processed meat, fibre, and dairy products. Points ranging from zero to four were assigned to the different quintiles, where high points were assigned to intake quintiles expected to be associated with decreased CRC risk, and low points assigned to intake quintiles expected to be associated with increased CRC risk. Finally, the index points were summed up to the CDQI, and were then divided into four groups; low CDQI (0–3 points); medium low CDQI (4–6 points); medium

high CDQI (7–9 points); and high CDQI (10–12 points). The four groups of the CDQI were then used as exposure categories.

**Tabel 1. Index points for the Colorectal Diet Quality Index**

Values are index points in the Colorectal Diet Quality Index assigned to different quintiles of food intake, where high index points were assigned to intakes expected to be inversely associated with risk of CRC, and low index points assigned to intakes expected to be associated with increased risk of CRC, based on earlier studies (2). Quintile 1= Lowest intake of food or nutrient

| Quintiles | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Processed red meat | 4 | 3 | 2 | 1 | 0 |
| Fibre | 0 | 1 | 2 | 3 | 4 |
| Dairy products | 0 | 1 | 2 | 3 | 4 |

HR was estimated for incident CRC, colon- and rectal cancer, depending on quintiles of energy-adjusted intakes (fibre, dairy products, and processed red meat), and CDQI. Adjustments for age, sex (when appropriate), season, and total energy intake, education, smoking status, alcohol intake, physical activity, BMI, NSAID-use, and when appropriate, for current use of MHT, were made. To test if the proportional hazard assumptions held, we tested interactions between the underlying time variable and examined covariates.

Test for interaction between sex and intakes of fibre, dairy products and processed meat and CDQI groups with regard to CRC incidence was performed by adding a multiplicative variable, e.g. [sex × CDQI group (treated as continuous variables)]. Exclusion of individuals with a reported dietary change in the past was performed as sensitivity analysis.

# Main results

## Paper 1

High fibre intake was associated with lower incidence of CRC (HR: 0.72 for highest compared with lowest quintile; 95% CI: 0.55, 0.94; p for trend = 0.026). Of the foods high in fibre, vegetable intake was associated with lower incidence of CRC (HR: 0.83 for highest compared with lowest quintile; 95% CI: 0.64, 1.07; p for trend = 0.048).

In separate analyses depending on tumour location, we observed a borderline interaction between sex and fibre intake (p = 0.052) and found that high fibre intake was inversely associated with incidence of colon cancer in women (HR: 0.51 for highest compared with lowest quintile; 95% CI: 0.31, 0.75; p for trend = 0.013), but not in men (p for trend = 0.69). In addition, women with high intake of fruits and berries had a significantly decreased risk of colon cancer (HR: 0.62 for highest compared with lowest quintile; 95% CI: 0.37, 0.98; p for trend = 0.022). No tendency of protective association was seen in men (p for trend = 0.72), even though the interaction between intake of fruits and berries and sex did not reach significance (p = 0.16).

When analysing intakes of fibre and fibre-rich foods and rectal cancer, no significant associations were seen in any of the sexes. However, we detected a significant interaction between vegetable intake and sex on rectal cancer (p = 0.039), and the risk of developing rectal cancer tended to increase with higher vegetable intake in women (HR: 2.22 for highest compared with lowest quintile; 95% CI: 1.07, 4.61; p for trend = 0.06). In contrast, a tendency of protective association between high vegetable intake and rectal cancer was seen in men (p for trend = 0.14). We also observed a significant interaction between fibre intake and sex on rectal cancer (p = 0.048), but although the associations in men and women seemed to reflect those for vegetable intake, the tendencies for fibre intake was less clear (p-values for trend $\geq$ 0.26).

Intake of fibre-rich cereal products was significantly associated with lower risk for N-stage 0 (p for trend = 0.015) and for M0 (p for trend = 0.046), but we did not observe any tendencies of associations with N-stage 1 and 2 or M-stage 1. When analysing women and men separately, the tendencies of different associations with intake of fibre-rich cereal products depending on N- and M-stage was mainly seen

in men, and the association between fibre-rich cereal products and stage N0 was significant for men (HR: 0.64 for highest quintile compared with lowest; 95% CI: 0.37; p for trend = 0.024). No other significant associations with tumours classified according to the TNM-system were found.

# Paper 2

High blood glucose levels were associated with CRC, and we observed a significant interaction with sex (p = 0.013). We found an association between high blood glucose levels and CRC in men (HR: 2.80 for highest compared with lowest quintile; 95% CI: 1.37, 5.70; p for trend = 0.001), but not in women (HR: 1.02 for highest compared with lowest quintile; 95% CI: 0.53, 1.95; p for trend = 0.74). We did not find any association between plasma insulin or HOMA-IR and CRC.

**Table 2. Hazard ratio (HR) of incident colorectal- and colon cancer associated with blood glucose for men and women in the Malmö Diet and Cancer Study cardiovascular cohort.**
Full model: Calculated with the Cox proportional hazard regression model. Adjusted for age (quartiles of age), BMI (≤ 25 kg/m2, > 25 kg/m2), and smoking (current, ex or never).
Values are quartile ranges, hazard ratios (HR), and 95% confidence intervals (CI).

| | | Colorectal cancer | | | Colon cancer | | |
|---|---|---|---|---|---|---|---|
| | | | Full model | | | Full model | |
| Quartiles of glucose | Min-max (mIU/l) | Cases/ person-years | HR | CI | Cases/ person-years | HR | CI |
| Men | | | | | | | |
| 1 | 3.3-4.5 | 5/4400 | 1.00 | | 2/4358 | 1.00 | |
| 2 | 4.6-4.8 | 10/7276 | 1.25 | 0.55, 2.85 | 4/7216 | 1.95 | 0.57, 6.70 |
| 3 | 4.9-5.2 | 16/9835 | 2.03 | 0.95, 4.32 | 9/9767 | 2.08 | 0.60, 7.17 |
| 4 | 5.3-16.8 | 40/10853 | 2.80 | 1.37, 5.70 | 22/19679 | 4.23 | 1.46, 13.44 |
| p for trend | | | 0.001 | | | 0.002 | |
| Women | | | | | | | |
| 1 | 3.4-4.5 | 20/13981 | 1.00 | | 12/13888 | 1.00 | |
| 2 | 4.6-4.8 | 20/14048 | 1.21 | 0.62, 2.34 | 9/13977 | 0.73 | 0.27, 1.96 |
| 3 | 4.9-5.2 | 18/12553 | 0.82 | 0.44, 1.55 | 13/12510 | 0.96 | 0.45, 2.09 |
| 4 | 5.3-12.2 | 16/8834 | 1.02 | 0.53, 1.95 | 10/8797 | 1.01 | 0.44, 2.34 |
| p for trend | | | 0.739 | | | 0.878 | |

# Paper 3

In the full multivariate model, beef intake was inversely associated with risk for CRC in women (HR: 0.65 for highest compared with lowest quintile; 95% CI: 0.45, 0.95; p for trend = 0.046), but not in men, and a borderline interaction between sex and beef intake was seen (p = 0.07). High pork intake was associated

with increased incidence for CRC (HR: 1.39 for highest compared with lowest quintile; 95% CI: 1.09, 1.78; p for trend = 0.023). The association was only significant in women (HR: 1.54 for highest compared with lowest quintile; 95% CI: 1.12, 2.15; p for trend = 0.003), but no significant interaction with sex was seen (p = 0.157). We found that higher intake of processed red meat was significantly associated with increased risk for CRC in men (HR: 1.23 for highest compared with lowest quintile; 95% CI: 0.87, 1.73; p for trend = 0.023), but not in women, and a borderline interaction was seen (p = 0.062).
High intake of beef was inversely associated with risk of colon cancer (HR: 0.60 for highest compared with lowest quintile; 95% CI: 0.44, 0.82; p for trend = 0.009). The inverse association was significant in women (HR: 0.60 for highest compared with lowest quintile; 95% CI: 0.37, 0.96; p for trend = 0.049), and a similar tendency was seen in men (p for trend = 0.069). High intake of pork was associated with increased risk of colon cancer (HR: 1.41 for highest compared with lowest quintile; 95% CI: 1.04, 1.90; p for trend = 0.021). We observed a borderline significant association between high intake of processed meat and increased risk of colon cancer in men (HR: 1.23 for highest compared with lowest quintile; 95% CI: 0.80, 1.90; p for trend = 0.053), but no significant interaction with sex was seen (p = 0.127).
High intake of beef was associated with increased risk of rectal cancer in men (HR: 1.82 for highest compared with lowest quintile; 95% CI: 1.02, 3.25, p for trend = 0.028), but not in women, and a significant interaction was seen between beef intake and sex (p = 0.025). High intake of fish was inversely associated with risk of rectal cancer in all (HR: 0.59 for highest compared with lowest quintile; 95% CI: 0.38, 0.92, p for trend = 0.025), and the association did not differ depending on sex (p for interaction = 0.597).
No significant interactions were found between the different types of meat intakes and BMI status (< 25 or ≥ 25) on CRC.

# Paper 4

No significant interactions were found between the different types of meat intakes and BMI status (< 25 or ≥ 25) on CRC In the full multivariate model, a high CDQI was associated with decreased risk of CRC (HR: 0.57 for highest compared with lowest quintile; 95% CI: 0.43, 0.75; p for trend < 0.001). Similar findings were seen for colon- and rectal cancer (HR: 0.58 for highest compared with lowest quintile; 95% CI: 0.41, 0.83; p for trend = 0.003 and HR: 0.58 for highest compared with lowest quintile; 95% CI: 0.36, 0.94; p for trend = 0.018, respectively). No significant interaction between sex and the CDQI was seen, and the risk only slightly differed between women and men, when analysed separately.

High intake of dairy products was associated with decreased risk of CRC (HR: 0.77 for highest compared with lowest quintile; 95% CI: 0.62, 0.96; p for trend = 0.008), colon cancer (HR: 0.81 for highest compared with lowest quintile; 95% CI: 0.61, 1.06; p for trend = 0.042), and rectal cancer (HR: 0.66 for highest compared with lowest quintile; 95% CI: 0.46, 0.94; p for trend = 0.019).
High intake of fibre was inversely associated with risk of CRC (HR: 0.77 for highest compared with lowest quintile; 95% CI: 0.61, 0.98; p for trend = 0.043). No significant association was seen between fibre intake and colon cancer or rectal cancer, although the risk estimates were similar to those in CRC.
High intakes of processed meat was associated with increased risk of CRC (HR: 1.31 for highest compared with lowest quintile; 95% CI: 1.05, 1.63; p for trend = 0.012). A borderline significant association was seen between processed meat and colon cancer (HR: 1.36 for highest compared with lowest quintile; 95% CI: 1.03, 1.78; p for trend = 0.06), as well as with rectal cancer (HR: 1.29 for highest compared with lowest quintile; 95% CI: 0.87, 1.83; p for trend = 0.05).
No significant interaction was seen between sex and dietary intake on CRC (p = 0.20-0.37) or rectal cancer (p = 0.91-0.95), but there was a tendency towards interaction between sex and fibre intake on colon cancer (p = 0.08). An inverse association between fibre intake and risk of colon cancer was seen in women (HR: 0.56 for highest compared with lowest quintile; 95% CI: 0.38, 0.82; p for trend = 0.007), but not in men (HR: 0.98 for highest compared with lowest quintile; 95% CI: 0.61, 1.57; p for trend = 0.66).

# General discussion

## Main findings and interpretation

### Fibre intake and colorectal cancer

In **Paper 1**, we studied intake of fibre, and fibre sources, in relation to incident CRC. We found that fibre intake was inversely associated with risk of CRC, as well as some of the fibre sources, as fruit and berry intake in women regarding colon cancer. In **Paper 4**, we studied intake of fibre again, but with prolonged follow-up time and 195 more cases of CRC. In **Paper 4**, compared with **Paper 1**, we found that the association between fibre intake and CRC still remained, and that the association between fibre intake and colon cancer in women were now even more pronounced.

In line with our findings several studies have found an association between fibre and CRC, and it can therefore be hypothesised that the association between fibre and CRC reflects a true association. Although, if the association really differs by sex, still needs to be replicated in more studies. However, as before mentioned, there might be different factors behind CRC development in women and men, and the differences in results may for example depend on different bowel transit-time in women and men. Women have a longer bowel transit-time (34), and may therefore potentially have a greater benefit from increase in fibre intake. Women might also benefit more from a higher fibre intake because of their decrease in oestrogen after menopause, whereas men have a later hormonal decrease, and might therefore not benefit as much. Oestrogen may strengthen the tight junctions in the epithelial barrier (129). When oestrogen levels decrease, increased permeability can occur (130), with increased translocation of carcinogenic compounds. Fibre, together with other anti-carcinogenic compounds found in fibre-rich foods, may counteract the effect of lower oestrogen levels.

Only individuals in the highest quintile had a reported fibre intake at or above the recommended intake of 3g/MJ in Sweden (131). When comparing the study population's intake with a large study on dietary intake in Sweden, the intake of vegetables and fruit has increased over the years, with a 30% increase for women and 15% increase for men between 1989 and 2011. Still, only one third of the population has a fibre intake above recommended intake, and the average intake is

20 gram fibre/day (91). This could also be an explanation to why the associations between men and women differ, if hypothesised that the increase in women represents an even greater intake of fibre mostly in women already eating a larger amount of fibre at the MDC baseline.

Fibre has many potentially anti-carcinogenic effects, in addition to the decrease in bowel transit-time. Increase in stool output, stool frequencies and dilution of faecal output are some of them, together with reduction of potential toxins and bile acids, increased fermentation in the bowel, and not least the increase of the production of SCFAs (132, 133), adding to the evidence of the inverse association between fibre intake and CRC.

It has also been found that wholegrain, which could be considered comparable to fibre-rich cereals, is associated with decreased risk of CRC (2). In **Paper 1**, we also studied intake of fibre, and its sources, in relation to classification of CRC. We found that intake of fibre-rich cereals was associated with decreased risk for stage M0 and N0. This indicates that when excluding more advanced cases of CRC, an association between intake of fibre-rich cereals and CRC might be present in the MDC as well. Intake of fibre-rich cereals is higher in Sweden (134), than in for example USA (135). Higher intakes may indicate that more individuals reach the threshold for when a potential anti-carcinogenic effect occur, making it difficult to compare groups with different intakes. As there is a shortfall of other prospective studies examining the association between fibre intake and tumour classification, further research is warranted.

Fruit and berry intake was inversely associated with colon cancer in women (**Paper 1**). The decreased risk of CRC associated with fruit and berries intake may in general be explained by their content of cytoprotectants, such as phenolic compounds. Phenolic compounds can protect against DNA damage repair, cell proliferation and differentiation, avoidance of apoptosis, mutations, invasion and forming of metastasis (136). The difference between women and men may reflect the relatively low intake of fruits and berries in men compared with women. In Sweden, the intake of fruit and berries is relatively low compared with other countries. This was for example found in the European Prospective Investigation into Cancer and Nutrition study (137).

In **Paper 1**, we found that high intake of vegetables was associated with decreased risk of CRC, but we also found a discrepancy in that high intake of vegetables tended to be associated with increased risk of rectal cancer in women, but tended to be associated with decreased risk for rectal cancer in men. The association between intake of vegetables and CRC were no longer present, both when excluding individuals with prevalent cancer and when excluding individuals with dietary change in the past. This might indicate that the intake of vegetables was a temporary effect of other diseases and an unstable intake of vegetables over time.

## Meat intake and colorectal cancer

In **Paper 3**, we found that high intakes of pork and processed meat were associated with increased risk of CRC. We also found that the association for processed meat was more pronounced in men. Although in **Paper 4**, with a longer follow-up time and more cases added, the interaction with sex seen in intake of processed meat found in **Paper 3** did not remain. In **Paper 3**, we found a discrepancy in that high intake of beef was associated with decreased risk of colon cancer, but also with increased risk of rectal cancer in men. Most studies have found that high intake of red meat is associated with CRC (2), but as there might be differences in meat production between countries, such as use of growth hormones and antibiotics, associations between meat intake and CRC might not be entirely comparable between countries. Traditionally, there are also high intakes of pork in the southernmost region of Sweden, compared with other regions in Sweden. This may affect the results, as the intake of beef may be eaten more sporadically making it more difficult for the individuals to report a correct intake.

Even if our findings indicate an association between meat intake and risk of developing CRC, and that the association differs depending on the type of meat, one must take the intake levels in the MDC cohort into consideration, and speculate on what the different intakes are representing. That beef, in our study, is protecting against colon cancer in women, might indicate a rather small intake level and that it was not large enough to give a negative effect, or just gave a positive effect by for example increasing levels of iron and other nutrients. Beef intake might also be a marker of a higher socioeconomic status, as beef is more expensive than for example pork, or a marker of more healthy food choices. That intake of pork and processed meat are associated with CRC is more in line with observations in several studies and suggested underlying mechanisms (2).

The difference on risk for colon and rectal cancer can be explained by different physiology throughout the colon and the rectum (12), and that the possible carcinogenic properties in food and mechanisms initiated by food have different time to affect. Meat, which mostly is absorbed in the small intestine, affects other systems in the digestion, such as the secretion of bile acids, which in turn can affect the development of cancer.

Similar to pork, the fish intake has been traditionally high in the southernmost region of Sweden and the fish intake seems to have been consistent over the years in Sweden (91). In **Paper 3**, we found that high intake of fish was inversely associated with rectal cancer. This has been found in other studies (100), giving our result a confirmative role. Particularly as fish contains nutrients that might have anti-carcinogenic properties, it confirms the result further. Fatty fish contains vitamin D in abundance, as well as omega-3 fatty acids, both of which have been found to have anti-carcinogenic and anti-inflammatory properties (101, 102).

## Intake of dairy products and colorectal cancer

Dairy products contain many vital nutrients and may be associated with decreased risk of CRC because of many factors. The most discussed factors are the dairy products contents of calcium and vitamin D. Both calcium and vitamin D have been associated with decreased risk of CRC (2). Calcium may bind to bile acids and ionised fatty acids in the lumen of the colon, through which it increases cell differentiation and induces apoptosis (86, 87). Vitamin D has been proposed to be both growth-inhibitory and antiproliferative (101). As both calcium and vitamin D are present in dairy products, they might work together to decrease the risk of CRC.

Dairy products were found to be inversely associated with CRC, irrespective of fat-content (138), which is comparable with our results on dairy products in **Paper 4**. In our study, all types of dairy products were included in the analysis, and there was a strong inverse association with CRC.

High intake of dairy products has been seen to be associated with higher nutrient density, as well as lower energy density (139). It has also been found to be inversely associated with obesity (140). High intake of milk has been found to be associated with overall healthier lifestyle choices and better socioeconomic status (141), or lower alcohol intake (142). Therefore, a high intake of milk might also reflect other factors involved in development of CRC. In **Paper 4**, we adjusted for socioeconomic status and leisure time physical activity, but cannot rule out residual confounding.

## The Colorectal cancer Dietary Quality Index

In **Paper 4**, we found that high CDQI was inversely associated with CRC. Previous studies examining the association between an overall healthy lifestyle, which included dietary recommendations from WCRF, and CRC have found an association between overall healthy lifestyle and CRC (143, 144). CDQI is a more disease-specific index, than other indexes examining the association between food intake and CRC. CDQI may contribute to the understanding of the complex association between foods, and possibly even to better understand the underlying mechanisms, as it is does not include as many components as in most other indexes.

Even though other studies have investigated the association between the WCRF's conclusions regarding lifestyle factors including diet and CRC, and found an inverse association, we chose to focus on foods which already have shown an association with CRC.

It is important to examine the combined effect of foods, since we do not eat a single food, but several together. Different foods may also interact with each other.

The CDQI indicated a much stronger risk, than any of the included components. It is further important to analyse all the included components of an index, to rule out that the association are not solely dependent on the component's correlation to each other (103, 104). Since intake of fibre, processed meat and dairy products all have shown to be associated with CRC, and very likely represent casual associations (2), they are valid components to insert into an index when examining associations with CRC. Since intake of processed red meat is strongly associated with intake of red meat, we chose to only include processed red meat in the analysis, although WCRF has concluded that there is strong evidence for an association between high intake of red meat and CRC (2).

## Overweight, meat intake and colorectal cancer

In **Paper 3**, we found that presence of overweight did not affect the associations between different intakes of meat. Overweight is a strong risk factor in the aetiology of CRC (40), and presence of overweight might more be associated with an overall high intake of food and low physical activity, than with intake of a specific nutrient or food. Obesity is also a strong risk factor for type 2 diabetes (145), and in **Paper 2**, we found that high blood glucose level was associated with CRC, especially in men. In the MDC cohort, the cases, compared with the non-cases, had a higher BMI and a wider waist, and they also more frequently developed type 2 diabetes. The question still remains if obesity is a factor that causes CRC, mainly via the rise in glucose levels that often occur in obesity, or whether other factors involved in obesity mediates observed associations between obesity and CRC.

## Hyperglycaemia, hyperinsulinemia, and insulin resistance and colorectal cancer

As other studies have found similar results (146) as we in **Paper 2**, between high blood glucose levels and risk of CRC, especially in men, it strengthens our conclusion that there is an association between high blood glucose levels and CRC. There also seems to be a causal effect, as glucose is an important part of the cancer tumour's energy utilisation. Hyperglycaemia in itself is causing inflammation, which in turn causes ROS and induces oxidative stress (66). An association solely in men and not in women might be explained by the difference in glucose metabolism (35).
Even though, we did not find a significant association between high plasma insulin levels and CRC, the risk estimates indicate a risk increase with higher plasma insulin levels, and there are plausible mechanisms that point towards an

association. Insulin has been found to be involved in the stimulation of the cell's proliferation via binding to the IGF-receptors (147). This in turn will raise the levels of circulating IGF-1, which has been found to increase the risk for CRC (148). Other studies have found an association between insulin resistance and CRC, but it has mostly been case-control studies (146).
Insulin resistance is affecting the metabolic pathways for the cell, and over-stimulates the pathway which induces cell mitosis and proliferation (64). In type 2 diabetes, the insulin production from pancreas increases to compensate for high blood glucose and insulin levels. The increased insulin levels are speculated to increase the epithelial cells activity, and thereby their transformation into EMT (149).
In our study, we found that more of the CRC-cases than the non-cases developed diabetes during follow-up, but also had a larger waist circumference. The question still remains whether it is the different components in the metabolic syndrome that causes CRC, or if it is the same mechanisms behind both diseases.
It has also been found that use of Metformin, an anti-diabetic medicine, is associated with decreased risk of CRC (150). Metformin reduces insulin resistance and improves glycaemic control. Unfortunately, we did not have access to information on use of Metformin. It would have been valuable to compare the results after excluding of Metformin users, with the sensitivity analysis excluding incident diabetes.
In **Paper 3**, we applied the knowledge that diabetes is a risk factor in CRC, and performed sensitivity analysis where we excluded individuals with diabetes. It did not affect the results to any major extent, except for that the association between intake of processed meat and CRC in men did not remain significant.

# Methodological considerations

Epidemiological observational studies on diet and disease are subject to several potential errors, such as reverse causation, confounding, and selection error. As randomisation to different exposures seldom is performed, it is difficult to investigate if the association reflects a causal association. One has to consider whether the association is a causal association or made by chance, error or confounding. It is therefore important to review all these options in explaining the association. Causality in epidemiological studies is hard to determine and is a question of judgement based on consistency of results from several epidemiological studies, the strength of association (151), and whether the results from studies on mechanistic levels are consistent with results in epidemiological studies. Choosing the right method of assessing diet is important, as it is key to minimize measurement error. Even though the diet assessment method in the

MDC shows high relative validity and reproducibility, no assessment method is without fault. As there is no golden standard in dietary assessment, the assessment method chosen may have the same errors as the reference method (152, 153). In dietary assessment, the same foods can contribute with different substances that may have positive as well as negative health effects, making it even more difficult to find the causal effect. Health effects can also differ in different populations depending on sex, age and nutritional status. As the exposure in observational studies often is measured only once, the assessment of the exposure is limited.
The MDC study was initiated to evaluate intakes of different foods and their effect on diseases such as cancer. The dietary data is of high relative validity (119, 124), and since it is a large population-based prospective study, we should have been able to minimize selection error and reverse causation. We also had extensive information on potential confounding factors and were able to exclude individuals who reported dietary changes in the past.

## Misclassification and measurement error

Misclassification of outcome could occur since CRC takes a long time to develop. In the TNM-classification, misclassification also might occur due to patient's delay, since the time for the diagnosis can affect the outcome of the classification, without knowing about the length of tumour growth. However, we are not aware of any previous study taking TNM-classification into consideration when examining associations between fibre intake and CRC, as we have done in **Paper 1**.
A selection of population towards a more health conscious population in the MDC than in the source population might have occurred since cancer incidence was higher before baseline examinations in participants in MDC compared with non-participants (154). This might imply that the results cannot be generalised to the source population. As seasonal variation in food intake occur, season is important to take into account when assessing dietary intake (155), to minimise the risk of measurement error.
As the MDC only measured the dietary intake once, one might speculate that the dietary habits might change over time. But as the average age at inclusion of the participants in MDC was 57 years, one might also speculate that the dietary habits were well established. It has been shown that repeated dietary measurements only have a minor influence on observed associations (156). In the MDC, it was possible to single out those who have previously been changing their dietary habits and it was more common to answer yes on the question "Have you substantially changed your food habits in the past due to illness or other reason?" if you were obese (157). In MDC, the individuals with dietary change in the past had most often changed their food habits because of the metabolic syndrome. In addition,

they had higher intakes of several foods considered healthy, than the ones who did not report a dietary change in the past (158). As we performed sensitivity analysis where we excluded individuals with dietary changes in the past, this could decrease error.
Obesity has been found to be associated with under-reporting of energy intake in previous research (159). In MDC, it was more common to be an under-reporter of energy intake if you were obese, than non-obese (157). Under-reporting of energy was defined as having a ratio of energy intake to basal metabolic rate below the 95% confidence interval limits of the calculated physical activity level (160). Misreporting of dietary intake may be affected by what is socially acceptable and can differ depending on sex, education level and awareness of health promotion messages (161). Almost 18% of the women and 12% of the men were classified as under-reporters of energy intake, whereas 2.8% of the women and 3.5% of the men were classified as over-reporters of their energy intake (160).
The Swedish cancer registry is considered to be a reliable registry when it comes to completeness and correctness (162, 163), and the risk of misclassification can therefore be considered as rather low.
We were able to include more cancer cases in **Paper 4** compared with, **Paper 1 and 3**, and this confirmed the results of previous analyses of fibre intake and intake of processed red meat regarding CRC. The results indicated an even clearer association. In addition, no differences were seen with sex when analysing processed meat in **Paper 4**.

## Energy adjustment

As measurement errors are common in dietary assessment, energy adjustment is important to minimise the effect of measurement errors associated with reported dietary intake (164, 165), as total energy intake is positively associated with most nutrients, and as errors tend to be correlated. In addition, absolute intakes are often determined by body size and physical activity. Body size and physical activity may therefore influence the association between food intake and disease, and energy intake may confound an association between food and disease (122). In **Paper 1 and 4**, we addressed this by dividing the food variables with non-alcoholic energy intake and by adding total energy intake to the multivariable statistical model. In **Paper 3**, we addressed this by the residual method (122). The fact that misreporting of energy was found in approximately 20% of the women and 15% of the men in the MDC cohort (160), highlights the importance of energy-adjustment, thus minimizing measurement errors by examining relative intakes.

## Confounding and correlated intakes

Confounding occurs when other factors, than the examined exposure contribute to the outcome. Confounding therefore creates an uncertainty about whether it was the exposure or the confounder which caused the outcome (166). A confounder is associated with both the disease and the exposure, and it is not an effect of the exposure. To handle confounding, one can either use: 1) randomisation, which is a very costly method to use in large epidemiological studies examining dietary exposures; 2) restriction, where you exclude individuals with the confounding factor; 3) matching; or 4) inclusion of the confounder in the statistical model.
It is of importance to adjust for confounding factors in order to avoid error. It has been proposed that there should be at least ten cases per variable adjusted within the model (167, 168), or the model will be influenced largely by random error.
Food intake correlates with energy intake, body composition and physical activity. Intake of different foods may also correlate with each other, and may in analysis create multicollinearity (169). The correlation between the food variables included in the multivariable analysis was examined in this thesis, and correlations between food intakes were found. In **Paper 1 and 3**, we addressed this problem by adjusting for intakes that were highly correlated with each other. In **Paper 2**, we adjusted for blood glucose levels and plasma insulin levels in their respective analysis, as glucose and insulin levels also correlate to each other.
In **Paper 4**, we examined the combined intake of three food components that have shown to be associated with CRC. This was partly because it can be difficult to see the significance of a single food or nutrient intake on disease development, as food intakes may be correlated and different foods or nutrients may interact.

## Residual confounding

Residual confounding is an expression for all the confounding caused by unmeasured confounders, or confounders used in the model but measured inaccurately (166). As information was missing on some known risk factors for CRC in our studies, which could affect both intake and risk of CRC, as for example IBD and family history of CRC (40), we cannot exclude that residual confounding has occurred, even if adjustments for possible confounders and known available risk factors were made. It is also important that the confounding variable is not affected by measurement errors, which are common in all type of self-reported data. In MDC, the variable physical activity, which is self-reported, might for example harbour measurement errors and affect observed estimates.

## Generalisability

Generalisability may be a problem, as only 41% of the eligible population participated (170). However, the participation rate of the MDC is similar or higher compared with other large-scale population-based studies (171), and regarding the generalisability of our results, the sociodemographic structure, weight distribution, and smoking habits were similar among participants in the MDSC and participants in a health survey in Malmö with a higher participation rate (75%) (171). Moreover, although the cancer incidence was somewhat higher among the non-participants, 2.6% of the participants in the MDC developed CRC during the first 15 years of follow-up (154), which is comparable with previously published data from other cohorts (172). Selection error might have occurred. However, internal and relative comparisons give a lesser risk for selection errors, but internal comparison decrease the chance for generalisability.
We cannot rule out detection errors in the study. Individuals with co-morbidity, such as diabetes or high blood pressure, and who eat a certain diet may be more prone to seek care. Their tumour may in that way be discovered sooner, giving the individual ostensibly higher risk. That may also cause some of the differences between men and women, when it comes to seeking health care and giving them a later or earlier diagnosis. Women seem to be more inclined to participate in studies and to contact the health care earlier in the disease process (173).

## Power

In the research program of MDC, power calculations have concluded that sufficient power (80% and $\alpha = 0.05$) was reached after inclusion of 283 cases of cancer, provided a validation coefficient of 0.6 and a "true" risk gradient from 1-3 over quintiles of nutrients. Power may be a problem in some of the stratified analyses, and it cannot be excluded that the analyses of for example some of the stages in the TNM-classification were underpowered due to fewer cases. New studies in larger populations are needed. It would for example be interesting to replicate the borderline association between fibre intake and risk of T-stage 3 and 4. Information on distal and proximal CRC was available in **Paper 4**, but only until 31 December 2008, and was therefore deemed to contribute too few cases for enough power, as we in **Paper 4** had access to cases until 31th December 2014. It would have been valuable to have information about distal and proximal CRC for all cancer cases, since the colon's physiology changes from distal to proximal colon.

# Conclusions

In this population of inhabitants in Malmö, we found different preconditions for associations between food intake and CRC, dependent on sex, meat subtype, fibre source, and the location of the tumour, but not for presence of overweight and diabetes.

We also found that high fibre intake was associated with lower risk for CRC, especially with lower risk for colon cancer in women, and that high intake of fruits and berries was associated with lower risk of CRC in women. Regarding meat intake, we found that high intakes of pork, as well as processed meat, were associated with increased risk of CRC. We also found that high intake of dairy products was inversely associated with CRC. In addition, we found that high fasting blood glucose was associated with higher risk of CRC, especially with colon cancer in men.

Finally, we found that high adherence to a predefined CRC-specific diet quality index, based on WCRF's conclusions regarding diet, was inversely associated with risk of CRC, and gave a stronger association with CRC, than when analysing the components of the CDQI individually.

# Clinical and future perspectives

The interest in foods' ability to cure or to prevent a disease has exploded the last decade among the public, as well as the will to choose foods based on beliefs and not science. The need to clarify the association between food and disease is therefore growing. As not all people react equally on all food intakes, it is important to find the persons most in need of change, and create the possibility to tailor their advice on how to increase their chances of a healthier lifestyle. As the public's knowledge mostly comes from non-scientific sources, and the knowledge of basic nutrition often is low, it is important to consider how you convey the result from your research. It is also important not to base your knowledge on a single study, but letting it guide you to the next one.

A change of dietary habits is not always possible, and a national screening for CRC in Sweden is getting closer. Even though this would probably decrease the incidence of CRC, it is still important to prevent CRC from ever arising. As the incidence of CRC is slowly increasing, together with other lifestyle-related diseases, the importance of a change in dietary habits is more crucial than ever before.

CRC is a disease that develops under a long period of time, and the prospective cohort study contributes with a large amount of knowledge, but there are many challenges in conducting well-designed prospective cohort studies. As diet is a highly complex exposure, which often is self-reported, there will always be difficulties to determine the effect on disease. Hopefully in the future, a more precise method to measure diet will be possible, together with reliable biomarkers for dietary intake. It would be interesting to repeat the analysis with total possible follow-up time, and to repeat the analysis taking into account the effect of survival bias.

This thesis has contributed with knowledge indicating that associations between foods and CRC are not straightforward. Further research in the field is needed to find the subgroups most in need of change in dietary habits and to continue study the links between diet and cancer. Further research connecting diet to type of cancer pathway, facilitating the choices in dietary advice, would take the primary care of CRC a step further. Clinical research on lifestyle changes in targeted groups, in combination with the knowledge of the epigenetic changes that occur within the cancer, might be the future.

# Populärvetenskaplig sammanfattning

## (Summary in Swedish)

Kolorektalcancer kallas på svenska även tjock- och ändtarmscancer. Det är en av våra vanligaste former av cancer, och utvecklas under flera år. Vår livsstil påverkar risken för att utveckla kolorektalcancer, där maten är en viktig del, men även tillstånd som diabetes och fetma.

I den här avhandlingen undersöktes samband mellan matintag, blodsocker, insulin och insulinets effektivitet i kroppen, s.k. insulinresistens, och kolorektalcancer. Vi undersökte även vad som kunde påverka sambanden, som t.ex. kön, var i tarmen cancern uppstår, och om övervikt spelar roll.

För att utföra studierna har vi använt oss av data från en stor befolkningsstudie som utfördes på 1990-talet, Malmö Kost Cancer (MKC). I MKC samlades data in om deltagarnas matintag, deras fysiska aktivitet och socioekonomiska status. Utöver det mättes även vikt och längd. Totalt fullföljde 28 098 individer alla undersökningarna. En mindre del av undersökningsgruppen, ca 6 000 individer, fick genomgå ytterligare undersökningar. Bland annat togs blodprov för att mäta blodsocker och plasmainsulin.

Efter att deltagarna medverkat i de inledande undersökningarna, följdes de under flera år via det svenska cancerregistret för att se om de utvecklat kolorektalcancer. Efter knappt 20 år hade 923 av deltagarna i studien utvecklat kolorektalcancer.

När vi jämförde matvanorna hos de som utvecklat kolorektalcancer, med matvanorna hos de som inte utvecklat kolorektalcancer, fann vi att fiber var kopplat till minskad risk för att utveckla kolorektalcancer, framför allt vad gäller kvinnor och risken att utveckla tjocktarmscancer. Högt intag av fläskkött var kopplat till ökad risk för kolorektalcancer och dessutom verkade män som åt mycket charkuterier ha en ökad risk. Vi fann även en koppling mellan högt intag av mjölkprodukter och minskad risk för kolorektalcancer.

Slutligen fann vi att de som äter mycket fiber och mjölkprodukter, samtidigt som de äter lite charkuterier har en minskad risk att utveckla kolorektalcancer. Vi såg att sambandet var starkare när alla tre kostfaktorerna slogs ihop, än sambandet mellan de enskilda ingående kostfaktorerna och kolorektalcancer. Vi såg även ett fortsatt samband mellan fiberintag och minskad risk för kolorektalcancer och att

sambandet mellan intag av charkuterier och ökad risk för kolorektalcancer förstärktes, när vi hade en längre uppföljningstid och fler cancerfall.
När vi jämförde blodsockervärdena hos de som utvecklat kolorektalcancer, med blodsockervärdena hos de som inte utvecklat kolorektalcancer, fann vi att höga blodsockervärden hos män är kopplat till en högre risk att utveckla kolorektalcancer.

# Slutsats

I denna befolkningsgrupp fann vi olika förutsättningar för samband mellan matintag och kolorektalcancer, som var beroende på kön, hur intagsmönstret för livsmedel och näringsämnen såg ut och var i tarmen cancern uppstod. Vi såg även att höga intag av fiber och mjölkprodukter verkade skydda mot kolorektalcancer, och att höga intag av charkuterier, höga intag av fläskkött och höga blodsockervärden kunde kopplas till ökad risk för kolorektalcancer.

# Acknowledgement

Tack alla deltagare i Malmö Kost Cancerstudien och alla ni som funnits där för mig under mina studier!

Thank you all participants of Malmö Diet and Cancer study and all of you who have been there for me during my studies!

Ett speciellt tack vill jag ge till:

Mina båda handledare **Bodil** och **Ulrika**, utan er hade det inte blivit något!

**Jonas**, för att du alltid kommer med bra kommentarer och stöttat mig i mitt arbete!

**Anders**, för att du alltid bistår mig med bra data och information om den!

**Elisabet**, för att du stöttat mig, både som kollega och chef!

**Micke** och **Malin**, för att jag har fått störa er med mina frågor, och för att ni stört mig med era!

**Camilla**, för all praktisk hjälp och för härliga pratstunder!

# References


1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 Lyon, France: International Agency for Reseach on Cancer; 2013 [cited 2018 29 January]. Available from: http://globocan.iarc.fr.
2. World Cancer Research Fund International, American Insitute for Cancer Reseach. Continuous Update project Report. Diet, Nutrition, Physical Activity, and Colorectal Cancer2017 [cited 2018 29 January]. Available from: http://wcrf.org/colorectal-cancer-2017.
3. Vander A, Sherman J, Luciano D. The digestion and absorption of food. Human physiology, the mechanisms of body funtion, 7th edition. Boston, USA: WCB Mc Graw-Hill; 1998. p. 550-89.
4. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. European journal of cancer. 2013;49(6):1374-403.
5. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010;116(3):544-73.
6. Socialstyrelsens cancerregister. Statistikdatabas för cancer 2017 [cited 2018 29 January]. Available from: http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer.
7. International Agency for Reserch on Cancer, World Helath Organization. Colorectal Cancer. Estimated Incidence, Mortality and Prevalence Worldwide in 2012 Lyon, France: IARC; 2012 [cited 2018 7 February]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx?cancer=colorectal.
8. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. CA Cancer J Clin. 2017;67(3):177-93.
9. Groff RJ, Nash R, Ahnen DJ. Significance of serrated polyps of the colon. Curr Gastroenterol Rep. 2008;10(5):490-8.
10. Alrawi SJ, Schiff M, Carroll RE, Dayton M, Gibbs JF, Kulavlat M, et al. Aberrant crypt foci. Anticancer research. 2006;26(1A):107-19.
11. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138(6):2073-87 e3.
12. Lee GH, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer? - a systematic review. European journal of surgical oncology: the journal of the European Society of

Surgical Oncology and the British Association of Surgical Oncology. 2015;41(3):300-8.

13. Tariq K, Ghias K. Colorectal cancer carcinogenesis: a review of mechanisms. Cancer Biol Med. 2016;13(1):120-35.
14. Koopman M, Kortman GA, Mekenkamp L, Ligtenberg MJ, Hoogerbrugge N, Antonini NF, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. British journal of cancer. 2009;100(2):266-73.
15. Armaghany T, Wilson JD, Chu Q, Mills G. Genetic alterations in colorectal cancer. Gastrointest Cancer Res. 2012;5(1):19-27.
16. Bae JM, Kim JH, Kang GH. Epigenetic alterations in colorectal cancer: the CpG island methylator phenotype. Histol Histopathol. 2013;28(5):585-95.
17. Union for International Cancer Control. TNM classification of Malignany Tumours. 7th ed. Chichester, West Sussex: John Wiley & Sons; 2009.
18. Boland CR. Clinical uses of microsatellite instability testing in colorectal cancer: an ongoing challenge. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2007;25(7):754-6.
19. Regionla cancercentrum i samverkan. Symtom, kliniska fynd och diagnostik. National care program for colorectal cancer. Sweden; 2016 [cited 2018 12 February]. Available from: https://www.cancercentrum.se/samverkan/cancerdiagnoser/tjocktarm-andtarm-och-anal/tjock--och-andtarm/vardprogram/gallande-vardprogram/6.-symtom-kliniska-fynd-och-diagnostik/.
20. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. Nat Rev Dis Primers. 2015;1:15065.
21. Riihimaki M, Hemminki A, Sundquist J, Hemminki K. Patterns of metastasis in colon and rectal cancer. Scientific reports. 2016;6:29765.
22. Bergman O, Hont G, Johansson E. Cancer i siffror [Internet page]. Socialstyrelsen, Cancerfonden; 2013 [cited 2018 February 18]. Available from: http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/19108/2013-6-5.pdf
23. Gatta G, Capocaccia R, Sant M, Bell CM, Coebergh JW, Damhuis RA, et al. Understanding variations in survival for colorectal cancer in Europe: a EUROCARE high resolution study. Gut. 2000;47(4):533-8.
24. Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. The New England journal of medicine. 1993;329(27):1977-81.
25. Blom J, Kilpelainen S, Hultcrantz R, Tornberg S. Five-year experience of organized colorectal cancer screening in a Swedish population - increased compliance with age, female gender, and subsequent screening round. J Med Screen. 2014;21(3):144-50.
26. Regionala cancercentrum i samverkan. Förebyggande och tidig upptäckt av cancer 2017 [cited 2018 18 February]. Available from: https://www.cancercentrum.se/samverkan/vara-uppdrag/prevention-och-tidig-upptackt/studie-tarmcancerscreening/.

27. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr., Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546-58.
28. Kune GA, Bannerman S, Watson LF. Attributable risk for diet, alcohol, and family history in the Melbourne Colorectal Cancer Study. Nutrition and cancer. 1992;18(3):231-5.
29. Erdrich J, Zhang X, Giovannucci E, Willett W. Proportion of colon cancer attributable to lifestyle in a cohort of US women. Cancer causes & control : CCC. 2015;26(9):1271-9.
30. Platz EA, Willett WC, Colditz GA, Rimm EB, Spiegelman D, Giovannucci E. Proportion of colon cancer risk that might be preventable in a cohort of middle-aged US men. Cancer causes & control: CCC. 2000;11(7):579-88.
31. Smith BD, Smith GL, Hurria A, Hortobagyi GN, Buchholz TA. Future of cancer incidence in the United States: burdens upon an aging, changing nation. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2009;27(17):2758-65.
32. Faggiano F, Partanen T, Kogevinas M, Boffetta P. Socioeconomic differences in cancer incidence and mortality. IARC scientific publications. 1997(138):65-176.
33. Doubeni CA, Laiyemo AO, Major JM, Schootman M, Lian M, Park Y, et al. Socioeconomic status and the risk of colorectal cancer: an analysis of more than a half million adults in the National Institutes of Health-AARP Diet and Health Study. Cancer. 2012;118(14):3636-44.
34. Sadik R, Abrahamsson H, Stotzer PO. Gender differences in gut transit shown with a newly developed radiological procedure. Scandinavian journal of gastroenterology. 2003;38(1):36-42.
35. Hall E, Volkov P, Dayeh T, Esguerra JL, Salo S, Eliasson L, et al. Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. Genome Biol. 2014;15(12):522.
36. Rettberg JR, Yao J, Brinton RD. Estrogen: a master regulator of bioenergetic systems in the brain and body. Front Neuroendocrinol. 2014;35(1):8-30.
37. Comitato R, Saba A, Turrini A, Arganini C, Virgili F. Sex hormones and macronutrient metabolism. Crit Rev Food Sci Nutr. 2015;55(2):227-41.
38. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA: the journal of the American Medical Association. 2002;288(3):321-33.
39. Limsui D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, et al. Postmenopausal hormone therapy and colorectal cancer risk by molecularly defined subtypes among older women. Gut. 2012;61(9):1299-305.
40. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. Cancer causes & control : CCC. 2013;24(6):1207-22.
41. Geiersbach KB, Samowitz WS. Microsatellite instability and colorectal cancer. Arch Pathol Lab Med. 2011;135(10):1269-77.

42. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895-2015. Nat Rev Cancer. 2015;15(3):181-94.
43. East JE, Atkin WS, Bateman AC, Clark SK, Dolwani S, Ket SN, et al. British Society of Gastroenterology position statement on serrated polyps in the colon and rectum. Gut. 2017;66(7):1181-96.
44. Khankari NK, Shu XO, Wen W, Kraft P, Lindstrom S, Peters U, et al. Association between Adult Height and Risk of Colorectal, Lung, and Prostate Cancer: Results from Meta-analyses of Prospective Studies and Mendelian Randomization Analyses. PLoS medicine. 2016;13(9):e1002118.
45. Thrift AP, Gong J, Peters U, Chang-Claude J, Rudolph A, Slattery ML, et al. Mendelian randomization study of height and risk of colorectal cancer. International journal of epidemiology. 2015;44(2):662-72.
46. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer. 2013;13(11):759-71.
47. Manzoni M, Mariucci S, Delfanti S, Rovati B, Ronzoni M, Loupakis F, et al. Circulating endothelial cells and their apoptotic fraction are mutually independent predictive biomarkers in Bevacizumab-based treatment for advanced colorectal cancer. Journal of cancer research and clinical oncology. 2012;138(7):1187-96.
48. Kornfeld D, Ekbom A, Ihre T. Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. Gut. 1997;41(4):522-5.
49. Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. Gastroenterology. 2004;126(2):451-9.
50. Risques RA, Lai LA, Himmetoglu C, Ebaee A, Li L, Feng Z, et al. Ulcerative colitis-associated colorectal cancer arises in a field of short telomeres, senescence, and inflammation. Cancer research. 2011;71(5):1669-79.
51. Flossmann E, Rothwell PM, British Doctors Aspirin Trial, the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. The Lancet. 2007;369(9573):1603-13.
52. Nishihara R, Lochhead P, Kuchiba A, Jung S, Yamauchi M, Liao X, et al. Aspirin use and risk of colorectal cancer according to BRAF mutation status. JAMA : the journal of the American Medical Association. 2013;309(24):2563-71.
53. Wang D, Xia D, Dubois RN. The Crosstalk of PTGS2 and EGF Signaling Pathways in Colorectal Cancer. Cancers (Basel). 2011;3(4):3894-908.
54. Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Rafaniello C, et al. Colorectal cancer association with metabolic syndrome and its components: a systematic review with meta-analysis. Endocrine. 2013;44(3):634-47.
55. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. Journal of the National Cancer Institute. 2005;97(22):1679-87.
56. Larsson SC, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. Am J Clin Nutr. 2007;86(3):556-65.

57. Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, et al. Obesity and risk of colorectal cancer: a systematic review of prospective studies. PloS one. 2013;8(1):e53916.
58. Nimptsch K, Pischon T. Body fatness, related biomarkers and cancer risk: an epidemiological perspective. Horm Mol Biol Clin Investig. 2015;22(2):39-51.
59. Riondino S, Roselli M, Palmirotta R, Della-Morte D, Ferroni P, Guadagni F. Obesity and colorectal cancer: role of adipokines in tumor initiation and progression. World journal of gastroenterology: WJG. 2014;20(18):5177-90.
60. Wang L, Cai S, Teng Z, Zhao X, Chen X, Bai X. Insulin therapy contributes to the increased risk of colorectal cancer in diabetes patients: a meta-analysis. Diagnostic pathology. 2013;8:180.
61. Lee W. The CEACAM1 expression is decreased in the liver of severely obese patients with or without diabetes. Diagnostic pathology. 2011;6:40.
62. Yuhara H, Steinmaus C, Cohen SE, Corley DA, Tei Y, Buffler PA. Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? The American journal of gastroenterology. 2011;106(11):1911-21; quiz 22.
63. Coleman MP, Forman D, Bryant H, Butler J, Rachet B, Maringe C, et al. Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995-2007 (the International Cancer Benchmarking Partnership): an analysis of population-based cancer registry data. The Lancet. 2011;377(9760):127-38.
64. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. The Journal of clinical investigation. 2000;105(3):311-20.
65. Yeh CS, Wang JY, Chung FY, Lee SC, Huang MY, Kuo CW, et al. Significance of the glycolytic pathway and glycolysis related-genes in tumorigenesis of human colorectal cancers. Oncology reports. 2008;19(1):81-91.
66. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.
67. Shaw E, Farris MS, Stone CR, Derksen JWG, Johnson R, Hilsden RJ, et al. Effects of physical activity on colorectal cancer risk among family history and body mass index subgroups: a systematic review and meta-analysis. BMC cancer. 2018;18(1):71.
68. Roberts CK, Little JP, Thyfault JP. Modification of insulin sensitivity and glycemic control by activity and exercise. Medicine and science in sports and exercise. 2013;45(10):1868-77.
69. Quadrilatero J, Hoffman-Goetz L. Physical activity and colon cancer. A systematic review of potential mechanisms. J Sports Med Phys Fitness. 2003;43(2):121-38.
70. Douchi T, Yamamoto S, Oki T, Maruta K, Kuwahata R, Yamasaki H, et al. The effects of physical exercise on body fat distribution and bone mineral density in postmenopausal women. Maturitas. 2000;35(1):25-30.
71. McTiernan A, Tworoger SS, Rajan KB, Yasui Y, Sorenson B, Ulrich CM, et al. Effect of exercise on serum androgens in postmenopausal women: a 12-month randomized clinical trial. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(7):1099-105.

72. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA: the journal of the American Medical Association. 2008;300(23):2765-78.
73. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer. 2003;3(10):733-44.
74. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, et al. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2011;22(9):1958-72.
75. Roswall N, Weiderpass E. Alcohol as a risk factor for cancer: existing evidence in a global perspective. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2015;48(1):1-9.
76. Sieri S, Krogh V, Saieva C, Grobbee DE, Bergmann M, Rohrmann S, et al. Alcohol consumption patterns, diet and body weight in 10 European countries. Eur J Clin Nutr. 2009;63 Suppl 4:S81-100.
77. Rittmueller SE, Corriveau A, Sharma S. Dietary quality and adequacy among Aboriginal alcohol consumers in the Northwest Territories, Canada. Int J Circumpolar Health. 2012;71:17341.
78. Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. Nat Rev Cancer. 2007;7(8):599-612.
79. Nishihara R, Wang M, Qian ZR, Baba Y, Yamauchi M, Mima K, et al. Alcohol, one-carbon nutrient intake, and risk of colorectal cancer according to tumor methylation level of IGF2 differentially methylated region. Am J Clin Nutr. 2014;100(6):1479-88.
80. Aune D, Lau R, Chan DS, Vieira R, Greenwood DC, Kampman E, et al. Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies. Gastroenterology. 2011;141(1):106-18.
81. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am J Clin Nutr. 2003;78(3 Suppl):559S-69S.
82. Raskov H, Burcharth J, Pommergaard HC. Linking Gut Microbiota to Colorectal Cancer. J Cancer. 2017;8(17):3378-95.
83. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. Gastroenterology. 2010;138(6):2029.
84. Yun J, Mullarky E, Lu C, Bosch KN, Kavalier A, Rivera K, et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science. 2015;350(6266):1391-6.
85. Keum N, Aune D, Greenwood DC, Ju W, Giovannucci EL. Calcium intake and colorectal cancer risk: dose-response meta-analysis of prospective observational studies. Int J Cancer. 2014;135(8):1940-8.
86. Buset M, Lipkin M, Winawer S, Swaroop S, Friedman E. Inhibition of human colonic epithelial cell proliferation in vivo and in vitro by calcium. Cancer research. 1986;46(10):5426-30.

87. Wargovich MJ, Eng VW, Newmark HL. Calcium inhibits the damaging and compensatory proliferative effects of fatty acids on mouse colon epithelium. Cancer Lett. 1984;23(3):253-8.
88. Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. Eur J Clin Nutr. 2003;57(1):1-17.
89. Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, et al. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. PloS one. 2011;6(6):e20456.
90. Bjerselius R, Konde ÅB, Färnstrand JS. Konsumtion av rött kött och charkuteriprodukter och samband med tjock- och ändtarmscancer av - risk- och nyttohanteringsrapport Uppsala, Sweden: Livsmedelsverket; 2014 [cited 2018 12 March]. Available from: http://www.livsmedelsverket.se/globalassets/matvanor-halsa-miljo/kostrad-matvanor/rott-kott-och-chark/livsmedelsverket.-konsumtion-av-rott-kott-och-charkuteriprodukter-och-samband-med-tjock--och-andtarmscancer.-rapport-20-2014.pdf?id=3573.
91. Livsmedelsverket. Riksmaten - vuxna 2010-11: Vad äter svenskarna? - Livsmedels- och näringsintag bland vuxna i Sverige. Uppsala, Sweden: Livsmedelsverket; 2012 [cited 2018 27 March]. Available from: http://www.slv.se/upload/dokument/rapporter/mat_naring/2012/riksmaten_2010_2011_kortversion.pdf.
92. Gueraud F, Tache S, Steghens JP, Milkovic L, Borovic-Sunjic S, Zarkovic N, et al. Dietary polyunsaturated fatty acids and heme iron induce oxidative stress biomarkers and a cancer promoting environment in the colon of rats. Free radical biology & medicine. 2015;83:192-200.
93. Bresgen N, Eckl PM. Oxidative stress and the homeodynamics of iron metabolism. Biomolecules. 2015;5(2):808-47.
94. Narisawa T, Magadia NE, Weisburger JH, Wynder EL. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. Journal of the National Cancer Institute. 1974;53(4):1093-7.
95. Chomchai C, Bhadrachari N, Nigro ND. The effect of bile on the induction of experimental intestinal tumors in rats. Diseases of the colon and rectum. 1974;17(3):310-2.
96. Knize MG, Salmon CP, Pais P, Felton JS. Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Advances in experimental medicine and biology. 1999;459:179-93.
97. Nagao M, Wakabayashi K, Ushijima T, Toyota M, Totsuka Y, Sugimura T. Human exposure to carcinogenic heterocyclic amines and their mutational fingerprints in experimental animals. Environ Health Perspect. 1996;104 Suppl 3:497-501.
98. NNR 2012, Sodium as salt, in Nordic Nutrition Recommendations 2012. In: Nordic council of ministers, editor. 2014. p. 515-34.
99. Kune GA, Kune S, Watson LF. Dietary sodium and potassium intake and colorectal cancer risk. Nutrition and cancer. 1989;12(4):351-9.

100. Wu S, Feng B, Li K, Zhu X, Liang S, Liu X, et al. Fish consumption and colorectal cancer risk in humans: a systematic review and meta-analysis. The American journal of medicine. 2012;125(6):551-9 e5.
101. Moukayed M, Grant WB. Molecular link between vitamin D and cancer prevention. Nutrients. 2013;5(10):3993-4021.
102. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br J Clin Pharmacol. 2013;75(3):645-62.
103. Willett W. Nutritional epidemiology. New York: Oxford university press; 2013. p. 305-32.
104. Jacobs DR, Jr., Tapsell LC. Food, not nutrients, is the fundamental unit in nutrition. Nutrition reviews. 2007;65(10):439-50.
105. Steck SE, Guinter M, Zheng J, Thomson CA. Index-based dietary patterns and colorectal cancer risk: a systematic review. Adv Nutr. 2015;6(6):763-73.
106. Fung TT, Hu FB, Wu K, Chiuve SE, Fuchs CS, Giovannucci E. The Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets and colorectal cancer. Am J Clin Nutr. 2010;92(6):1429-35.
107. Bamia C, Lagiou P, Buckland G, Grioni S, Agnoli C, Taylor AJ, et al. Mediterranean diet and colorectal cancer risk: results from a European cohort. European journal of epidemiology. 2013;28(4):317-28.
108. Reedy J, Wirfalt E, Flood A, Mitrou PN, Krebs-Smith SM, Kipnis V, et al. Comparing 3 dietary pattern methods--cluster analysis, factor analysis, and index analysis--With colorectal cancer risk: The NIH-AARP Diet and Health Study. American journal of epidemiology. 2010;171(4):479-87.
109. Shivappa N, Prizment AE, Blair CK, Jacobs DR, Jr., Steck SE, Hebert JR. Dietary inflammatory index and risk of colorectal cancer in the Iowa Women's Health Study. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2014;23(11):2383-92.
110. Tabung FK, Steck SE, Ma Y, Liese AD, Zhang J, Caan B, et al. The association between dietary inflammatory index and risk of colorectal cancer among postmenopausal women: results from the Women's Health Initiative. Cancer causes & control: CCC. 2015;26(3):399-408.
111. Wirth MD, Shivappa N, Steck SE, Hurley TG, Hebert JR. The dietary inflammatory index is associated with colorectal cancer in the National Institutes of Health-American Association of Retired Persons Diet and Health Study. The British journal of nutrition. 2015;113(11):1819-27.
112. Jarvandi S, Davidson NO, Schootman M. Increased risk of colorectal cancer in type 2 diabetes is independent of diet quality. PloS one. 2013;8(9):e74616.
113. Moskal A, Freisling H, Byrnes G, Assi N, Fahey MT, Jenab M, et al. Main nutrient patterns and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition study. British journal of cancer. 2016;115(11):1430-40.
114. Shin S, Saito E, Sawada N, Ishihara J, Takachi R, Nanri A, et al. Dietary patterns and colorectal cancer risk in middle-aged adults: A large population-based prospective cohort study. Clin Nutr. 2017.

115. Mehta RS, Song M, Nishihara R, Drew DA, Wu K, Qian ZR, et al. Dietary Patterns and Risk of Colorectal Cancer: Analysis by Tumor Location and Molecular Subtypes. Gastroenterology. 2017;152(8):1944-53 e1.
116. Park Y, Lee J, Oh JH, Shin A, Kim J. Dietary patterns and colorectal cancer risk in a Korean population: A case-control study. Medicine (Baltimore). 2016;95(25):e3759.
117. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. Journal of internal medicine. 1993;233(1):45-51.
118. Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. Diabet Med. 2000;17(4):299-307.
119. Wirfalt E, Mattisson I, Johansson U, Gullberg B, Wallstrom P, Berglund G. A methodological report from the Malmo Diet and Cancer study: development and evaluation of altered routines in dietary data processing. Nutrition journal. 2002;1:3.
120. Livsmedelsverket. Livsmedelsverkets livsmedelsdatabas version 2015-05-28. 2015 [cited 2018 11 March]. Available from: http://www7.slv.se/SokNaringsinnehall/.
121. Livsmedelsverket. Vikttabell. Uppsala: the Swedish national Food Adminitration; 1999.
122. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. American journal of epidemiology. 1986;124(1):17-27.
123. Riboli E, Elmstahl S, Saracci R, Gullberg B, Lindgarde F. The Malmo Food Study: validity of two dietary assessment methods for measuring nutrient intake. International journal of epidemiology. 1997;26 Suppl 1:S161-73.
124. Elmstahl S, Riboli E, Lindgarde F, Gullberg B, Saracci R. The Malmo Food Study: the relative validity of a modified diet history method and an extensive food frequency questionnaire for measuring food intake. Eur J Clin Nutr. 1996;50(3):143-51.
125. Li C, Aronsson CA, Hedblad B, Gullberg B, Wirfalt E, Berglund G. Ability of physical activity measurements to assess health-related risks. Eur J Clin Nutr. 2009;63(12):1448-51.
126. Diabetes Trial Unit, the Oxford Centre for Diabetes, Endocrinology and metabolism. 2016 HOMA2 calculator Oxford, UK: The University of Oxford; 2016 [cited 2017 22 October].
127. Wangefjord S, Manjer J, Gaber A, Nodin B, Eberhard J, Jirstrom K. Cyclin D1 expression in colorectal cancer is a favorable prognostic factor in men but not in women in a prospective, population-based cohort study. Biol Sex Differ. 2011;2:10.
128. Margetts BM, Nelson, M. Design Concepts in Nutritional Epidemiology. Norfolk: Oxford University Press, printed by Biddles LTd King's Lynn. 1997.
129. Braniste V, Leveque M, Buisson-Brenac C, Bueno L, Fioramonti J, Houdeau E. Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. The Journal of physiology. 2009;587(Pt 13):3317-28.

130. Vrachnis N, Zygouris D, Iliodromiti Z, Daniilidis A, Valsamakis G, Kalantaridou S. Probing the impact of sex steroids and menopause-related sex steroid deprivation on modulation of immune senescence. Maturitas. 2014;78(3):174-8.
131. Nordic co-operation. Nordic Nutrition Recommendations 2012. Part 1: Summary, principles and use In: Nordic council of ministers, editors. Oct 03, 2013 ed. Copenhagen2012.
132. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutrition research reviews. 2010;23(1):65-134.
133. Azcarate-Peril MA, Sikes M, Bruno-Barcena JM. The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer? American journal of physiology Gastrointestinal and liver physiology. 2011;301(3):G401-24.
134. Kyro C, Skeie G, Dragsted LO, Christensen J, Overvad K, Hallmans G, et al. Intake of whole grain in Scandinavia: intake, sources and compliance with new national recommendations. Scandinavian journal of public health. 2012;40(1):76-84.
135. Koh-Banerjee P, Franz M, Sampson L, Liu S, Jacobs DR, Jr., Spiegelman D, et al. Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. Am J Clin Nutr. 2004;80(5):1237-45.
136. Duthie SJ. Berry phytochemicals, genomic stability and cancer: evidence for chemoprotection at several stages in the carcinogenic process. Molecular nutrition & food research. 2007;51(6):665-74.
137. Agudo A, Slimani N, Ocke MC, Naska A, Miller AB, Kroke A, et al. Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. Public health nutrition. 2002;5(6B):1179-96.
138. Murphy N, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, et al. Consumption of dairy products and colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). PloS one. 2013;8(9):e72715.
139. O'Sullivan TA, Bremner AP, Bremer HK, Seares ME, Beilin LJ, Mori TA, et al. Dairy product consumption, dietary nutrient and energy density and associations with obesity in Australian adolescents. J Hum Nutr Diet. 2015;28(5):452-64.
140. Feeney EL, O'Sullivan A, Nugent AP, McNulty B, Walton J, Flynn A, et al. Patterns of dairy food intake, body composition and markers of metabolic health in Ireland: results from the National Adult Nutrition Survey. Nutr Diabetes. 2017;7(2):e243.
141. Fahlman MM, McCaughtry N, Martin J, Shen B. Racial and socioeconomic disparities in nutrition behaviors: targeted interventions needed. J Nutr Educ Behav. 2010;42(1):10-6.
142. Kesse E, Clavel-Chapelon F, Slimani N, van Liere M, Group EN. Do eating habits differ according to alcohol consumption? Results of a study of the French cohort of the European Prospective Investigation into Cancer and Nutrition (E3N-EPIC). Am J Clin Nutr. 2001;74(3):322-7.
143. Turati F, Bravi F, Di Maso M, Bosetti C, Polesel J, Serraino D, et al. Adherence to the World Cancer Research Fund/American Institute for Cancer Research

recommendations and colorectal cancer risk. European journal of cancer. 2017;85:86-94.

144. Romaguera D, Vergnaud AC, Peeters PH, van Gils CH, Chan DS, Ferrari P, et al. Is concordance with World Cancer Research Fund/American Institute for Cancer Research guidelines for cancer prevention related to subsequent risk of cancer? Results from the EPIC study. Am J Clin Nutr. 2012;96(1):150-63.
145. Abdullah A, Peeters A, de Courten M, Stoelwinder J. The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies. Diabetes Res Clin Pract. 2010;89(3):309-19.
146. Xu J, Ye Y, Wu H, Duerksen-Hughes P, Zhang H, Li P, et al. Association between markers of glucose metabolism and risk of colorectal cancer. BMJ open. 2016;6(6):e011430.
147. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. Journal of the National Cancer Institute. 2002;94(13):972-80.
148. Chi F, Wu R, Zeng YC, Xing R, Liu Y. Circulation insulin-like growth factor peptides and colorectal cancer risk: an updated systematic review and meta-analysis. Molecular biology reports. 2013;40(5):3583-90.
149. Ezuka A, Sakai E, Kawana K, Nagase H, Kakuta Y, Uchiyama S, et al. Association between factors associated with colorectal cancer and rectal aberrant crypt foci in humans. Oncology letters. 2015;10(6):3689-95.
150. Cardel M, Jensen SM, Pottegard A, Jorgensen TL, Hallas J. Long-term use of metformin and colorectal cancer risk in type II diabetics: a population-based case-control study. Cancer medicine. 2014;3(5):1458-66.
151. Potischman N, Weed DL. Causal criteria in nutritional epidemiology. Am J Clin Nutr. 1999;69(6):1309S-14S.
152. Day N, McKeown N, Wong M, Welch A, Bingham S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. International journal of epidemiology. 2001;30(2):309-17.
153. Kipnis V, Freedman LS. Impact of exposure measurement error in nutritional epidemiology. Journal of the National Cancer Institute. 2008;100(23):1658-9.
154. Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, et al. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation. 2001;10(6):489-99.
155. Subar AF, Frey CM, Harlan LC, Kahle L. Differences in reported food frequency by season of questionnaire administration: the 1987 National Health Interview Survey. Epidemiology. 1994;5(2):226-33.
156. Goldbohm RA, van 't Veer P, van den Brandt PA, van 't Hof MA, Brants HA, Sturmans F, et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. Eur J Clin Nutr. 1995;49(6):420-9.

157. Sonestedt E, Wirfalt E, Gullberg B, Berglund G. Past food habit change is related to obesity, lifestyle and socio-economic factors in the Malmo Diet and Cancer Cohort. Public health nutrition. 2005;8(7):876-85.
158. Sonestedt E, Gullberg B, Wirfalt E. Both food habit change in the past and obesity status may influence the association between dietary factors and postmenopausal breast cancer. Public health nutrition. 2007;10(8):769-79.
159. Lissner L. Measuring food intake in studies of obesity. Public health nutrition. 2002;5(6A):889-92.
160. Mattisson I, Wirfalt E, Aronsson CA, Wallstrom P, Sonestedt E, Gullberg B, et al. Misreporting of energy: prevalence, characteristics of misreporters and influence on observed risk estimates in the Malmo Diet and Cancer cohort. The British journal of nutrition. 2005;94(5):832-42.
161. Maurer J, Taren DL, Teixeira PJ, Thomson CA, Lohman TG, Going SB, et al. The psychosocial and behavioral characteristics related to energy misreporting. Nutrition reviews. 2006;64(2 Pt 1):53-66.
162. Pukkala E, Engholm G, Hojsgaard Schmidt LK, Storm H, Khan S, Lambe M, et al. Nordic Cancer Registries - an overview of their procedures and data comparability. Acta Oncol. 2017:1-16.
163. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27-33.
164. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997;65(4 Suppl):1220S-8S; discussion 9S-31S.
165. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing with dietary measurement error in nutritional cohort studies. Journal of the National Cancer Institute. 2011;103(14):1086-92.
166. Rothman KJ. Epidemiology: An introduktion. 2nd ed. USA: Oxford university press; 2012.
167. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995;48(12):1503-10.
168. Concato J, Peduzzi P, Holford TR, Feinstein AR. Importance of events per independent variable in proportional hazards analysis. I. Background, goals, and general strategy. J Clin Epidemiol. 1995;48(12):1495-501.
169. Katz MH. Relationships of independent variables to one another. Multivariable analysis: A practical guide for clinicians and public health researchers. New York, USA: Cambridge university press; 2011. p. 88-92.
170. Manjer J, Elmstahl S, Janzon L, Berglund G. Invitation to a population-based cohort study: differences between subjects recruited using various strategies. Scandinavian journal of public health. 2002;30(2):103-12.
171. Boeing H, Korfmann A, Bergmann MM. Recruitment procedures of EPIC-Germany. European Investigation into Cancer and Nutrition. Annals of nutrition & metabolism. 1999;43(4):205-15.

172. Howlader N, Noone A, Krapcho M, Garshell J, Miller D, Altekruse S, et al. SEER Cancer Statistics Review, 1975–2011 Bethesda: National Cancer Institute; 2014 [cited 2018 17 March]. Available from: http://seer.cancer.gov/archive/csr/1975_2011/results_merged/sect_06_colon_rectum.pdf.
173. Briscoe ME. Why do people go to the doctor? Sex differences in the correlates of GP consultation. Social science & medicine. 1987;25(5):507-13.

# Paper 1

*British Journal of Nutrition*, page 1 of 11
doi:10.1017/S0007114515002743


# Fibre intake and incident colorectal cancer depending on fibre source, sex, tumour location and Tumour, Node, Metastasis stage

Alexandra Vulcan[1]*, Jenny Brändstedt[2], Jonas Manjer[3], Karin Jirström[4], Bodil Ohlsson[5] and Ulrika Ericson[6]

[1]*Department of Clinical Sciences, Division of Gastroenterology, Lund University, Skåne University Hospital, Malmö, Sweden*
[2]*Department of Clinical Sciences, Division of Surgery, Lund University, Skåne University Hospital, Malmö, Sweden*
[3]*Department of Clinical Sciences, Division of Plastic Surgery, Lund University, Skåne University Hospital, Malmö, Sweden*
[4]*Department of Clinical Sciences, Division of Pathology, Lund University, Skåne University Hospital, Lund, Sweden*
[5]*Department of Clinical Sciences, Division of Internal Medicine, Lund University, Skåne University Hospital, Malmö, Sweden*
[6]*Diabetes and Cardiovascular Disease, Genetic Epidemiology, Lund University, Skåne University Hospital, Malmö, Sweden*

*(Submitted 12 December 2014 – Final revision received 18 June 2015 – Accepted 25 June 2015)*

## Abstract

Studies on fibre intake and incident colorectal cancer (CRC) indicate inverse associations. Differences by tumour stage have not been examined. We examined associations between fibre intake and its sources, and incidental CRC. Separate analyses were carried out on the basis of sex, tumour location and the Tumour, Node, Metastasis (TNM) classification. The Malmö Diet and Cancer Study is a population-based cohort study, including individuals aged 45–74 years. Dietary data were collected through a modified diet history method. The TNM classification was obtained from pathology/clinical records and re-evaluated. Among 27 931 individuals (60 % women), we found 728 incident CRC cases during 428 924 person-years of follow-up. Fibre intake was inversely associated with CRC risk ($P_{trend}=0{\cdot}026$). Concerning colon cancer, we observed borderline interaction between fibre intake and sex ($P=0{\cdot}052$) and significant protective association restricted to women ($P_{trend}=0{\cdot}013$). Intake of fruits and berries was inversely associated with colon cancer in women ($P_{trend}=0{\cdot}022$). We also observed significant interactions between intakes of fibre ($P=0{\cdot}048$) and vegetables ($P=0{\cdot}039$) and sex on rectal cancer, but no significant associations were seen between intake of fibre, or its sources, in either of the sexes. Except for inverse associations between intake of fibre-rich cereal products and N0- and M0-tumours, we did not observe significant associations with different TNM stages. Our findings suggest different associations between fibre intake and CRC depending on sex, tumour site and fibre source. High fibre intake, especially from fruits and berries, may, above all, prevent tumour development in the colon in women. No clear differences by TNM classification were detected.

**Key words: Colorectal cancer: Fibre: Sex: Tumour, Node, Metastasis classification: Malmö Diet and Cancer Study**

Colorectal cancer (CRC) is estimated to be one of the most common forms of cancer in the western world[1,2]. In Sweden, it is the fourth most common form of cancer and constitutes >7 % of the cancer cases[3]. The results from epidemiological studies are not wholly consistent, but most epidemiological studies indicate inverse association between fibre intake and incidental CRC[4]. For example, The European Prospective Investigation into Cancer and Nutrition (EPIC) found the risk of getting CRC to be 17 % lower at a high fibre intake[5]. The World Cancer Research Fund (WCRF) has, together with the American Institute for Cancer Research, concluded that there is convincing evidence that high intake of fibre, and its sources, is associated with a lower risk for CRC[4].

There may be several potential mechanisms behind these observations[6]. The protective effect of fibre on CRC development has been considered to be the fibre's effect on modulation of colonic transit time, alteration of bile acid metabolism or increase in the production of SCFA[7]. It is under debate whether it is mainly total fibre intake that may be of benefit, or subtypes of fibre in specific fibre-rich food sources such as vegetables, fruits, berries or fibre-rich cereal products. Besides fibre, other bioactive components present in fibre-rich foods may have contributed to previously observed associations between fibre intake and CRC. In a large meta-analysis, where higher fibre intake was associated with reduced risk for CRC, the strongest association was seen with whole grain intake, but no evidence of associations of fruit and vegetable fibre was seen[8]. Similar findings regarding whole grain were reported by the WCRF, but they also stated that findings regarding fruit and vegetable fibre were in the same direction,

**Abbreviations:** CRC, colorectal cancer; HR, hazard ratio; MDCS, Malmö Diet and Cancer Study; TNM, Tumour, Node, Metastasis classification.

* **Corresponding author:** A. Vulcan, fax +46 40 337 489, email alexandra.vulcan@med.lu.se

British Journal of Nutrition

British Journal of Nutrition

although they did not reach statistical significance[4]. In line with this, high intake of fruit and vegetables has been associated with decreased risk of CRC[9].

Previous observations have indicated that associations with fibre from fruits and vegetables may differ depending on tumour site[5]. In addition, and similar to observations regarding anthropometric risk factors for CRC, the importance of fibre intake may also vary by different clinical tumour stages[10]. The association between dietary intake and different stages of CRC has, to our knowledge, not previously been examined.

The objective of the present study was to examine the association between fibre intake and its sources, and incidental CRC, and whether the association differs depending on sex, tumour location and the Tumour, Node, Metastasis (TNM) classification.

## Methods

The study was approved by the ethical committee at Lund University (50.91, 2013/803).

### *Participants*

The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study in Malmö, Sweden. All men and women living in Malmö between 1991 and 1996, born between 1923 and 1950, were invited to participate. Altogether, 28 098 participants (40 %) completed all of the baseline examinations after having given their written, informed consent. Of them, 167 had CRC before or at baseline examination, and were therefore excluded from the study.

### *Data collection*

At baseline, the participants were asked to fill out questionnaires on socioeconomic, lifestyle and dietary factors. They also registered their cooked meals and underwent a diet history interview. Weight was measured using a balance-beam scale, with subjects wearing only light clothes and no shoes. Height was measured using a fixed stadiometer, calibrated in centimetres. Waist circumference was measured midway between the iliac crest and the lowest rib margin. Body composition was estimated with a single-frequency bio-impedance methodology (BIA 103; RJL Systems). Body fat percentage was calculated using an algorithm provided by the manufacturer.

### *Dietary data*

Dietary data were gathered through a modified diet history method with a 7-d menu book for registration of meals that varied from day to day, most likely lunch and dinner, cold beverages and nutrient supplements. In addition, the participants were given a 168-item questionnaire for assessment of consumption frequencies and portion sizes of foods that were not covered in the menu book. Finally, a 45-min interview completed the dietary assessment.

The diet analyses were adjusted for the variables called 'method version' and 'season'. Method version was used because of altered coding routines of dietary data introduced in September 1994 in order to shorten the interview time (from 1 h to 45 min). This resulted in two slightly different method versions, before and after September 1994, but did not have any major influence on the ranking of individuals[11]. The variable season was divided into spring, summer, autumn and winter depending on when in the year the baseline examination was executed. Dietary change in the past (yes, no) was based on the question 'Have you substantially changed your eating habits because of illness or some other reasons?' The relative validity of the MDCS method was evaluated in the Malmö Food study 1984–1985[12,13]. The Pearson correlation coefficients, adjusted for total energy, between the reference method and the MDCS method were 0·53/0·54 (proteins), 0·69/0·64 (fats), 0·70/0·74 (carbohydrates), 0·69/0·74 (fibres), 0·58/0·50 (breads), 0·73/0·74 (cereals), 0·24/0·35 (rice and pastas), 0·77/0·60 (fruits) and 0·53/0·65 (vegetables) in women and men, respectively.

We used the following variables for daily nutrient intake in this study: total energy (MJ), non-alcoholic energy (MJ), carbohydrates (percentage of energy (En%)), fat (En%), protein (En%), fibre (g/MJ), fibre (g), vitamin D (μg), Ca (mg) and folate (mg). The following daily intakes of foods were examined: vegetables (g/MJ), fruits and berries (g/MJ), fibre-rich cereal products (portions of fibre-rich bread and breakfast cereals/MJ) and red meat (g).

Portions, instead of grams, were used to analyse the sum of fibre-rich cereal products because of different water content and because they usually are consumed in different weights. Standard portion sizes from the National Food Agency in Sweden were used: fibre-rich soft bread (50 g/portion), fibre-rich crisp bread (30 g/portion) and fibre-rich breakfast cereals (25 g/portion). Energy-adjusted variables were calculated by dividing dietary intakes by non-alcohol energy intake. Quintiles of the dietary variables were used as exposure categories.

### *Cancer cases*

We identified 728 cases of CRC from the Swedish Cancer Registry, of which 463 were colon cancer and 265 were rectal cancer, during 428 924 person-years of follow-up. Follow-up time was defined as the time from date of enrolment until the date of CRC diagnosis, death, migration or end of follow-up (December 2010), whichever came first. The mean duration of follow-up was 15·4 years.

### *Tumour characteristics*

Classification of the CRC cases was done by examining clinical and/or pathology records. In addition, the histopathological examination was re-evaluated by a senior pathologist. Colorectal tumours were classified according to the TNM system. The method used to identify the tumour characteristics of CRC has been described elsewhere and the clinicopathological characteristics did not differ between CRC tumours in the MDCS and those in the EPIC cohort[14]. The cases identified, until the end of 2008, were examined and gave a total of 635 cases of CRC. Of them,

British Journal of Nutrition

363 were colonic cancers and 272 were rectal cancers. Of the cancer cases where cancer staging was possible, 113 were tumour (T)-stage 1 and 2, 405 were T-stage 3 and 4, 292 were node-negative (N0) disease, 193 were lymph node positive (N1 and N2) disease, 451 were non-metastatic (M0) disease and 116 were metastatic (M1) disease. Mean follow-up was 13·7 years for the classified cancer cases.

### Other variables

Age was obtained from personal identification numbers. Smokers were divided into three categories: current smokers, ex-smokers and non-smokers. Irregular smoking was defined as current smoking. Physical activity was estimated by asking the subjects to estimate how many minutes per week they spent on seventeen different activities. The duration was multiplied with an activity-specific intensity coefficient, and an overall leisure-time physical activity score was created. The individuals were then divided into quintiles. The level of education was divided into four different categories: ≤8 years, 9–10 years or 11–13 years of education, and university degree. Alcohol intake was divided into four categories: zero, <15 g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, and >30 g/d for women and >40 g/d for men. The BMI was calculated from measured weight and length. Current use of menopausal hormonal replacement therapy (MHT) was divided into non-users and users. Regular use of non-steroid anti-inflammatory drugs (NSAID) was divided into users and non-users.

### Statistical analyses

The SPSS statistics (version 22; IBM Corporation) was used for all statistical analyses. Food variables – that is, vegetables, fruits and berries, and fibre-rich cereal products – were log-transformed (e-log) to normalise the distribution before analysis. A very small amount (0·0001) was added before transformation, to handle zero intakes. The general linear model was used when examining baseline continuous characteristics in the different fibre quintiles, and adjustments were made for age, method version and season. The $\chi^2$ test was used for categorical variables.

When examining baseline characteristics in cases and non-cases, a general linear model was used for the continuous variables and adjustments were made for age and sex when applicable. Additional adjustments for method version and season were made when dietary variables were examined. The $\chi^2$ test was used for categorical variables. The Cox proportional hazard regression model was used when estimating hazard ratios (HR) of incident CRC, colon and rectal cancer, and TNM stages, depending on energy-adjusted quintiles of fibre, vegetables, fruit and berries and fibre-rich cereal products. The basic model was adjusted for age, sex (when applicable), season, method version and total energy intake. The full model was additionally adjusted for level of education, smoking, alcohol intake, physical activity, BMI and current use of MHT, when appropriate. These covariates were identified from the literature and indicated potential confounding due to their association with CRC. We also performed the multivariate model excluding BMI, as it might be an intermediate between dietary habits and disease. Finally, we made additional adjustments for regular use of NSAID, and for intakes of folate, red meat, vitamin D or Ca. Years of follow-up were used as the underlying time variable. A test for interaction between sex and fibre or food component with regard to CRC incidence was performed by adding a multiplicative variable (sex × diet quintile (treated as continuous variables)) to the full model. In sensitivity analyses, individuals with a reported dietary change in the past or prevalent cancer (except cervix cancer *in situ*) were excluded. All tests were two-sided, and statistical significance was assumed at $P < 0·05$.

## Results

### Baseline characteristics

Altogether, 16 944 women (60·7 %) and 10 987 men completed the baseline examinations. Age and reported intake of protein, carbohydrates and folate increased with higher energy-adjusted fibre intake, whereas intakes of fat, vitamin D and alcohol decreased. The men had a higher BMI than the women, but the mean BMI indicated overweight for both sexes. The men also had a higher fibre intake, but when energy-adjusted the women had a higher intake. There were fewer smokers, and more individuals with higher levels of education and physical activity in the highest fibre quintile compared with the lowest (online Supplementary Table S1).

The cases were, compared with non-cases, older and had a higher BMI and a wider waist. Fewer cases had high education, but a higher percentage of cases had a high physical activity level, compared with non-cases. Current use of MHT was less common among cases than among non-cases (Table 1).

### Dietary intake and colorectal cancer

The different statistical models resulted in very similar findings, and therefore only the full multivariate model was chosen when presenting data. High fibre intake was associated with a lower incidence of CRC (HR: 0·72 for highest compared with lowest quintile; 95 % CI 0·55, 0·94; $P$ for trend = 0·026) (Table 2). Of the foods high in fibre, vegetable intake was associated with a lower incidence of CRC (HR: 0·83 for highest compared with lowest quintile; 95 % CI 0·64, 1·07; $P$ for trend = 0·048). Intakes of fruits and berries or fibre-rich cereal products were not significantly associated with a lower incidence of CRC. Additional adjustments for current use of NSAID and for intakes of folate, red meat, vitamin D or Ca did not change the outcome (data not shown).

In separate analyses depending on tumour location, fibre intake was inversely associated with incidence of colon cancer in women (HR: 0·51 for highest compared with lowest quintile; 95 % CI 0·31, 0·75; $P$ for trend = 0·013), but not in men ($P$ for trend = 0·69), and we observed a borderline interaction between sex and fibre intake ($P = 0·052$) (Table 3). In addition, women with high intake of fruits and berries had a significantly decreased risk for colon cancer (HR: 0·62 for highest compared with lowest quintile; 95 % CI 0·37, 0·98; $P$ for trend = 0·022). No tendency of protective association was seen in men

**Table 1.** Baseline characteristics of cases and non-cases of incident colorectal cancer in the Malmö Diet and Cancer Study cohort (Mean values and standard deviations or percentages)

| | | Non-cases (*n* 27 203) | | Cases (*n* 728) | | | Non-cases women (*n* 16 572) | | Cases women (*n* 372) | | | Non-cases men (*n* 10 631) | | Cases men (*n* 356) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | SD | Mean | SD | *P** | Mean | SD | Mean | SD | *P** | Mean | SD | Mean | SD | *P** |
| Age (years) | 27 931 | 58·0 | 7·6 | 61·4 | 7·0 | <0·001 | 57·3 | 7·9 | 61·7 | 7·1 | 0·001 | 59·1 | 7·0 | 61·1 | 6·8 | 0·001 |
| BMI (kg/m$^2$) | 27 886 | 25·7 | 4·0 | 26·4 | 4·0 | 0·014 | 25·4 | 4·2 | 26·0 | 4·3 | 0·355 | 26·2 | 3·5 | 26·8 | 3·6 | 0·006 |
| Waist (cm) | 27 878 | 84·0 | 12·9 | 87·5 | 13·6 | 0·010 | 77·8 | 10·5 | 79·6 | 11·2 | 0·185 | 93·6 | 10·1 | 95·8 | 10·5 | 0·001 |
| Body fat (%) | 27 760 | 26·8 | 7·0 | 26·5 | 7·2 | 0·069 | 30·7 | 5·0 | 31·6 | 4·9 | 0·428 | 20·7 | 5·0 | 21·2 | 5·0 | 0·100 |
| Fibre intake (g/MJ) | 27 931 | 2·2 | 0·6 | 2·12 | 0·6 | 0·192 | 2·3 | 0·7 | 2·3 | 0·6 | 0·117 | 2·1 | 0·6 | 2·07 | 0·6 | 0·828 |
| Fibre intake (g) | 27 931 | 20·1 | 7·1 | 20·0 | 6·9 | 0·186 | 19·1 | 6·4 | 18·8 | 6·4 | 0·283 | 21·7 | 7·8 | 21·2 | 7·1 | 0·414 |
| Fat intake (En%) | 27 931 | 38·3 | 6·2 | 38·5 | 6·3 | 0·475 | 37·7 | 6·1 | 37·6 | 6·0 | 0·937 | 39·1 | 6·3 | 39·4 | 6·4 | 0·275 |
| Protein intake (En%) | 27 931 | 15·5 | 2·5 | 15·3 | 2·4 | 0·275 | 15·6 | 2·5 | 15·5 | 2·4 | 0·357 | 15·3 | 2·5 | 15·1 | 2·4 | 0·517 |
| Carbohydrate intake (En%) | 27 931 | 46·3 | 6·2 | 46·3 | 6·2 | 0·777 | 46·6 | 6·1 | 47·0 | 5·9 | 0·646 | 45·7 | 6·3 | 45·5 | 6·5 | 0·399 |
| Vitamin D (μg) | 27 931 | 8·7 | 4·5 | 8·7 | 4·0 | 0·025 | 8·1 | 4·2 | 7·9 | 3·8 | 0·098 | 9·8 | 4·7 | 9·5 | 4·1 | 0·128 |
| Folate (mg) | 27 931 | 290·9 | 307·1 | 284·6 | 388·2 | 0·445† | 286·8 | 310·9 | 265·8 | 119·0 | 0·122† | 297 | 301·0 | 304·3 | 541·3 | 0·647† |
| Ca (mg) | 27 931 | 1144·8 | 425·7 | 1097·5 | 406·8 | 0·007 | 1112·2 | 393·2 | 1084·9 | 398·2 | 0·615 | 1195·8 | 467·4 | 1107·6 | 416·1 | 0·002 |
| Vegetable intake (g) | 27 931 | 181·4 | 100·4 | 175·8 | 102·0 | 0·778†‡ | 187·0 | 100·4 | 182·9 | 97·7 | 0·060†‡ | 172·7 | 99·8 | 169·5 | 106·0 | 0·977†‡ |
| Fruit and berry intake (g) | 27 931 | 194·4 | 125·6 | 187·7 | 121·6 | 0·237†‡ | 208·3 | 124·1 | 203·6 | 109·7 | 0·167†‡ | 172·9 | 127·3 | 171·1 | 131·1 | 0·673†‡ |
| Intake of fibre-rich cereal products (portions/d) | 27 931 | 0·987 | 1·022 | 0·978 | 0·965 | 0·714†‡ | 0·982 | 0·872 | 0·929 | 0·941 | 0·341†‡ | 1·078 | 1·214 | 1·029 | 0·988 | 0·775†‡ |
| Intake of red meat (g) | 27 931 | 59·0 | 35·3 | 60·2 | 36·7 | 0·130†‡ | 49·6 | 27·5 | 48·7 | 27·1 | 0·090†‡ | 73·7 | 40·8 | 72·2 | 41·3 | 0·770†‡ |
| Alcohol intake (g) | 27 931 | 10·7 | 12·7 | 11·2 | 14·4 | 0·312†‡ | 7·7 | 8·7 | 6·4 | 8·4 | 0·382†‡ | 15·5 | 16·0 | 16·2 | 17·4 | 0·122†‡ |
| Smoking, ex/current (%) | 27 919 | 33·7/28·4 | | 38·3/26·2 | | 0·172 | 27·8/28·1 | | 28·0/26·9 | | 0·693 | 43·0/28·8 | | 38·3/26·2 | | 0·220 |
| Education (>10 years) (%) | 27 931 | 31·9 | | 27·3 | | 0·040 | 30·3 | | 23·9 | | 0·011 | 34·3 | | 30·9 | | 0·174 |
| Physical activity high (%)§ | 27 740 | 19·9 | | 23·0 | | 0·043 | 18·7 | | 20·8 | | 0·314 | 21·8 | | 25·2 | | 0·126 |
| Regular use of NSAID (%) | 27 931 | 2·7 | | 2·0 | | 0·070 | 2·2 | | 1·7 | | 0·278 | 3·3 | | 2·3 | | 0·109 |
| Menopausal hormone therapy women (%) | 27 931 | | | | | | 19·4 | | 15·3 | | 0·047 | | | | | |

En%, percentage energy; NSAID, non-steroid anti-inflammatory drugs.
* Adjusted for sex and age when appropriate. Calculated with the general linear model for continuous values and $\chi^2$ test for categorical values.
† Calculated with log-transformed values.
‡ Adjusted for age, method, and season, and for sex when appropriate.
§ Physical activity was defined as high when being in the highest quintile of the whole group.

**Table 2.** Hazard ratios (HR) of colorectal cancer associated with intakes of fibres and fibre-rich foods in the Malmö Diet and Cancer cohort (Quintile ranges, hazard ratios and 95 % confidence intervals)

| | All | | | Women | | | Men | | |
|---|---|---|---|---|---|---|---|---|---|
| Quintiles for all | Cases/person-years | HR*† | 95 % CI | Cases/person-years | HR† | 95 % CI | Cases/person-years | HR† | 95 % CI |
| Fibre (g/MJ) | | | | | | | | | |
| 0·5–1·7 | 145/83 146 | 1·00 | | 59/38 020 | 1·00 | | 86/45 126 | 1·00 | |
| 1·7–2·0 | 159/85 385 | 1·06 | 0·85, 1·34 | 75/47 379 | 0·97 | 0·68, 1·37 | 84/38 005 | 1·12 | 0·83, 1·52 |
| 2·0–2·3 | 152/86 290 | 1·00 | 0·79, 1·27 | 71/52 886 | 0·80 | 0·56, 1·15 | 81/33 403 | 1·20 | 0·88, 1·64 |
| 2·3–2·7 | 161/87 418 | 1·05 | 0·83, 1·33 | 89/60 183 | 0·87 | 0·62, 1·23 | 72/27 234 | 1·26 | 0·91, 1·75 |
| 2·7–8·0 | 111/88 920 | 0·72 | 0·55, 0·94 | 78/67 097 | 0·66 | 0·47, 0·97 | 33/21 823 | 0·72 | 0·47, 1·10 |
| *P* for trend | | 0·026 | | | 0·078 | | | 0·316 | |
| *P* for interaction sex and fibre intake | | 0·683 | | | | | | | |
| Vegetables (g/MJ) | | | | | | | | | |
| 0–11·1 | 153/83 108 | 1·00 | | 46/33 753 | 1·00 | | 107/49 355 | 1·00 | |
| 11·1–15·9 | 167/85 453 | 1·10 | 0·88, 1·37 | 71/45 748 | 1·14 | 0·78, 1·65 | 96/39 702 | 1·08 | 0·82, 1·43 |
| 15·9–21·1 | 143/86 900 | 0·95 | 0·75, 1·20 | 78/53 960 | 1·08 | 0·74, 1·56 | 65/32 940 | 0·86 | 0·63, 1·84 |
| 21·1–28·7 | 137/87 167 | 0·94 | 0·74, 1·20 | 87/62 585 | 1·13 | 0·78, 1·63 | 50/24 582 | 0·87 | 0·62, 1·23 |
| 28·7–206·7 | 128/88 530 | 0·83 | 0·64, 1·07 | 90/69 520 | 1·09 | 0·75, 1·59 | 38/19 010 | 0·75 | 0·50, 1·11 |
| *P* for trend | | 0·048 | | | 0·832 | | | 0·179 | |
| *P* for interaction sex and vegetable intake | | 0·565 | | | | | | | |
| Fruits and berries (g/MJ) | | | | | | | | | |
| 0–9·9 | 159/84 371 | 1·00 | | 43/31 532 | 1·00 | | 116/52 838 | 1·00 | |
| 9·9–16·1 | 149/85 289 | 0·92 | 0·74, 1·16 | 59/43 266 | 0·97 | 0·65, 1·46 | 90/42 023 | 0·91 | 0·67, 1·20 |
| 16·1–23·1 | 135/86 188 | 0·84 | 0·66, 1·07 | 75/54 984 | 0·90 | 0·61, 1·32 | 60/31 204 | 0·80 | 0·58, 1·10 |
| 23·1–33·1 | 158/87 608 | 0·94 | 0·74, 1·19 | 103/63 812 | 1·01 | 0·69, 1·47 | 55/23 796 | 0·89 | 0·64, 1·24 |
| 33·1–224·2 | 127/87 701 | 0·82 | 0·64, 1·06 | 92/71 971 | 0·84 | 0·57, 1·24 | 35/15 730 | 0·86 | 0·58, 1·28 |
| *P* for trend | | 0·111 | | | 0·205 | | | 0·382 | |
| *P* for interaction sex and fruit and berry intake | | 0·898 | | | | | | | |
| Fibre-rich cereal products (portions/MJ) | | | | | | | | | |
| 0–0·02 | 141/82 963 | 1·00 | | 61/42 768 | 1·00 | | 80/40 195 | 1·00 | |
| 0·02–0·06 | 139/85 826 | 1·06 | 0·85, 1·340 | 68/52 041 | 0·91 | 0·64, 1·31 | 71/33 785 | 1·07 | 0·77, 1·47 |
| 0·06–0·11 | 157/86 636 | 1·00 | 0·79, 1·27 | 88/56 072 | 1·10 | 0·78, 1·52 | 69/30 563 | 1·12 | 0·81, 1·55 |
| 0·11–0·18 | 141/87 238 | 1·05 | 0·83, 1·33 | 76/58 106 | 0·88 | 0·62, 1·23 | 65/29 131 | 1·07 | 0·77, 1·49 |
| 0·18–1·29 | 150/88 495 | 0·72 | 0·55, 0·94 | 79/56 578 | 0·89 | 0·61, 1·23 | 71/31 917 | 1·04 | 0·75, 1·45 |
| *P* for trend | | 0·115 | | | 0·277 | | | 0·318 | |
| *P* for interaction sex and intake of fibre-rich cereal products | | 0·497 | | | | | | | |

* Adjusted for sex (man, woman)

† Adjusted for age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex or never), alcohol intake (zero, <15 g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), physical activity (quintiles of physical activity), BMI (continuous) and when appropriate current use of menopausal hormonal replacement therapy (yes, no).

**Table 3.** Hazard ratios (HR) of colon cancer associated with intakes of fibres and fibre-rich foods in the Malmö Diet and Cancer cohort (Quintile ranges, hazard ratios and 95 % confidence intervals)

| | All | | | Women | | | Men | | |
|---|---|---|---|---|---|---|---|---|---|
| Quintiles for all | Cases/person-years | HR*† | 95 % CI | Cases/person-years | HR† | 95 % CI | Cases/person-years | HR† | 95 % CI |
| Fibre (g/MJ) | | | | | | | | | |
| 0–1·7 | 86/82 340 | 1·00 | | 43/37 740 | 1·00 | | 43/44 601 | 1·00 | |
| 1·7–2·0 | 102/84 395 | 1·13 | 0·84, 1·51 | 48/46 950 | 0·83 | 0·54, 1·25 | 54/37 445 | 1·41 | 0·94, 2·11 |
| 2·0–2·3 | 101/85 376 | 1·07 | 0·80, 1·45 | 47/52 438 | 0·67 | 0·43, 1·02 | 54/32 938 | 1·57 | 1·05, 2·37 |
| 2·3–2·7 | 106/86 520 | 1·09 | 0·81, 1·47 | 62/59 666 | 0·75 | 0·50, 1·13 | 44/26 853 | 1·45 | 0·94, 2·24 |
| 2·7–8·0 | 68/87 892 | 0·68 | 0·48, 0·96 | 47/66 401 | 0·51 | 0·31, 0·75 | 21/21 492 | 0·90 | 0·52, 1·54 |
| *P* for trend | | 0·028 | | | 0·013 | | | 0·689 | |
| *P* for interaction sex and fibre | | 0·052 | | | | | | | |
| Vegetables (g/MJ) | | | | | | | | | |
| 0·5–11·1 | 96/82 155 | 1·00 | | 36/33 469 | 1·00 | | 60/48 686 | 1·00 | |
| 11·1–15·9 | 105/84 490 | 1·08 | 0·82, 1·43 | 46/45 283 | 0·93 | 0·60, 1·44 | 59/39 207 | 1·17 | 0·82, 1·69 |
| 15·9–21·1 | 95/86 026 | 1·01 | 0·76, 1·36 | 53/53 534 | 0·92 | 0·60, 1·41 | 42/32 492 | 1·02 | 0·68, 1·53 |
| 21·1–28·8 | 86/86 253 | 0·96 | 0·71, 1·30 | 57/62 065 | 0·90 | 0·59, 1·39 | 29/24 188 | 0·94 | 0·60, 1·48 |
| 28·8–206·8 | 81/87 599 | 0·89 | 0·64, 1·24 | 55/68 844 | 0·79 | 0·50, 1·25 | 26/18 755 | 1·02 | 0·62, 1·66 |
| *P* for trend | | 0·235 | | | 0·115 | | | 0·782 | |
| *P* for interaction sex and vegetable intake | | 0·401 | | | | | | | |
| Fruit and berries (g/MJ) | | | | | | | | | |
| 0–9·9 | 97/83 608 | 1·00 | | 33/31 414 | 1·00 | | 64/5 219 595 | 1·00 | |
| 9·9–16·1 | 90/84 347 | 0·88 | 0·66, 1·18 | 37/42 904 | 0·79 | 0·49, 1·29 | 53/4 144 292 | 0·95 | 0·66, 1·40 |
| 16·1–23·0 | 98/85 130 | 0·94 | 0·70, 1·25 | 58/54 482 | 0·86 | 0·55, 1·35 | 40/30 648 | 0·94 | 0·63, 1·40 |
| 23·0–33·1 | 100/86 667 | 0·90 | 0·66, 1·21 | 63/63 136 | 0·76 | 0·49, 1·20 | 37/23 531 | 1·04 | 0·68, 1·58 |
| 33·1–224·2 | 78/86 771 | 0·74 | 0·54, 1·03 | 56/71 259 | 0·62 | 0·37, 0·98 | 22/15 513 | 0·95 | 0·57, 1·56 |
| *P* for trend | | 0·041 | | | 0·022 | | | 0·721 | |
| *P* for interaction sex and fruit and berry intake | | 0·164 | | | | | | | |
| Fibre-rich cereal products (portions/MJ) | | | | | | | | | |
| 0–0·02 | 88/82 145 | 1·00 | | 37/42 379 | 1·00 | | 51/39 766 | 1·00 | |
| 0·02–0·06 | 93/84 895 | 1·07 | 0·79, 1·44 | 50/51 640 | 1·10 | 0·71, 1·79 | 43/33 255 | 1·03 | 0·68, 1·54 |
| 0·06–0·11 | 98/85 614 | 1·11 | 0·83, 1·48 | 58/55 458 | 1·19 | 0·79, 1·81 | 40/30 156 | 1·00 | 0·66, 1·52 |
| 0·11–0·18 | 92/86 332 | 0·98 | 0·73, 1·32 | 51/57 550 | 0·94 | 0·61, 1·44 | 41/28 782 | 1·02 | 0·67, 1·56 |
| 0·18–1·29 | 92/87 536 | 0·90 | 0·67, 1·22 | 51/56 167 | 0·90 | 0·58, 1·39 | 41/31 369 | 0·93 | 0·61, 1·14 |
| *P* for trend | | 0·095 | | | 0·141 | | | 0·426 | |
| *P* for interaction sex and intake of fibre-rich cereal products | | 0·652 | | | | | | | |

* Adjusted for sex (man, woman).

† Adjusted for age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex or never), alcohol intake (zero, <15 g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), physical activity (quintiles physical activity), BMI (continuous) and when appropriate current use of menopausal hormonal replacement therapy (yes, no).

British Journal of Nutrition

($P$ for trend = 0·72), but the interaction between intake of fruits and berries and sex did not reach significance ($P$ = 0·16).

When analysing intakes of fibre and fibre-rich foods and rectal cancer, no significant associations were seen in either of the sexes. However, the risk of developing rectal cancer tended to increase with higher vegetable intake in women (HR: 2·22 for highest compared with lowest quintile; 95 % CI 1·07, 4·61; $P$ for trend = 0·06) (Table 4). In contrast, a tendency of protective association between high vegetable intake and rectal cancer was seen in men ($P$ for trend = 0·14), and we detected a significant interaction between vegetable intake and sex on rectal cancer ($P$ = 0·039). We also observed a significant interaction between fibre intake and sex on rectal cancer ($P$ = 0·048), but although the associations in men and women seemed to reflect those for vegetable intake the tendencies for fibre intake were less clear ($P$ values for trend ≥0·26).

### *Fibre intake and relative risk for colorectal cancer of different Tumour, Node, Metastasis stages*

High fibre intake had a tendency towards association with lower risk for T-stage 3 or 4, together with N-stage 0, especially in women (data not shown), but no significant association was seen in the final model regarding high fibre intake and association with risk for CRC (Table 5). Intake of fibre-rich cereal products was significantly associated with lower risk for N-stage 0 ($P$ for trend = 0·015) and for M0 ($P$ for trend = 0·046), but we did not observe any tendencies of associations with N-stage 1 and 2 or M-stage 1. When analysing women and men separately, the tendencies of different associations with intake of fibre-rich cereal products depending on N- and M-stage were mainly seen in men, and the association between fibre-rich cereal products and stage N0 was significant for men (HR: 0·64 for highest quintile compared with lowest; 95 % CI 0·37; $P$ for trend = 0·024). No other significant associations with tumours classified according to the TNM system were found.

### *Sensitivity analysis*

When excluding individuals reporting dietary change in the past, the association between fibre intake and risk for CRC was no longer significant, but the risk estimate did not change (HR: 0·72 for highest quintile compared with lowest; 95 % CI 0·52, 1·00, $P$ for trend 0·21). However, the previously inverse association between vegetable intake and risk for CRC disappeared (HR: 1·05 for highest quintile compared with lowest; 95 % CI 0·77, 1·42; $P$ for trend = 0·51). For colon cancer, the inverse associations with intakes of fibre ($P$ for trend = 0·027) and fruits and berries ($P$ for trend = 0·013) remained significant in women. The association between intake of fibre-rich cereals and N0-stage was no longer significant (HR: 0·62 for highest quintile compared with lowest; 95 % CI 0·32, 1·23; $P$ for trend = 0·42).

When excluding individuals with a prevalent cancer, the association between fibre intake and risk for CRC remained significant ($P$ for trend = 0·042), but the association between vegetables and risk for CRC disappeared ($P$ for trend = 0·58).

## Discussion

The results from this large prospective cohort study indicate that a high fibre intake is associated with a lower risk for CRC, or more specifically with a lower risk for colon cancer, in women. When intakes of different fibre-rich foods were analysed, high fruit and berry intake was associated with a lower risk for colon cancer in women. No significant associations were found between fibre intake and its sources, and different clinical stages, except for high intake of fibre-rich cereal products and lower risk for N-stage 0 and M-stage 0.

The present study confirms the results from other studies that show an association between high fibre intake and lower risk for CRC[5,8], but in contrast this study did not show a significant overall association with high intake of fibre-rich cereal products. The last may be because of somewhat higher intake levels of whole grain in Sweden[15] than, for example, in the USA[16], resulting in that most MDCS participants may reach a potential threshold level for protective effects on CRC.

The present study showed a borderline interaction between fibre intake and sex regarding risk for colon cancer, indicating that an inverse association is restricted to women. Other studies have not detected interactions between sex and total fibre intake[17]. However, the results from the present study are in analogy with a meta-analysis by Riboli and Norat, which only indicated an association between fruit intake and risk for colon cancer in women[18]. The observed association between high intake of fruits and berries and lower risk for colon cancer in women in the present study may be a reflection of the relatively low intake of fruit and berries in Sweden; as seen in the EPIC study other populations have a higher estimated intake[19] and may have intakes well above a potentially protective level. It cannot be ruled out that the differing observations in men and women could be explained by variation in intake levels and accuracy of dietary reporting[20,21]. A Swedish national dietary survey showed that women had a higher estimated fibre intake (25 g/10 MJ per d) than did men (23 g/10 MJ per d)[22]. The potentially protective role of dietary fibre may also vary depending on exposure to other lifestyle factors of importance in CRC development. In the MDCS cohort, women and men may, for example, have been exposed to different amounts and types of carcinogens, because Malmö is historically a city with a high proportion of workers, and with a high proportion of women being housewives. Another explanation for sex differences may be that the oestrogen levels are lowered in women after menopause, whereas men have another endocrine equilibrium. The intestinal epithelium is an important barrier for luminal factors, and oestrogen has been shown to be important to strengthen the tight junctions in the intercellular spaces[23]. Lower oestrogen levels after menopause might increase permeability in the colon, and thus implicate increased risk for penetration of luminal irritants inducing chronic mucosal inflammation, and in the long-term maybe even cancer[24]. Anti-carcinogenic compounds, potentially including dietary fibre, may therefore be of greater importance to women.

The mechanisms behind the inverse association between fibre intake and CRC are much thought to be local mechanisms in the colon – for example – reduction of colonic transit time, increase in stool output and frequency, increase in water content of the stool, dilution of colonic content, reduction of

**Table 4.** Hazard ratios (HR) of rectal cancer associated with intakes of fibres and fibre-rich foods in the Malmö Diet and Cancer cohort (Quintile ranges, hazard ratios and 95 % confidence intervals)

| | All | | | Women | | | Men | | |
|---|---|---|---|---|---|---|---|---|---|
| Quintiles for all | Cases/person-years | HR*† | 95 % CI | Cases/person-years | HR† | 95 % CI | Cases/person-years | HR† | 95 % CI |
| Fibre (g/MJ) | | | | | | | | | |
| 0–1·7 | 46/81 286 | 1·00 | | 20/57 913 | 1·00 | | 26/23 373 | 1·00 | |
| 1·7–2·0 | 58/84 108 | 1·18 | 0·79, 1·76 | 34/57 380 | 1·74 | 0·98, 3·10 | 24/26 727 | 0·78 | 0·44, 1·37 |
| 2·0–2·3 | 48/84 915 | 0·92 | 0·60, 1·54 | 25/54 710 | 1·28 | 0·68, 2·39 | 23/30 205 | 0·66 | 0·37, 1·18 |
| 2·3–2·7 | 56/86 269 | 1·01 | 0·66, 1·54 | 23/50 535 | 1·26 | 0·65, 2·42 | 33/35 733 | 0·77 | 0·44, 1·36 |
| 2·7–8·0 | 57/87 796 | 0·88 | 0·55, 1·42 | 23/41 424 | 1·36 | 0·67, 2·78 | 34/46 372 | 0·59 | 0·31, 1·12 |
| *P* for trend | | 0·525 | | | 0·660 | | | 0·263 | |
| *P* for interaction sex and fibre intake | | 0·048 | | | | | | | |
| Vegetables (g/MJ) | | | | | | | | | |
| 0·5–11·1 | 58/80 879 | 1·00 | | 17/44 410 | 1·00 | | 41/36 469 | 1·00 | |
| 11·1–15·9 | 48/84 578 | 0·83 | 0·56, 1·22 | 20/50 819 | 1·89 | 0·90, 3·96 | 28/33 759 | 0·75 | 0·46, 1·21 |
| 15·9–21·1 | 45/84 854 | 0·82 | 0·55, 1·22 | 24/53 103 | 1·65 | 0·78, 3·49 | 21/31 750 | 0·62 | 0·37, 1·06 |
| 21·1–28·8 | 54/86 288 | 1·01 | 0·69, 1·48 | 28/56 185 | 1·97 | 0·95, 4·07 | 26/30 103 | 0·82 | 0·50, 1·36 |
| 28·8–206·8 | 60/87 775 | 1·09 | 0·74, 1·60 | 36/57 446 | 2·22 | 1·07, 4·61 | 24/30 330 | 0·71 | 0·42, 1·21 |
| *P* for trend | | 0·738 | | | 0·062 | | | 0·138 | |
| *P* for interaction sex and vegetable intake | | 0·039 | | | | | | | |
| Fruit and berries (g/MJ) | | | | | | | | | |
| 0–9·9 | 62/82801 | 1·00 | | 17/38 567 | 1·00 | | 45/44 224 | 1·00 | |
| 9·9–16·1 | 49/83 799 | 0·83 | 0·57, 2·21 | 15/48 606 | 1·48 | 0·69, 3·15 | 34/35 193 | 0·91 | 0·58, 1·43 |
| 16·1–23·0 | 43/84 665 | 0·73 | 0·49, 1·08 | 23/53 858 | 0·92 | 0·42, 2·03 | 20/30 807 | 0·62 | 0·36, 1·06 |
| 23·0–33·1 | 49/86 465 | 0·78 | 0·53, 1·17 | 29/60 390 | 1·81 | 0·89, 3·69 | 20/26 075 | 0·69 | 0·40, 1·19 |
| 33·1–224·2 | 62/86 645 | 1·04 | 0·71, 1·52 | 41/60 533 | 1·59 | 0·77, 3·29 | 21/26 111 | 0·75 | 0·43, 1·30 |
| *P* for trend | | 0·960 | | | 0·291 | | | 0·344 | |
| *P* for interaction sex and fruit and berry intake | | 0·053 | | | | | | | |
| Fibre-rich cereal products (portions/MJ) | | | | | | | | | |
| 0–0·02 | 50/81 861 | 1·00 | | 22/44 322 | 1·00 | | 28/37 538 | 1·00 | |
| 0·02–0·06 | 45/84 371 | 0·94 | 0·62, 1·42 | 22/55 595 | 0·63 | 0·34, 1·18 | 23/28 777 | 1·05 | 0·60, 1·84 |
| 0·06–0·11 | 54/84 913 | 1·14 | 0·77, 1·69 | 29/57 761 | 0·96 | 0·55, 1·67 | 25/27 151 | 1·26 | 0·73, 2·16 |
| 0·11–0·18 | 60/86 244 | 1·23 | 0·84, 1·80 | 25/57 297 | 0·79 | 0·45, 1·41 | 35/28 947 | 1·62 | 0·98, 2·68 |
| 0·18–1·29 | 56/86 986 | 0·99 | 0·67, 1·48 | 27/46 988 | 0·88 | 0·50, 1·54 | 29/39 997 | 0·95 | 0·56, 1·63 |
| *P* for trend | | 0·696 | | | 0·823 | | | 0·554 | |
| *P* for interaction sex and intake of fibre-rich cereal products | | 0·612 | | | | | | | |

* Adjusted for sex (man, woman).

† Adjusted for age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex or never), alcohol intake (zero, <15 g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), physical activity (quintiles of physical activity), BMI (continuous) and when appropriate current use of menopausal hormonal replacement therapy (yes, no).

**Table 5.** Hazard ratios (HR) of colorectal cancer stages associated with intakes of fibre and fibre-rich foods in the Malmö Diet and Cancer cohort (Quintile ranges, hazard ratios and 95 % confidence intervals)

| | HR of T-stage 1 and 2 | | | HR of T-stage 3 and 4 | | | HR of N-stage 0 | | | HR of N-stage 1 and 2 | | | HR of M-stage 0 | | | HR of M-stage 1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Quintiles for all | Cases/ person-years | HR* | 95 % CI | Cases/ person-years | HR* | 95 % CI | Cases/ person-years | HR* | 95 % CI | Cases/ person-years | HR* | 95 % CI | Cases/ person-years | HR* | 95 % CI | Cases/ person-years | HR* | 95 % CI |
| Fibre (g/MJ) | | | | | | | | | | | | | | | | | | |
| 0·5–1·7 | 22/74 605 | 1·00 | | 75/75 333 | 1·00 | | 57/75 118 | 1·00 | | 34/74 877 | 1·00 | | 82/75 417 | 1·00 | | 22/74 618 | 1·00 | |
| 1·7–2·0 | 24/75 199 | 1·08 | 0·60, 1·94 | 101/76 264 | 1·26 | 0·93, 1·71 | 67/77 5888 | 1·17 | 0·82, 1·67 | 47/75 696 | 1·18 | 0·77, 1·83 | 107/76 312 | 1·23 | 0·92, 1·65 | 26/75 229 | 1·20 | 0·67, 2·12 |
| 2·0–2·3 | 21/75 628 | 0·96 | 0·52, 1·78 | 88/76 536 | 1·06 | 0·77, 1·46 | 70/76 343 | 1·17 | 0·82, 1·67 | 33/75 897 | 0·85 | 0·53, 1·36 | 93/76 605 | 1·03 | 0·76, 1·40 | 25/75 692 | 1·15 | 0·64, 2·07 |
| 2·3–2·7 | 30/75 898 | 1·40 | 0·78, 2·51 | 75/76 547 | 0·89 | 0·64, 1·25 | 58/76 411 | 0·98 | 0·67, 1·44 | 40/76 190 | 0·95 | 0·60, 1·51 | 98/76 832 | 1·10 | 0·81, 1·50 | 20/75 771 | 0·89 | 0·47, 1·69 |
| 2·7–8·0 | 16/76 063 | 0·75 | 0·37, 1·51 | 66/76 733 | 0·79 | 0·55, 1·12 | 40/76 395 | 0·67 | 0·43, 1·03 | 39/76 484 | 0·95 | 0·60, 1·53 | 71/76 782 | 0·77 | 0·55, 1·09 | 23/76 082 | 1·11 | 0·59, 2·10 |
| *P* for trend | | 0·661 | | | 0·086 | | | 0·090 | | | 0·708 | | | 0·159 | | | 0·787 | |
| Vegetables (g/MJ) | | | | | | | | | | | | | | | | | | |
| 0–11·1 | 22/74 319 | 1·00 | | 80/74 906 | 1·00 | | 56/74 879 | 1·00 | | 39/74 678 | 1·00 | | 81/75 145 | 1·00 | | 29/74 447 | 1·00 | |
| 11·1–15·9 | 19/75 136 | 0·91 | 0·49, 1·70 | 88/76 116 | 0·90 | 0·64, 1·27 | 68/75 789 | 1·25 | 0·88, 1·79 | 39/75 490 | 1·05 | 0·68, 1·63 | 96/76 167 | 1·23 | 0·91, 1·66 | 26/75 209 | 0·98 | 0·57, 1·67 |
| 15·9–21·1 | 22/75 816 | 1·12 | 0·61, 2·07 | 85/76 453 | 1·03 | 0·74, 1·42 | 60/76 426 | 1·17 | 0·81, 1·70 | 41/76 185 | 0·98 | 0·62, 1·54 | 101/76 893 | 1·31 | 0·97, 1·77 | 12/75 634 | 0·49 | 0·25, 0·97 |
| 21·1–28·7 | 34/75 850 | 1·77 | 1·00, 3·13 | 72/76 984 | 1·01 | 0·73, 1·39 | 55/76 255 | 1·13 | 0·76, 1·67 | 36/76 085 | 1·10 | 0·70, 1·72 | 91/76 628 | 1·26 | 0·92, 1·73 | 21/75 676 | 0·90 | 0·50, 1·63 |
| 28·7–206·7 | 16/76 272 | 0·92 | 0·46, 1·85 | 80/76 954 | 0·93 | 0·67, 1·29 | 53/76 806 | 1·14 | 0·76, 1·72 | 39/76 705 | 0·98 | 0·60, 1·59 | 81/77 117 | 1·12 | 0·80, 1·57 | 28/76 405 | 1·24 | 0·69, 2·23 |
| *P* for trend | | 0·575 | | | 0·987 | | | 0·914 | | | 0·913 | | | 0·559 | | | 0·922 | |
| Fruits and berries (g/MJ) | | | | | | | | | | | | | | | | | | |
| 0–9·9 | 28/74 144 | 1·00 | | 85/76 165 | 1·00 | | 65/74 670 | 1·00 | | 39/74 423 | 1·00 | | 96/75 035 | 1·00 | | 25/74 091 | 1·00 | |
| 9·9–16·1 | 16/75 212 | 0·55 | 0·29, 1·02 | 81/76 053 | 0·91 | 0·67, 1·24 | 52/75 757 | 1·11 | 0·75, 1·63 | 41/75 668 | 0·97 | 0·63, 1·49 | 86/76 154 | 0·84 | 0·63, 1·37 | 23/75 299 | 1·04 | 0·56, 1·94 |
| 16·1–23·1 | 22/75 663 | 0·76 | 0·43, 1·35 | 79/76 670 | 0·89 | 0·65, 1·22 | 58/76 223 | 0·83 | 0·56, 1·23 | 38/75 984 | 0·82 | 0·53, 1·29 | 85/76 527 | 0·86 | 0·63, 1·16 | 25/75 679 | 0·93 | 0·50, 1·72 |
| 23·1–33·1 | 28/76 245 | 0·94 | 0·53, 1·65 | 80/76 336 | 0·87 | 0·63, 1·21 | 59/76 794 | 0·96 | 0·66, 1·39 | 40/76 608 | 0·86 | 0·55, 1·35 | 99/77 231 | 0·96 | 0·71, 1·29 | 21/76 202 | 1·00 | 0·55, 1·81 |
| 33·1–224·2 | 19/76 130 | 0·71 | 0·38, 1·34 | 80/77 159 | 0·92 | 0·66, 1·29 | 58/76 712 | 0·95 | 0·66, 1·37 | 35/76 461 | 0·79 | 0·49, 1·28 | 85/77 002 | 0·86 | 0·62, 1·18 | 22/76 120 | 0·85 | 0·46, 1·55 |
| *P* for trend | | 0·928 | | | 0·207 | | | 0·555 | | | 0·219 | | | 0·449 | | | 0·369 | |
| Fibre-rich cereal products (portions/MJ) | | | | | | | | | | | | | | | | | | |
| 0–0·02 | 26/74 940 | 1·00 | | 79/75 684 | 1·00 | | 68/75 613 | 1·00 | | 29/75 128 | 1·00 | | 94/75 862 | 1·00 | | 18/74 824 | 1·00 | |
| 0·02–0·06 | 24/75 444 | 0·91 | 0·52, 1·60 | 74/76 086 | 0·95 | 0·69, 1·32 | 51/75 858 | 0·81 | 0·56, 1·17 | 40/75 744 | 1·22 | 0·76, 1·97 | 86/76 253 | 0·92 | 0·68, 1·24 | 21/75 367 | 1·24 | 0·66, 2·34 |
| 0·06–0·11 | 23/75 471 | 0·86 | 0·49, 1·51 | 91/76 411 | 1·17 | 0·86, 1·59 | 70/76 167 | 1·06 | 0·76, 1·49 | 38/75 822 | 1·31 | 0·82, 2·10 | 96/76 470 | 1·03 | 0·77, 1·37 | 28/75 579 | 1·64 | 0·90, 2·99 |
| 0·11–0·18 | 18/75 907 | 0·65 | 0·35, 1·19 | 84/76 792 | 1·02 | 0·75, 1·40 | 53/76 423 | 0·77 | 0·53, 1·10 | 41/76 354 | 1·33 | 0·83, 2·11 | 90/76 839 | 0·91 | 0·68, 1·22 | 22/75 933 | 1·23 | 0·66, 2·31 |
| 0·18–1·29 | 22/75 631 | 0·74 | 0·41, 1·33 | 77/76 440 | 0·84 | 0·61, 1·16 | 50/76 095 | 0·67 | 0·46, 0·97 | 45/76 094 | 1·21 | 0·76, 1·93 | 84/76 525 | 0·77 | 0·57, 1·04 | 27/75 688 | 1·36 | 0·73, 2·52 |
| *P* for trend | | 0·126 | | | 0·212 | | | 0·015 | | | 0·573 | | | 0·046 | | | 0·813 | |

* Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex or never), alcohol intake (zero, <15 g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), physical activity (quintiles of leisure-time physical activity score) and BMI (continuous).

toxins and bile acids, increase in colonic fermentation and an increase in colonic SCFA[6,25]. This may explain why our finding in general indicates intakes of fibre, and its food sources, to be inversely associated with tumours of the colon, but not of the rectum. However, we cannot exclude that it is a power issue, and we actually observed a tendency of inverse associations between high vegetable intake and risk for rectal cancer in men.

At a more cellular level in CRC development, cytoprotectants in fruits and berry, including vitamins, minerals, polyphenol flavonoids and anthocyanins, have been shown to have anticancer activity in colon cancer cell lines *in vitro*[26]. The antiproliferative effect of CRC cells by different berry juices was not correlated to the antioxidant level, but induced cell-cycle arrest in the $G_1$ phase[27]. The phenolic compounds vary with species, but there is strong evidence that these compounds modulate numerous cellular processes by up- or down-regulation of key proteins involved in cell signalling pathways that control gene expression, cell proliferation, cell differentiation, DNA damage repair, apoptosis, malignant transformation and inhibition of cell invasiveness and metastasis[28]. Our finding of a reduced risk for colon cancer in women with high intake of fruits and berries, in contrast to fibres from other sources, may be explained by the effect of phenolic compounds.

Participants who reported dietary change may have unstable food habits. Their reported dietary habits may reflect a short period of their lives, and may therefore have less influence on the development of future morbidity. However, as our low-risk estimates for CRC at higher intakes of fibre remained virtually unchanged after excluding those with dietary change, the loss of significance could probably be explained by a lower number of individuals (75·6 %) in the sensitivity analysis.

The strengths of this study are that the MDCS is a large study, with long follow-up. As it is a population-based prospective study, we should have been able to minimise selection bias and reverse causation. One of the main objectives of the MDCS was to examine fibre intake and estimated intakes of fibre, and its food sources have shown a high relative validity[12,13]. It was also possible to identify and exclude individuals with unstable food habits. In addition, we are not aware of any previous study taking TNM classification into consideration when examining associations between fibre intake and CRC. The participation rate of the MDCS is similar or higher compared with other large-scale population-based studies[29], and regarding the generalisability of our results it is important to point out that the socio-demographic structure, weight distribution and smoking habits were similar among participants in the MDSC and participants in a health survey in Malmö with a higher participation rate (75 %)[29]. Moreover, although the cancer incidence was somewhat higher among non-participants, 2·6 % of the participants in the MDCS developed CRC during 15-year follow-up[30], which is comparable to previously published data on other cohorts[31].

In analyses of different TNM stages, we observed significant associations only between high intake of fibre-rich cereals and lower risk for N-stage 0 and M-stage 0. A possible interpretation is that potential effects of fibre on tumour initiation may differ from effects on tumour invasiveness and metastasis, and phenolic compounds referred to above may influence several steps in carcinogenesis[28]. However, a limitation of the study is the small number of cases in some of the stratified analyses and it cannot be excluded that the analyses of the other stages were underpowered because of fewer cases, and new studies in large populations are needed. It would, for example, be interesting to replicate the borderline association between fibre intake and risk for T-stage 3 and 4. Patient delay may also be a source of error in the TNM classification of the cases, as the time for the diagnosis can affect the outcome of the classification, without knowledge about the length of the tumour growth. There may also be a different pattern in when men and women visit healthcare services, thus giving them an earlier or later diagnosis. Women seem to have a tendency to be more inclined to participate in studies and to contact the healthcare service earlier in the disease process[32]. Another weakness of the study is that the collection of intake data was measured only once. As the results from a Swedish national survey on dietary intake has shown an increase in intakes of vegetables, fruit and berries[18], repeated measurements would have been valuable. Because of different structuring after data gathering, it was not possible in the present study to estimate intakes of fibre from different fibre sources. Moreover, taking family history of CRC into account in our analyses would have been valuable, as it is a contributing factor in the development of CRC[33], but information on the risk factor was missing. We have chosen not to adjust for multiple testing, as dietary intakes are highly correlated and the analyses could not be treated as independent. However, it should be noted that some of our subgroup associations could have occurred because of chance, as a consequence of multiple tests. Finally, despite adjustments for possible confounders and known risk factors, occurrence of some residual confounding cannot be excluded.

## Conclusion

In conclusion, our findings indicate that a high fibre intake is associated with a lower risk for CRC, especially with a lower risk for colon cancer, in women. Regarding intakes of different fibre-rich foods, our results indicate that only high intakes of fruits and berries are associated with lower risk for colon cancer in women. Our findings suggesting that high intakes of fibre-rich cereal products may lower the risk of developing N0- and M0-stage need to be replicated in future studies.

## Acknowledgements

This research was funded by the Development foundation of Region Skåne and Dir Albert Påhlsson's Foundation (B. O.) and by the Malmö Hospital Foundation for Cancer Prevention. The Development foundation of Region Skåne and Dir Albert Påhlsson's Foundation had no role in the design, analysis or writing of this article.

A. V., B. O., J. M. and U. E. designed the research. A. V., B. O. and U. E. wrote the paper. A. V. performed the statistical analysis. K. J. and J. B. provided essential materials. U. E. had primary responsibility for the final content. All authors read and approved the final manuscript.

There are no conflicts of interest.

British Journal of Nutrition

## Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/http://dx.doi.org/doi:10.1017/S0007114515002743

## References


1. Ferlay J, Parkin DM & Steliarova-Foucher E (2010) Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* **46**, 765–781.
2. Edwards BK, Ward E, Kohler BA, *et al.* (2010) Annual Report to the Nation on the Status of Cancer, 1975-2006, featuring colorectal trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer* **116**, 544–573.
3. Bergman O, Hont G & Johansson E Cancer i siffror. Socialstyrelsen Cancerfonden; 2013. 20130605. http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/19108/2013-6-5.pdf (accessed June 2014).
4. World Cancer Research Fund and American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. (2011).
5. Murphy N, Norat T, Ferrari P, *et al.* (2012) Dietary fibre intake and risks of cancers of the colon and rectum in the European Prospective Investigation into Cancer and Nutrition (EPIC). *PLOS ONE* **7**, e39361.
6. Azcarate-Peril MA, Sikes M & Bruno-Barcena JM (2011) The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer? *Am J Physiol Gastrointest Liver Physiol* **301**, G401–G424.
7. Chan AT & Giovannucci EL (2010) Primary prevention of colorectal cancer. *Gastroenterology* **138**, 2029–2043.
8. Aune D, Chan DSM, Lau R, *et al.* (2011) Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ* **343**, d6617.
9. Aune D, Lau R, Chan DS, *et al.* (2011) Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies. *Gastroenterology* **141**, 106–118.
10. Brandstedt J, Wangefjord S, Borgquist S, *et al.* (2013) Influence of anthropometric factors on tumour biological characteristics of colorectal cancer in men and women: a cohort study. *J Transl Med* **11**, 293.
11. Wirfalt E, Mattisson I, Johansson U, *et al.* (2002) A methodological report from the Malmo Diet and Cancer study: development and evaluation of altered routines in dietary data processing. *Nutr J* **1**, 3.
12. Elmstahl S, Riboli E, Lindgarde F, *et al.* (1996) The Malmo Food Study: the relative validity of a modified diet history method and an extensive food frequency questionnaire for measuring food intake. *Eur J Clin Nutr* **50**, 143–151.
13. Riboli E, Elmstahl S, Saracci R, *et al.* (1997) The Malmo Food Study: validity of two dietary assessment methods for measuring nutrient intake. *Int J Epidemiol* **26**, Suppl. 1, S161–S173.
14. Wangefjord S, Manjer J, Gaber A, *et al.* (2011) Cyclin D1 expression in colorectal cancer is a favorable prognostic factor in men but not in women in a prospective, population-based cohort study. *Biol Sex Differ* **2**, 10.
15. Kyro C, Skeie G, Dragsted LO, *et al.* (2012) Intake of whole grain in Scandinavia: intake, sources and compliance with new national recommendations. *Scand J Public Health* **40**, 76–84.
16. Koh-Banerjee P, Franz M, Sampson L, *et al.* (2004) Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. *Am J Clin Nutr* **80**, 1237–1245.
17. Park Y, Hunter DJ, Spiegelman D, *et al.* (2005) Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. *JAMA* **294**, 2849–2857.
18. Riboli E & Norat T (2003) Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr* **78**, Suppl., 559S–569S.
19. Agudo A, Slimani N, Ocke MC, *et al.* (2002) Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. *Public Health Nutr* **5**, 1179–1196.
20. Wirfalt E, Hedblad B, Gullberg B, *et al.* (2001) Food patterns and components of the metabolic syndrome in men and women: a cross-sectional study within the Malmo Diet and Cancer cohort. *Am J Epidemiol* **154**, 1150–1159.
21. Hebert JR, Ma Y, Clemow L, *et al.* (1997) Gender differences in social desirability and social approval bias in dietary self-report. *Am J Epidemiol* **146**, 1046–1055.
22. Livsmedelsverket. Riksmaten – vuxna 2010–11: Vad äter svenskarna? – Livsmedels- och näringsintag bland vuxna i Sverige (Riksmaten - Adults 2010-11. What do the Swedes eat? Food and nutrition intake among adults in Sweden). Uppsala, Sweden: Livsmedelsverket; 2012. http://www.slv.se/upload/dokument/rapporter/mat_naring/2012/riksmaten_2010_2011_kortversion.pdf. (accessed 27 August 2014).
23. Braniste V, Leveque M, Buisson-Brenac C, *et al.* (2009) Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. *J Physiol* **587**, pt 13, 3317–3328.
24. Vrachnis N, Zygouris D, Iliodromiti Z, *et al.* (2014) Probing the impact of sex steroids and menopause-related sex steroid deprivation on modulation of immune senescence. *Maturitas* **78**, 174–178.
25. Fardet A (2010) New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr Res Rev* **23**, 65–134.
26. Brown EM, Gill CI, McDougall GJ, *et al.* (2012) Mechanisms underlying the anti-proliferative effects of berry components in in vitro models of colon cancer. *Curr Pharm Biotechnol* **13**, 200–209.
27. Boivin D, Blanchette M, Barrette S, *et al.* (2007) Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFkappaB by edible berry juice. *Anticancer Res* **27**, 937–948.
28. Duthie SJ (2007) Berry phytochemicals, genomic stability and cancer: evidence for chemoprotection at several stages in the carcinogenic process. *Mol Nutr Food Res* **51**, 665–674.
29. Boeing H, Korfmann A & Bergmann MM (1999) Recruitment procedures of EPIC-Germany. European investigation into cancer and nutrition. *Ann Nutr Metab* **43**, 205–215.
30. Manjer J, Carlsson S, Elmstahl S, *et al.* (2001) The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev* **10**, 489–499.
31. Howlader N, Noone A, Krapcho M, *et al.* (2014) *SEER Cancer Statistics Review, 1975–2011.* Bethesda: National Cancer Institute; http://seer.cancer.gov/archive/csr/1975_2011/results_merged/sect_06_colon_rectum.pdf (accessed 17 June 2015).
32. Briscoe ME (1987) Why do people go to the doctor? Sex differences in the correlates of GP consultation. *Soc Sci Med* **25**, 507–513.
33. Butterworth AS, Higgins JP & Pharoah P (2006) Relative and absolute risk of colorectal cancer for individuals with a family history: a meta-analysis. *Eur J Cancer* **42**, 216–227.

# Paper 2

Vulcan et al. BMC Cancer (2017) 17:842
DOI 10.1186/s12885-017-3874-4

BMC Cancer

RESEARCH ARTICLE Open Access

# High blood glucose levels are associated with higher risk of colon cancer in men: a cohort study

Alexandra Vulcan[1*], Jonas Manjer[2] and Bodil Ohlsson[3]

## Abstract

**Background:** High levels of blood glucose are thought to be associated with colorectal cancer (CRC) and hyperinsulinemia, an interstage in the development of CRC. The purpose of this study was to examine associations between incident CRC and blood glucose; plasma insulin; and the homeostasis model assessment for insulin resistance (HOMA2-IR), respectively, and to determine whether these associations were dependent on sex and cancer site.

**Methods:** The Malmö Diet and Cancer cardiovascular cohort comprises 6103 individuals. During 81,781 person-years of follow-up, 145 cases of CRC were identified. The hazard ratio of measured blood glucose and plasma insulin and calculated HOMA2-IR were estimated with Cox proportional hazard regression.

**Results:** An association was found between high levels of blood glucose and risk of CRC (HR: 1.72 for the highest compared with the lowest quartile; 95% CI: 1.05, 2.84; $p_{trend} = 0.044$), and colon cancer (HR: 1.70 for the highest compared with the lowest quartile; 95% CI: 0.87, 3.33; $p_{trend} = 0.032$). In men, an association was found between blood glucose and CRC (HR: 2.80 for the highest compared with the lowest quartile; 95% CI: 1.37, 5.70; $p_{trend} = 0.001$), and colon cancer (HR: 4.48 for the highest compared with the lowest quartile; 95% CI: 1.27, 15.84; $p_{trend} = 0.007$), but this was not found in women. No associations between plasma insulin, or HOMA2-IR, and CRC, were found.

**Conclusion:** High levels of blood glucose in men are associated with risk of colon cancer. The findings contribute to facilitating to identify those most in need of prevention and screening.

**Keywords:** Blood glucose, Colorectal cancer (CRC), Homeostasis model assessment of insulin resistance (HOMA2-IR), Malmö diet and cancer study, Plasma insulin, Sex

## Background

Colorectal cancer (CRC) is one of the most common cancer forms in the Western world [1]. The increased incidence of CRC is associated with the increased incidence of lifestyle-related diseases, e.g., metabolic syndrome, overweight, obesity, and type 2 diabetes [2–4].

The latter diseases are characterized by hyperglycaemia, hyperinsulinemia, and insulin resistance [5]. The interaction between these diseases and CRC is being discussed, and molecular and etiological mechanisms are being sought. Hyperglycaemia might be associated with CRC [6], and the association may differ depending on different cancer sites and sex [4, 7]. Involvement of reactive oxygen species (ROS) and the accumulation of advanced glycation end products (AGE) are hypothesized to stimulate carcinogenic pathways [8–10]. Other proposals are that hyperinsulinemia drives the carcinogenic process through influence on the growth of cancer cells, stimulation of proliferation, decrease of apoptosis, and promotion of intestinal carcinogenesis [11]. Another hypothesis is that insulin resistance is responsible for the increased cancer risk. Although several studies have been conducted in the field, most of them have been case-control studies, and there has been discussion of whether further cohort studies are warranted [7].

## Method

The primary aim of this study was to examine the associations between incident CRC and blood glucose levels;

* Correspondence: alexandra.vulcan@med.lu.se
[1]Lund University, Skåne University Hospital, Department of Clinical Sciences, Division of Internal Medicine, Jan Waldenströms gata 14, 205 02 Malmö, Sweden
Full list of author information is available at the end of the article

plasma insulin levels; and homeostasis model assessment for insulin resistance (HOMA2-IR), respectively, and incident CRC. The secondary aim was to study whether the associations were dependent on sex and cancer site.

The study was approved by the Regional Ethical Review Board in Lund (50–91, 2013/804).

### Study population

The Malmö Diet and Cancer study (MDCS) is a large population-based study, conducted in the period 1991–1996 in Malmö, where all men and women, born between 1923 and 1950, were invited to participate. Altogether, 28,098 participants completed the baseline examinations after having given their written informed consent. The method of MDCS has previously been described by Manjer et al. [12]. The Malmö Diet and Cancer cardiovascular cohort (MDC-CC) comprises 6103 individuals randomly selected from MDCS. Out of the 6103 participants in MDC-CC, 5540 individuals accepted and were re-scheduled for blood sampling. 396 individuals did not leave a blood glucose and plasma insulin sample and were therefore excluded from the present study, leaving 5144 individuals (2117 men). From the remaining individuals, prevalent diabetes was found in 219 individuals (123 men), and prevalent CRC in 14 individuals (2 men), at baseline, all of whom were also excluded from the study, leaving 4910 individuals (1992 men).

### Cases of CRC

In the study, 145 cases of CRC (71 men) were identified from the Swedish Cancer Registry, of which 81 cases were colon cancer (37 men) and 64 were rectal cancer (34 men), during 81,781 person-years of follow-up. Follow-up time was defined as the time from the date of enrolment until the date of CRC diagnosis, death, migration or end of follow-up (31 December 2010), whichever came first. The mean follow-up was 16.7 ± 3.7 years.

### Plasma insulin, blood glucose and insulin resistance

In the MDC-CC all the blood and plasma samples were collected at the MDC-CC baseline by a trained nurse in the morning after 12 h of fasting, and plasma was separated and immediately frozen at −20 °C until analysed. Analyses were performed according to the clinical routines of the Department of Clinical Chemistry. Blood glucose was analysed using a routine hexokinase method. Insulin levels were measured in mIU/ml by a radioimmunoassay. The lowest limit for detection was 3 mIU/ml.

HOMA2-IR was calculated with the use of a HOMA2-IR calculator [13]. In the analysis of HOMA2-IR, extreme values of blood glucose (< 3.5 or >25 mmol/l) were excluded, as were extreme values of plasma insulin (< 3 or >57.5 mIU/ml), thus leaving 4451 individuals for analysis of HOMA2-IR. From the remaining individuals 135 cases of CRC, 76 cases colon cancer, and 59 cases of rectal cancer were found.

### Other variables

At MDCS baseline, age was obtained from personal identification numbers. Body Mass Index (BMI) was calculated from measured weight and length.

A self-administered structured questionnaire was used for the assessment of level of education, physical activity, alcohol consumption and smoking. The level of education was divided into four different categories: ≤ 8 years; 9–10 years; 11–13 years of education; and university degree. The subjects were asked to estimate their physical activity in terms of how many minutes per week they spent on 17 different activities. The duration was multiplied with an activity-specific intensity coefficient and an overall leisure-time physical activity score was created [14]. Alcohol intake was divided into four categories: zero; < 15 g/d for women and <20 g/d for men; 15–30 g/d for women and 20–40 g/d for men; and >30 g/d for women and >40 g/d for men. Smokers were divided into three categories: current smokers; ex-smokers; and non-smokers. Irregular smoking was counted as current smoking. The result was divided into quartiles.

Prevalent diabetes was determined by self-reported illness based on physician's diagnosis or treatment with anti-diabetes medicine, or information from medical data registries indicating a date of diagnosis before inclusion in the MDC-CC. Incident diabetes diagnosis was obtained either from the Regional Diabetes 2000 register of Scania, the Malmö HbA1C register or the Swedish National Diabetes Register until 31st of December 2010. In the MDC-CC, 716 incident cases of diabetes (350 men) were found. In those with incident diabetes, 31 cases of incident CRC (22 men) were found.

### Statistics

The SPSS statistics (version 23; IBM Corporation, Armonk, NY, USA) was used for all statistical analyses.

Chi-square test was used when examining baseline categorical characteristics, and T-test when examining baseline continuous characteristics, in the cases and the non-cases.

The Cox proportional hazard regression model was used when estimating hazard ratios (HR) of incident CRC, colon cancer, and rectal cancer, depending on quartiles of blood glucose, plasma insulin and HOMA2-IR. The proportionality assumption was tested for all the adjustment factors with a Kaplan-Maier curve before analysis. Two models are presented: an unadjusted model, and a full model. The full model was adjusted for the background variables indicating a difference between the cases and the non-cases ($p < 0.2$), i.e. age, sex (when appropriate), BMI, and smoking status.

A test for interaction between sex and blood glucose; plasma insulin levels; and HOMA2-IR, respectively, with regard to CRC incidence was performed by adding a multiplicative variable (i.e. sex × blood glucose−/insulin-/HOMA2-IR quartiles (treated as continuous variables)) to the full model. If a significant interaction was found, subgroup analysis based on sex was performed.

In the sensitivity analysis, we excluded individuals with incident diabetes, and apart from that we made one additional model, full model glucose/insulin. The full model insulin was only used when estimating HR for quartiles of glucose and was adjusted for age, sex, BMI, smoking status, and insulin. The full model glucose was only used when estimating HR for quartiles of insulin and adjusted for age, sex, BMI, smoking status, and glucose. In addition, we excluded individuals with CRC diagnosis within two years of inclusion.

All tests were two-sided and statistical significance was assumed at $p < 0.05$.

## Results

Compared with the non-cases, the cases were older (Table 1), had larger waist circumference (86.9 ± 13.5 cm and 83.2 ± 12.6 cm, respectively, $p = 0.019$), and higher plasma insulin levels (9.6 ± 18.9 mIU/ml and 7.7 ± 7.1 mIU/ml, respectively, $p = 0.012$) at inclusion. More of the cases than non-cases developed diabetes during the follow-up (21.4% and 14.6%, respectively).

### Blood glucose levels and colorectal, colon, or rectal cancer

An association between high levels of blood glucose and risk of CRC and colon cancer was found (HR: 1.72 for the highest compared with the lowest quartile; 95% CI: 1.05, 2.84, p for trend = 0.044) and (HR: 1.70 for the highest compared with the lowest quartile; 95% CI: 0.87, 3.33, p for trend = 0.032) (Table 2), respectively. With regard to blood glucose levels, an interaction between sex was found in CRC ($p = 0.013$) and in colon cancer ($p = 0.032$), but not in rectal cancer ($p = 0.130$). A significant association between high levels of blood glucose and CRC was found in men (HR: 2.80 for the highest compared with the lowest quartile; 95% CI: 1.37, 5.70; p for trend = 0.001), but not in women (HR: 1.02 for the highest compared with the lowest quartile; 95% CI: 0.53, 1.95; p for trend = 0.739) (Table 3). Also, a significant association was found between high levels of blood glucose and colon cancer in men (HR: 4.23 for the highest compared with the lowest quartile; 95% CI: 1.46, 13.44; p for trend = 0.002), but not in women (HR: 1.01 for the highest compared with the lowest quartile; 95% CI: 0.44, 2.34; p for trend = 0.878) (Table 4).

### Plasma insulin levels and colorectal, colon, or rectal cancer

No significant associations were found between plasma insulin levels and CRC, colon, or rectal cancer (Table 2). No interaction between sex and plasma insulin levels was found in CRC ($p = 0.142$), colon cancer ($p = 0.358$), or in rectal cancer ($p = 0.280$).

**Table 1** Baseline characteristics of cases and non-cases in the Malmö Diet and Cancer Study cardiovascular cohort

| | Cases $n = 145$ (%) | Non-cases $n = 4765$ (%) | $p$-value[a] |
|---|---|---|---|
| Sex | | | 0.037 |
| - Men | 71 (49.0) | 1921 (40.1) | |
| - Women | 74 (51.0) | 2844 (59.9) | |
| Age (years) | 61.3 ± 7.0 | 57.4 ± 5.9 | <0.001 |
| Age quartiles | | | <0.001 |
| 45.8–52.4 years | 22 (15.2) | 1205 (25.3) | |
| 52.4–57.8 years | 26 (17.9) | 1202 (25.2) | |
| 57.8–62.7 years | 34 (23.4) | 1194 (25.1) | |
| 62.7–68.1 years | 63 (43.5) | 1164 (24.4) | |
| Body Mass Index (kg/ $m^2$) | 26.2 ± 3.9 | 25.6 ± 3.9 | 0.032 |
| Body Mass Index ≤25 kg/$m^2$ > | | | 0.155 |
| - ≤ 25 kg/$m^2$ | 63 (43.4) | 2356 (49.4) | |
| - > 25 kg/$m^2$ | 82 (56.6) | 2409 (50.6) | |
| Education | | | 0.732 |
| - > 10 years | 42 (29.0) | 1335 (28.0) | |
| - ≤ 10 years | 103 (71.0) | 3425 (71.9) | |
| - Missing | 0 (0) | 5 (0.1) | |
| Physical activity | | | 0.439 |
| - High[c] | 38 (27.3) | 1182 (24.5) | |
| - Lower[c] | 105 (71.3) | 3555 (74,9) | |
| - Missing | 2 (1.4) | 28 (0.6) | |
| Alcohol intake | | | 0.832 |
| - Zero | 26 (17.9) | 742 (15.6) | |
| - < 15 g/d for women and <20 g/d for men | 91 (62.8) | 3088 (64.8) | |
| - 15–30 g/d for women and 20–40 g/d for men | 21 (14.5) | 757 (15.9) | |
| - > 30 g/d for women and >40 g/d for men | 7 (4.8) | 173 (3.6) | |
| - Missing | 0 (0) | 5 (0.1) | |
| Smoking | | | 0.023 |
| - Current | 31 (21.4) | 1307 (27.4) | |
| - Ex | 65 (44.8) | 1556 (32.7) | |
| - Never | 49 (33.8) | 1900 (39.9) | |
| - Missing | 0 (0) | 2 (0.04) | |

[a]Calculated with Chi-square test for categorical variables and with T-test for the continuous variables

Physical activity was defined as high when in the highest quartile of the whole group, and lower when in the three lower quartiles [14]

Values are number of individuals and percentage or mean and standard deviation. $p < 0.05$ was considered statistically significant

**Table 2** Hazard ratio (HR) of incident colorectal-, colon, and rectal cancer associated with blood glucose, plasma insulin and Homeostasis Model Assessment for Insulin Resistance (HOMA2-IR) in the Malmö Diet and Cancer Study cardiovascular cohort

| | | | Colorectal cancer | | | | Colon cancer | | | | | Rectal cancer | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Crude model | | Full model | | | Crude model | | Full model | | | Crude model | | Full model | |
| Quartiles | Min-max | Cases/person-years | HR | 95% CI | HR | 95% CI | Cases/person-years | HR | 95% CI | HR | 95% CI | Cases/person-years | HR | 95% CI | HR | 95% CI |
| Blood glucose (mmol/l) *n* = 4910 | | | | | | | | | | | | | | | | |
| 1 | 3.3–4.5 | 25/18381 | 1.00 | | 1.00 | | 14/18246 | 1.00 | | 1.00 | | 11/18215 | 1.00 | | 1.00 | |
| 2 | 4.6–4.8 | 30/21325 | 1.04 | 0.61, 1.76 | 0.94 | 0.55, 1.60 | 13/21193 | 0.80 | 0.38, 1.70 | 0.71 | 0.33, 1.51 | 17/21197 | 1.33 | 0.62, 2.83 | 1.21 | 0.56, 2.60 |
| 3 | 4.9–5.2 | 34/22388 | 1.12 | 0.67, 1.87 | 0.99 | 0.59, 1.68 | 22/22277 | 1.29 | 0.66, 2.52 | 1.11 | 0.56, 2.21 | 12/22163 | 0.89 | 0.39, 2.03 | 0.78 | 0.34, 1.81 |
| 4 | 5.3–16.8 | 56/19687 | 2.11 | 1.31, 3.37 | 1.72 | 1.05, 2.84 | 32/19476 | 2.17 | 1.15, 4.06 | 1.70 | 0.87, 3.33 | 24/19360 | 2.05 | 1.00, 4.18 | 1.62 | 0.75, 3.45 |
| p for trend | | | 0.003 | | 0.044 | | | 0.005 | | 0.032 | | | 0.165 | | 0.530 | |
| p for interaction between sex and blood glucose | | | | | 0.013 | | | | | 0.032 | | | | | 0.130 | |
| Plasma insulin (mIU/l) n = 4910 | | | | | | | | | | | | | | | | |
| 1 | <3–4 | 33/23177 | 1.00 | | 1.00 | | 22/2047 | 1.00 | | 1.00 | | 11/22922 | 1.00 | | 1.00 | |
| 2 | 5–6 | 32/19306 | 1.16 | 0.72, 1.89 | 1.09 | 0.67, 1.78 | 17/19190 | 0.93 | 0.49, 1.75 | 0.85 | 0.45, 1.60 | 15/19154 | 1.63 | 0.75, 3.55 | 1.58 | 0.72, 3.45 |
| 3 | 7–8 | 33/16926 | 1.37 | 0.84, 2.22 | 1.25 | 0.76, 2.04 | 20/16800 | 1.25 | 0.68, 2.29 | 1.09 | 0.59, 2.03 | 13/16710 | 1.62 | 0.72, 3.06 | 1.55 | 0.69, 3.52 |
| 4 | 9–224 | 47/22372 | 1.48 | 0.95, 2.32 | 1.21 | 0.75, 1.96 | 22/22154 | 1.05 | 0.58, 1.89 | 0.80 | 0.42, 1.50 | 25/21149 | 2.35 | 1.16, 4.78 | 2.12 | 0.99, 4.53 |
| p for trend | | | 0.064 | | 0.411 | | | 0.645 | | 0.635 | | | 0.024 | | 0.078 | |
| p for interaction between sex and plasma insulin | | | | | 0.142 | | | | | 0.350 | | | | | 0.316 | |
| HOMA2-IR *n* = 4451 | | | | | | | | | | | | | | | | |
| 1 | 0.3–0.5 | 23/13702 | 1.00 | | 1.00 | | 16/13623 | 1.00 | | 1.00 | | 7/13514 | 1.00 | | 1.00 | |
| 2 | 0.5–0.8 | 30/20111 | 0.98 | 0.59, 1.62 | 0.91 | 0.55, 1.50 | 16/19996 | 0.80 | 0.42, 1.53 | 0.72 | 0.37, 1.38 | 14/19966 | 1.33 | 0.60, 2.92 | 1.27 | 0.58, 2.82 |
| 3 | 0.8–1.2 | 37/20527 | 1.19 | 0.74, 1.91 | 1.06 | 0.65, 1.72 | 22/20387 | 1.08 | 0.59, 1.96 | 0.92 | 0.50, 1.70 | 15/20288 | 1.40 | 0.64, 3.04 | 1.33 | 0.60, 2.95 |
| 4 | 1.2–7.3 | 45/19776 | 1.51 | 0.96, 2.37 | 1.23 | 0.75, 2.01 | 22/19574 | 1.13 | 0.62, 2.06 | 0.84 | 0.44, 1.33 | 23/19553 | 2.23 | 1.09, 4.57 | 2.03 | 0.93, 4.42 |
| p for trend | | | 0.066 | | 0.429 | | | 0.527 | | 0.733 | | | 0.040 | | 0.116 | |
| p for interaction between sex and HOMA2-IR | | | | | 0.099 | | | | | 0.211 | | | | | 0.789 | |

Full model: Calculated with the Cox proportional hazard regression model. Adjusted for sex (man, woman), age (quartiles of age), BMI (≤ 25 kg/m$^2$, > 25 kg/m$^2$), and smoking (current, ex or never). Values are quartile ranges, hazard ratios (HR), and 95% confidence intervals (CI)

**Table 3** Hazard ratio (HR) of incident colorectal- and colon cancer associated with blood glucose for men and women in the Malmö Diet and Cancer Study cardiovascular cohort

| | | Colorectal cancer $n_{men} = 1992$ $n_{women} = 2981$ | | | | | Colon cancer $n_{men} = 1958$ $n_{women} = 2888$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Crude model | | Full model | | | Crude model | | Full model | |
| Quartiles of glucose | Min-max (mIU/l) | Cases/person-years | HR | 95% CI | HR | 95% CI | Cases/person-years | HR | 95% CI | HR | 95% CI |
| Men | | | | | | | | | | | |
| 1 | 3.3–4.5 | 5/4400 | 1.00 | | 1.00 | | 2/4358 | 1.00 | | 1.00 | |
| 2 | 4.6–4.8 | 10/7276 | 1.28 | 0.57, 2.90 | 1.25 | 0.55, 2.85 | 4/7216 | 2.05 | 0.50, 7.01 | 1.95 | 0.57, 6.70 |
| 3 | 4.9–5.2 | 16/9835 | 2.10 | 0.99, 4.45 | 2.03 | 0.95, 4.32 | 9/9767 | 2.28 | 0.67, 7.79 | 2–08 | 0.60, 7.17 |
| 4 | 5.3–16.8 | 40/10853 | 3.08 | 1.54, 6.15 | 2.80 | 1.37, 5.70 | 22/19679 | 5.40 | 1.84, 15.87 | 4.23 | 1.46, 13.44 |
| p for trend | | | <0.001 | | 0.001 | | | 0.001 | | 0.002 | |
| Women | | | | | | | | | | | |
| 1 | 3.4–4.5 | 20/13981 | 1.00 | | 1.00 | | 12/13888 | 1.00 | | 1.00 | |
| 2 | 4.6–4.8 | 20/14048 | 1.24 | 0.64, 2.38 | 1.21 | 0.62, 2.34 | 9/13977 | 0.77 | 0.29, 2.05 | 0.73 | 0.27, 1.96 |
| 3 | 4.9–5.2 | 18/12553 | 0.89 | 0.47, 1.66 | 0.82 | 0.44, 1.55 | 13/12510 | 1.08 | 0.50, 2.34 | 0.96 | 0.45, 2.09 |
| 4 | 5.3–12.2 | 16/8834 | 1.16 | 0.62, 2.17 | 1.02 | 0.53, 1.95 | 10/8797 | 1.22 | 0.54, 2.71 | 1.01 | 0.44, 2.34 |
| | | | 0.908 | | 0.739 | | | 0.562 | | 0.878 | |

Full model: Calculated with the Cox proportional hazard regression model. Adjusted for age (quartiles of age), BMI (≤ 25 kg/m$^2$, > 25 kg/m$^2$), and smoking (current, ex or never)
Values are quartile ranges, hazard ratios (HR), and 95% confidence intervals (CI)

### HOMA2-IR and colorectal, colon, or rectal cancer

No significant associations between HOMA2-IR and CRC, colon, or rectal cancer were found in the full model (Table 2). There was a borderline interaction between sex and HOMA2-IR in CRC ($p = 0.099$), but not in colon cancer ($p = 0.211$), or rectal cancer ($p = 0.789$). The associations between HOMA2-IR and CRC did not reach statistical significance in men (HR: 1.62 for the highest compared with the lowest quartile; 95% CI: 0.81, 3.25; p for trend = 0.132), or in women (HR: 0.77 for the

**Table 4** Hazard ratio (HR) of incident colorectal cancer associated with Homeostasis Model Assessment for Insulin Resistance (HOMA2-IR) for men and women in the Malmö Diet and Cancer Study cardiovascular cohort

| | | | Unadjusted model | | Full model | |
|---|---|---|---|---|---|---|
| Quartiles of HOMA2-IR | Min-max (mIU/l) | Cases/person-years | HR | 95% CI | HR | 95% CI |
| Men ($n = 1824$) | | | | | | |
| 1 | 0.35–0.63 | 6/3938 | 1.00 | | 1.00 | |
| 2 | 0.64–0.90 | 9/7615 | 0.66 | 0.30, 1.47 | 0.64 | 0.29, 1.44 |
| 3 | 0.91–1.28 | 19/8618 | 1.35 | 0.69, 2.63 | 1.29 | 0.65, 2.56 |
| 4 | 1.29–6.85 | 30/9434 | 1.80 | 0.95, 3.41 | 1.62 | 0.81, 3.25 |
| p for trend | | | 0.042 | | 0.132 | |
| Women ($n = 2627$) | | | | | | |
| 1 | 0.34–0.51 | 17/9764 | 1.00 | | 1.00 | |
| 2 | 0.52–0.77 | 21/12496 | 1.26 | 0.67, 2.37 | 1.11 | 0.59, 2.09 |
| 3 | 0.78–1.06 | 18/11908 | 1.15 | 0.60, 2.18 | 0.97 | 0.50, 1.89 |
| 4 | 1.07–7.30 | 15/10309 | 0.97 | 0.49, 1.89 | 0.77 | 0.37, 1.59 |
| p for trend | | | 0.760 | | 0.779 | |

HR was calculated with the Cox proportional hazard regression model
Unadjusted model: No adjustments made
Full model: Adjusted for age (quartiles of age), BMI (≤ 25 kg/m$^2$, > 25 kg/m$^2$), and smoking (current, ex or never)
Values are quartile ranges, hazard ratios (HR), and 95% confidence intervals (CI)

highest compared with the lowest quartile; 95% CI: 0.37, 1.59; p for trend = 0.779) (Table 4).

### Sensitivity analysis

After excluding those with incident diabetes, the association between blood glucose and CRC was no longer significant (HR: 1.74 for the highest compared with the lowest quartile; 95% CI: 1.00, 3.07, p for trend = 0.134). The same was true for the association between blood glucose and colon cancer (HR: 1.71 for the highest compared with the lowest quartile; 95% CI: 0.80, 3.68, p for trend = 0.128). In men, the association between blood glucose and colon cancer had borderline significance (HR: 3.47 for the highest compared with the lowest quartile; 95% CI: 0.88, 13.74, p for trend = 0.099).

In excluding those with diagnosis of CRC within two years from inclusion in the study (13 individuals, where of eight were men), the association between blood glucose and CRC had borderline significance (HR: 1.71 for the highest compared with the lowest quartile; 95% CI: 1.01, 2.89, p for trend = 0.090), and the risk estimate for the association between blood glucose and rectal cancer in men increased (HR: 5.20 for the highest compared with the lowest quartile; 95% CI: 1.47, 18.43, p for trend = 0.006).

In additionally adjusting for insulin in the full model for glucose, neither the risk estimate, nor the significance substantially changed. Nor did they substantially change when additionally adjusting for glucose in the full model for insulin (data not shown).

## Discussion

The results from the present study indicate that high levels of blood glucose are associated with risk of CRC, more specifically in men. No associations were found for insulin or HOMA2-IR and risk of CRC.

Our results are in agreement with other studies, which also found an association between high glucose levels and CRC, with significant associations in men, but not in women, and in colon cancer, but not in rectal cancer [7]. Glucose induces expressions of Amphiregulin, through transcriptional regulation of the MAX-like protein X [15], suggesting that one part of the tumorigenesis in CRC might be glycolysis [16]. Furthermore, hyperglycaemia gives energy to malignant cells for their proliferation [16], and thus favours cancer growth and neoangiogenesis. Since there can be an insulin-independent glucose uptake in cancer cells, this may have an impact on the association between glucose, insulin and risk of CRC.

In hyperglycaemia, the cells' production of reactive oxygen intermediates increases, and is therefore speculated to be a part of the induction of apoptosis in endothelial cells [17]. Chronic inflammation, present for example during hyperglycaemia, leads to imbalance between production and restoration of ROS, leading to oxidative stress within the target tissue, which may damage DNA and reduce DNA repair [18].

Glucose and insulin levels are closely linked, which makes it difficult to separate the association, although they influence the cancer development through different pathways. Insulin stimulates the proliferation of cells partly through binding insulin to insulin- or insulin-like growth factor 1 (IGF-1) receptors, and partly through inhibition of IGF-binding proteins, thus increasing the availability for IGF-1 to bind to IGF-1 receptors [19]. Circulating IGF-1 is thought to increase the risk of CRC [20]. In a meta-analysis by Xu et al. [7], an association between plasma insulin levels and risk of CRC was found in case-cohort studies, but not in cohort studies, in line with the present cohort study.

Insulin resistance, hyperinsulinemia, and hyperglycaemia are important factors in metabolic syndrome. When insulin resistance develops in type 2 diabetes, the pancreatic insulin secretion is increased in compensation [21]. It is speculated that excess of insulin might enhance colonic epithelial activity and induce the formation of aberrant cryptic foci [22]. Insulin resistance affects the metabolic pathways, over-stimulates the mitogenic pathways and stimulates cell proliferation [23]. However, in the present study no association was found between HOMA2-IR and CRC, but this might be due to the small number of cases in the analysis.

In the present study, more of the cases than the non-cases developed diabetes. They also had a larger waist circumference. Some research has suggested that the association between diabetes and CRC may not be causal; rather the two diseases just share the same preconditions, such as obesity [24]. This might be a reason why only high glucose levels, and not insulin and insulin resistance, show risk association. Metformin reduces insulin resistance and improves glycaemic control [25]. High intake of metformin seems to be protective against CRC [26]. The hypothesis is that metformin slows the progression and growth of the tumour [27]. In the present study, it was not possible to determine whether metformin was used between inclusion and the end of study, and this might have affected the results. However, in excluding incident diabetes in the sensitivity analysis, the effect on cancer development might have been avoided, but also excluding those with higher blood glucose, and decreasing the possible number of cases in the analysis.

As seen in our previous and present study on the MDCS, associations with CRC might not be straightforward, but may be dependent on sex and cancer site [28]. CRC may differ in nature depending on whether it is a distal or proximal CRC, with more micro instability in the distal CRC and more chromosomal instability in the proximal cancer [29]. Whether this difference can explain the variations in associations between rectal and

colon cancer remains to be determined. There is a difference in the DNA methylation based on sex. An association between altered expressions and insulin secretion in the human pancreatic islet has been found, where women have a higher glucose-induced insulin secretion [30]. Oestrogen also plays an important role in the glucose metabolism in the body. Oestrogen facilitates the transport of glucose to the brain and promotes neural aerobic glycolysis [31]. In men, 5-DHEA, an adrenal steroid hormone which modulates glucose uptake, is more elevated than in women [32]. As there are differences in the glucose metabolism for men and women, high glucose levels may affect the risk differently, as seen in the present study.

The strength of the present research is that it is a cohort study with a long follow-up. A limitation of the study is the small number of cases, and it cannot be excluded that some of the stratified analyses may be underpowered. Taking family history of CRC or inflammatory bowel disease into account in our analyses would have been valuable, since they are contributing factors in the development of CRC [33], but information on these risk factors was missing, as was information on family history of diabetes and blood lipid profile. Even if adjustments for possible confounders and known risk factors were made, the occurrence of some residual confounding cannot be excluded.

## Conclusion

High levels of blood glucose are associated with risk of CRC, mainly with colon cancer in men. The findings contribute to facilitating to identify those most in need of prevention and screening.

#### Abbreviations

AGE: Advanced Glycation End products; BMI: Body Mass Index; HOMA2-IR: Homeostasis Model Assessment for Insulin Resistance; IGF-1: Insulin-like Growth Factor 1; MDC-CC: The Cardiovascular Cohort in the Malmö Diet and Cancer study; MDCS: Malmö Diet and Cancer study; ROS: Reactive Oxygen Species

#### Acknowledgments

Professional writing service was provided by Anchor English.

#### Funding

This study was funded by grants from Development Foundation of Region Skåne, Direktör Albert Påhlsson's Foundation, the Malmö Hospital Foundation for Cancer Prevention and the South Region of healthcare. The funders had no role in the design, analysis or writing of this article.

#### Availability of data and materials

The data that supports the finding of this study are available from the Malmö Diet and Cancer Study, but restrictions apply to the availability of these data, which are used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Malmö Diet and cancer study.

#### Authors' contributions

AV contributed to the study design, performed the data analysis and the interpretation of the results and wrote the manuscript. BO and JM contributed to the study design and interpretation of the results. BO financed the study. All authors contributed to the manuscript writing with constructive criticism.

#### Ethics approval and consent to participate

All procedures performed in the studies involving human participants were in accordance with the ethical standard of the ethical committee at Lund University (50–91, 2013/804) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

#### Competing interest

The authors declare that they have no competing interests.

#### Consent for publication

Not applicable.

## Publisher's Note



#### Author details

[1]Lund University, Skåne University Hospital, Department of Clinical Sciences, Division of Internal Medicine, Jan Waldenströms gata 14, 205 02 Malmö, Sweden. [2]Lund University, Skåne University Hospital, Department of Clinical Sciences, Division of Surgery, Inga Marie Nilssons gata 47, 205 02 Malmö, Sweden. [3]Lund University, Skåne University Hospital, Department of Clinical Sciences, Division of Internal Medicine, Jan Waldenströms gata 15, 205 02 Malmö, Sweden.

Received: 12 April 2017 Accepted: 4 December 2017
Published online: 12 December 2017

#### References

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013; doi:10.1016/j.ejca.2012.12.027.
2. Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, Qin H. Obesity and risk of colorectal cancer: a systematic review of prospective studies. PLoS One. 2013; doi:10.1371/journal.pone.0053916.
3. Kramer HU, Schottker B, Raum E. Brenner H (2012) type 2 diabetes mellitus and colorectal cancer: meta-analysis on sex-specific differences. Eur J Cancer. 2012; doi:10.1016/j.ejca.2011.07.010.
4. Yuhara H, Steinmaus C, Cohen SE, Corley DA, Tei Y. Buffler PA (2011) is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? Am J Gastroenterol. 2011; https://doi.org/10.1038/ajg.2011.301.
5. Maitra A. Endocrine system. In: Kumar V, Abbas A, J. A, editors. Robbins Basic Pathology, 9th edition. Philadelphia, USA; Elsevier Saunders; 2013. p 715–763.
6. Shin HY, Jung KJ, Linton JA. Jee SH (2014) association between fasting serum glucose levels and incidence of colorectal cancer in Korean men: the Korean cancer prevention study-II. Metab Clin Exp. 2014; doi:10.1016/j.metabol.2014.07.006.
7. Xu J, Ye Y, Wu H, Duerksen-Hughes P, Zhang H, Li P, Huang J, Yang J, Wu Y, Xia D. Association between markers of glucose metabolism and risk of colorectal cancer. BMJ Open. 2016; doi:10.1136/bmjopen-2016-011430.
8. Van Guilder GP, Hoetzer GL, Greiner JJ, Stauffer BL, Desouza CA. Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. Obesity. 2006; doi:10.1038/oby.2006.248.
9. Wang L, Cai S, Teng Z, Zhao X, Chen X, Bai X. Insulin therapy contributes to the increased risk of colorectal cancer in diabetes patients: a meta-analysis. Diagn Pathol. 2013; doi:10.1186/1746-1596-8-180.
10. Lee W. The CEACAM1 expression is decreased in the liver of severely obese patients with or without diabetes. Diagn Pathol. 2011; doi:10.1186/1746-1596-6-40.
11. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. The Journal of nutrition 2001; 131 Supplement 11:3109S–3120S.
12. Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, Mattisson I, Berglund G. The Malmo diet and cancer study: representativity, cancer incidence and mortality in participants and non-participants. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation. 2001;10:489–99.

13. The Oxford Centre for Diabetes, Endocinology, and Metabolism, Diabetes Trials Unit. The University of Oxford. 2016. https://www.dtu.ox.ac.uk/homacalculator. Acessed 15 Nov 2016.
14. Li C, Aronsson CA, Hedblad B, Gullberg B, Wirfalt E, Berglund G. Ability of physical activity measurements to assess health-related risks. Eur J Clin Nutr. 2009; doi:0.1038/ejcn.2009.69.
15. Nam SO, Yotsumoto F, Miyata K, Fukagawa S, Yamada H, Kuroki M. Miyamoto S (2015) Warburg effect regulated by amphiregulin in the development of colorectal cancer. Cancer medicine. 2015; doi:10.1002/cam4.416.
16. Yeh CS, Wang JY, Chung FY, Lee SC, Huang MY, Kuo CW, Yang MJ, Lin SR. Significance of the glycolytic pathway and glycolysis related-genes in tumorigenesis of human colorectal cancers. Oncol Rep. 2008;19:81–91.
17. Du X, Stocklauser-Farber K, Rosen P. Generation of reactive oxygen intermediates, activation of NF-kappaB, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? Free Radic Biol Med. 1999;27:752–63.
18. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; doi:10.1038/nature01322.
19. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. J Natl Cancer Inst. 2002;94:972–80.
20. Chi F, Wu R, Zeng YC, Xing R, Liu Y. Circulation insulin-like growth factor peptides and colorectal cancer risk an updated systematic review and meta-analysis. Mol Biol Rep. 2013; doi:10.1007/s11033-012-2432-z.
21. Akbaraly TN, Kumari M, Head J, Ritchie K, Ancelin ML, Tabak AG, Brunner E, Chaudieu I, Marmot MG, Ferrie JE, Shipley MJ, Kivimaki M. Glycemia, insulin resistance, insulin secretion, and risk of depressive symptoms in middle age. Diabetes Care. 2013; doi:10.2337/dc12-0239.
22. Ezuka A, Sakai E, Kawana K, Nagase H, Kakuta Y, Uchiyama S, Ohkubo H, Higurashi T, Nonaka T, Endo H, Takahashi H, Nakajima A. Association between factors associated with colorectal cancer and rectal aberrant crypt foci in humans. Oncol Lett. 2015; doi:10.3892/ol.2015.3763.
23. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest. 2000; doi:10.1172/JCI7535.
24. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. Diabetes Care. 2010; https://doi.org/10.2337/dc10-0666.
25. Bordini HP, Kremer JL, Fagundes TR, Melo GP, Conchon-Costa I, da Silva SS, Cecchini AL, Panis C, Luiz RC. Protective effect of metformin in an aberrant crypt foci model induced by 1,2-dimethylhydrazine: modulation of oxidative stress and inflammatory process. Mol Carcinog. 2016; doi:10.1002/mc.22545.
26. Cardel M, Jensen SM, Pottegard A, Jorgensen TL, Hallas J. Long-term use of metformin and colorectal cancer risk in type II diabetics: a population-based case-control study. Cancer medicine. 2014; doi:10.1002/cam4.306.
27. Hosono K, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, Suzuki K, Iida H, Sakamoto Y, Yoneda K, Koide T, Tokoro C, Abe Y, Inamori M, Nakagama H, Nakajima A. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. Cancer Prev Res. 2010; doi:10.1158/1940-6207.CAPR-10-0186.
28. Vulcan A, Brandstedt J, Manjer J, Jirstrom K, Ohlsson B, Ericson U. Fibre intake and incident colorectal cancer depending on fibre source, sex, tumour location and tumour, node, metastasis stage. Br J Nutr. 2015; doi:10.1017/S0007114515002743.
29. Lee GH, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer? - a systematic review. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2015; doi:10.1016/j.ejso.2014.11.001.
30. Hall E, Volkov P, Dayeh T, Esguerra JL, Salo S, Eliasson L, Ronn T, Bacos K, Ling C. Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. Genome Biol. 2014; doi:10.1186/s13059-014-0522-z.
31. Rettberg JR, Yao J, Brinton RD. Estrogen: a master regulator of bioenergetic systems in the brain and body. Front Neuroendocrinol. 2014; doi:10.1016/j.yfrne.2013.08.001.
32. Comitato R, Saba A, Turrini A, Arganini C, Virgili F. Sex hormones and macronutrient metabolism. Crit Rev Food Sci Nutr. 2015; doi:10.1080/10408398.2011.651177.
33. 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; doi:10.1016/j.ejca.2005.09.023.

# Paper 3

FOOD & NUTRITION RESEARCH, 2017
VOL. 61, 1341810
https://doi.org/10.1080/16546628.2017.1341810

Taylor & Francis
Taylor & Francis Group

ARTICLE



# Intake of different types of red meat, poultry, and fish and incident colorectal cancer in women and men: results from the Malmö Diet and Cancer Study

Alexandra Vulcan[a], Jonas Manjer[b], Ulrika Ericson[c] and Bodil Ohlsson[a]

[a]Division of Internal Medicine, Department of Clinical Science, Lund University, Skåne University Hospital, Malmö, Sweden; [b]Division of Surgery, Department of Clinical Science, Lund University, Skåne University Hospital, Malmö, Sweden; [c]Diabetes and Cardiovascular Disease, Genetic Epidemiology, Lund University, Skåne University Hospital, Malmö, Sweden

**ABSTRACT**

***Background***: Colorectal cancer (CRC) is considered one of the most common forms of cancer in the Western world. High intake of red and processed meat is considered to increase CRC development.

***Objective***: This study examined associations between intake of red meats, poultry, and fish and incident CRC, and if weight status modifies the associations.

***Design***: In the Malmö Diet and Cancer Study, dietary data was collected through a modified diet history method. Via the Swedish Cancer Registry, 728 cases of CRC were identified during 428 924 person-years of follow-up of 16 944 women and 10 987 men.

***Results***: Beef intake was inversely associated with colon cancer. However, in men high intake of beef was associated with increased risk of rectal cancer. High intake of pork was associated with increased incidence of CRC, and colon cancer. Processed meat was associated with increased risk of CRC in men. Fish intake was inversely associated with risk of rectal cancer. No significant interactions were found between different types of meat and weight status.

***Conclusions***: Findings suggest that associations between meat intake and CRC differ depending on meat type, sex, and tumor location in the bowel. Weight status did not modify observed associations.

**ARTICLE HISTORY**
Received 22 December 2016
Accepted 31 May 2017

**KEYWORDS**
BMI; colorectal cancer; colon cancer; rectal cancer; sex; red meat

## Introduction

Colorectal cancer (CRC) is estimated to be one of the most common forms of cancer in the Western world [1,2]. In Sweden, it is the fourth most common form of cancer and around 6500 persons per year develop the disease [3].

Several lifestyle factors are considered to be associated with the development of CRC. Higher body mass index (BMI) and waist circumference are positively associated with risk of CRC, independent of location, sex. or geographic area, as seen in a meta-analysis by Ma et al. [4]. Other lifestyle factors, such as diet, are also considered to be associated with risk of CRC. The World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) have concluded that there is convincing evidence that high intakes of red and processed meat are associated with increased risk of CRC [5]. A meta-analysis indicated that the risk increases by 17% per 100 g daily intake of red meat and by 18% per 50 g daily intake of processed red meat [6]. According to the guidelines from the National Food Agency in Sweden, the intake of red meat should not exceed 500 g per week [7].

The risk associations may differ between gender and tumor location in the bowel [8]. In a meta-analysis by Alexander et al. [9], the association between high intake of processed meat and CRC was mainly seen in men. Although rather few studies have examined specific types of red meat in relation to colorectal cancer, a recent meta-analysis (2015) indicate that the associations differ depending on meat subtypes and sub-sites of CRC. High intakes of beef associated with risk of colon cancer, but not with rectal cancer. Lamb intake was suggested to associate with increased risk of CRC. Intake of poultry was not associated with CRC, but the authors concluded that more studies on pork intake are warranted [10]. Findings regarding high fish intake indicate an inverse association with risk of CRC [11].

**CONTACT** Alexandra Vulcan alexandra.vulcan@med.lu.se Skåne University Hospital, Malmö Inga Marie Nilssons Gata 46 205 02, Malmö, Sweden.
Supplemental data for this article can be accessed here.

The primary objective of the present study was to examine if intake of red meat, considering different subgroups, such as beef and pork, unprocessed and processed red meat, fish, and poultry, is associated with incident CRC, colon cancer, and rectal cancer in women and men from the Malmö Diet and Cancer Study (MDCS). Since obesity may promote development of CRC, our second objective was to investigate whether weight status may modify the association between meat intakes and CRC.

## Subjects and methods

The study was approved by the Regional Ethical Review Board in Lund (50–81, 2013/804).

### Subjects

MDCS is a population-based prospective cohort study in Malmö, Sweden. All men and women living in Malmö between 1991 and 1996, born between 1923 and 1950, were invited to participate. Altogether, 28 098 participants completed all of the baseline examinations after having given their written informed consent. Of those having completed the baseline examination, 167 had been diagnosed with CRC before or at baseline examination, and were therefore excluded from the present study.

### Data collection

At baseline, the participants were asked to fill out questionnaires on socioeconomic, lifestyle, and dietary factors. They also recorded their cooked meals and underwent a diet history interview. Weight, height, and waist circumference were measured by trained nurses. Body composition was estimated with a single-frequency bio-impedance methodology (BIA 103, RJL systems, Detroit, USA). Body fat percentage was calculated using an algorithm provided by the manufacturer.

### Dietary data

Dietary data was gathered through a modified diet history method with a seven-day menu book for registration of meals that varied from day to day, most likely lunch and dinner, cold beverages, and nutrient supplements. In addition, the participants were given a 168-item questionnaire for assessment of consumption frequencies and portion sizes of foods that were not covered in the menu book. Finally, a 45-minute interview completed the dietary assessment. The merged data from the above mentioned methods was then coded using the Swedish Food Data Base [12]. The MDC diet assessment method has been described in detail elsewhere [13,14].

The diet analyses were adjusted for the variables called 'method version' and 'season'. Method version was used because altered coding routines of dietary data were introduced in September 1994 in order to shorten the interview time (from one hour to 45 minutes). This resulted in two slightly different method versions, before and after September 1994, but did not have any major influence on ranking of individuals [13]. The variable season was divided into spring, summer, autumn, and winter depending on when in the year the baseline examination was executed. Dietary change in the past (yes, no) was based on the question 'Have you substantially changed your eating habits because of illness or some other reasons?' The relative validity of the MDCS method was evaluated in the Malmö Food study 1984–1985 in a sample of Malmö residents, 105 women and 101 men, 50–69 years old. An 18-day weighted food record was used as the reference method, three days every second month during a year [15,16]. The Pearson correlation coefficients, adjusted for total energy, between the reference method and the MDCS method were 0.70/0.74 (carbohydrates), 0.69/0.64 (fats), 0.53/0.54 (proteins), 0.69/0.74 (fibers), 0.70/0.35 (fish), 0.51/0.43 (low fat meat) and 0.80/0.40 (high fat meat), in women and men, respectively.

The following variables for nutrient intake were used in this study: total energy (MJ), non-alcoholic energy (MJ), carbohydrates (percentage of energy (E %)), fat (E%), protein (E%), saturated fat (g), fiber (g), calcium (mg), folate (mg), iron (mg), and zinc (mg). The following daily intakes of foods were used in this study: red meat (g), unprocessed red meat (g) processed red meat (sausages and cured meat) (g), beef (g), pork (g), poultry (g), fish (g), dairy products (portions of milk, yogurt sour milk, and cheese), fruit and berries (g), and sugar-sweetened beverages (g). Red meat was defined as pork, beef and game. Total red meat included both processed and unprocessed red meat. Intakes of pork and beef were mainly based on non-processed meat, as distinction between pork and beef was not possible for all processed meats based on items included in the food questionnaire. The fish variable consisted of both processed and unprocessed fish. Portions, instead of grams, were used in order to analyse the sum of dairy products, because of different water content and because they usually are consumed in different weights. Standard portion sizes from the national Food Agency in Sweden were used to define portions for dairy products [17].

Energy-adjusted variables were obtained by regressing the food intakes on non-alcohol energy intake

[18]. Quintiles of the food residuals were used as exposure categories.

### Cancer cases

In the study, 728 cases of CRC were identified from the Swedish Cancer Registry, of which 463 were colon cancer and 265 rectal cancers, during 428 924 person-years of follow-up. Follow-up time was defined as the time from date of enrolment until date of CRC-diagnosis, death, migration, or end of follow-up (31 December 2010), whichever came first. Information on date of death was collected from The Swedish Cause-of-death registry. Mean follow-up was 15.4 years.

### Other variables

Prevalent diabetes diagnosis was determined from self-reported diagnosis, self-reported medication for diabetes, or information from medical data registries indicating a date of diagnosis before inclusion in the MDCS. Incident diabetes diagnosis was obtained either from the Regional Diabetes 2000 register of Scania, the Malmö HbA1C register or the Swedish National Diabetes Register. In the MDCS, 3245 incident cases of diabetes and 1183 prevalent cases of diabetes were found. In all those with diabetes, 185 cases of incident CRC were found.

Age was obtained from personal identification numbers. Smokers were divided into three categories: current smokers; ex-smokers; and non-smokers. Irregular smoking was counted as current smoking. The subjects estimated their physical activity in minutes and the results were divided into quintiles. The level of education was divided into four different categories: ≤ 8 years; 9–10 years; 11–13 years of education; and university degree. Alcohol intake was divided into four categories: zero; < 15 g/d for women and < 20 g/d for men; 15–30 g/d for women and 20–40 g/d for men; and > 30 g/d for women and > 40 g/d for men. Current use of menopausal hormonal replacement therapy (HRT) and regular use of non-steroid anti-inflammatory drugs (NSAID) were divided into non-users and users. BMI was calculated from measured weight and length. BMI was divided into high (≥ 25 kg/m$^2$) and normal and low (< 25 kg/m$^2$), after the World Health Organization's classification of overweight [19].

### Statistical methods

The SPSS statistics package (version 22; IBM Corporation, Armonk, USA) was used for all statistical analyses. The food variables were log-transformed (e-log) to normalize the distribution before analysis. A very small amount (0.0001) was added before transformation, to handle zero intakes [20].

The general linear model was used when examining baseline continuous characteristics in the cases and non-cases, and also adjusting for age and sex, when appropriate, and season and method version for the food variables.

The Cox proportional hazard regression model was used when estimating hazard ratios (HR) of incident CRC, colon cancer, and rectal cancer, depending on quintiles of energy-adjusted food intakes (red meat, beef, pork, unprocessed red meat, processed red meat, poultry, and fish). The basic model included adjustments for age, sex (when appropriate), season, and total energy intake. In addition, the full model was also adjusted for level of education, smoking status, alcohol intake, physical activity, BMI, NSAID use, and when appropriate, for current use of HRT. We also performed the multivariate model excluding BMI, since it might be an intermediate between diet and CRC. Additional models were constructed with further adjustments for diabetes (prevalent and incident) or potential dietary confounders found in previous studies (intake of: fiber; protein; saturated fat; calcium; folate; iron; zinc; fruits and vegetables; milk products; and sugar-sweetened beverages) [5].

Spearman's correlation matrix was used to examine the correlation between energy-adjusted food intakes (total red meat, beef, pork, unprocessed red meat, processed red meat, poultry, and fish). For intakes where a correlation over 0.40 was found (Supplementary Table 1), additional models were constructed with mutual adjustment in the full models.

A test for interaction between sex or BMI status (< 25 and ≥ 25) and dietary intakes with regard to CRC incidence was performed by adding a multiplicative variable (e.g. sex × diet quintile (treated as continuous variables)) to the full model.

In sensitivity analysis, we excluded individuals with a reported dietary change in the past, all forms of prevalent cancer except cervix cancer in situ or incident and prevalent diabetes. All tests were two-sided and statistical significance was assumed at $p < 0.05$.

## Results

### Baseline characteristics

Altogether, 16 944 (60.7%) women and 10 987 men completed the baseline examinations, after exclusion of individuals with prevalent CRC. The cases of CRC were, compared with non-cases, older, had a larger waist circumference, and a higher BMI (Table 1). They also had a lower intake of calcium, zinc, beef,

and dairy products and a higher intake of pork. Fewer cases had a high level of education, but more of them were highly physically active. Diabetes was more common among the cases, especially in men. In women, the use of HRT was higher among the non-cases.

### Dietary intake and colorectal cancer

In the full multivariate model, beef intake was inversely associated with risk for CRC in women (HR: 0.65 for highest compared with lowest quintile; 95% CI: 0.45, 0.95; p for trend = 0.046), but not in men, and a borderline interaction between sex and beef intake was seen (p = 0.068) (Table 2). High pork intake was associated with increased incidence for CRC (HR: 1.39 for highest compared with lowest quintile; 95% CI: 1.09, 1.78; p for trend = 0.023). The association was only significant in women (HR: 1.54 for highest compared with lowest quintile; 95% CI: 1.12, 2.15; p for trend = 0.003), but no significant interaction with sex was seen (p = 0.157). The trend for intake of processed red meat was significantly associated with increased risk for CRC in men (HR: 1.23 for highest compared with lowest quintile; 95% CI: 0.87, 1.73; p for trend = 0.023), but not in women, and a borderline interaction was seen (p = 0.062).

### Dietary intake and colon cancer

High intake of beef was inversely associated with risk of colon cancer (HR: 0.60 for highest compared with lowest quintile; 95% CI: 0.44, 0.82; p for trend = 0.009) (Table 3). The inverse association was also significant in women (HR: 0.60 for highest compared with lowest quintile; 95% CI: 0.37, 0.96; p for trend = 0.049) and similar tendencies were seen in men (p for trend = 0.069). High intake of pork was associated with increased risk of colon cancer (HR: 1.41 for highest compared with lowest quintile; 95% CI: 1.04, 1.90; p for trend = 0.021). The association was only significant in women (HR: 1.56 for highest compared with lowest quintile; 95% CI: 1.12, 2.15; p for trend = 0.003), but no significant interaction with sex was seen (p = 0.146). We observed a borderline significant association between high intake of processed meat and increased risk of colon cancer in men (HR: 1.23 for highest compared with lowest quintile; 95% CI: 0.80, 1.90; p for trend = 0.053), but no significant interaction with sex was seen (p = 0.127).

### Dietary intake and rectal cancer

High intake of beef was associated with increased risk of rectal cancer in men (HR: 1.82 for highest compared with lowest quintile; 95% CI: 1.02, 3.25, p for trend = 0.028), but not in women (Table 4), and a significant interaction was seen between beef intake and sex (p = 0.025). High intake of fish was inversely associated with risk of rectal cancer in all (HR: 0.59 for highest compared with lowest quintile; 95% CI: 0.38, 0.92, p for trend = 0.025), and the association did not differ depending on sex (p for interaction = 0.597).

### Complementary models

In the analysis of CRC, colon and rectal cancer, additional adjustments for potential dietary confounders (intake of: fiber; protein; saturated fat; calcium; folate; iron; zinc; fruits and vegetables; milk products; and sugar-sweetened beverages) did not affect the results and were therefore excluded from further analysis (data not shown).

In the analysis of CRC, colon, and rectal cancer, additional mutual adjustments for the correlated food intakes (total red meat – beef; total red meat – pork; total red meat – unprocessed red meat; total red meat – processed red meat; unprocessed red meat – beef; unprocessed red meat – pork), did not affect the results to any major extent (data not shown).

### Dietary intake, colorectal, colon, and rectal cancer, and body mass index

Excluding BMI from the multivariate model gave virtually similar results (data not shown). Moreover, no significant interactions were found between the different types of meat intakes and BMI status (< 25 or ≥ 25) on CRC (Supplementary Table 2).

### Sensitivity analyses

#### Exclusion of subjects with dietary change

When excluding individuals reporting dietary change in the past, the association between intake of pork and CRC was only borderline significant (HR: 1.15 for highest compared with lowest quintile; 95% CI: 0.86, 1.52; p for trend = 0.074). Apart from that, we did not observe any major changes in any of the results.

#### Exclusion of subjects with prevalent cancer

When excluding individuals with any type of prevalent cancer at baseline, except for cervix cancer in situ, the inverse association between intake of beef and colon

**Table 1.** Baseline characteristics of cases and non-cases of incident colorectal cancer in the Malmö Diet and Cancer Study cohort.

| | Non-cases n = 27 203 | Cases n = 728 | p-value[a] | Non-cases Women n = 16 572 | Cases Women n = 372 | p-value[a] | Non-cases Men n = 10 631 | Cases men n = 356 | p-value[a] |
|---|---|---|---|---|---|---|---|---|---|
| Age (years) | 58.0 ± 7.6 | 61.4 ± 7.0 | <0.001 | 57.3 ± 7.9 | 61.7 ± 7.1 | <0.001 | 59.1 ± 7.0 | 61.1 ± 6.8 | <0.001* |
| BMI (kg/m$^2$) | 25.7 ± 4.0 | 26.4 ± 4.0 | 0.014 | 25.4 ± 4.2 | 26.0 ± 4.3 | 0.355 | 26.2 ± 3.5 | 26.8 ± 3.6 | 0.006 |
| Waist (cm) | 84.0 ± 12.9 | 87.5 ± 13.6 | 0.010 | 77.8 ± 10.5 | 79.6 ± 11.2 | 0.185 | 93.6 ± 10.1 | 95.8 ± 10.5 | <0.001 |
| Body fat (%) | 26.8 ± 7.0 | 26.5 ± 7.2 | 0.069 | 30.7 ± 5.0 | 31.6 ± 4.9 | 0.428 | 20.7 ± 5.0 | 21.2 ± 5.0 | 0.100 |
| Energy intake (MJ) | 9.5 ± 2.7 | 9.6 ± 2.7 | 0.535 | 8.5 ± 2.1 | 8.4 ± 2.1 | 0.996 | 11.1 ± 2.5 | 10.8 ± 2.8 | 0.406 |
| Fat intake (E%) | 38.3 ± 6.2 | 38.5 ± 6.3 | 0.475 | 37.7 ± 6.1 | 37.6 ± 6.0 | 0.937 | 39.1 ± 6.3 | 39.4 ± 6.4 | 0.275 |
| Protein intake (E%) | 15.5 ± 2.5 | 15.3 ± 2.4 | 0.275 | 15.6 ± 2.5 | 15.5 ± 2.4 | 0.357 | 15.3 ± 2.5 | 15.1 ± 2.4 | 0.517 |
| Protein (g) | 84.0 ± 23.4 | 83.5 ± 22.8 | 0.154 | 76.5 ± 18.8 | 75.2 ± 19.0 | 0.822 | 92.1 ± 25.1 | 92.1 ± 23.1 | 0.093 |
| Carbohydrate intake (E%) | 46.3 ± 6.2 | 46.3 ± 6.2 | 0.777 | 46.6 ± 6.1 | 47.0 ± 5.9 | 0.646 | 45.7 ± 6.3 | 45.5 ± 6.5 | 0.399 |
| Saturated fat (g) | 40.9 ± 17.5 | 41.0 ± 17.4 | 0.434 | 36.6 ± 14.6 | 36.0 ± 13.9 | 0.824 | 47.5 ± 19.5 | 46.2 ± 19.1 | 0.408 |
| Iron (mg) | 18.1 ± 11.9 | 17.5 ± 4.5 | 0.177 | 16.7 ± 12.8 | 12.3 ± 6.6 | 0.130 | 20.1 ± 10.0 | 19.8 ± 9.6 | 0.778 |
| Zinc (mg) | 13.0 ± 6.0 | 12.5 ± 5.2 | 0.033 | 12.2 ± 6.3 | 11.5 ± 5.1 | 0.128 | 14.2 ± 5.4 | 13.5 ± 5.2 | 0.108 |
| Calcium (mg) | 1144.8 ± 425.7 | 1097.5 ± 406.8 | 0.007 | 1112.2 ± 393.2 | 1084.9 ± 398.2 | 0.615 | 1195. 8 ± 467.4 | 1107.6 ± 416.1 | 0.002 |
| Folate (mg) | 290.9 ± 307.1 | 284.6 ± 388.2 | 0.445[e] | 286.8 ± 310.9 | 265.8 ± 119.0 | 0.122[e] | 297 ± 301.0 | 304.3 ± 541.3 | 0.647[e] |
| Red meat (g) | 97.6 ± 51.2 | 102.1 ± 53.5 | 0.147[b,e] | 80.7 ± 39.0 | 80.5 ± 38.0 | 0.439[b,e] | 124.0 ± 57.6 | 124.6 ± 57.9 | 0.262[b,e] |
| Red meat (g/MJ) | 10.3 ± 4.5 | 10.6 ± 4.4 | 0.058[b,e] | 9.6 ± 4.3 | 9.7 ± 4.2 | 0.369[b,e] | 11.3 ± 4.6 | 11.6 ± 4.4 | 0.084[b,e] |
| • Beef (g) | 24.6 ± 20.6 | 23.2 ± 18.8 | 0.021[b,e] | 21.1 ± 17.1 | 18.8 ± 16.3 | 0.013[b,e] | 29.9 ± 24.1 | 27.7 ± 20.1 | 0.443[b,e] |
| • Beef (g/MJ) | 2.6 ± 2.1 | 2.5 ± 1.9 | 0.024[b,e] | 2.5 ± 2.0 | 2.3 ± 1.9 | 0.013[b,e] | 2.8 ± 2.2 | 2.7 ± 4.6 | 0.495[b,e] |
| • Pork (g) | 39.6 ± 27.8 | 42.8 ± 28.7 | 0.007[b,e] | 34.3 ± 22.9 | 36.7 ± 22.4 | 0.005[b,e] | 48.0 ± 32.2 | 49.2 ± 32.9 | 0.381[b,e] |
| • Pork (g/MJ) | 4.2 ± 2.8 | 4.5 ± 2.8 | 0.006[b,e] | 4.1 ± 2.7 | 4.5 ± 2.7 | 0.005[b,e] | 4.4 ± 2.8 | 4.6 ± 2.9 | 0.320[b,e] |
| Unprocessed red meat (g) | 59.0 ± 35.3 | 60.2 ± 36.7 | 0.130[b,e] | 49.6 ± 27.5 | 48.7 ± 27.1 | 0.090[b,e] | 73.7 ± 40.8 | 72.2 ± 41.3 | 0.770[b,e] |
| Unprocessed red meat (g/MJ) | 6.2 ± 3.4 | 6.4 ± 3.3 | 0.098[b,e] | 6.0 ± 3.2 | 6.0 ± 3.2 | 0.085[b,e] | 6.8 ± 3.5 | 6.7 ± 3.4 | 0.617[b,e] |
| Processed red meat (g) | 38.6 ± 30.5 | 41.9 ± 32.4 | 0.124[b,e] | 31.1 ± 23.3 | 31.8 ± 24.3 | 0.091[b,e] | 50.3 ± 36.2 | 52.4 ± 36.3 | 0.691[b,e] |
| Processed red meat (g/MJ) | 4.0 ± 2.9 | 4.3 ± 2.9 | 0.101[b,e] | 3.7 ± 2.7 | 3.8 ± 2.7 | 0.086[b,e] | 4.5 ± 3.0 | 4.8 ± 3.0 | 0.591[b,e] |
| Poultry (g) | 12.1 ± 16.7 | 12.8 ± 16.9 | 0.186[b,e] | 11.6 ± 15.2 | 11.8 ± 15.0 | 0.096[b,e] | 13. 1 ± 18.7 | 13.8 ± 18.6 | 0.909[b,e] |
| Poultry (g/MJ) | 1.3 ± 1.8 | 1.4 ± 1.8 | 0.173[b,e] | 1.4 ± 1.9 | 1.4 ± 1.9 | 0.094[b,e] | 1.2 ± 1.8 | 1.3 ± 1.8 | 0.796[b,e] |
| Fish (g) | 42.2 ± 32.2 | 43.2 ± 31.2 | 0.516[b,e] | 39.5 ± 28.3 | 40.2 ± 25.9 | 0.237[b,e] | 46.4 ± 37.1 | 46.3 ± 35.7 | 0.586[b,e] |
| Fish (g/MJ) | 4.6 ± 3.5 | 4.7 ± 3.4 | 0.461[b,e] | 4.8 ± 3.5 | 4.9 ± 3.2 | 0.611[b,e] | 4.3 ± 3.5 | 4.5 ± 4.3 | 0.601[b,e] |
| Fruits and vegetables (g) | 375.8 ± 185.3 | 363.5 ± 182. 5 | 0.401[b,e] | 395.3 ± 183.8 | 385.4 ± 174.2 | 0.299[b,e] | 345.5 ± 183.6 | 340.6 ± 188.3 | 0.847[b,e] |
| Sugar sweetened beverages (g) | 77.3 ± 149.2 | 81.3 ± 147.5 | 0.557[b,e] | 66.1 ± 126.8 | 67.7 ± 124.5 | 0.213[b,e] | 94.7 ± 177.3 | 95.6 ± 167.3 | 0.787[b,e] |
| Dairy products (portions[c]) | 6.4 ± 3.9 | 6.2 ± 3.8 | 0.027[b,e] | 6.0 ± 3.4 | 5.8 ± 3.1 | 0.743[b,e] | 7.1 ± 4.5 | 6.6 ± 4.3 | 0.009[b,e] |
| Alcohol intake (g) | 10.7 ± 12.7 | 11.2 ± 14.4 | 0.312[b,e] | 7.7 ± 8.7 | 6.4 ± 8.4 | 0.382[b,e] | 15.5 ± 16.0 | 16.2 ± 17.4 | 0.122[b,e] |
| Smoking, ex/current (%) | 33.7/28.4 | 38.3/26.2 | 0.172 | 27.8/28.1 | 28.0/26.9 | 0.693 | 43.0/28.8 | 38.3/26.2 | 0.220 |
| Education (> 10 years) (%) | 31.9 | 27.3 | 0.040 | 30.3 | 23.9 | 0.011 | 34.3 | 30.9 | 0.174 |
| Physical activity high (%)[d] | 19.9 | 23.0 | 0.043 | 18.7 | 20.8 | 0.314 | 21.8 | 25.2 | 0.126 |
| Incident diabetes (%) | 11.6 | 13.7 | 0.071 | 9.4 | 9.4 | 0.994 | 14.9 | 18.3 | 0.080 |
| Diabetes (All) (%) | 15.8 | 19.6 | 0.005 | 12.7 | 13.4 | 0.664 | 20.5 | 26.1 | 0.010 |
| Menopausal hormone therapy, women (%) | | | | 19.4 | 15.3 | 0.047 | | | |

[a]Adjusted for sex and age when appropriate. Calculated with the general linear model for continuous values and Chi$^2$-test for categorical values.
[b]Adjusted for age, method, and season, and for sex when appropriate
[c]Standard portion sizes of milk, yoghurt, sour milk and cheese from the National Food Agency in Sweden
[d]Physical activity was defined as high when being in the highest quintile of the whole group
[e]*Calculated with log transformed values*
*Values are means ± SD or percentages. P < 0.05 was considered statistically significant*

**Table 2.** Hazard ratio (HR) of colorectal cancer associated with intakes of different foods in the Malmö Diet and Cancer study cohort.

| | | | All | | | | | Women | | | | | Men | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Basic model[a] | | Full model[b] | | | Basic model[a] | | Full model[b] | | | Basic model[a] | | Full model[b] | |
| Median | | Cases/person-years | HR | CI | HR | CI | Cases/person-years | HR | CI | HR | CI | Cases/person-years | HR | CI | HR | CI |
| **Total Red meat (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **43.6** | **127/86 428** | 1.00 | | 1.00 | | **94/64 056** | 1.00 | | 1.00 | | **33/22 373** | 1.00 | | 1.00 | |
| **2** | **69.3** | **152/85 820** | 1.19 | 0.94, 1.51 | 1.16 | 0.91, 1.48 | **86/57 761** | 1.03 | 0.77, 1.39 | 1.02 | 0.76, 1.37 | **66/28 060** | 1.59 | 1.04, 2.41 | 1.52 | 1.00, 2.32 |
| **3** | **87.9** | **142/86 102** | 1.10 | 0.86, 1.41 | 1.07 | 0.84, 1.37 | **75/54 421** | 0.97 | 0.71, 1.32 | 0.95 | 0.70, 1.30 | **67/31 681** | 1.45 | 0.96, 2.21 | 1.39 | 0.91, 2.11 |
| **4** | **107.8** | **146/85 610** | 1.14 | 0.90, 1.46 | 1.09 | 0.85, 1.40 | **61/49 284** | 0.91 | 0.65, 1.26 | 0.89 | 0.64, 1.24 | **85/36 325** | 1.62 | 1.08, 2.43 | 1.51 | 1.00, 2.28 |
| **5** | **146.2** | **161/84 979** | 1.33 | 1.04, 1.69 | 1.24 | 0.97, 1.59 | **56/38 777** | 1.17 | 0.83, 1.65 | 1.14 | 0.81, 1.62 | **105/46 203** | 1.70 | 1.14, 2.53 | 1.55 | 1.04, 2.33 |
| **p for trend** | | | 0.058 | | 0.203 | | | 0.769 | | 0.902 | | | 0.025 | | 0.098 | |
| **Beef (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **0.3** | **174/86 560** | | | 1.00 | | **102/56 377** | 1.00 | | 1.00 | | **72/30 184** | 1.00 | | 1.00 | |
| **2** | **8.0** | **139/84 121** | 0.85 | 0.67, 1.06 | 0.84 | 0.67, 1.05 | **78/54 288** | 0.84 | 0.62, 1.13 | 0.83 | 0.61, 1.13 | **61/29 833** | 0.85 | 0.61, 1.20 | 0.86 | 0.61, 1.21 |
| **3** | **15.1** | **157/85 252** | 0.99 | 0.79, 1.23 | 0.98 | 0.79, 1.23 | **88/54 065** | 1.03 | 0.77, 1.38 | 1.03 | 0.76, 1.38 | **69/31 186** | 0.94 | 0.68, 1.31 | 0.95 | 0.68, 1.32 |
| **4** | **24.0** | **140/86 252** | 0.89 | 0.71, 1.12 | 0.88 | 0.70, 1.11 | **62/52 425** | 0.78 | 0.56, 1.07 | 0.77 | 0.56, 1.07 | **78/33 827** | 1.02 | 0.74, 1.41 | 1.01 | 0.73, 1.40 |
| **5** | **42.6** | **118/86 739** | 0.81 | 0.63, 1.03 | 0.79 | 0.62, 1.01 | **42/47 127** | 0.66 | 0.45, 0.96 | 0.65 | 0.45, 0.95 | **76/39 612** | 0.93 | 0.67, 1.29 | 0.91 | 0.65, 1.27 |
| **p for trend** | | | 0.226 | | 0.169 | | | 0.055 | | 0.046 | | | 0.928 | | 0.962 | |
| **Pork (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **3.9** | **117/87 256** | 1.00 | | 1.00 | | **68/57 804** | 1.00 | | 1.00 | | **49/29 452** | 1.00 | | 1.00 | |
| **2** | **14.2** | **157/84 767** | 1.32 | 1.03, 1.68 | 1.31 | 1.03, 1.67 | **69/52 604** | 1.04 | 0.74, 1.46 | 1.04 | 0.74, 1.46 | **88/32 163** | 1.63 | 1.15, 2.32 | 1.61 | 1.13, 2.29 |
| **3** | **23.7** | **134/85 538** | 1.12 | 0.87, 1.44 | 1.10 | 0.86, 1.41 | **67/52 784** | 1.03 | 0.73, 1.44 | 1.02 | 0.72, 1.44 | **67/32 754** | 1.21 | 0.84, 1.76 | 1.19 | 0.82, 1.72 |
| **4** | **34.2** | **153/85 826** | 1.28 | 1.00, 1.63 | 1.25 | 0.98, 1.60 | **79/52 329** | 1.25 | 0.90, 1.74 | 1.24 | 0.89, 1.73 | **74/33 507** | 1.30 | 0.90, 1.88 | 1.26 | 0.87, 1.82 |
| **5** | **55.2** | **167/85 526** | 1.45 | 1.14, 1.85 | 1.39 | 1.09, 1.78 | **89/48 760** | 1.57 | 1.13, 2.17 | 1.54 | 1.12, 2.15 | **78/36 766** | 1.34 | 0.93, 1.93 | 1.26 | 0.87, 1.82 |
| **p for trend** | | | 0.007 | | 0.023 | | | 0.002 | | 0.003 | | | 0.555 | | 0.857 | |
| **Unprocessed red meat (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **21.3** | **141/85 679** | 1.00 | | 1.00 | | **89/60 792** | 1.00 | | 1.00 | | **52/24 888** | 1.00 | | 1.00 | |
| **2** | **39.1** | **147/85 967** | 1.02 | 0.81, 1.28 | 1.00 | 0.82, 1.22 | **82/56 278** | 0.99 | 0.73, 1.34 | 0.98 | 0.72, 1.33 | **65/29 689** | 1.05 | 0.73, 1.51 | 1.02 | 0.71, 1.47 |
| **3** | **52.1** | **150/85 640** | 1.07 | 0.85, 1.35 | 0.88 | 0.68, 1.14 | **79/53 682** | 1.05 | 0.77, 1.42 | 1.03 | 0.76, 1.40 | **71/31 959** | 1.10 | 0.77, 1.58 | 1.06 | 0.74, 1.52 |
| **4** | **67.0** | **146/85 624** | 1.07 | 0.85, 1.35 | 0.98 | 0.80, 1.20 | **67/51 105** | 0.98 | 0.71, 1.35 | 0.96 | 0.69, 1.33 | **79/34 519** | 1.17 | 0.82, 1.67 | 1.11 | 0.78, 1.58 |
| **5** | **96.3** | **144/86 014** | 1.07 | 0.84, 1.37 | 1.08 | 1.06, 1.09 | **55/42 426** | 1.06 | 0.75, 1.49 | 1.03 | 0.73, 1.46 | **89/43 588** | 1.09 | 0.77, 1.54 | 1.00 | 0.70, 1.43 |
| **p for trend** | | | 0.305 | | 0.589 | | | 0.524 | | 0.632 | | | 0.494 | | 0.863 | |
| **Processed red meat (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **6.7** | **120/87 060** | 1.00 | | 1.00 | | **65/58 622** | 1.00 | | 1.00 | | **55/28 439** | 1.00 | | 1.00 | |
| **2** | **20.7** | **156/86 124** | 1.26 | 0.99, 1.60 | 1.25 | 0.98, 1.59 | **100/53 716** | 1.64 | 1.19, 2.26 | 1.62 | 1.18, 2.24 | **56/32 409** | 0.87 | 0.60, 1.26 | 0.87 | 0.60, 1.26 |
| **3** | **31.7** | **142/85 707** | 1.15 | 0.90, 1.47 | 1.13 | 0.88, 1.45 | **75/52 840** | 1.27 | 0.90, 1.78 | 1.25 | 0.89, 1.76 | **67/32 867** | 1.01 | 0.70, 1.44 | 1.00 | 0.70, 1.43 |
| **4** | **44.0** | **154/85 824** | 1.23 | 0.96, 1.56 | 1.20 | 0.94, 1.53 | **70/51 459** | 1.18 | 0.83, 1.67 | 1.18 | 0.83, 1.67 | **84/34 366** | 1.23 | 0.87, 1.73 | 1.20 | 0.85, 1.69 |
| **5** | **67.0** | **156/84 209** | 1.25 | 0.98, 1.60 | 1.20 | 0.94, 1.53 | **62/47 647** | 1.15 | 0.80, 1.65 | 1.13 | 0.79, 1.62 | **94/36 562** | 1.28 | 0.91, 1.80 | 1.23 | 0.87, 1.73 |
| **p for trend** | | | 0.041 | | 0.104 | | | 0.824 | | 0.887 | | | 0.009 | | 0.023 | |
| **Poultry (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **0.0** | **141/86 418** | 1.00 | | 1.00 | | **43/35 511** | 1.00 | | 1.00 | | **104/50,907** | 1.00 | | 1.00 | |
| **2** | **0.0** | **147/85 274** | 0.96 | 0.74, 1.25 | 0.94 | 0.72, 1.22 | **86/66 149** | 0.97 | 0.64, 1.47 | 0.97 | 0.64, 1.46 | **50/19 125** | 1.11 | 0.77, 1.62 | 1.07 | 0.73, 1.56 |
| **3** | **3.1** | **150/84 483** | 1.04 | 0.82, 1.32 | 1.02 | 0.80, 1.31 | **77/55 813** | 1.13 | 0.76, 1.68 | 1.14 | 0.76, 1.69 | **62/19 125** | 1.01 | 0.73, 1.39 | 0.99 | 0.72, 1.36 |
| **4** | **16.5** | **146/86 634** | 0.96 | 0.75, 1.23 | 0.95 | 0.74, 1.22 | **98/59 689** | 1.29 | 0.88, 1.89 | 1.28 | 0.87, 1.88 | **35/26 945** | 0.62 | 0.42, 0.91 | 0.62 | 0.42, 0.91 |
| **5** | **31.9** | **144/86 115** | 1.27 | 1.01, 1.58 | 1.24 | 0.99, 1.55 | **68/47 120** | 1.22 | 0.82, 1.81 | 1.21 | 0.81, 1.81 | **105/38 995** | 1.34 | 1.02, 1.76 | 1.29 | 0.98, 1.70 |
| **p for trend** | | | 0.110 | | 0.132 | | | 0.124 | | 0.128 | | | 0.422 | | 0.536 | |
| **Fish (g/day)** | | | | | | | | | | | | | | | | |
| **1** | **6.2** | **135/85 629** | 1.00 | | 1.00 | | **57/49 962** | 1.00 | | 1.00 | | **78/35 667** | 1.00 | | 1.00 | |
| **2** | **23.2** | **150/86 126** | 1.00 | 0.79, 1.26 | 1.00 | 0.79, 1.27 | **79/54 549** | 1.11 | 0.78, 1.57 | 1.12 | 0.79, 1.59 | **71/31 578** | 0.93 | 0.67, 1.28 | 0.93 | 0.67, 1.28 |
| **3** | **36.2** | **134/86 220** | 0.84 | 0.66, 1.07 | 0.85 | 0.67, 1.08 | **81/54 109** | 1.08 | 0.76, 1.53 | 1.09 | 0.77, 1.54 | **53/32 111** | 0.65 | 0.46, 0.93 | 0.65 | 0.46, 0.93 |
| **4** | **51.9** | **151/86 070** | 0.91 | 0.72, 1.15 | 0.91 | 0.72, 1.16 | **85/55 296** | 1.02 | 0.72, 1.45 | 1.03 | 0.73, 1.46 | **66/30 774** | 0.83 | 0.59, 1.15 | 0.82 | 0.59, 1.14 |
| **5** | **81.8** | **158/84 879** | 0.90 | 0.71, 1.14 | 0.90 | 0.71, 1.14 | **70/50 366** | 0.88 | 0.61, 1.26 | 0.88 | 0.61, 1.27 | **88/34 513** | 0.95 | 0.70, 1.30 | 0.93 | 0.68, 1.28 |
| **p for trend** | | | 0.308 | | 0.282 | | | 0.357 | | 0.393 | | | 0.609 | | 0.506 | |

[a]Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), and total energy (continuous)

[b]Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex, or never), alcohol intake (zero, <15g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), use of non-steroid anti-inflammatory drugs (yes, no), and physical activity (quintiles of physical activity)

Values are quintile medians, hazard ratios, and 95% confidence intervals. $p < 0.05$ was considered statistically significant. HR was calculated by using Cox proportional hazard risk model and was adjusted for energy with the residual method.

**Table 3.** Hazard ratio (HR) of colon cancer associated with intakes of different foods in the Malmö Diet and Cancer Study cohort.

| Quintiles for all | | | All | | | | | | Women | | | | | | Men | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Basic model[a] | | | Full model[b] | | | | Basic model[a] | | | Full model[b, c] | | | | Basic model[a] | | | Full model[b] | | |
| Total | Red meat (g/day) | Cases/ person-years | HR | CI | | HR | CI | | Cases/person-years | HR | CI | | HR | CI | | Cases/person-years | HR | CI | | HR | CI | |
| **1** | **43.5** | **87/86 036** | 1.00 | | | 1.00 | | | **65/63 765** | 1.00 | | | 1.00 | | | **22/22 272** | 1.00 | | | 1.00 | | |
| **2** | **69.2** | **91/85 236** | 1.03 | 0.77, | 1.39 | 1.00 | 0.74, | 1.35 | **52/57 427** | 0.88 | 0.60, | 1.27 | 0.87 | 0.60, | 1.26 | **39/27 809** | 1.40 | 0.83, | 2.36 | 1.35 | 0.80, | 2.28 |
| **3** | **87.9** | **91/85 628** | 1.02 | 0.76, | 1.38 | 0.99 | 0.73, | 1.34 | **50/54 228** | 0.90 | 0.62, | 1.31 | 0.89 | 0.61, | 1.30 | **41/31 400** | 1.33 | 0.79, | 2.24 | 1.28 | 0.76, | 2.16 |
| **4** | **107.7** | **94/85 141** | 1.09 | 0.81, | 1.46 | 1.04 | 0.77, | 1.40 | **44/49 143** | 0.94 | 0.64, | 1.40 | 0.93 | 0.63, | 1.38 | **50/35 997** | 1.42 | 0.86, | 2.36 | 1.34 | 0.80, | 2.24 |
| **5** | **146.0** | **100/84 499** | 1.23 | 0.91, | 1.66 | 1.16 | 0.85, | 1.57 | **36/38 648** | 1.07 | 0.70, | 1.64 | 1.06 | 0.69, | 1.63 | **64/45 850** | 1.55 | 0.95, | 2.54 | 1.44 | 0.87, | 2.38 |
| **p for trend** | | | 0.255 | | | 0.471 | | | | 0.884 | | | 0.941 | | | | 0.179 | | | 0.336 | | |
| **Beef (g/day)** | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **0.4** | **124/86 051** | 1.00 | | | 1.00 | | | **70/56 066** | 1.00 | | | 1.00 | | | **54/29 985** | 1.00 | | | 1.00 | | |
| **2** | **8.0** | **91/83 696** | 0.78 | 0.59, | 1.03 | 0.77 | 0.59, | 1.02 | **54/54 090** | 0.86 | 0.60, | 1.24 | 0.83 | 0.58, | 1.21 | **37/29 606** | 0.69 | 0.45, | 1.04 | 0.68 | 0.45, | 1.04 |
| **3** | **15.1** | **94/84 767** | 0.78 | 0.66, | 1.14 | 0.86 | 0.66, | 1.13 | **58/53 819** | 0.93 | 0.65, | 1.33 | 1.00 | 0.70, | 1.44 | **39/30 948** | 0.71 | 0.47, | 1.08 | 0.71 | 0.47, | 1.08 |
| **4** | **24.0** | **88/85 754** | 0.81 | 0.61, | 1.07 | 0.79 | 0.60, | 1.05 | **39/52 220** | 0.64 | 0.43, | 0.96 | 0.73 | 0.49, | 1.09 | **49/33 534** | 0.85 | 0.58, | 1.26 | 0.84 | 0.57, | 1.24 |
| **5** | **42.6** | **63/86 256** | 0.61 | 0.45, | 0.84 | 0.60 | 0.44, | 0.82 | **26/46 999** | 0.43 | 0.27, | 0.70 | 0.60 | 0.37, | 0.96 | **37/39 256** | 0.59 | 0.38, | 0.90 | 0.57 | 0.37, | 0.88 |
| **p for trend** | | | 0.015 | | | 0.009 | | | | 0.0003 | | | 0.049 | | | | 0.090 | | | 0.069 | | |
| **Pork (g/day)** | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **3.7** | **80/86 908** | 1.00 | | | 1.00 | | | **46/57 590** | 1.00 | | | 1.00 | | | **34/29 318** | 1.00 | | | 1.00 | | |
| **2** | **14.1** | **90/84 160** | 1.12 | 0.83, | 1.52 | 1.12 | 0.82, | 1.52 | **40/52 329** | 1.04 | 0.74, | 1.46 | 1.04 | 0.74, | 1.46 | **50/31 831** | 1.40 | 0.90, | 2.18 | 1.38 | 0.89, | 2.15 |
| **3** | **23.7** | **82/85 023** | 1.01 | 0.74, | 1.39 | 1.00 | 0.73, | 1.37 | **42/52 552** | 1.03 | 0.73, | 1.44 | 1.02 | 0.73, | 1.44 | **40/32 471** | 1.07 | 0.67, | 1.71 | 1.05 | 0.66, | 1.68 |
| **4** | **34.2** | **98/85 355** | 1.22 | 0.90, | 1.64 | 1.19 | 0.88, | 1.62 | **58/52 171** | 1.25 | 0.90, | 1.74 | 1.24 | 0.89, | 1.73 | **40/33 184** | 1.07 | 0.67, | 1.71 | 1.04 | 0.65, | 1.67 |
| **5** | **55.2** | **113/85 077** | 1.45 | 1.08, | 1.95 | 1.41 | 1.04, | 1.90 | **61/48 553** | 1.57 | 1.14, | 2.17 | 1.56 | 1.12, | 2.15 | **52/36 524** | 1.32 | 0.84, | 2.06 | 1.26 | 0.80, | 1.97 |
| **p for trend** | | | 0.010 | | | 0.021 | | | | 0.002 | | | 0.003 | | | | 0.687 | | | 0.885 | | |
| **Unprocessed red meat (g/day)** | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **21.3** | **98/85 263** | 1.00 | | | 1.00 | | | **62/60 513** | 1.00 | | | 1.00 | | | **36/24 750** | 1.00 | | | 1.00 | | |
| **2** | **39.1** | **91/85 432** | 0.83 | 0.61, | 1.14 | 0.85 | 0.62, | 1.17 | **50/55 999** | 0.76 | 0.50, | 1.16 | 0.75 | 0.49, | 1.15 | **41/29 433** | 0.85 | 0.48, | 1.53 | 0.89 | 0.50, | 1.60 |
| **3** | **52.1** | **91/85 056** | 0.85 | 0.59, | 1.22 | 0.89 | 0.61, | 1.28 | **52/53 401** | 0.78 | 0.45, | 1.36 | 0.76 | 0.43, | 1.34 | **39/31 654** | 0.81 | 0.45, | 1.46 | 0.90 | 0.50, | 1.64 |
| **4** | **66.9** | **91/85 163** | 0.75 | 0.48, | 1.17 | 0.80 | 0.51, | 1.27 | **44/50 929** | 0.58 | 0.27, | 1.22 | 0.56 | 0.26, | 1.21 | **47/34 234** | 0.83 | 0.44, | 1.58 | 0.95 | 0.49, | 1.86 |
| **5** | **95.3** | **92/85 609** | 0.76 | 0.40, | 1.44 | 0.83 | 0.43, | 1.60 | **39/42 352** | 0.57 | 0.19, | 1.70 | 0.53 | 0.17, | 1.65 | **53/43 257** | 0.87 | 0.37, | 2.05 | 1.05 | 0.43, | 2.59 |
| **p for trend** | | | 0.097 | | | 0.201 | | | | 0.358 | | | 0.058 | | | | 0.453 | | | 0.835 | | |
| **Processed red meat (g/day)** | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **6.7** | **76/86 650** | 1.00 | | | 1.00 | | | **42/58 392** | 1.00 | | | 1.00 | | | **34/28 258** | 1.00 | | | 1.00 | | |
| **2** | **20.7** | **105/85 682** | 1.32 | 1.32, | 1.20 | 1.32 | 0.98, | 1.78 | **73/53 504** | 1.89 | 1.28, | 2.79 | 1.75 | 1.19, | 2.58 | **32/32 178** | 0.82 | 0.50, | 1.32 | 0.82 | 0.51, | 1.33 |
| **3** | **31.7** | **86/85 182** | 1.07 | 1.07, | 1.14 | 1.07 | 0.78, | 1.46 | **47/52 575** | 1.26 | 0.82, | 1.92 | 1.15 | 0.75, | 1.77 | **39/32 607** | 0.96 | 0.60, | 1.52 | 0.97 | 0.61, | 1.53 |
| **4** | **43.8** | **97/85 229** | 1.17 | 0.42, | 1.00 | 1.17 | 0.86, | 1.56 | **45/51 226** | 1.20 | 0.78, | 1.84 | 1.12 | 0.73, | 1.73 | **52/34 002** | 1.22 | 0.79, | 1.88 | 1.21 | 0.78, | 1.87 |
| **5** | **66.5** | **99/83 776** | 1.16 | 0.35, | 1.22 | 1.16 | 0.85, | 1.58 | **40/47 497** | 1.09 | 0.70, | 1.71 | 1.05 | 0.67, | 1.64 | **59/36 283** | 1.26 | 0.82, | 1.94 | 1.23 | 0.80, | 1.90 |
| **p for trend** | | | 0.254 | | | 0.385 | | | | 0.712 | | | 0.602 | | | | 0.036 | | | 0.053 | | |
| **Poultry (g/day)** | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **0.0** | **87/85 887** | 1.00 | | | 1.00 | | | **24/35 345** | 1.00 | | | 1.00 | | | **63/50 542** | 1.00 | | | 1.00 | | |
| **2** | **0.0** | **96/84 946** | 1.33 | 0.98, | 1.79 | 0.98 | 0.71, | 1.35 | **64/65 950** | 1.00 | 0.65, | 1.55 | 0.97 | 0.62, | 1.51 | **32/18 997** | 0.82 | 0.46, | 1.49 | 0.84 | 0.46, | 1.52 |
| **3** | **3.0** | **95/84 132** | 1.09 | 0.79, | 1.48 | 0.93 | 0.64, | 1.36 | **55/55 680** | 0.89 | 0.48, | 1.64 | 0.85 | 0.45, | 1.58 | **40/28 452** | 0.84 | 0.46, | 1.51 | 0.89 | 0.49, | 1.63 |
| **4** | **16.4** | **85/86 169** | 1.19 | 0.88, | 1.61 | 0.73 | 0.45, | 1.18 | **62/59 361** | 0.60 | 0.25, | 1.44 | 0.56 | 0.23, | 1.36 | **23/26 807** | 0.69 | 0.36, | 1.33 | 0.76 | 0.39, | 1.48 |
| **5** | **31.9** | **100/85 389** | 1.20 | 0.88, | 1.63 | 0.74 | 0.37, | 1.50 | **42/46 860** | 0.80 | 0.22, | 2.87 | 0.69 | 0.18, | 2.59 | **28/38 530** | 0.63 | 0.26, | 1.50 | 0.70 | 0.28, | 1.74 |
| **p for trend** | | | 0.176 | | | 0.292 | | | | 0.353 | | | 0.265 | | | | 0.265 | | | 0.501 | | |

(*Continued*)

**Table 3.** (Continued).

| Quintiles for all | | | All | | | | | | Women | | | | | | Men | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Basic model[a] | | | Full model[b] | | | | Basic model[a] | | | Full model[b, c] | | | | Basic model[a] | | | Full model[b] | | |
| Total | Red meat (g/day) | Cases/ person-years | HR | CI | | HR | CI | | Cases/person-years | HR | CI | | HR | CI | | Cases/person-years | HR | CI | | HR | CI | |
| **Fish (g/day)** | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **6.1** | **73/85 032** | 1.00 | | | 1.00 | | | **35/49 778** | 1.00 | | | 1.00 | | | **38/35 254** | 1.00 | | | 1.00 | | |
| **2** | **23.4** | **91/85 589** | 0.86 | 0.63, | 1.18 | 0.88 | 0.64, | 1.21 | **47/54 293** | 0.89 | 0.57, | 1.36 | 0.88 | 0.57, | 1.36 | **44/31 296** | 0.75 | 0.42, | 1.35 | 0.76 | 0.43, | 1.37 |
| **3** | **36.2** | **86/85 795** | 0.88 | 0.61, | 1.28 | 0.92 | 0.63, | 1.33 | **55/53 869** | 0.92 | 0.51, | 1.66 | 0.90 | 0.49, | 1.65 | **31/31 926** | 0.75 | 0.42, | 1.34 | 0.80 | 0.45, | 1.44 |
| **4** | **51.7** | **103/85 634** | 0.67 | 0.42, | 1.06 | 0.70 | 0.44, | 1.13 | **60/55 058** | 0.56 | 0.24, | 1.30 | 0.54 | 0.23, | 1.28 | **43/30 576** | 0.65 | 0.34, | 1.23 | 0.71 | 0.37, | 1.37 |
| **5** | **81.4** | **110/84 475** | 0.73 | 0.37, | 1.42 | 0.77 | 0.39, | 1.53 | **50/50 198** | 0.85 | 0.25, | 2.89 | 0.77 | 0.22, | 2.75 | **60/34 276** | 0.62 | 0.26, | 1.45 | 0.69 | 0.28, | 1.67 |
| **p for trend** | | | 0.098 | | | 0.173 | | | | 0.524 | | | 0.241 | | | | 0.149 | | | 0.316 | | |

[a]Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), and total energy (continuous)

[b]Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex or never), alcohol intake (zero, <15g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), use of non-steroid anti-inflammatory drugs (yes, no), and physical activity (quintiles of physical activity).

[c]Adjusted for current use of hormonal replacement therapy (yes/no)

Values are quintile medians, hazard ratios, and 95% confidence intervals. $p < 0.05$ was considered statistically significant. HR was calculated by using Cox proportional hazard risk model and was adjusted for energy with the residual method.

**Table 4.** Hazard ratio (HR) of rectal cancer associated with intakes of different foods in the Malmö Diet and Cancer study cohort.

| | | | All | | | | | | | Women | | | | | | | Men | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Basic model[a] | | | Full model[b] | | | | Basic model[a] | | | Full model[b, c] | | | | Basic model[a] | | | Full model[b] | | | |
| Quintiles for all Total Red meat (g/day) | | Cases/person-years | HR | CI | | HR | CI | | Cases/person-years | HR | CI | | HR | CI | | Cases/person-years | HR | CI | | HR | CI | | |
| **1** | **12.0** | **40/85 673** | 1.00 | | | 1.00 | | | **29/63 513** | 1.00 | | | 1.00 | | | **11/22 159** | 1.00 | | | 1.00 | | | |
| **2** | **24.9** | **61/84 893** | 1.56 | 1.04, | 2.34 | 1.53 | 1.02, | 2.29 | **34/57 244** | 1.39 | 0.84, | 2.30 | 1.38 | 0.83, | 2.29 | **27/27 650** | 1.99 | 0.98, | 4.01 | 1.91 | 0.94, | 3.85 |
| **3** | **33.8** | **51/85 110** | 1.30 | 0.85, | 1.98 | 1.25 | 0.82, | 1.91 | **25/53 883** | 1.11 | 0.64, | 1.91 | 1.09 | 0.63, | 1.89 | **26/31 227** | 1.73 | 0.85, | 3.51 | 1.63 | 0.80, | 3.31 |
| **4** | **45.6** | **52/84 644** | 1.26 | 0.82, | 1.93 | 1.20 | 0.78, | 1.84 | **17/48 445** | 0.82 | 0.44, | 1.53 | 0.81 | 0.43, | 1.51 | **35/35 799** | 1.98 | 0.99, | 3.93 | 1.82 | 0.91, | 3.64 |
| **5** | **69.2** | **61/84 ,071** | 1.57 | 1.04, | 2.39 | 1.45 | 0.95, | 2.22 | **20/38 495** | 1.39 | 0.77, | 2.49 | 1.31 | 0.72, | 2.38 | **41/45 576** | 2.07 | 1.05, | 4.08 | 1.85 | 0.93, | 3.68 |
| **p for trend** | | | 0.097 | | | 0.236 | | | | 0.746 | | | 0.887 | | | | 0.053 | | | 0.142 | | | |
| **Beef (g/day)** | | **Interaction** | | | | 0.025 | | | | | | | | | | | | | | | | | |
| **1** | **0.4** | **50/85 393** | 1.00 | | | 1.00 | | | **32/55 706** | 1.00 | | | 1.00 | | | **18/29 686** | 1.00 | | | 1.00 | | | |
| **2** | **8.0** | **48/83 313** | 1.00 | 0.67, | 1.50 | 1.00 | 0.67, | 1.49 | **24/53 850** | 0.81 | 0.47, | 1.39 | 0.81 | 0.47, | 1.39 | **24/29 464** | 1.37 | 0.74, | 3.53 | 1.38 | 0.75, | 2.55 |
| **3** | **15.1** | **60/84 279** | 1.28 | 0.88, | 1.88 | 1.28 | 0.87, | 1.87 | **30/53 519** | 1.08 | 0.65, | 1.79 | 1.08 | 0.65, | 1.79 | **30/30 760** | 1.64 | 0.91, | 2.96 | 1.66 | 0.92, | 3.00 |
| **4** | **24.1** | **52/85 349** | 1.20 | 0.81, | 1.76 | 1.18 | 0.75, | 1.75 | **23/52 039** | 0.86 | 0.50, | 1.48 | 0.86 | 0.50, | 1.48 | **29/33 311** | 1.69 | 0.94, | 3.02 | 1.69 | 0.94, | 3.03 |
| **5** | **42.7** | **55/86 040** | 1.28 | 0.87, | 1.90 | 1.23 | 0.83, | 1.83 | **16/46 850** | 0.77 | 0.42, | 1.42 | 0.76 | 0.41, | 1.40 | **39/39 190** | 1.82 | 1.03, | 3.24 | 1.82 | 1.02, | 3.25 |
| **p for trend** | | | 0.242 | | | 0.266 | | | | 0.509 | | | 0.487 | | | | 0.025 | | | 0.028 | | | |
| **Pork (g/day)** | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **21.3** | **37/86 474** | 1.00 | | | 1.00 | | | **22/57 359** | 1.00 | | | 1.00 | | | **15/29 115** | 1.00 | | | 1.00 | | | |
| **2** | **39.2** | **67/84 758** | 1.77 | 1.18, | 2.64 | 1.76 | 0.92, | 2.25 | **29/52 233** | 1.32 | 0.75, | 2.32 | 1.33 | 0.75, | 2.34 | **38/32 355** | 2.28 | 1.25, | 4.15 | 2.26 | 1.24, | 4.13 |
| **3** | **52.3** | **52/84 758** | 1.43 | 0.94, | 2.18 | 1.39 | 0.69, | 1.91 | **25/52 403** | 1.24 | 0.70, | 2.20 | 1.24 | 0.70, | 2.21 | **27/32 355** | 1.59 | 0.85, | 3.00 | 1.55 | 0.82, | 2.93 |
| **4** | **67.0** | **55/84 853** | 1.49 | 0.98, | 2.27 | 1.43 | 0.57, | 1.86 | **21/51 778** | 1.06 | 0.58, | 1.93 | 1.04 | 0.57, | 1.91 | **34/33 075** | 1.88 | 1.01, | 3.47 | 1.79 | 0.97, | 3.33 |
| **5** | **95.3** | **54/84 418** | 1.58 | 1.04, | 2.41 | 1.47 | 0.47, | 2.44 | **28/48 190** | 1.56 | 0.88, | 2.75 | 1.50 | 0.84, | 2.67 | **24/36 288** | 1.45 | 0.76, | 2.75 | 1.34 | 0.70, | 2.56 |
| **p for trend** | | | 0.265 | | | 0.439 | | | | 0.278 | | | 0.356 | | | | 0.649 | | | 0.906 | | | |
| **Unprocessed red meat (g/day)** | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **21.2** | **43/84 844** | 1.00 | | | 1.00 | | | **27/60 282** | 1.00 | | | 1.00 | | | **16/24 561** | 1.00 | | | 1.00 | | | |
| **2** | **38.8** | **56/85 003** | 1.48 | 0.94, | 2.33 | 1.59 | 1.00, | 2.50 | **32/55 763** | 1.31 | 0.72, | 2.40 | 1.42 | 0.77, | 2.61 | **24/29 240** | 1.26 | 0.67, | 2.38 | 1.23 | 0.65, | 2.33 |
| **3** | **53.3** | **59/84 716** | 1.16 | 0.70, | 1.91 | 1.28 | 0.76, | 2.13 | **27/53 175** | 1.20 | 0.56, | 2.56 | 1.35 | 0.62, | 2.92 | **32/31 541** | 1.63 | 0.89, | 2.98 | 1.56 | 0.85, | 2.86 |
| **4** | **70.5** | **55/84 710** | 1.04 | 0.58, | 1.84 | 1.19 | 0.66, | 2.16 | **23/50 ,640** | 1.14 | 0.43, | 3.01 | 1.31 | 0.49, | 3.56 | **32/34 070** | 1.52 | 0.83, | 2.78 | 1.44 | 0.78, | 2.64 |
| **5** | **103.0** | **52/85 102** | 0.89 | 0.40, | 1.97 | 1.07 | 0.47, | 2.45 | **16/42 103** | 1.23 | 0.30, | 4.93 | 1.47 | 0.35, | 6.14 | **36/42 998** | 1.45 | 0.80, | 2.64 | 1.32 | 0.72, | 2.44 |
| **p for trend** | | | 0.316 | | | 0.700 | | | | 0.656 | | | 0.935 | | | | 0.080 | | | 0.195 | | | |
| **Processed red meat (g/day)** | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **6.7** | **44/86 373** | 1.00 | | | 1.00 | | | **23/58 278** | | | | 1.00 | | | **21/28 095** | 1.00 | | | 1.00 | | | |
| **2** | **20.7** | **51/85 055** | 1.55 | 0.99, | 2.45 | 1.67 | 1.06, | 2.64 | **27/53 024** | 1.36 | 0.75, | 2.48 | 1.45 | 0.79, | 2.65 | **24/32 031** | 1.99 | 0.85, | 4.69 | 2.11 | 0.89, | 4.99 |
| **3** | **31.7** | **56/84 840** | 1.20 | 0.72, | 2.01 | 1.34 | 0.80, | 2.26 | **28/52 404** | 1.27 | 0.60, | 2.70 | 1.40 | 0.66, | 2.99 | **28/32 435** | 1.18 | 0.48, | 2.87 | 1.28 | 0.52, | 3.14 |
| **4** | **43.9** | **57/84 781** | 1.28 | 0.72, | 2.28 | 1.50 | 0.83, | 2.71 | **25/50 943** | 1.38 | 0.54, | 3.56 | 1.56 | 0.60, | 4.07 | **32/33 839** | 1.22 | 0.49, | 3.04 | 1.39 | 0.55, | 3.53 |
| **5** | **66.6** | **57/83 324** | 1.25 | 0.57, | 2.75 | 1.54 | 0.68, | 3.50 | **22/47 314** | 1.96 | 0.52, | 7.41 | 2.25 | 0.58, | 8.70 | **35/36 010** | 0.99 | 0.32, | 3.01 | 1.20 | 0.38, | 3.80 |
| **p for trend** | | | 0.592 | | | 0.941 | | | | 0.742 | | | 0.972 | | | | 0.242 | | | 0.490 | | | |
| **Poultry (g/day)** | | | | | | | | | | | | | | | | | | | | | | | |
| **1** | **0.0** | **60/85 562** | 1.00 | | | 1.00 | | | **19/35 299** | | | | 1.00 | | | **41/50 263** | 1.00 | | | 1.00 | | | |
| **2** | **0.0** | **40/84 333** | 1.48 | 0.93, | 2.35 | 1.59 | 1.00, | 2.53 | **22/65 530** | 1.64 | 0.85, | 3.20 | 1.80 | 0.91, | 3.55 | **18/18 803** | 1.49 | 0.65, | 3.39 | 1.56 | 0.68, | 3.57 |
| **3** | **3.2** | **44/83 503** | 1.31 | 0.78, | 2.19 | 1.46 | 0.86, | 2.46 | **22/55 265** | 1.95 | 0.81, | 4.69 | 2.24 | 0.91, | 5.50 | **22/28 239** | 0.98 | 0.42, | 2.28 | 1.04 | 0.45, | 2.44 |
| **4** | **16.5** | **48/85 815** | 1.12 | 0.61, | 2.05 | 1.30 | 0.69, | 2.42 | **36/59 108** | 1.81 | 0.55, | 5.95 | 2.16 | 0.64, | 7.35 | **12/26 707** | 0.84 | 0.35, | 2.02 | 0.93 | 0.38, | 2.27 |
| **5** | **31.9** | **73/85 161** | 1.18 | 0.51, | 2.72 | 1.43 | 0.60, | 3.42 | **26/46 762** | 3.92 | 0.72, | 21.23 | 4.96 | 0.87, | 28.2 | **47/38 400** | 0.65 | 0.22, | 1.93 | 0.76 | 0.25, | 2.33 |
| **p for trend** | | | 0.851 | | | 0.680 | | | | 0.734 | | | 0.476 | | | | 0.207 | | | 0.407 | | | |

(*Continued*)

Table 4. (Continued).

| | All | | | | | | Women | | | | | | Men | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Basic model[a] | | | Full model[b] | | | Basic model[a] | | | Full model[b, c] | | | Basic model[a] | | | Full model[b] | |
| Quintiles for all Total Red meat (g/day) | Cases/person-years | HR | CI | | HR | CI | Cases/person-years | HR | CI | | HR | CI | Cases/person-years | HR | CI | | HR | CI |
| **Fish (g/day)** | | | | | | | | | | | | | | | | | | |
| **1** | **62/84 902** | 1.00 | | | 1.00 | | **22/49 631** | 1.00 | | | 1.00 | | **40/35 271** | 1.00 | | | 1.00 | |
| **2** | **59/85 245** | 0.83 | 0.55, | 1.24 | 0.84 | 0.56, 1.26 | **32/54 088** | 1.14 | 0.62, | 2.08 | 1.14 | 0.62, 2.09 | **27/31 158** | 0.66 | 0.38, | 1.17 | 0.67 | 0.38, 1.18 |
| **3** | **48/85 385** | 0.66 | 0.43, | 1.01 | 0.67 | 0.44, 1.02 | **26/53 618** | 0.92 | 0.49, | 1.73 | 0.92 | 0.49, 1.73 | **22/31 767** | 0.51 | 0.28, | 0.93 | 0.52 | 0.28, 0.95 |
| **4** | **48/85 016** | 0.65 | 0.43, | 1.00 | 0.65 | 0.43, 1.00 | **25/54 719** | 0.86 | 0.42, | 1.52 | 0.78 | 0.41, 1.50 | **23/30 297** | 0.60 | 0.34, | 1.06 | 0.61 | 0.34, 1.09 |
| **5** | **48/83 827** | 0.60 | 0.39, | 0.92 | 0.59 | 0.38, 0.92 | **20/49 908** | 0.71 | 0.36, | 1.39 | 0.67 | 0.34, 1.32 | **28/33 919** | 0.55 | 0.31, | 0.98 | 0.57 | 0.32, 1.03 |
| **p for trend** | | 0.024 | | | 0.025 | | | 0.224 | | | 0.171 | | | 0.052 | | | 0.075 | |

[a]Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), and total energy (continuous)
[b]Adjusted for sex (man, woman), age (continuous), method version (before or after September 1994), season (winter, spring, summer, autumn), total energy (continuous), education (<8 years, 9–10 years, 11–13 years or university degree), smoking (current, ex or never), alcohol intake (zero, <15g/d for women and <20 g/d for men, 15–30 g/d for women and 20–40 g/d for men, >30 g/d for women and >40 g/d for men), use of non-steroid anti-inflammatory drugs (yes, no), and physical activity (quintiles of physical activity)
[c]Adjusted for current use of hormonal replacement therapy (yes/no). Values are quintile medians, hazard ratios, and 95% confidence intervals. $p < 0.05$ was considered statistically significant. HR was calculated by using Cox propoonal hazard risk model and was adjusted for energy with the residual method.

cancer became significant also in men (HR: 0.56 for highest compared with lowest quintile; 95% CI: 0.36, 0.88; p for trend = 0.035).

### *Exclusion of subjects with prevalent or incident diabetes*

The association between high intake of processed red meat and risk of CRC in men, did not remain significant (HR: 1.05 for highest compared with lowest quintile; 95% CI: 0.71, 1.56; p for trend = 0.155) when excluding individuals with diabetes.

## Discussion

In the present study, high intake of pork was associated with increased risk of CRC, and especially with colon cancer in women. In contrast, high intake of beef was associated with decreased risk of colon cancer, whereas it was associated with increased risk of rectal cancer in men. Furthermore, there was a trend for increased risk of CRC with higher intake of processed meat among the men, mainly driven by colon cancer. Fish intake was inversely associated with rectal cancer.

In line with our findings, Bernstein et al. [8] did not observe high intakes of unprocessed red meat to be associated with a substantially increased risk of CRC when recent results from the Nurses' Health Study and the Health professionals Follow-up Study were pooled. Similarly to the meta-analysis by Alexander et al. [9], our results indicate a positive association between intake of processed red meat and CRC in men, but not in women, whereas other meta-analyses have not shown differing associations depending on sex [21,22]. Previous meta-analyses have also indicated significant associations between high intake of processed meat and colon cancer, but not with rectal cancer [6,8], although Bernstein et al. [8] concluded that they could not find evidence to show that the associations differed with colon or rectal cancers, but that the intake of processed red meat was especially associated with increased risk of distal colon cancer. Few studies have examined subsites of colon malignancies, but comparable findings were seen in a meta-analysis of three earlier prospective studies [23], whereas positive associations with both proximal and distal colon cancer were seen in a Norwegian study [24]. In a recent meta-analysis (2015) comparing different meat subtypes, high intakes of beef and lamb, but not pork and poultry, were associated with increased risk of CRC [10]. However, when excluding one of the included cohorts due to heterogeneity between the studies,

meta-analysis of four prospective studies indicated, similar to our results, an increased risk of CRC at high intakes of pork [10].

The diverse findings depending on type of meat, gender, and tumor site may reflect the complexity of colorectal cancer. Proximal colon and distal colon (including rectum) arise from different embryonic tissues. They also serve different functions, and the mucosal properties and microenvironment differ between segments [25]. As the fecal content is degraded by the microbiota and water and minerals are reabsorbed during the colonic passage, the production of short-chain fatty acids and metabolites varies, and fecal content changes its properties, in distal direction. Traditionally, we consider colon cancers as one disease. This may be misleading, since proximal colon cancer more often have microsatellite instability, CpG island methylator phenotype, and *KRAS* mutations, whereas rectal and distal colon cancers more often have chromosomal instability and *TP53* and *APC* mutations [26]. So far, the incidence of distal cancer has been higher than the incidence of proximal cancer [27]. In future studies, subgroup analysis of proximal or distal CRC ought to be done in addition to analysis of colon and rectal cancer, which is more common in epidemiological studies and therefore more comparable.

Associations between meat intake and CRC may differ depending on the type of meat most frequently consumed in a different population, as well as on intake levels. In the southernmost district in Sweden, where participants of the MDCS cohort were enrolled, pork intake is by tradition high. Although the intake data on meat could be considered to be satisfactory, it is worth noting, that the main part of the meat intake was recorded in the MDCS seven-day menu book, and that fewer days are needed to capture intakes of food consumed more frequently, compared to those consumed more seldom, indicating that intake data on pork may be more valid, compared to that on beef. This may partly explain why beef was not found to associate with higher risk of colon cancer in this study, while such association has been observed in other populations [9,10]. On the other hand, difference in meat production, with fewer antibiotics and growth factors used in Sweden, compared with for example USA, may affect the association between beef intake and risk of CRC differently. It is stated that increased risk for CRC starts at an intake of 500 g/week for red meat and for processed meat only by eating it [5]. In the present study, the estimated median intake in the lowest (44 g/day), and the highest (146 g/day) quintile of red meat, was well below, and respectively above, the stated threshold level.

The gender difference in observed associations may have several explanations. The prolonged gastrointestinal transit time in women compared with men may lead to a prolonged exposure of the mucosa to carcinogens [28]. On the other hand, this may be counteracted by higher meat intake in men compared with that in women in the MDCS. If meat has carcinogen-inducing properties in the bowel, a difference in transit time may decrease the difference in risk between genders caused by difference in meat intake.

Several mechanisms may lie behind observed associations between red meats and CRC. One difference between beef and pork is the amount and composition of fat [29]. The generally higher fat concentration in pork may enhance the excretion of bile acids, which may promote tumorigenesis [30,31]. During the passage down the colon, the bile acids are modified and dehydroxylated [25], leading to different properties along the colon mucosa. Thus, the malignancy risk may vary between different colon segments. The heme iron in red meat is another plausible explanation for the association between red meats and CRC [29], as heme iron damages the colon's lining [32]. However, the inverse association between beef intake and risk of colon cancer in the present study, and the association between high pork intake and increased risk of CRC, do not support this explanation.

Consumption of red meat may result in exposure to carcinogens through cooking methods, such as cooking meat at a high temperature by barbequing or smoking, or through preservation with nitrite. Heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), and N-nitroso compounds (NOCs) are thought to be factors in development of CRC [33,34]. In addition, processed meat often contains a high amount of salt [35] and it has been discussed if salt may be a risk factor for CRC [36].

Previous indications of protective associations with high fish intake have, consistent with our findings, above all been seen for rectal cancer [11]. Intake of marine omega-3 is thought to be inversely associated with CRC [37]. Mechanisms are thought to be the reducing effect omega-3 has on the omega-6 polyunsaturated fatty acid production of eicosanoids, and inhibition of cyclo-oxygenase-2 [38], but when analyzing associations between omega-3 intake and risk of CRC, no clear association was seen [5]. The high content of vitamin D and selenium has also been suggested as potential mechanisms [39]. This may explain the inverse association between fish and rectal cancer seen in the present study.

Obesity is considered to be an established risk factor for CRC [4]. Low grade inflammation and changes in

microbiota are associated with obesity and have been discussed as possible causes [4], along with insulin-like growth factor-1 and leptin [40]. The observed associations in this study did not clearly differ between individuals with BMI above or below 25, and exclusion of BMI in the multivariate model did not substantially change our finding, suggesting that weight status does not have any modifying or mediating effects on the associations between meat intakes and CRC. Yet, we cannot exclude modification if further discriminating between overweight and obese in more well-powered studies.

Diabetes may promote development of CRC, and red and processed meats have in studies shown to be associated with increased risk of diabetes [41,42]. Exclusion of patients with diabetes did not significantly affect the association between intake of pork or beef and risk of CRC, but the association between intake of processed red meat and CRC did not remain significant in men and the risk estimate in the highest quintile changed from 1.23 to 1.05. Loss of power may lie behind this observation; especially since diabetes is more common among men (26% were excluded). Future well-powered studies may reveal if the presence of diabetes has any modifying effect.

The strength of the present study is dietary data of high relative validity [13,15]. As it is a large population-based prospective study with long follow-up, selection bias and reverse causation were minimized. We had also extensive information on potential confounding factors and were able to exclude individuals who reported dietary changes in the past. There are several limitations of this study. Power may be a problem in some of the gender specific analyses. Family history of CRC and inflammatory bowel disease, seen as strong risk factors in development of CRC [22], were not included in the study as information on these risk factors were missing. Furthermore, neither information on the source of some of the processed meat, which may have influenced the findings regarding intakes of beef and pork and their association with risk of CRC, nor information about intake of heme iron was available.

Despite adjustments for possible confounders and known risk factors, occurrence of residual confounding cannot be completely excluded and we have not adjusted for multiple testing, since dietary intakes are highly correlated and the analyses could not be treated as independent.

## Conclusion

In conclusion, our findings suggest that type of meat as well as sex and tumor location in the bowel influence associations between meat intake and risk of CRC. The findings support previous studies indicating that high intake of processed meat above all is associated with increased risk of CRC in men and that high intake of pork may be associated with an increased risk of CRC. Beef intake was in contrast to previous observations inversely associated with colon cancer, but in men associated with increased risk of rectal cancer. Fish intake was inversely associated with rectal cancer. Presence of overweight did not seem to have any major impact on the findings.

## Acknowledgements

This study was funded by grants from Development Foundation of Region Skåne, Direktör Albert Påhlsson's Foundation, and the Malmö Hospital Foundation for Cancer Prevention. The funders had no role in the design, analysis or writing of this article.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## ORCID

Jonas Manjer http://orcid.org/0000-0002-3158-5289
Ulrika Ericson http://orcid.org/0000-0003-4629-4318
Bodil Ohlsson http://orcid.org/0000-0002-9142-5244

## References

[1] Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer. 2010;46(4):765–781.
[2] Edwards BK, Ward E, Kohler BA, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010;116(3):544–573.
[3] Bergman O, Hont G, Johansson E Cancer i siffror [Internet page]. Socialstyrelsen Cancerfonden; 2013 [cited 2013 september 18]. 20130605: Available from: http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/19108/2013-6-5.pdf
[4] Ma Y, Yang Y, Wang F, et al. Obesity and risk of colorectal cancer: a systematic review of prospective studies. Plos One. 2013;8(1):e53916.
[5] World Cancer Research Fund and American Institute for Cancer Research. Continuous update project report. Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. 2011.
[6] Chan DSM, Lau R, Aune D, et al. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. Plos One. 2011;6(6):e20456.
[7] Bjerselius R, Konde ÅB, Färnstrand JS. Konsumtion av rött kött och charkuteriprodukter och samband med tjock- och ändtarmscancer av - risk- och nyttohanteringsrapport. Uppsala, Sweden: Livsmedelsverket; 2014.

Available from: http://www.livsmedelsverket.se/globalassets/matvanor-halsa-miljo/kostrad-matvanor/rott-kott-och-chark/livsmedelsverket.-konsumtion-av-rott-kott-och-charkuteriprodukter-och-samband-med-tjock–och-andtarmscancer.-rapport-20-2014.pdf?id=3573

[8] Bernstein AM, Song M, Zhang X, et al. Processed and unprocessed red meat and risk of colorectal cancer: analysis by tumor location and modification by time. Plos One. 2015;10(8):e0135959.

[9] Alexander DD, Miller AJ, Cushing CA, et al. Processed meat and colorectal cancer: a quantitative review of prospective epidemiologic studies. European Journal Cancer Prevention: Official Journal European Cancer Prevention Organisation. 2010;19(5):328–341.

[10] Carr PR, Walter V, Brenner H, et al. Meat subtypes and their association with colorectal cancer: systematic review and meta-analysis. Int J Cancer. 2015;138(2):293–302.

[11] Wu S, Feng B, Li K, et al. Fish consumption and colorectal cancer risk in humans: a systematic review and meta-analysis. Am J Med. 2012;125(6):551–9e5.

[12] Livsmedelsverk S. Livsedelstabeller. Stockholm: Liber tryck AB; 1986.

[13] Wirf?Lt E, Mattisson I, Johansson U, et al. A methodological report from the Malm? Diet and Cancer study: development and evaluation of altered routines in dietary data processing. Nutr J. 2002;1:3.

[14] Callmer E, Riboli E, Saracci R, et al. Dietary assessment methods evaluated in the Malmö food study. J Intern Med. 1993;233(1):53–57.

[15] Elmståhl S, Riboli E, Lindgärde F, et al. The Malmö Food Study: the relative validity of a modified diet history method and an extensive food frequency questionnaire for measuring food intake. Eur J Clin Nutr. 1996;50(3):143–151.

[16] Riboli E, Elmståhl S, Saracci R, et al. The Malmö Food Study: validity of two dietary assessment methods for measuring nutrient intake. Int J Epidemiol. 1997;26 (Suppl 1):S161–73.

[17] The Swedish National Food Adminstration (SLV). Vikttabell. Uppsala: The Swedish national Food Adminitration; 1999.

[18] Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol. 1986;124(1):17–27.

[19] WHO. BMI classification 2006. Updated 26 Novmeber 2015. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html

[20] Margetts BM, Nelson M. Design Concepts in Nutritional Epidemiology:Norfolk:Oxford University Press, printed by Biddles LTd King's Lynn;1997.

[21] Xu X, Yu E, Gao X, et al. Red and processed meat intake and risk of colorectal adenomas: a meta-analysis of observational studies. Int J Cancer. 2013;132(2):437–448.

[22] Johnson CM, Wei C, Ensor JE, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control. 2013;24(6):1207–1222.

[23] 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.

[24] Parr CL, Hjartåker A, Lund E, et al. Meat intake, cooking methods and risk of proximal colon, distal colon and rectal cancer: the Norwegian Women and Cancer (NOWAC) cohort study. Int J Cancer. 2013;133(5):1153–1163.

[25] Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer. 2002;101(5):403–408.

[26] Lee GH, Malietzis G, Askari A, et al. Is right-sided colon cancer different to left-sided colorectal cancer? - a systematic review. Eur J Surg Oncol. 2015;41(3):300–308.

[27] Beart RW, Melton LJ 3rd, Maruta M, et al. Trends in right and left-sided colon cancer. Dis Colon Rectum. 1983;26(6):393–398.

[28] Sadik R, Abrahamsson H, Stotzer PO. Gender differences in gut transit shown with a newly developed radiological procedure. Scand J Gastroenterol. 2003;38(1):36–42.

[29] Livsmedelsverket. Livsmedelsverkets livsmedelsdatabas version 2015-05-28. 2015 [cited 2015 11 november]. Available from: http://www7.slv.se/SokNaringsinnehall/

[30] Narisawa T, Magadia NE, Weisburger JH, et al. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. J Natl Cancer Inst. 1974;53(4):1093–1097.

[31] Chomchai C, Bhadrachari N, Nigro ND. The effect of bile on the induction of experimental intestinal tumors in rats. Dis Colon Rectum. 1974;17(3):310–312.

[32] Gu?Raud F, Tach? S, Steghens J-P, et al. Dietary polyunsaturated fatty acids and heme iron induce oxidative stress biomarkers and a cancer promoting environment in the colon of rats. Free Radic Biol Med. 2015;83:192–200.

[33] Knize MG, Salmon CP, Pais P, et al. Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Adv Exp Med Biol. 1999;459:179–193.

[34] Wakabayashi K, Nagao M, Esumi H, et al. Food-derived mutagens and carcinogens. Cancer Res. 1992;52 (7):2092s–8s.

[35] NNR. Sodium as salt, in Nordic nutrition recommendations 2012. In: ministers Nco, editor. Vol. 2014, 515–534. Copenhagen: Nordic Council of Ministers; 2012.

[36] Kune GA, Kune S, Watson LF. Dietary sodium and potassium intake and colorectal cancer risk. Nutr Cancer. 1989;12(4):351–359.

[37] Song M, Nishihara R, Wu K, et al. Marine ω-3 polyunsaturated fatty acids and risk for colorectal cancer according to microsatellite instability. JNCI: J Natl Cancer Inst. 2015;107:4.

[38] Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br J Clin Pharmacol. 2013;75(3):645–662.

[39] Baena R, Salinas P. Diet and colorectal cancer. Maturitas. 2015;80(3):258–264.

[40] Riondino S. Obesity and colorectal cancer: role of adipokines in tumor initiation and progression. World J Gastroenterol. 2014;20(18):5177–5190.

[41] Aune D, Ursin G, Veierød MB. Meat consumption and the risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. Diabetologia. 2009;52 (11):2277–2287.

[42] Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. Am J Clin Nutr. 2011;94 (4):1088–1096.