The University Of Sheffield.

Thesis Title

Diet, dietary patterns and colorectal adenoma

By

Abeir El Mogassabi

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

The University of Sheffield
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Abstract

Background: Colorectal cancer (CRC) is the 3rd most common cancer in the UK. It is estimated that 95% of CRC develop from sporadic colorectal adenoma. Colorectal adenoma is usually detected and removed during cancer screening but recurrence is common. According to the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR), around 45% of CRC cases could be prevented by adopting a healthy lifestyle. The aim of this project was *"To describe the dietary characteristics of patients newly diagnosed with high-risk colorectal adenoma and to explore the association between diet, dietary patterns and colorectal adenoma profile and the risk of recurrence"*.

Methodology: This is a secondary data analysis research project used data collected from colorectal adenoma patients recruited to the seAFOod trial, through the Bowel Cancer Screening Programme (BCSP) and data from the FACT study. Both used EPIC food frequency questionnaires (EPIC-FFQ) to assess diet and FETA dietary analysis software was used to extract the mean daily intake of foods and nutrients. Two approaches of dietary pattern analysis were used for the seAFOod trial data data-driven dietary patterns and the predefined pattern, the Dietary Inflammatory Index (DII). For the FACT study data, DII was calculated and adherence score to the cancer prevention recommendation of WCRF/AICR was assessed. SPSS software was used to perform calculations and statistical tests.

Findings: For the seAFOod trial, the mean age of the population was 65 years, over 80% were overweight or obese. Analysis of dietary data for 674 patients showed that at baseline, diet was high in alcohol, red and processed meat and iron, and was low in fibre and vitamin D. Three dietary patterns were generated (High-energy pattern, the Healthy pattern and Alcohol and nuts pattern) and 76.7% of the patients had a proinflammatory DII score. During the 12 months after diagnosis, only males significantly reduced their intake of energy, red and processed meat. No association was found between dietary behaviour at baseline and the risk of colorectal adenoma in the 156 patients allocated to the placebo arm. No association between diet and colorectal adenoma profile at baseline or exit. For the FACT study analysis, 79.5% of the participants did not adhere to the WCRF/AICR cancer prevention recommendations and no association was found between adherence to these recommendations and markers of cell crypt proliferation, endocrine cells or keratin in colon biopsies.

Conclusions: In this sample recruited through the BCSP, diet at baseline did not meet the recommendation for healthy diet provided by the WCRF/AICR and Public Health England recommendations, however, this analysis found no association between dietary behaviour at baseline and the risk of colorectal adenoma recurrence after 12 months. There was no association between adherence to the WCRF/AICR cancer prevention recommendations and the assessed cellular biomarkers.

Acknowledgements

First I would like to express my deep and sincere gratitude to my supervisors **Dr Liz Williams and Prof Bernard Corfe**. Thank you for sharing your experience, and for keeping the balance between providing support, guidance and motivation while allowing me to work independently. My thanks goes to the Molecular Gastroenterology Research Group, especially my friends **Claire, Sabeen and Esme** for all the support throughout this journey.

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Thanks are also due to **Angela Mulligan** for supporting me in using FETA software and performing analysis for omega-3 fatty acids, to **Prof Jeremy Dawson** for his guidance and support in statistical analysis, and to **Dr Nitin Shivappa** for assisting in dietary inflammatory index validation.

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Finally, I dedicated this thesis to my husband and sons. **Kafi** and **Wahab**, thank you for all the help and encouragement and I hope I have still been a good mother during the period of this research. **Osama,** thank you for believing in me, for all the support, encouragement, sacrifices and love. Thank you for giving up so much so I can achieve, I could not have done this without you. Thank you.

Intellectual Property and Publication Statements

I, the author, confirm that the Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not been previously been presented for an award at this, or any other, university.

The following was jointly-authored published conference abstracts:

El-Mogassabi A, Corfe B, Hull M, Williams E (2020) Do people change their diet after colorectal adenoma diagnosis?' Proc Nutr Soc, (OCE2)

List of abbreviation

APC	**Adenomatous Polyposis Coli**
BCSP	Bowel Cancer Screening Programme
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CCQ	Cross-Check Questions
CI	Confidence Interval
CgA	Chromogranin A
CL	Confidence Level
CPPC	The Calcium Polyp Prevention Study
CRC	Colorectal Cancer
CRF	Case Report Form
CUP	Continuous Update Project
DII	Dietary Inflammatory Index
DRV	Dietary Reference Value
EEC	Enteroendocrine cells
ECP	European Cancer Prevention study
EPIC	European Prospective Investigation into Cancer and Nutrition
FAP	Familial Adenomatous Polyposis
FETA	Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis
FFQ	Food Frequency Questionnaires
EPA	Eicosapentaenoic Acid
GTE	Green Tea Extract
HR	Hazard Ratio
LRNI	Lower Reference Nutrient Intake
MUFA	Mono Unsaturated Fatty Acid
NDNS	National Diet and Nutrition Survey
NSP	Non-Starch Polysaccharides
OR	Odds Ratio
PAL	Physical Activity Level
PCA	Principal Component Analysis
PHE	Public Health England
PPT	Polyp Prevention Trial
PUFA	Poly Unsaturated Fatty Acid
RCT	Randomised Clinical Trial
RR	Relative Risk
SACN	Scientific Advisory Committee on Nutrition
SAT	Saturated Fatty Acids
seAFOod Trial	Systematic Evaluation of Aspirin and Fish Oil Bowel Polyp Prevention Trial
SCFA	Short Cain Fatty Acid
Sel/Cel Trial	The Selenium and Celecoxib Trial
TPPT	Toronto Polyp Prevention Trial
ukCAP Trial	The United Kingdom Colorectal Adenoma Prevention Trial
VAT	Visceral Adipocyte Tissues
WBFT	Wheat Bran Fibre Trial
WCS	Women's Cohort study
WCRF/AICR	World Cancer Research Fund/American Institute for Cancer Research
WF	Weighting Factor

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Introduction, literature review, aim and objectives

Chapter 1 Introduction, literature review, aim and objectives

1.1 General introduction

Colorectal Cancer (CRC) is cancer that develops in the colon and the rectal region of the large intestine. Worldwide, CRC is the 3rd most commonly diagnosed cancer; it is the 2nd cause of cancer-related deaths for females after breast cancer and the 3rd cause of cancer-related deaths for males after lung and prostate cancers (1).

Less than 10% of CRC cases are caused by inherited mutations in common cancer-related genes and the majority of the cases are sporadic (2). The risk of the disease increases with age (3) and with particular lifestyle factors such as physical inactivity, smoking and specific dietary behaviours. These risk factors are thought to integrate and promote an accumulation of genetic and epigenetic changes in the mucosa of the large intestine that lead over time to histological and morphological changes, and the development of polyps within the lumen of the large intestine (2).

In general, most polyps remain benign, but evidence suggests that early detection and removal of these lesions reduces the risk of CRC incidence and mortality (2,4). At the early stage, the disease has no symptoms and, a non-invasive biomarker for early detection has not yet been identified (5). Therefore, several countries have implemented CRC screening as a prevention measure (6). The screening programmes aim to detect and remove the lesions before malignant transformation takes place or to detect the lesion at the early stages of malignancy, where cure is still possible, but there is a high risk that the lesion will reoccur (7).

The risk of polyp recurrence and the risk of progression to cancer are associated with the polyp histological structure, size, location and number. Therefore, according to the screening outcome, individuals are classified as at low, intermediate, or high risk of recurrence with each group has a different surveillance strategy (4). According to their histological structure, polyps are classified into two types, neoplastic and non-neoplastic; the neoplastic type is classified into adenoma (benign) and carcinoma (malignant) (8). The non-neoplastic types do not typically transform to the malignant lesion and will not be further discussed.

The association between diet and CRC was first proposed in 1971 when Denis P. Burkitt observed that high fibre intake was associated with a lower risk of CRC (9). This was further evidenced by the increase

in CRC risk in Japanese migrants in the USA who changed their dietary habits (10). Nowadays, there is a significant body of evidence showing the association between dietary factors and the disease, yet, inconsistency in the evidence is noticeable. The association is complex and understanding the role of foods or nutrients require special consideration because many factors limit the quality of the evidence. For example, i) the disease is a multistage condition, that develops over a long time asymptomatically due to interaction between different factors, in which diet is only one of the risk factors (11). ii) food and nutrients may exhibit as a promotor or inhibitor factor at different stages of the disease (12). iii) dietary intake is characterised by being dynamic and varies during different stages of the disease (13). iv) the fact that people do not consume isolated foods or nutrients so identification of the role of each, is not possible (12). Hence, several studies investigated the association between diet and colorectal tumorigenesis stages by exploring the dietary patterns of the patients instead of exploring the role of each food or nutrient separately.

Subsequent sections will describe the biology and epidemiology of colorectal tumorigenesis, the risk factors associated with different stages of colorectal tumorigenesis and some of the putative mechanisms that associate the diet with the disease. The final section will provide the evidence about the role of foods, nutrients and dietary patterns on the risk of colorectal adenoma and CRC development and the risk of colorectal adenoma recurrence.

1.2 Literature review

The literature review was conducted in four sections as shown in **Figure 1.1.** The first section will introduce the colorectal tumourigenesis process by providing a brief overview of the mucosa of the large intestine in the normal state and the molecular changes that lead to the development of the disease. Section 2 will describe the epidemiology, section 3 is a summary for the non-dietary risk factors and section 4 will collate the evidence of the association between diet and colorectal tumorigenesis. This final section is divided into three subsections i) some of the proposed mechanisms ii) the evidence of the association between diet and the risk of colorectal adenoma and CRC and ii) the evidence of the association between diet and the risk of colorectal adenoma recurrence.

1. Colorectal tumorigenesis

- Large intestine, an overview.
- Molecular mechanism of colorectal tumorigenesis
- Classifications of colorectal adenoma

2. Colorectal cancer epidemiology

- Incidence, trends and mortality rates
- Colorectal cancer survival and screening
- Risk of colorectal adenoma recurrence

3. Non-dietary risk factors for CRC, adenoma and adenoma recurrence

- Risk factors for CRC and colorectal adenoma development
- Risk factors for colorectal adenoma recurrence

4. Diet and colorectal tumorigenesis

- Mechanisms underlying the role of diet on colorectal tumorigenesis
- Foods and nutrients and the risk of:
  - CRC and adenoma development
  - Adenoma recurrence
- Dietary patterns and the risk of:
  - CRC and adenoma development
  - Adenoma recurrence

Figure 1-1. A summary of the structure of the literature review section

1.2.1 Colorectal tumorigenesis

1.2.1.1 Large intestine, an overview.

The large intestine is a large organ of the digestive system, it starts from the ileocecal valve and ends with the anus. Its total length is about 1.5m and is divided into five sections: the caecum, the colon, the rectum, the anal canal and the anus **(Fig 1-2)**. The large intestine harbour a complex and active population of microorganisms that offers a mutually beneficial relationship with the host. They are involved in the synthesis of essential nutrients, protect from opportunistic pathogens and host immunity regulation. However, alteration in the microbial composition leads to a disturbance of these activities (14). The main function of the large intestine is to recover the water and electrolytes, absorb the bacterial metabolites and store, temporarily, the food residue before elimination as faeces (15).

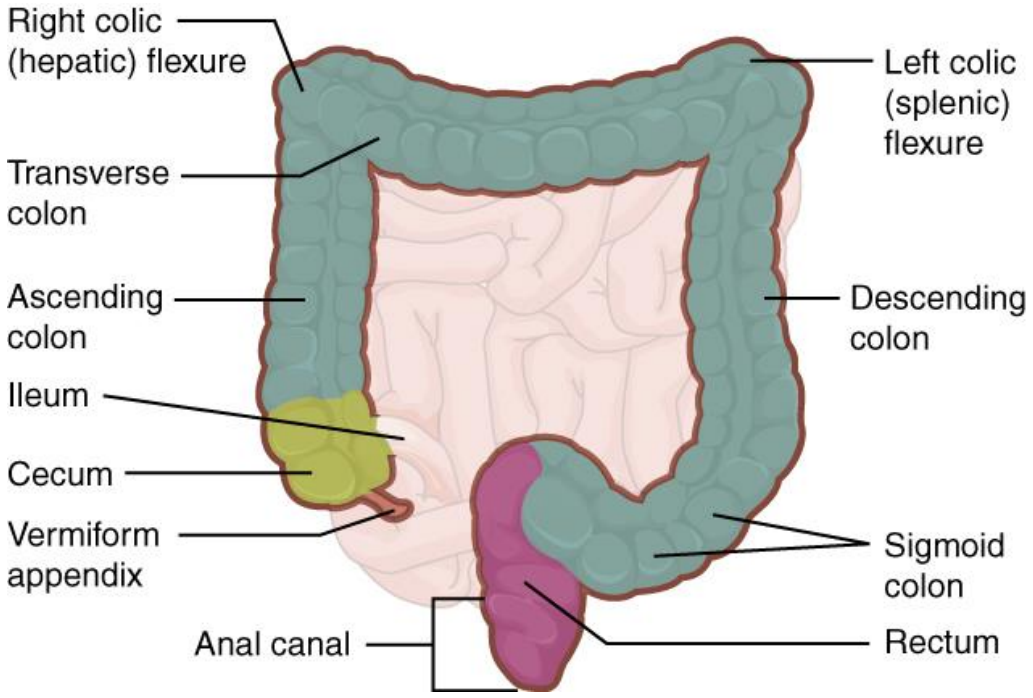

Figure 1-2. Diagram shows different sections of the large intestine by OpenStax

https://openstax.org/books/anatomy-and-physiology/pages/23-5-the-small-and-large-intestines

The colon is the largest part of the large intestine and is divided into sections according to the way which food is travelling into it to ascending, transverse and descending colon and the final part is the sigmoid colon **(Fig 1-2).** The colon wall has 4 layers, the outer layer is the serosa, it acts as a protective outer skin, the next layer is the muscular layer (muscularis), then the submucosa layer, which comprise of loose connective tissue, nerves, blood vessels and lymphatics. The innermost is the mucosa, which lines the lumen of the colon. The mucosa consists of a flat continuous sheet of simple columnar epithelium cells punctuated by crypts **(Fig 1-3)**. It contains a combination of absorptive colonocytes, mucin-producing goblet cells and endocrine cells(16). In addition to its role in the absorption of water, vitamins, and electrolytes, the mucosa forms a vital physiochemical barrier that controls molecules' movement and prevents microorganisms and toxins from reaching the circulation(17). The life span of epithelial cells is from three to 6 days, and a continuous supply of new cells is provided by the division of stem cells within the crypt. This division produces daughter cells which in turn multiply and migrate from the base of the crypts to the surface of the mucosa to replace the old enterocyte. The equilibrium between cell production and loss is tightly controlled and imbalance may result in impaired mucosa and disease development. Overall, changes in cell proliferation patterns and uncontrolled apoptosis are considered one of the earliest events in colorectal carcinogenesis (18,19) and the expression of genes that control cell proliferation is usually different between healthy and tumour tissues (20).

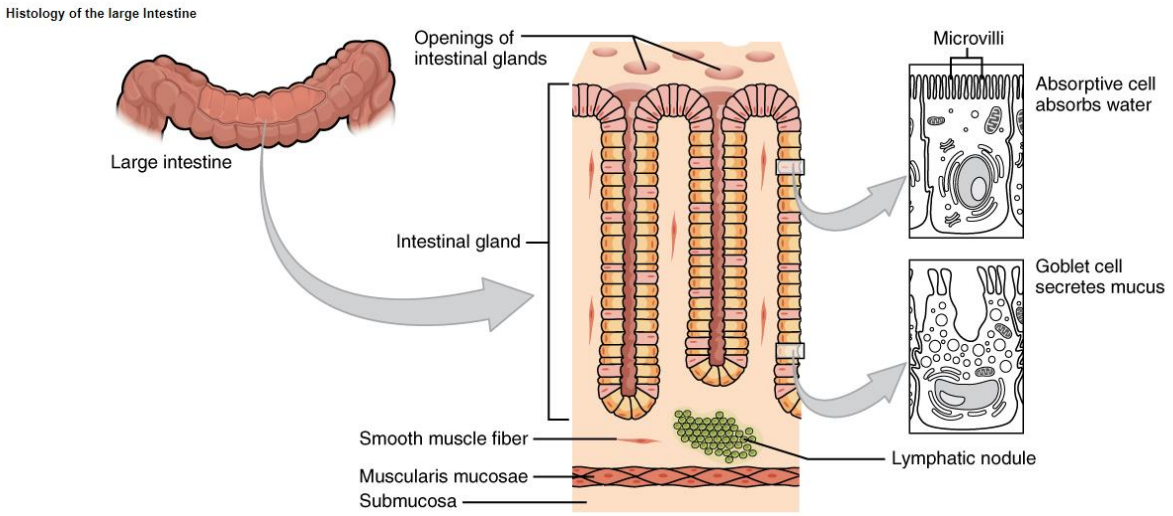

Figure 1-3. A diagram to show the histological structure of the large intestine. It shows the different layers of the mucosa and the distribution of enterocytes and goblet cells among the crypt.by OpenStax

https://openstax.org/books/anatomy-and-physiology/pages/23-5-the-small-and-large-intestines

1.2.1.2 Molecular mechanism of colorectal tumorigenesis

Progression of the healthy tissue to adenomas to malignant tissues is associated with the accumulation of genetic mutations and epigenetic events and is typically takes decades **(Figure 1-4).** Fearon and Vogelstein were the first to provide a CRC development model in 1990 (21). The model suggested that transformation from adenoma to carcinoma require mutations in the Adenomatous Polyposis Coli (APC) genes and the TP53 genes (21). These mutations affect both cell division and cell adhesion and lead to the formation of unicryptal adenoma. Accumulation of these small dysplastic growths over time, results in the development of adenoma (22). The proposed mutations in the model are common between different types of CRC. However, in recent years, the increase in using lower endoscopy and advanced instrument provided the opportunity to collect samples from different stages of the disease and investigate the molecular pathways involved in each one (23).

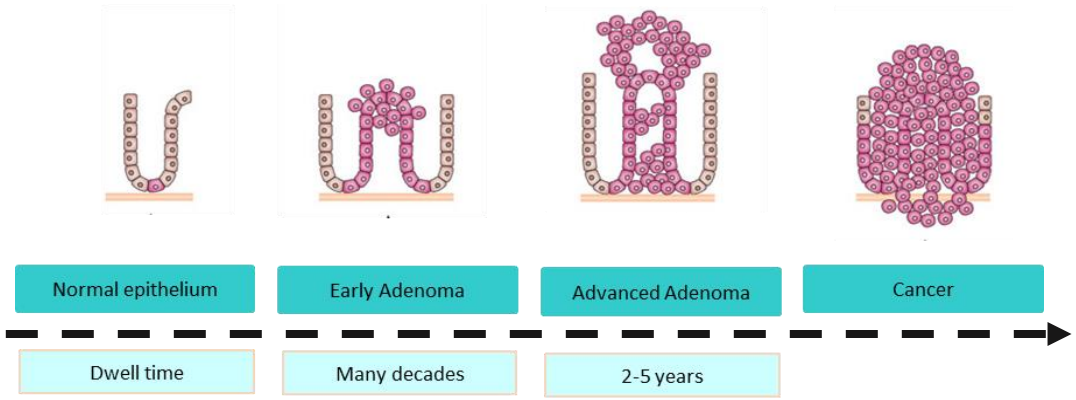

Figure 1-4. Simplified model for adenoma-carcinoma progression.

Modified with permission from R. Justin Davies *et.al* (2005)

Three molecular pathways of CRC pathogenesis have been identified:

1. Chromosomal Instability Pathway (CIN): This is the most common pathway found in about 85% of sporadic CRC cases. In CRC patients classified with this pathway, cell function is affected by a mutation in the chromosomes of the somatic genes. The mutation occurs due to chromosomal loss, gain or rearrangement (23).
2. Microsatellite Instability Pathway (MSI): In this pathway, a mutation occurs in the DNA in one of the mismatch repair genes. Cells affected by this mutation lose their ability to correct errors that occur

during DNA replication. With time, impaired cells multiply, producing cells characterised by impaired cell cycle control mechanisms. This mutation is found in about 15% of sporadic CRC cases (24).

3. Serrated pathway: The molecular profiling of this type of cancer is heterogeneous and overlaps with molecular profiling found in the CIN and MSI pathways. This pathway is identified in 10% to 20% of sporadic CRC cases and cells of this type are characterised by hyperproliferation and impaired apoptosis (25).

According to the genetic pathway involved the time required for the disease to progress from the initiation stage to cancer is estimated to be between 30 to 70 years (23).

1.2.1.3 Classifications of colorectal adenoma

The term colorectal adenoma used to describe a group of lesions that have broad characteristics and features. Adenomas are classified according to their morphology, size, location, number and the presence or absence of a stalk (peduncle). The morphology of these lesions varies from a flat, sessile, elevated, or even depressed lesion (**Figure 1-5**). However, the hallmark of adenoma is the epithelial dysplasia within these lesions. As the size of the adenoma increases the architecture changes from tubular, which is less prone to malignant changes, to villous or tubulovillous architecture, which has more chance of becoming more malignant. Therefore, assessment for adenoma characteristics is essential, the patients' follow-up plan after polypectomy (which is the procedure of polyp removal during colonoscopy screening) is usually set up according to these features (2,26) .

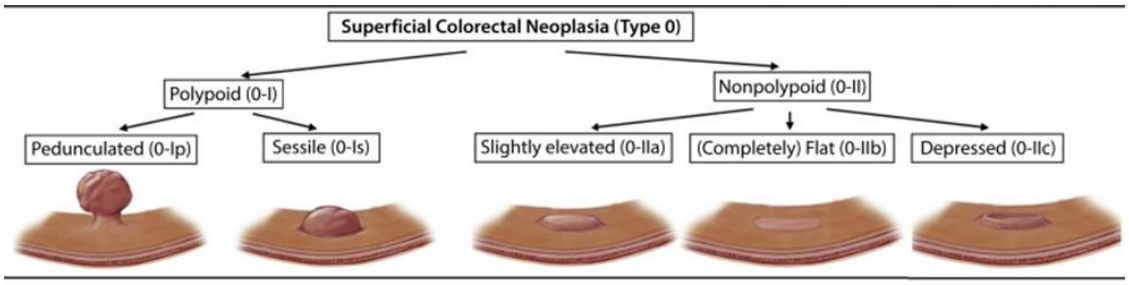

Figure 1-5. Morphological classification of colorectal adenoma.

Modified with permission from Soetikno et al (2021)

The World Health Organization (WHO) classified adenoma according to its histopathological structure into 4 types: tubular adenoma, villus adenoma, a mixed tubulovillous adenoma, and the serrated adenoma. About 85% of sporadic CRC develop through the CIN pathway from tubular, villous, or tubulovillous adenoma. Villous adenoma has a higher percentage of carcinoma in all ages followed by the combined adenoma in young patients and the serrated adenoma in > 60 years patients. A serrated adenoma is characterised by being flat with a saw-teeth appearance and usually grow in the proximal colon(27).

Previously, serrated adenomas were considered benign, however recent understanding of the molecular profile and morphological features has revealed that this lesion accounts for 15% to 30% of CRC cases. It is subdivided into three types; hyperplastic polyps, traditional serrated adenoma, and sessile serrated adenoma/ or polyp (SSA/P) (27,28). The term SSA/P is used by the pathologist to describe the histological features of the sessile serrated adenoma; however, this term is not recommended by the British Society of Gastroenterology (BSG) since it does not clarify the presence or absence of cytological dysplasia in the lesion. In the UK, it is recommended to use the term Sessile Serrated Lesion with or without dysplasia. These two terms account for both the morphology and pathology of the lesion and clearly distinguish between high-risk lesion (with dysplasia) and benign lesion (without dysplasia) (29).

1.2.2 Colorectal cancer epidemiology

1.2.2.1 Incidence, trends and mortality rates

Globally, CRC is the fourth most diagnosed cancer, incidence and mortality vary widely between countries (up to 8-fold), with rates higher in developed countries(30) **(Fig 1-6).** The world age-standardised incidence rates in 2018 was 19.7 per 100,000 for both sexes, with the incidence is higher in males than females 23.6 and 16.3 per 100,000 respectively (31). In the UK, CRC is also ranked as the 3rd most common cancer for both males and females and the incidence rate has been stable over the last 10 years (32). In England in 2017, the age-standardised incidence rates per 100,000 was 68 for both sexes, with a higher incidence in males (83.2) than in females (55.2) (33) . A recent study aimed to assess the differences in the trends in the incidence and mortality of CRC worldwide included data from 36 country revealed that the disease has continued to increase in younger populations and in countries that are classified as medium to high Human Development Index (HDI) (34). HDI is an indicator used to grade countries according to human development based on education, income and life expectancy (35). In the

UK this study revealed an increase in colon cancer incidence among persons younger than 50 years, however, the analysis also revealed a decrease in the disease incidence in people older than 50 years. The study suggested that this increase in young population in developing countries may be related to the change in their lifestyle (34).

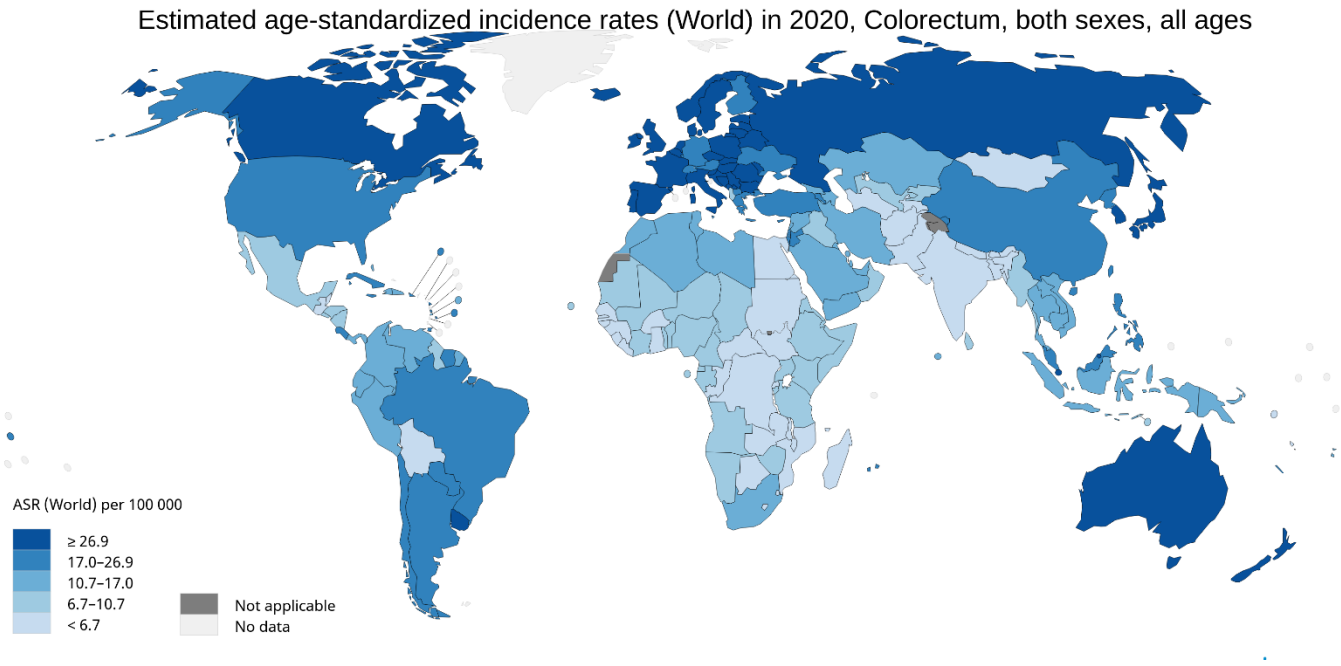

Figure 1-6. Age-standardised incidence rates for colorectal cancer in 2020, for males and females.

Data source: GLOBOCAN 2020. Graph production: IARC (http://gco.iarc.fr/today).World Health Organization

1.2.2.2 Colorectal cancer survival and screening

According to the clinical description of the tumor's size, type and spread to other organs, CRC is classified into categories of stages and grades according to an internationally agreed system. The grade is to describe the type of the cells i.e., l*ow grades* tumor comprise of cells that are look like normal cells, while the stage is used to describe if the tumor is local or it has spread to lymph nodes or other organs (36). The survival rate of CRC largely depends on the stage at which the disease is diagnosed. According to data available from England for the period from 2013 and 2017, the survival rate for 5 years is 90%, if the disease was diagnosed at stage one and decreases to only 10% if the disease was diagnosed in stage four (37).

Many countries have implemented CRC screening programmes targeting the high-risk age group, in England the target age group ranging from 60 to 74 years (38). The slow nature of the disease

development and the effectiveness of the treatment at early stages were behind the development of these programmes.

In England, the Bowel Cancer Screening Program (BCSP) began in 2006. It is a multi-stage programme that starts with the non-invasive test, the Faecal Occult Blood Test (FOBT), which detect blood traces in the stool, patients with positive results are invited to have a colonoscopy examination (6).

Despite the benefit of the screening programmes in reducing the incidence and mortality of the disease, some factors may affect their effectiveness; these factors may affect the response to the programme as a whole or affect the endoscopy examination procedure quality.

Comparing with other cancer screening programmes, such as breast and cervical cancer screening, the response to CRC screening is low. Only 57.9% of people responded to the FOBT between 2012 and 2015. Non-responders to FOBT are usually males, from diverse ethnic groups with lower socioeconomic status (39). For individuals with positive FOBT who were invited to colonoscopy the response rate was 79% (40). A recent study reported that ethnic group and religion may influence the bowel cancer screening uptake(41). The study analysed data obtained from two rounds of bowel screening for 1.7 million individuals in Scotland between 2007 and 2013. The uptake rate of White Scottish population was used as a reference for screening uptake of individuals from different ethnic groups. The results showed that the screening uptake of the Chinese and other White British populations were higher than the White Scottish population while individuals from South Asia regions such as India, Pakistan and Bangladesh, had a lower screening uptake when compared with the White Scottish population. Regarding religion, the screening uptake of individuals identified their current religion as Church of Scotland was used as a reference for screening uptake of individuals from other religions. The analysis revealed a difference in uptake between males and females. For males, the screening uptake of other Christian religion was higher than the reference population, while Muslim, Hindu and Sikh had a lower screening uptake than the reference population. Small difference in uptake was observed between males identified their religion as Jewish, Roman Catholic or with no religion and the reference population. For females, except from females identified their religion as Other Christian, low uptake was observed among Hindu, Muslim and Sikh and Roman Catholic women compared with the reference population (41). The invasiveness nature of the colonoscopy procedure and the fear of possible outcome are possible reasons for not attending the colonoscopy session (39).

It was reported that most CRC cases developed after having colonoscopy were due to failing to detect lesions during the first examination rather than due to new growths (42). Many factors could affect the quality of the procedure i.e. the qualification and experience of the endoscopist and the bowel preparation quality. In the BCSP, quality indicators are used to ensure high-quality endoscopy outcome (39). For example the Adenoma Detection Rate (ADR) which is defined as the "proportion of patients with at least one colorectal adenoma detected among all patients examined by an endoscopist" (43). Two studies explored the relationship between ADR with incidence and mortality of interval cancer (Interval cancer is identified as CRC that is diagnosed within 5 years after a negative colonoscopy examination)(44). The first study included 136 endoscopists who performed 314872 colonoscopy examination (45), and the secondary included 294 endoscopists who performed 146860 colonoscopy examination (46). Both studies reported that ADR is inversely associated with the risk of incidence and mortality of interval CRC (45,46). Poor bowel preparation is associated with poor detection of polyps smaller than 10mm, it is estimated that 25% of the patients undergo colonoscopy examination had an inadequate bowel preparation which affect the quality of the procedure outcome (47).

1.2.2.3 Risk of colorectal adenoma recurrence

Two meta-analyses have assessed the risk of colorectal adenoma recurrence in patients following an index colonoscopy and polypectomy. The first included seven studies with a total of 11783 patients who had a follow up period between 2 to 5 years (48). The analysis revealed that comparing with those with no adenomas at baseline, the relative risk of adenoma at screening was 1.8 in the cases of the adenoma diagnosed at baseline was classified as low-risk adenoma (48). The secondary included data from 10139 individuals who were included in eight studies from 2006 to 2015 and had a follow up period between 3 to 10 years. The analysis showed that in cases that adenoma diagnosed at baseline was classified as low-risk adenomas, the risk of developing advanced neoplasm during the follow-up period was 4.9%, however the risk increase to 17.1% in cases where adenoma diagnosed at baseline was classified as advanced adenoma (49).

1.2.3 Non-dietary risk factors for CRC, adenoma and adenoma recurrence

1.2.3.1 CRC and adenoma

Non-modifiable risk factors

He *et al.* (2018) investigated the association between risk factors for CRC and the risk factors of different types of colorectal adenoma by analysing data for 141,143 participants involved in three large prospective studies. After assessing 13 risk factors, they found that different types of adenoma share many of the risk factors of CRC, however, some of the CRC risk factors are strongly associated with the development of one or other type of colorectal adenoma (11). This section highlights the non-dietary risk factors for CRC and colorectal adenoma in general.

Age and sex: CRC is more common in people > 50 years old. In the UK, 40% of CRC cases are diagnosed in people aged ≥75 years (3). Age is also a primary risk factor for adenoma prevalence. In the USA, a study reported that adenoma is prevalent in 50% of people aged >70 years (findings from autopsies) and in 25–40 % of individuals over 50 years (findings from colonoscopy) (50). The analysis for the first 1 million BCSP tests (both FBOT and colonoscopy) performed in England shows that the prevalence of CRC and advanced colorectal adenoma were higher in males (11.6% CRC and 12% high-risk adenoma) than females (7.8% CRC and 6.2% advanced colorectal adenoma) (40).

Family history and genetics: About 25% of cases have family history of the disease but the mutations do not occur in one gene(51). Evidence shows that the risk of the disease is doubled in individuals with a first-degree family member who were diagnosed with the disease and were diagnosed at a young age (<60 years). No association was found if the family history of the disease was found in a second-degree relative. The risk also increases by family history of advanced adenoma, but the risk is not associated with family history of non-advanced adenoma (52).

Less than 10% of CRC cases are considered as hereditary types of CRC and caused by well-defined inherited mutations from birth, such as Lynch Syndrome and familial adenomatous polyposis(52).

Lynch Syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disease results from mutations in the mismatch repair genes which has a role in DNA correction during cell division. Individuals affected by this mutation has a 20% higher risk of developing CRC by the age of 50 years, the risk is further increases with age to reach 80% by the age of 85 years (52). The 2nd

hereditary syndrome is the familial adenomatous polyposis. It is caused by an autosomal dominant mutation in the adenomatous polyposis coli (APC) gene which is a tumour suppressor gene has a role in cell division and DNA replication. This mutation leads to the development of large number (hundreds) of polyps in young age and there is a high risk that these polyps progress to cancer at young age, especially, if the disease was not diagnosed or treated (51).

Certain health conditions: The risk of developing CRC and adenoma is higher in individuals diagnosed with diabetes. A meta-analysis was conducted in 2013 included 29 observational studies concluded that the risk of developing CRC is 22% higher in patients with diabetes when compared with individuals without diabetes (Relative Risk (RR): 1.22, 95% CI: 1.19–1.26) (53). In 2016, a meta-analysis was conducted to explore the association between type 2 diabetes and colorectal adenoma (54). The study found that the risk of developing colorectal adenoma is 52% higher in patients diagnosed with type 2 diabetes (RR: 1.52; 95 % CI: 1.29–1.80). Two mechanisms are proposed for the relationship between diabetes and colorectal tumorigenesis, the first is that slower bowel movement in diabetic patients increases the duration of the contact between colonocytes and potentially carcinogenic substances in food residues, and the 2nd is that elevated insulin levels might stimulate tumour growth by stimulating cell proliferation and extending cell survival (54,55). However, other studies proposed that common risk factors between the two diseases such as age, sedentary lifestyle, high fat diet, obesity, alcohol and tobacco use are behind this association (56,57).

The risk of CRC is higher in people diagnosed with inflammatory bowel diseases (IBD). The frequent mucosa inflammation episodes in both ulcerative colitis and Crohn's disease are associated with the development of abnormal tissues and risk of dysplasia, which may progress to cancer over time. Factors may increase the risk of CRC development in patients diagnosed with IBD, such as, age at IBD diagnosis (younger age is associated with higher risk of developing CRC), the length of affected segment of the colon and the severity of inflammation (58).

Modifiable risk factors

Doll and Peto (1981) estimated, based on epidemiological evidence, the proportion of cancer deaths in the U.S. that are related to modifiable risk factors. They found that about 20% of all cancer associated with occupational exposures (i.e., asbestos exposure), about 30% associated with tobacco use and 10% attributable to infection (59). In 2018, Brown and colleagues estimated the relative risk of different types

of cancer in the UK by calculating the attributable fractions for modifiable risk factors with sufficient convincing evidence (60). For bowel cancer, they found that about 54% of the cases in the UK are attributable to lifestyle factors. Smoking is estimated to cause 7% of the cases, overweight and obesity causes 11% of bowel cancer cases and too little physical activity is estimated to cause 5% of the cases.

The World Cancer Research Fund (WCRF) is a non-profit organization that was established in 1982. It collaborates with different international cancer prevention charities aiming to provide a practical guideline for health professionals, key organizations, and the general public about the association between diet, body weight, physical activity and cancer. Their method includes a process of analysing and interpreting available evidence and by funding new research. In 1997, the WCRF network published the first Expert Report that confirmed the association between lifestyle and cancer risk, the 2nd report was published in 2007 and the 3rd and last report in 2018. The 3rd report summarises the findings in 10 cancer prevention recommendations related to modifiable risk factors such as body weight, physical activity, diet, supplement use and breastfeeding **(Figure 1-7)** (61). These findings are based on data obtained from 51 million people including 3.5 million cancer patients. Over 20 independent studies showed that adherence to these recommendations lower the risk of developing cancer. In 2020, a prospective longitudinal cohort study was conducted within the frame of the PREDIMED study (in Spanish: PREvención con DIeta MEDiterránea) included data from 7216 elderly individuals revealed a significant linear negative association between adherence to these recommendations and the risk of CRC (62).

Figure 1-7. The 10 Cancer Prevention Recommendations published by the WCRF/AICR

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To keep up with the continuously published research associated modifiable risk factors with cancer, the WCRF has established the Continuous Update Project (CUP). CUP is a real time database includes ongoing evidence, it contains about 100,000 publications for 17 cancers, it provides a trusted scientific resource for cancer prevention and survival (63). CUP has published 17 cancer specific reports between 2008 and 2018, each report was based on a systematic review of the literature for the association between modifiable risk factors and a specific cancer type. For CRC, the latest CUP report was published in 2017 and revised in 2018 (63). The report assessed the evidence on the association between diet, nutrition, physical activity and CRC using data from 99 studies from around the world. The studies involved 29 million adults and including 247000 CRC cases (63). The link/findings between the lifestyle factors and the disease in this report were categorised according to the strength of the available evidence as either “strong convincing”, “strong probable” or “limited” evidence (**Fig 1-8).**

The mechanisms by which these factors modify disease risk are not entirely understood as the evidence is based mainly on epidemiological studies.

The modifiable risk factors of colorectal adenoma and CRC will be discussed within the findings of the WCRF/AICR report (2018) on diet, nutrition, physical activity and colorectal cancer. For each risk factor, the evidence from the WCRF/AICR regarding CRC will be summarised and will be followed by the evidence about the association between the risk factor and colorectal adenoma development.

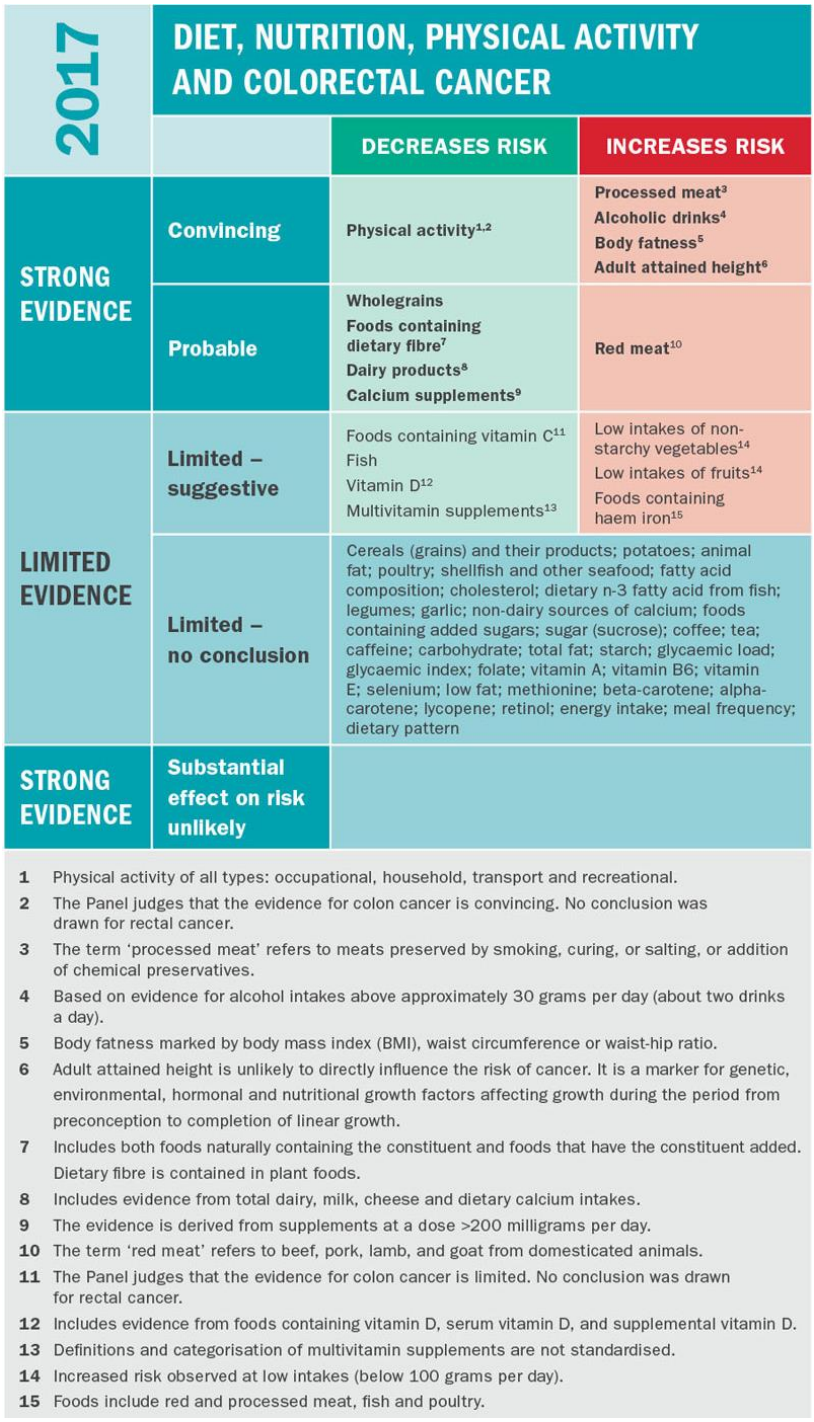
Body fatness: According to the WCRF/AICR there is 'strong convincing evidence' that body fatness increases the risk of CRC (63). A meta-analysis explored the association between the Body Mass Index (BMI) and risk of adenoma included 36 studies (29,860 cases of adenoma, age ranged from 30 to 84 years) reported that a 19% increase in the risk of adenoma in the colon region was observed with an increase of BMI by 5 units. This association was not affected by the sex or race of the patient (64).

Physical activity: WCRF report categorised physical activity as a reducing factor for CRC based on strong scientific evidence. The same association was observed for colorectal adenoma in a meta-analysis that included 20 studies, the analysis showed that physical activity could reduce the risk of advanced colorectal adenoma but the evidence are limited (65).

Smoking: Smoking has been associated with increased risk of CRC and colorectal adenoma. It is estimated that the risk of CRC increases by 40% in individual smoking 40 cigarettes per day (66), and the risk of colorectal adenoma increases by more than two-folds in individuals smoking >30 pack of cigarette per year (11).

1.2.3.2 Risk factors for colorectal adenoma recurrence

Higher risk of adenoma recurrence is associated with older age and high BMI. Also, location, size, histological structure, multiplicity and degree of dysplasia of the adenoma removed in the first colonoscopy, are a collection of characteristics that are used to predict the risk of adenoma recurrence (67,68).



2017	DIET, NUTRITION, PHYSICAL ACTIVITY AND COLORECTAL CANCER		
		DECREASES RISK	INCREASES RISK
STRONG EVIDENCE	Convincing	Physical activity[1,2]	Processed meat[3] Alcoholic drinks[4] Body fatness[5] Adult attained height[6]
	Probable	Wholegrains Foods containing dietary fibre[7] Dairy products[8] Calcium supplements[9]	Red meat[10]
LIMITED EVIDENCE	Limited – suggestive	Foods containing vitamin C[11] Fish Vitamin D[12] Multivitamin supplements[13]	Low intakes of non-starchy vegetables[14] Low intakes of fruits[14] Foods containing haem iron[15]
	Limited – no conclusion	Cereals (grains) and their products; potatoes; animal fat; poultry; shellfish and other seafood; fatty acid composition; cholesterol; dietary n-3 fatty acid from fish; legumes; garlic; non-dairy sources of calcium; foods containing added sugars; sugar (sucrose); coffee; tea; caffeine; carbohydrate; total fat; starch; glycaemic load; glycaemic index; folate; vitamin A; vitamin B6; vitamin E; selenium; low fat; methionine; beta-carotene; alpha-carotene; lycopene; retinol; energy intake; meal frequency; dietary pattern	
STRONG EVIDENCE	Substantial effect on risk unlikely		

1 Physical activity of all types: occupational, household, transport and recreational.
2 The Panel judges that the evidence for colon cancer is convincing. No conclusion was drawn for rectal cancer.
3 The term 'processed meat' refers to meats preserved by smoking, curing, or salting, or addition of chemical preservatives.
4 Based on evidence for alcohol intakes above approximately 30 grams per day (about two drinks a day).
5 Body fatness marked by body mass index (BMI), waist circumference or waist-hip ratio.
6 Adult attained height is unlikely to directly influence the risk of cancer. It is a marker for genetic, environmental, hormonal and nutritional growth factors affecting growth during the period from preconception to completion of linear growth.
7 Includes both foods naturally containing the constituent and foods that have the constituent added. Dietary fibre is contained in plant foods.
8 Includes evidence from total dairy, milk, cheese and dietary calcium intakes.
9 The evidence is derived from supplements at a dose >200 milligrams per day.
10 The term 'red meat' refers to beef, pork, lamb, and goat from domesticated animals.
11 The Panel judges that the evidence for colon cancer is limited. No conclusion was drawn for rectal cancer.
12 Includes evidence from foods containing vitamin D, serum vitamin D, and supplemental vitamin D.
13 Definitions and categorisation of multivitamin supplements are not standardised.
14 Increased risk observed at low intakes (below 100 grams per day).
15 Foods include red and processed meat, fish and poultry.

Figure 1-8. The effect of diet, nutrition and physical activity on CRC risk, factors with strong and limited evidence.

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1.2.4 Diet and colorectal tumourigenesis

Diet is thought to play a major role in the pathogenesis of CRC and is involved at each stage of the tumorigenesis process, initiation, promotion and progression (12). In their review, Doll and Peto (1981) estimated the cancer death related to diet in the U.S. is about 35% of all the cases (59), however, in 2004, after reviewing epidemiological evidence on diet and cancer, Key *et.al*. suggested that this is an over estimated figure, and they found that, after tobacco, obesity is the most important preventable risk factor for all cancers (69). In the UK, Brown and colleagues estimated that 6% of bowel cancer cases may be prevented by drinking less alcohol, cutting down on processed meat could prevent 13% of the cases, and eating a high fibre diet is could prevent 28% of bowel cancer cases in the UK (60).

This section is divided into three sections, the first will summarise the biological mechanisms underlying the associations between diet and colorectal tumorigenesis. The *2nd section* will summarise the evidence about the association between foods/nutrients and the risk of (i) development of colorectal adenoma and CRC (ii) colorectal adenoma recurrence. The 3rd *section* is about the association between dietary pattern and the risk of (i) development colorectal adenoma and CRC (ii) colorectal adenoma recurrence.

1.2.4.1 Mechanisms underlying the role of diet on colorectal tumorigenesis.

The association between diet and colorectal tumorigenesis is complex. **Figure 1-9** shows some of the proposed mechanism, for example, (i)effect of the foods and nutrients on the general health (i.e., availability of essential nutrients), (ii)direct contact with mucosa (i.e., effect on pathways related to inflammation and DNA methylation due to interaction between toxins and the mucosa) (iii) effect on the local environment in the lumen (i.e., change in pH content due to high amount of bile acids in the lumen due consumption of high fat diet) (iv) direct interaction with the gut microbiota. **Table 1-1** shows some examples of foods/nutrients and proposed mechanisms in promoting or inhibiting colorectal tumorigenesis.

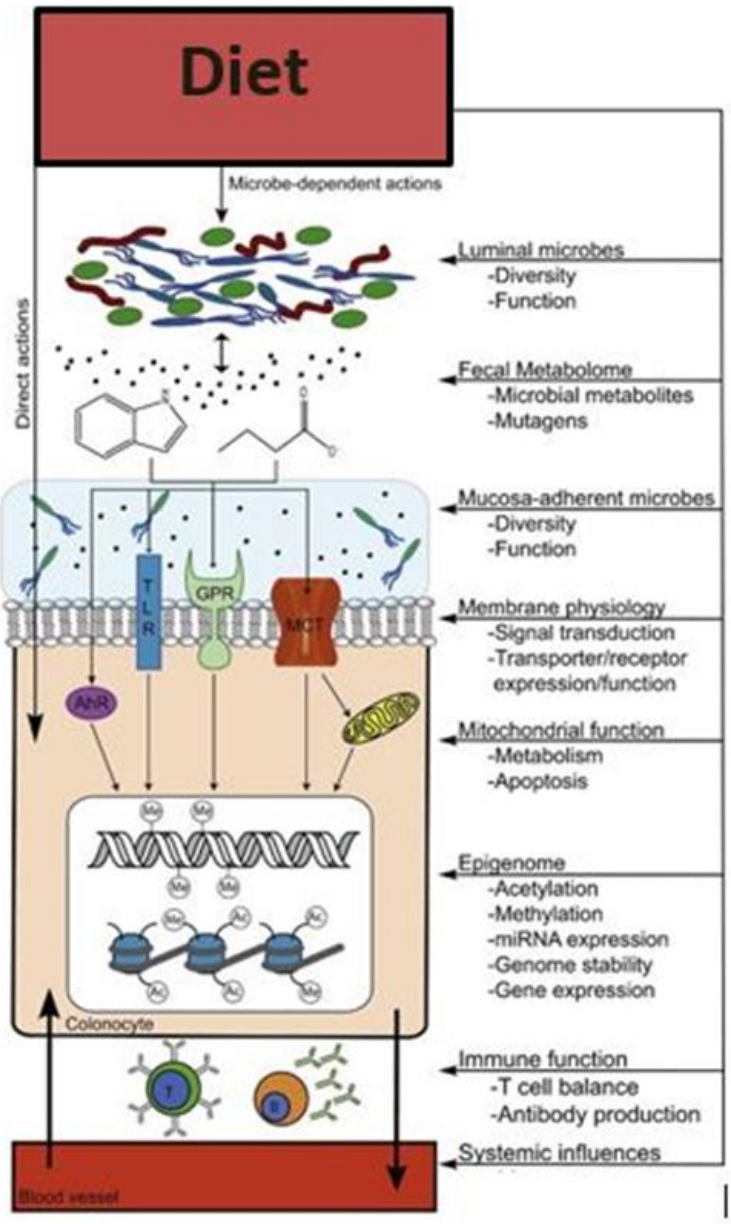


Figure 1-9. Example of different mechanisms by which diet may influence the colorectal carcinogenesis.

Modified with permission from Seidel *et.al* (2017).

Table 1-1. Examples of foods and nutrients that alter CRC risk and proposed mechanisms.

Food/nutrient (Effect on CRC risk)	***Proposed mechanisms***
*Alcohol **(+)***	*Acetaldehyde is an alcohol metabolite that is associated with the generation of ROS*, promotion of proliferation and DNA damage (70).* *High alcohol affects folate metabolism and reduces its serum levels which affect DNA methylation (71).*
*Red and processed meat **(+)***	*The high protein content is associated with limited digestibility; therefore, more protein reaches the colon and is subject to fermentation and formation of toxic metabolites and interaction with ROS (72).* *Chemicals used to preserve meat (e.g. nitrites and nitrates) and formed from processing meat at high temperature (e.g. heterocyclic amines and polycyclic aromatic hydrocarbons) are identified as carcinogens (73–75).* *High haem iron content (mechanism below).* *High-fat content is associated with a large amount of bile acids reaching the colon that are further metabolite to secondary bile acids (76,77).*
*Fish and fish products **(-)***	*Fish rich in ω-3 PUFA is associated with lower risk due to its anti-inflammatory role (78).*
*Dairy products **(-)***	*The protective effect of dairy products is linked to being rich in calcium and vitamin D.* *Calcium might be protective by stimulating apoptosis (79).*
*Fruits and vegetables **(-)***	*Source of fibre (mechanism below).* *Source of folic acid***
*Dietary fibre **(-)***	*Increasing the quantity of the stool leads to a decrease in the concentration of the toxins (80) .* *Increasing the motility of the intestine and decrease the contact time between these toxins and the mucosa (80).* *Altering the composition and variety of the gut flora.* *Fibre fermentation controls the production of SCFA***, which has a protective role through inducing apoptosis and gene expression regulation (81).*
*Food contains haem iron **(+)***	*High haem iron causes mucosal surface damage which leads to induces cellular proliferation(82).*
*Vitamin C **(-)***	*Its effect as an antioxidant in reducing the ROS (83).*
*Vitamin D **(-)***	*Vitamin D is associated with cell proliferation and differentiation, detoxification metabolism, and inhibition of angiogenesis (79,84).*

**Reactive Oxygen Species (ROS) are associated with a reduction in DNA repair capability and damage of mucosa by reaction with the protein and lipid of the cell membrane. ** The role of folic acid is complex. Its role in DNA methylation is important to maintain DNA stability, however, high folate consumption might stimulate the growth of colorectal tumours. Therefore, evidence shows that the association is complex because it has a protective role in healthy individuals but may act as a promoter in individuals with pre-existing tumour. ***SCFA=Short chain Fatty acids.(-) Decreases the risk, (+) Increases the risk.*

A review conducted by Yang and Yu (2018) suggested that the association between dietary pattern and the risk of CRC tumorigenesis is through the interaction between diet and the gut microbiota(85). The gut microbiota is rich and complex, it consists of both commensal and pathogenic microorganisms in a proportion that is specific for each individual. An alteration in the diversity and numbers of the commensal microorganisms is associated with chronic diseases such as IBD, obesity, and cancer (73,86). Besides age, genetics and medication, diet may alter the composition, the number and function of gut microbiota. In healthy individuals, about 10% of the ingested food reaches the large bowel. It consists of undigested complex carbohydrate, protein, primary bile acids, the chemicals used in, or formed during, food processing mixed with water vitamins and electrolytes (85). As **Figure 1-10** shows. adherence to a dietary pattern that is characterised by high amount of complex carbohydrate, fruits and vegetables and low amounts of meat and processed food (healthy dietary pattern) leads to a large amount of complex fibre reach the lumen. When metabolised by the gut microbiota, these components yield products with anti-inflammatory and anticarcinogenic effect, such as the short chain fatty acids (87). By contrast, diet that is rich in meat, fat and processed food, low in whole grains, fruits and vegetables (western dietary pattern) leads to large amounts of protein, fat and bile acids reach the lumen of the large intestine. Sulphated compounds (i.e., high protein) may enhance sulphate-reducing bacteria. This bacteria is associated with high risk of distal CRC is according to analysis from the Health Professionals Follow-up Study, however, the mechanism is not yet understood (88). The high saturated fat associated with western diet leads to high bile acids reach the colon. These bile acids are metabolised by the gut microbiota to secondary bile acids which has cytotoxic activity (89)

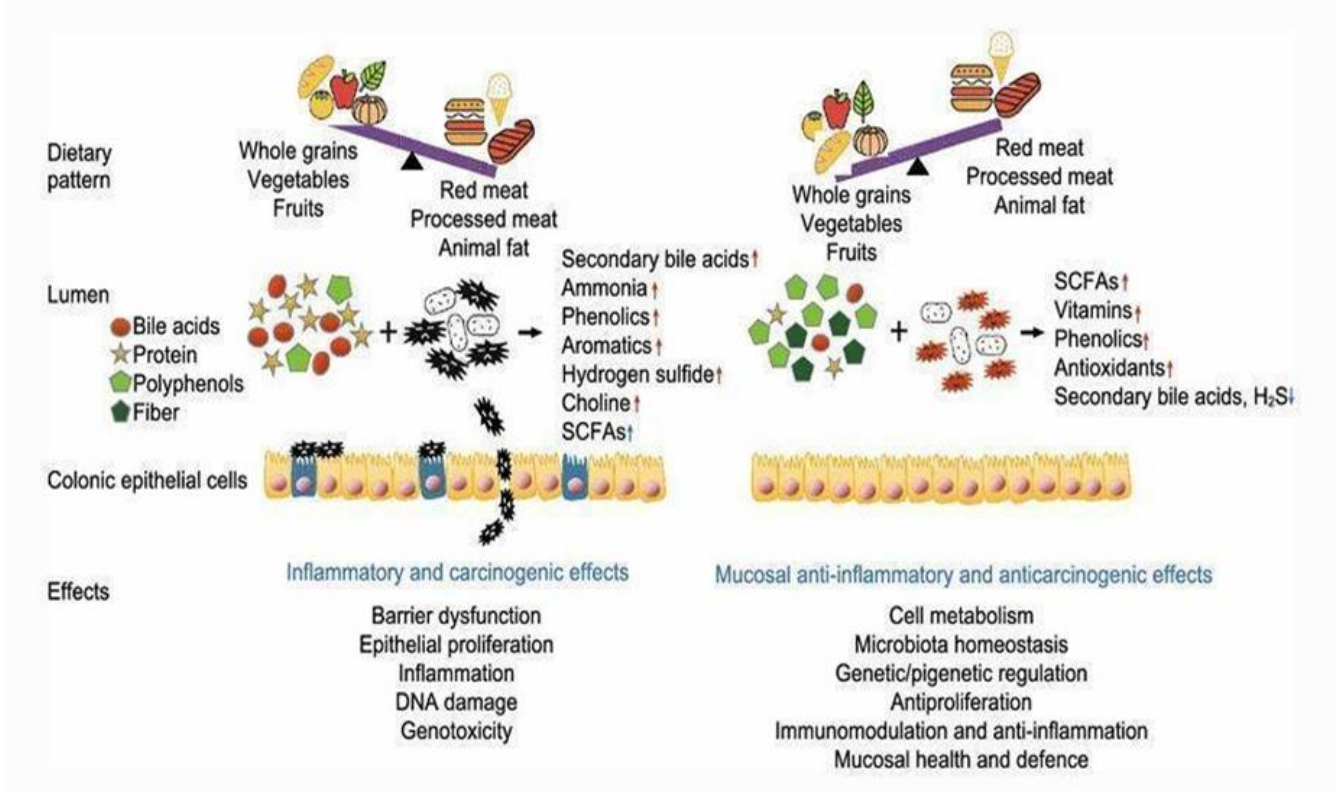


Figure 1-10. Summary of possible mechanisms underlying the role of dietary patterns in the development of CRC. The figure was taken with permission from Yang and Yu (2018)

1.2.4.2 Foods, nutrients and the risk of colorectal tumourigenesis

1.2.4.2.1 Food, nutrients and the risk of colorectal adenoma and CRC development, evidence from epidemiological studies

The association between foods and nutrients and the risk of developing colorectal adenoma and CRC will be discussed within the context of the findings of the WCRF/AICR report (2018) about diet, nutrition, physical activity and colorectal cancer (90). For each food/nutrient, the evidence about the association with CRC from the WCRF/AICR report will be summarised with results from observational study that was published after 2018 (if any) and will be followed by an evidence for the association between the dietary component and adenoma development. For each dietary risk factor if any studies provided an estimate for the effective amount of food will also be included.

Alcohol: The WCRF/AICR panel concluded that drinking alcohol increases the risk of CRC but the association is not linear and the positive association was observed in individual consuming ≥30g of ethanol per day(90).

For the association between alcohol and colorectal adenoma, a meta-analysis included 25 observational studies that reported that the risk of developing adenoma is 17% higher in all drinkers compared with non-drinker or occasional drinkers(91).

Red and processed meat: The WCRF/AICR report indicated that processed meat increases the risk of CRC based on strong evidence, but red meat is a probable cause of CRC(90). Epidemiological studies of the European Prospective Investigation into Cancer and Nutrition (EPIC) found that the risk increases in people consuming high amount of red meat (160g/day) when compared with individual consuming low amounts (<20g/day)(92).

A meta-analysis included data from 26 observational studies that found that the risk of colorectal adenoma is higher in people consuming high amounts of red and processed meat (93).

Fish: The WCRF/AICR report indicates that the protective role of fish consumption from CRC is based on based on limited evidence (90). Like the red meat, the role of fish may depend on the amount consumed. It was found by EPIC study that individuals consuming a high amount of fish (>80 g/day) had a significantly lower risk of CRC when compared with individuals consuming less amount (<10 g/day) (92). In contrast, no association was found between fish intake at adolescent age and the risk of developing colorectal adenoma later in life (94). The association was examined in 19,771 females who participated in the Nurses' Health Study II (94). However, the dietary data during their adolescent age was collected, retrospectively, when the participants were between the ages of 34 to 51 years which may lead to potential data inaccuracies due to recall bias.

Dairy products: according to the WCRF/AICR report, strong evidence suggest that dairy products intake is a probable protective factor from developing CRC (90), however, the effect might be sight specific, since a meta-analysis was published in 2012, included 19 cohort studies concluded that high consumption of milk and dairy products are associated with a lower risk of cancer of the colon but not the rectum (95). The association between milk products and colorectal adenoma was explored in a pooled case-control study included 807 colorectal adenoma cases and 2,185 controls and revealed no

association between milk and milk products consumption and risk of developing colorectal adenoma (96).

Fruits and vegetables: Overall, results regarding the association between risk of CRC and consumption of fruits and vegetables are inconsistent but there is limited evidence suggesting that low consumption of fruit and non-starchy vegetables increase the risk of CRC (90) . A meta-analysis published in 2018 included 23 studies for vegetables and 21 studies for fruits found an inverse association between high consumption of fruits and vegetables and the risk of colorectal cancer (97). A more recent prospective study used the data from the UK Biobank published in 2020 included 175 402 individuals, 2609 of them developed CRC. The study revealed no association between consumption of fruits and vegetables and the risk of CRC(98). A meta-analysis of 22 studies explored the association between consumption of fruits and vegetables and the risk of adenoma included 11,696 colorectal adenoma cases found a protective effect for fruits but not for vegetables (99).

Fibre: High Consumption of foods containing dietary fibre probably protects against CRC as per the WCRF/AICR findings (90). The association between consumption of dietary fibre and CRC was explored within the EPIC study that included 519978 individuals and was published in 2003. After a follow-up period of 1939011 person-years, CRC was detected in 1065 participants. The study concluded that doubling the amount of fibre intake in a population with low fibre intake may reduce the risk of CRC by 40% (100).

A meta-analysis included 20 studies and 10,948 colorectal adenoma cases found an inverse association between fibre intake and the risk the adenoma, and sub-analysis revealed that this association is significant when the source of fibre were fruits and cereals but not significant for vegetables' fibre (101). These findings may suggest that the source of fibre and the baseline intake might play a role in modifying the risk and should be considered when investigating the association.

Foods containing haem iron: The WCRF/AICR panel found limited evidence suggesting that foods containing haem iron increase the risk of CRC (90). Two case-control studies explored the association between iron and colorectal adenoma, however, both studies used serum ferritin levels as an indicator for body iron store instead of measuring dietary intake. Both studies reported a positive association between body iron storage and the risk of adenoma (102,103). One cohort study investigated the association between dietary intake of heam iron and the risk of colorectal adenoma in women and found

that the association was positive for adenoma in the colorectal and colon but not associated with adenoma of the rectum, however, they found that the association depends on the ratio between dietary intake of haem iron to the intake of antioxidants (104).

Vitamin C: Limited evidence suggest that consumption of food rich in vitamin C may decrease the risk of colon cancer (WCRF/AICR) (90). The association between vitamin C and the risk of colorectal adenoma was explored in a meta-analysis for 13 observational studies included 3832 cases of colorectal adenoma patients. The study found that the risk of colorectal adenoma is 22% lower in the individual with high intake of dietary vitamin C when compared with low intake. The association was not modified after adjusting for energy, BMI or smoking status (105).

Vitamin D: Based on the 2018 report by WCRF/AICR, limited evidence suggests that vitamin D decreases the risk of CRC (90). These findings were based on studies explored the role of dietary and supplementary vitamin D and also on serum concentration of 25-hydroxyvitamin D. A more recent meta-analysis that was published in 2021 explored the association between dietary and supplemental vitamin D and the risk of CRC. The study revealed that high intake of vitamin D is associated with lower risk of CRC (106). No association was found between dietary intake of vitamin D and the risk of colorectal adenoma in an a case (516), control (4804) study (107).

It is estimated that between 50% to 90% of vitamin D is produced by the skin after exposure to ultraviolet radiation from the sun (108), hence, serum concentration might provide a better measure for vitamin D status.

For the association between serum concentration of 25-hydroxyvitamin D and CRC, a dose-response analysis was conducted in a meta-analysis that included 15 observational studies. The study revealed that the risk of CRC is 33% lower in individuals with serum concentration of 25-hydroxyvitamin D of 30ng/ml and 60% lower in individuals with serum concentration of 25-hydroxyvitamin D of 50ng/ml, when compared with the risk in people with a concentration of 5ng/ml (109). For the association between serum concentration of 25-hydroxyvitamin D and colorectal adenoma, a significant inverse association was found when comparing the individual in the highest category with the lowest category of circulatory vitamin D in meta-analysis for 16 observational studies (110).

In view of an absence of meta-analyses and reports regarding diet and adenoma recurrence, a systematic approach was taken to review and synthesize the evidence from Randomised Controlled Trials (RCT) investigating the impact of food/nutrients on the risk of adenoma recurrence.

Nutritional Randomised Controlled Trials for the prevention of sporadic colorectal adenoma recurrence. A literature review using a systematic approach

Method

Inclusion criteria

Inclusion criteria were determined by three researchers (Corfe, Williams and El Mogassabi). Articles were considered for inclusion if they were RCTs of a nutrient or if they aimed to modify the dietary intake of participants in a randomised manner, had the primary outcome of colorectal adenoma recurrence and were published as full papers in English. When the RCT included a non-nutritional treatment arm (i.e. drug), only information related to the nutritional intervention was extracted and included in the results of this review.

Search strategy

The following four search terms were used: the first was related to adenoma ("adenoma "OR "polyp "OR "adenoma*"), the 2nd was related to the location of adenoma ("colorectal" OR "colon" OR "rectum"), the 3rd terms related to reoccurrence ("recurrence" OR "recurrent" OR "metachronous"), and the final was related to diet and nutritional supplement ("diet" OR "nutrition" OR "nutrition*" OR "nutrient" OR "food" OR "vitamin" OR "Alcohol" OR "Supplement"). A librarian was consulted to verify the searching strategy before combining and entering these terms in the following three databases: PubMed, Scopus, and Web of Science. The search was for RCTs that met the inclusion criteria of this review and were published from inception to 16 July 2021.

Outcome

The primary outcome was colorectal adenoma recurrence. Further outcomes of interest were the effect of nutritional/dietary intervention on adenoma characteristics (size, location, number, or type), and findings from sub-group analysis that may reveal factors that may influence the outcome of the intervention.

Study eligibility and selection process

As shown in **figure 1-11** primary results for searching the three databases identified 952 articles, 323 duplications were identified and deleted. The title and abstract of the remaining 629 articles were screened and identified 48 relevant articles. Study eligibility was assessed by full-text reading of the selected 48 articles. This identified 16 RCTs that fulfilled the inclusion criteria for this review.

Data extraction

The retrieved RCTs were classified into four groups according to the intervention used: (a) Food and food extracts (n=6), (b) Calcium and vitamin D (n=4), (c) Antioxidants (n=4), and (d) Folic acid group (n=3). The following data from the eligible studies were extracted: (1) data related to the study [Author, trial name, year, country], (2) the population [age, gender, sample size, how they were selected], (3) [inclusion and exclusion criteria], (4) participants' baseline data [smoking, supplement use, NSAID use], (4) data related to the intervention and placebo [diet or nutrient, dose, placebo, duration of the intervention] (5) main findings [rates of adenoma reoccurrences in both intervention and placebo group], (6) results of further analysis, when available, to identify any differences within each group. **Table 1-2** contains basic data related to the studies, population and intervention.

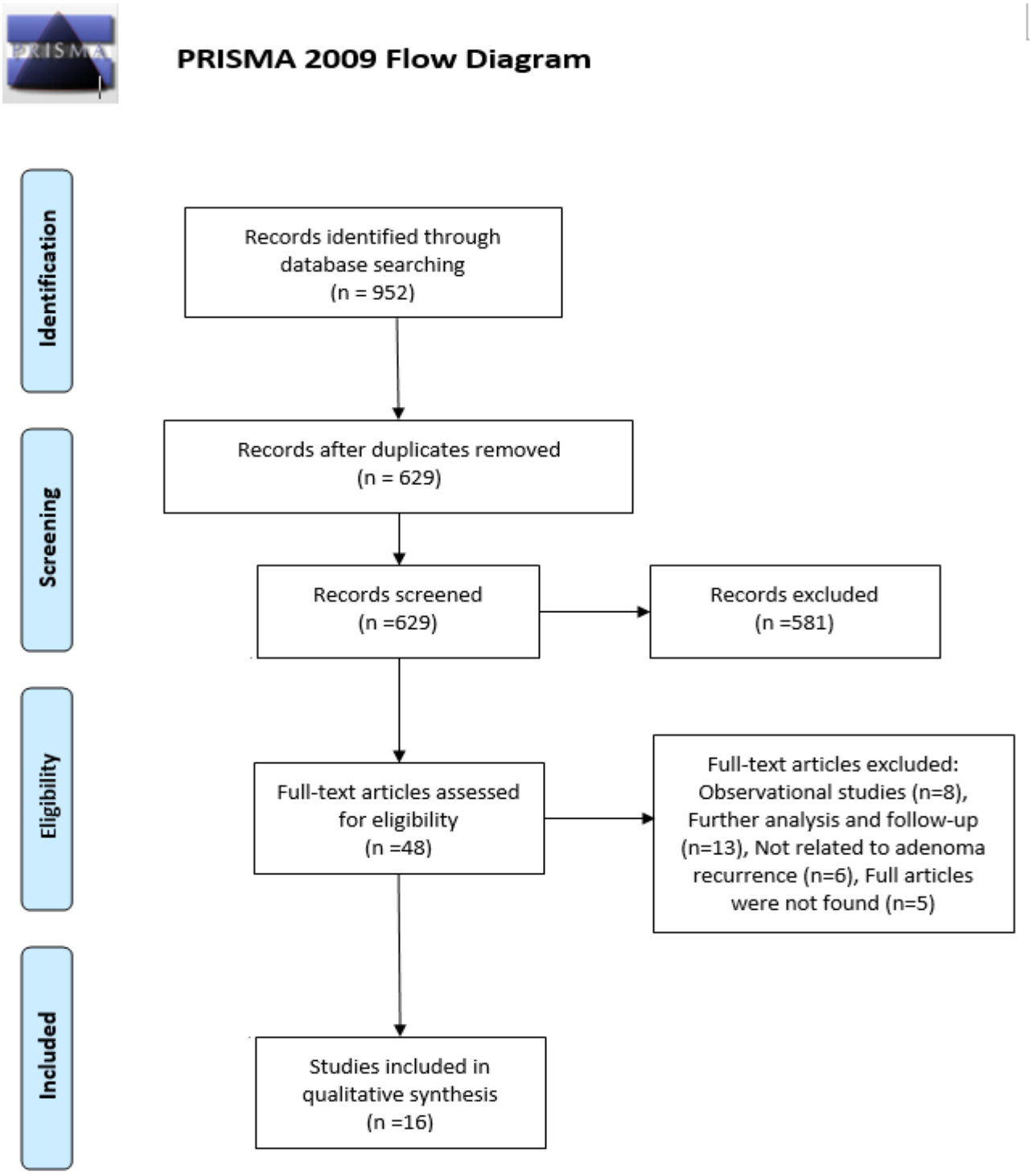


Figure 1-11. Search and selection process for nutritional randomised controlled trials for the prevention of sporadic colorectal adenoma recurrence included in the systematic review accordance to PRISMA.
(Moher et al. 2009)

General characteristics of studies and participants included

The total number of participants in the RCTs included in this review is 11390 colorectal adenoma patients. Mean age ranged from 57.8 to 66.2 years. The percentage of males in the included studies, in general, was higher than females, except for the study used folic acid (111), in which the percentage of males was under 38%. All patients had at least one adenoma removed within 3 to 6 months of randomisation . Duration of intervention and follow-up ranged from 12 months to 6.5 years. As interventions used dietary supplements or modified dietary intake, blinding was not always possible. However, to minimise bias, open labelled studies reported blinding the endoscopist to treatment allocation.

Main findings

The results are presented according to the intervention as follow: food and food extracts, calcium and or vitamin D, antioxidants and folic acid. **Figure 1-12** shows the percentage of patients with adenoma recurrence in the intervention and control groups of the included studies. For each intervention, the relative risk of colorectal adenoma recurrence will be presented with 95% Confidence Interval (CI). When available, the results of sub-analysis for the effect of each intervention on adenoma characteristics and the role of sex, age and smoking if any will be reported.

Table 1-2. Summary of nutritional RCTs for the prevention of sporadic colorectal adenoma recurrence included in this systematic review

Trial name (Author, year)	***Population***	***Sample size***	***Mean age (yrs)***	***% of Males***	***Nutrient***	***Duration of intervention***
(McKeown-Eyssen et al. 1988) *(112)*	*Canada*	*137*	*57.8*	*65.6*	*Antioxidants (C and E)*	*12 to 30 mos.*
(Roncucci, 1993) *(113)*	*Italy*	*209*	*59.2*	*62.2*	*Antioxidants (A,C,andE) or Lactulose*	*18 mos.*
TPPT (McKeown-Eyssen et al. 1994) *(114)*	*Canada*	*165*	*57.8*	*52.9*	*Fat and fibre*	*24 mos.*
CPPS (Baron et al. 1999) *(115)*	*USA*	*832*	*60.9*	*72*	*Calcium*	*36 mos.*
WBFT (Alberts et al. 2000) *(116)*	*USA*	*1303*	*66.2*	*67*	*Insoluble fibre*	*36 mos.*
ECP (Bonithon-Kopp et al. 2000) (117)	*Europe and Israel*	*552*	*59*	*63*	*Calcium and fibre*	*36 mos.*
PPT (Schatzkin et al. 2000) *(118)*	*USA*	*1905*	*61*	*64.5*	*Fat and fibre*	*48 mos.*
ukCAP (Logan et al. 2008) *(119)*	*UK and Denmark*	*419*	*58.8*	*56*	*Folic acid*	*36 mos.*
(Jaszewski et al. 2008) *(120)*	*UK*	*94*	*60*	*92.5*	*Folic acid*	*36 mos.*
(Wu et al. 2009) *(111)*	*USA*	*475*	*65*	*37.6*	*Folic acid*	*3 to 6.5 yr.*
(Bonelli et al. 2013) *(121)*	*Italy*	*330*	*57.5*	*63.6*	*Antioxidants (vitamin A, C, E, selenium, and zinc)*	*48 mos.*
(Baron et al. 2015) *(122)*	*USA*	*2259*	*57*	*58*	*Calcium and vit D*	*36 to 60 mos.*
(Pommergaard et al. 2016) *(123)*	*UK, Europe, Russia, and USA*	*427*	*60*	*58*	*Calcium and Calcitriol*	*36 mos.*
Sel/Cel (Thompson et al. 2016) (124)	*USA*	*1824*	*62*	*64*	*Selenium*	*36 to 60 mos.*
(Shin et al. 2017) *(125)*	*Korea*	*143*	*59.6*	*67.8*	*Green Tea Extract*	*12 mos.*
(Hull et al. 2018) *(126)*	*England*	*316*	*65*	*79*	*Eicosapentaenoic acid*	*12 mos.*

Abbreviation: CPPS, Calcium Polyp Prevention Study; ECP, European Cancer Prevention Study; PPT, Polyp Prevention Trial; Sel/Cel, The Selenium and Celecoxib Trial; TPPT, Toronto Polyp Prevention Trial; ukCAP, United Kingdom Colorectal Adenoma Prevention; WBFT, Wheat Bran Fibre Trial. mos.=months, yr=years.

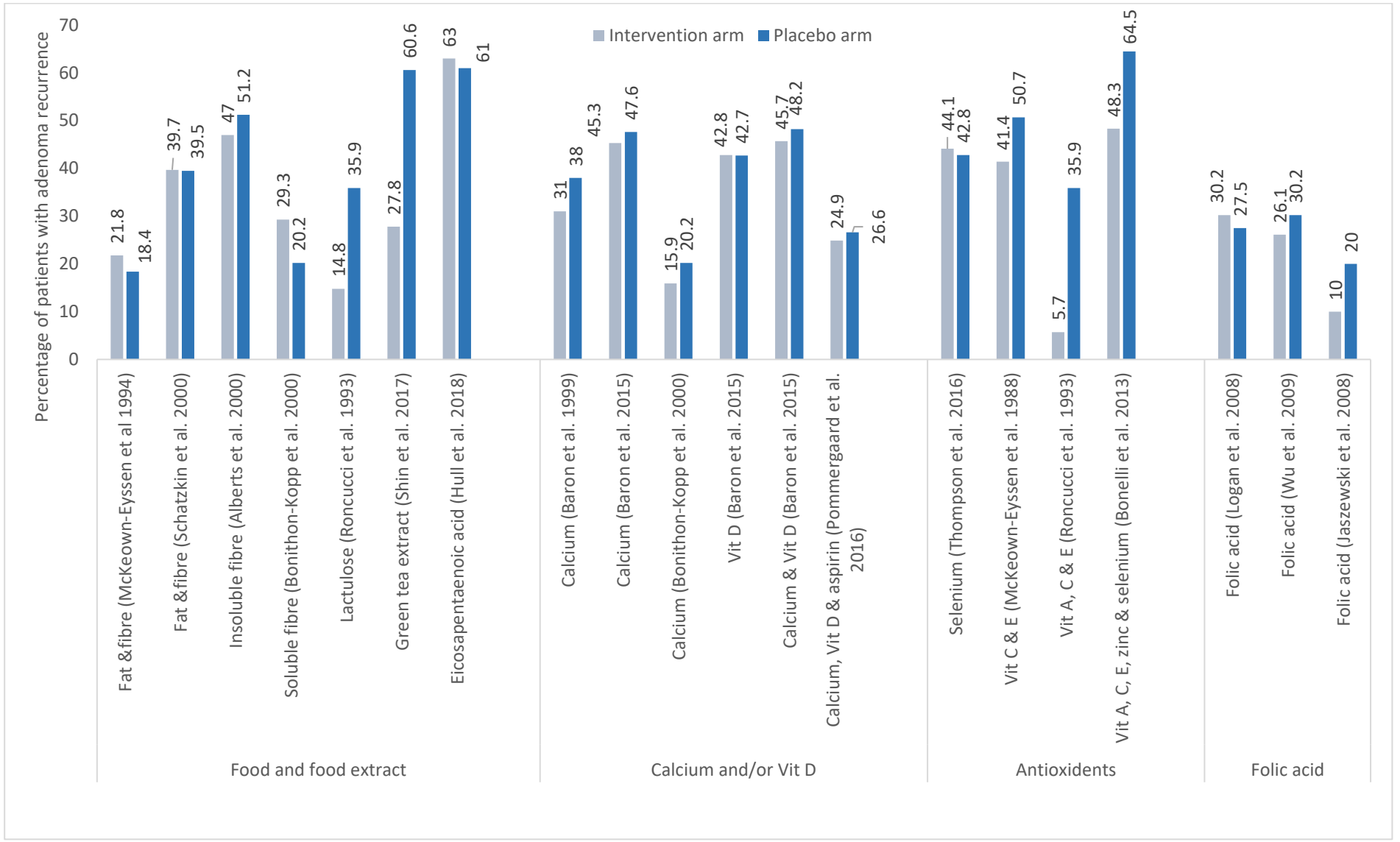


Figure 1-12. Percentage of patients with recurrent adenoma in the intervention and the placebo groups in the included studies

Intervention with food and food extract.

Seven studies investigated the effect of food or food extract on adenoma recurrence. As **table 1-3** shows four studies examined the effect of fibre or fibre and fat, the Toronto Polyp Prevention Trial (TPPT) (114), the Polyp Prevention Trial (PPT) (118), the Wheat Bran Fibre Trial (WBFT) (116), and the European cancer prevention organization intervention study (ECP) (117). One study (the seAFOod polyp prevention trial) (126) explored the effect of polyunsaturated omega three fatty acid Eicosapentaenoic acid (ω-3 PUFA EPA) in one of its intervention arms. One study examined the effect of Green Tea Extract (GTE) supplement (125), and one study (113) investigated the effect of lactulose in one of its intervention arms.

Fat and Fibre

Increasing fibre intake by 75% and reducing fat intake from about 35% to 24% for up to 4 years did not reduce the risk of colorectal adenoma recurrence as per the PPT study (118) results; the unadjusted risk ratio (RR) was 1 (95%, CI, 0.90 to1.12). Similar results were obtained from insoluble fibre supplement (13.5 g/day of WBF) for about 3 years as shown by the WBFT (116), with a multivariate-adjusted odds ratio (OR) of 0.88 (95% CI, 0.70 to 1.11, *p*=.28) in the high fibre group when compared with the low fibre group. An intention to treat analysis for the TPPT results indicated that doubling the amount of fibre intake and reducing fat intake from 35% to 23% for up to two years has no significant effect on the risk of adenoma recurrence; incidence ratio of 1.2 (95% CL, 0.6-2.2). However, further analysis performed on data from the group who received extensive dietary counselling and follow-up, revealed that males and females respond differently to this intervention (as shown later). The supplement of soluble fibre (3.5 g/day of ispaghula husk) for up to 3 years in the ECP study, revealed an adverse effect on adenoma recurrence; (OR 1.63, 95% CI 1.01-2.64]. Interestingly, this adverse effect was stronger in participants with higher calcium intake(117).

Results from WBFT show that more people in the high fibre group had three or more adenomas comparing with the low fibre group (*p*=.03). The size and histological structure of adenoma were not affected by either increasing fibre intake or modifying diet to contain more fibre and less fat (116–118). There was an increase in adenoma recurrence in both the left side and the right side of the colon in the soluble fibre arm in the ECP study; adjusted OR 1.70(95% CI, 0.95-3.00) and 1.39 (95% CI, 0.72-2.68), respectively.

It appears that the response to increasing dietary fibre may be sex-specific, with all studies reporting differential responses in men and women. Increasing dietary intake of insoluble fibre in the WBFT study was associated with a reduced risk of adenoma recurrence in men, but not in women. In the PPT study, the high fibre and low-fat intervention was associated with an increased risk of adenoma in women and did not affect men. Conversely, the TPPT study suggested a possible adverse effect of following a high fibre and low-fat diet in men, although this failed to reach significance with a RR of 1.9 (95% CI, 0.8-4.4). In contrast, there was a trend towards a beneficial effect in women in the TPPT study (114).

Eicosapentaenoic acid (EPA)

One RCT in England published in 2018 found that consumption of 2g of EPA for 12 months after polypectomy of high-risk colorectal adenoma has no effect on adenoma recurrence risk. A sub analysis revealed that no effect of EPA on number, type or location of adenoma in the case of recurrence (126).

Green Tea Extract (GTE)

One RCT performed in Korea in 2017 found a protective effect of daily consumption of 0.9g of GTE for one year against the risk of adenoma recurrence (RR= .46; 95% CI, 0.30-0.70) (p= .001). GTE intake significantly reduced the number of adenomas compared with the control group (0.5±0.9 vs. 1.5±2.0, p< .001) (125).

Lactulose

One study in Italy investigated the consumption of about 20 g of lactulose per day for about one year in one of its treatment arms. Results indicate a significant reduction in adenoma recurrence; (chi squared=6.2; p=.01, lactulose group vs. control group). There was no effect of lactulose on adenoma characteristics and no report of any differences between sexes (113).

Table 1-3. Results for Intervention RCTs that investigated the effect of food extracts on the risk of colorectal adenoma recurrence

Nutrient (s) /dose/duration/ sample size (Intervention/Placebo)	***Country/ Reference***	***Effect of intervention on risk of adenoma recurrence***	***Effect on intervention on adenoma characteristics***	***Results of further analysis***	***Notes and limitations***	***Reoccurrence % Intervention: placebo***
Fat (25% vs.33% and fibre 35g vs.16g) /24months/ (78/87)	*Canada/ (McKeown-Eyssen et al 1994)(114)*	*No significant effect*	*Not reported*	*Significantly lower recurrence in women and significant higher recurrence in men, compared with the normal diet group.*	*Sex-specific response. In the intervention group, an increase in the faecal bile acid concentration in men. Study has little statistical power to detect a gender-specific effect/no information about the fat source*	*21.8% : 18.4%)*
LFHF diet (fat provides 20% of energyand18 g of fibre per 1000kcal+3.5 servings of fruit and vegetables per 1000Kcal) /4years/(958/947)	*USA/(Schatzkin et al. 2000) (118)*	*No significant effect*	*No significant effect*	*Lower recurrence in men and higher in women in the intervention group comparing with the control group.*	*Sex-specific response. No information about the source of fat in the diet.*	*39.7% : 39.5%*
13.5 g of Wheat Bran Fibre (Insoluble fibre)/ 3years/(719/584)	*USA/(Alberts et al. 2000) (116)*	*No significant effect*	*Significantly higher number of adenomas in the intervention group, No effect on adenoma size and histology*	*Lower recurrence in men in the intervention group / no effect on women*	*Indicates a sex-specific response.*	*47% : 51.2%*

3.5 g of ispaghula husk (Soluble fibre)/3years/ (198/178)	*Europe/ (Bonithon-Kopp et al. 2000) (117)*	*Intervention with ispaghula husk may have an adverse effect on adenoma recurrence.*	*No significant effect*	*Adverse effect was significantly stronger in patients with high baseline dietary calcium intake.*		*29.3%: 20.2%*
GTE (0.9g)/1year/ (72/71)	*Korea/(Shin et al. 2017) (125)*	*Lower recurrence in intervention group*	*Significantly lower in number.*	*No effect on BMI, serum fasting glucose, lipid, or CRP.*	*Short intervention/ risk of toxicity if used over a long time*	*27.8%: 60.6%*
Lactulose (20g per day)/3years/ (61/78)	*Italy/ (Roncucci et al. 1993)(113)*	*Lower adenoma recurrence in the intervention group*	*No effect*	*Not reported*	*Lactulose may cause discomfort and diarrhoea/ small sample size.*	*14.75%: 35.9%*
Eicosapentaenoic acid (2g per day)/12 months/ (153/163)	*England/ (Hull et al. 2018) (126)*	*No effect*	*No effect.*	*Not reported.*	*Short intervention, high proportion of males and the RR was not calculated*	*63%:61%*

CI= Confidence Interval; CL=Confidence Level; GTE= Green Tea Extracts; LFHF= Low Fat High Fibre, CRP=C-reactive protein.

Intervention with calcium and vitamin D

As **table 1-4** shows, four RCTs examined the effect of calcium and vitamin D on the risk of adenoma recurrence. Two RCTs examined the effect of calcium supplementation, the Calcium Polyp Prevention Study (CPPS) (115) and the European Cancer Prevention Organization (ECP) intervention Study (117). The study examined the effect of calcium and vitamin D using a 2*2 factorial design (122). The final study investigated the effect of a combination of calcium, vitamin D, and aspirin on the risk of adenoma recurrence comparing with a placebo group (123).

Calcium

In the CPPS there was a significant reduction in the risk of adenoma recurrence after supplementation of 1.2 g of calcium per day for up to four years; the adjusted RR was 0.81 (95% CI, 0.67 to 0.99; *p*=.04). This effect was irrespective of baseline fat or calcium intake (115). However, the ECP trial found no effect on adenoma recurrence risk with a higher dose of calcium for a shorter period (2 g for three years); adjusted OR 0.66(95% CI 0.38-1.17; *p*=.16) (117). Similarly, Baron *et al* (2015) found no significant effect on adenoma recurrence after supplementation of 1.2g of calcium for up to 5 years; adjusted RR was 0.95 (95% CI, 0.85 to 1.06) (122). Further analysis of the ECP study revealed an association between initial adenoma location and calcium, since they found a significant risk reduction when inclusion adenoma was on the right side of the colon, but no effect when it was in the left side of the colon (117).

Vitamin D

Baron *et al* (2015) investigated the effect of 1000IU/d of vitamin D_3 supplement in one of its treatment arms. No significant effect was found for this dose on adenoma recurrence when consumed for up to 5 years compared with not taking vitamin D_3; the adjusted RR was 0.99 (95% CI, 0.89 to 1.09). Further analysis found no effect of baseline serum 25 hydroxycholecalciferol on the result (122).

Calcium and vitamin D

Two RCTs investigated the combined effect of vitamin D and calcium, the first investigated the effect of 1.2 g of calcium with 1000 IU of vitamin D3 for 3 to 5 years (122), and the 2nd examined the effect of 1.25 g of calcium with 0.5 µg of calcitriol and 75 mg of aspirin (123). Both concluded no effect on adenoma recurrence; adjusted RR was 0.93 (95% CI, 0.80 to 1.08) and (0.95, 95% CI, 0.61-1.48) respectively (122,123). There was no relation between calcium and vitamin D intake and the adenoma size, location and degree of dysplasia in either study (122,123).

Table 1-4. Results from intervention RCTs that investigated the effect of calcium and vitamin D supplementation on the risk of colorectal adenoma recurrence.

Nutrient (s) dose/duration/sample size (Intervention/Placebo)	***Country, Reference***	***Effect on risk of adenoma recurrence***	***Effect on adenoma characteristics***	***Results of further analysis***	***Notes and limitations***	***reoccurrence in Intervention : placebo***
1.2 g Calcium, 4years, (409/423)	*USA/ (Baron et al. 1999)*	*Significant reduction in adenoma recurrence*	*No effect*	*No role for age, sex, or baseline nutrient level*		*31%: 38%*
1.2 g Calcium, 4years, (762/761)	*USA/ (Baron et al. 2015)*	*No significant effect on risk of adenoma recurrence*	*No effect on advanced adenoma or the number of adenomas.*	*No effect for 25(OH)D serum level on the response to the treatment.*	*35% of patients were obese. Response to calcium intervention was affected by BMI. The lower the BMI the better the effect of calcium.*	*45.3%: 47.6%*
2 g Calcium, 3years,(176/178)	*Europe and Israel/ (Bonithon-Kopp et al. 2000)*	*No significant effect on risk of adenoma recurrence*	*Patients with right colon adenoma at inclusion had a significant reduction in adenoma recurrence*	*The effect of calcium supplement was not affected by dietary calcium intake.*	*Indicates a possible association between adenoma location calcium.*	*15.9%: 20.2%*
1000 IU Vitamin D3, 4years, (1024/1035)	*USA/ (Baron et al. 2015)*	*No significant effect on risk of adenoma recurrence*	*No effect on advance adenoma or number of adenomas*	*No effect for 25(OH)D level on the response to the treatment*	*More than 35% of patients were obese*	*42.8%: 42.7%*

1.2 g Calcium and 1000 IU vitamin D3, 4years, (381/380)	*USA/ (Baron et al. 2015)*	*No significant effect on risk of adenoma recurrence*	*No effect on advance adenoma or number of adenomas*	*No effect for 25(OH) D level on the response to the treatment.*	*35% of patients were obese*	*45.7%: 48.2%*
1.25 g Calcium 0.5 µg calcitriol, and 75mg Aspirin,3years, (209/218)	*Europe, USA, UK, Russia/ (Pommer gaard et al. 2016)*	*No significant effect on risk of adenoma recurrence*	*No difference between groups*	*Smoking may play a role in the treatment effect.*		*24.9%: 26.6%*

25(OH)D= 25-hydroxycholecalciferol

Intervention with antioxidants.

Four RCTs examined the effect of antioxidants (vitamins and /or trace minerals) in the prevention of colorectal adenoma recurrence. As **table 1-5** shows, the first RCT was the Selenium and Celecoxib Trial (Sel/Cel) in which selenium was tested in one of its treatment arms (124). The 2nd study examined the effect of vitamins C and E (112). The 3rd RCT examined the effect of a mix of Vitamins A, C, and E (113). The last one used a combination of vitamins A, C, and E with zinc and selenium (121).

The Sel/Cel study showed that 200 µg of selenium for about 3 years was not effective in the prevention of colorectal adenoma recurrence. Adenoma was detected in 44.1% in the intervention and in 42.8% of control groups, respectively; (RR= 1.03, 95% CI = 0.91 to 1.16, *p* = .68) (124). There was a small, but not significant, reduction in adenoma recurrence after supplementation with 400mg of each vitamin C and E for a period of 12 to 30 months; RR 0.86 with 95% CL 0.51 to 1.45 (112). The reduction of adenoma recurrence was more pronounced in the next study which used a supplement contained 30,000 IU of vitamin A, 1g of vitamin C and 70mg of vitamin E per day for up to 18 months. The percentage of recurrence in the intervention arm was 5.7 % comparing with 35.9% in the placebo arm (113). In the 3rd study, the supplement contained: 200 µg selenium,30 mg zinc, 2 mg vitamin A, 180 mg vitamin C and 30 mg vitamin E. Using this combination for about four years showed a significant reduction in adenoma recurrence; adjusted HR = 0.61; 95 % CI 0.41–0.92 (121).

No effect of daily consumption of 400mg of both vitamin C and E on adenoma recurrence risk RR=.85(95% CL, 0.45 to 1.60). Adding vitamin A to this mixture did not appear to have any influence on both size or histological structure as shown in the 2nd study (113). By contrast, the compound of vitamins, selenium, and zinc in the 3rd study, reduced the risk of developing small tubular adenoma (adjusted HR = 0.61; 95 % CI 0.37–0.99) and advanced adenomas (adjusted HR = 0.50; 95 % CI 0.24–1.01) (121). Selenium supplement in the Sel/Cel study has no effect on advanced adenoma recurrence (RR=1.02, 95% CI=0.74 to 1.43, *p*=.89) (124). Only the Sel/Cel study reported the effect of the intervention on adenoma number. It showed that 200 µg of selenium significantly increased the number of adenomas when compared with the placebo group (RR=1.47, 95% CI=1.08 to 2.02, *p*=.02) (124).

The Sel/Cel study also detected a sex-specific response, where multiple adenomas was statistically significantly higher in men (RR=1.64, 95% CI=1.17 to 2.31, *p*=.004) but not in women (124).

Table 1-5. Results for intervention RCT that investigated the effect of antioxidant supplementation on the risk of adenoma recurrence.

Nutrient (s) /dose/duration/ sample size(Intervention/ Placebo)	***Country/ Reference***	***Effect on the risk of adenoma recurrence***	***Effect on adenoma characteristics***	***Results of further analysis***	***Notes and limitations***	***% of reoccurrence in Intervention: placebo***
200µg Selenium /3years/ (786/791)	*USA/ (Thompson et al. 2016)*	*Not effective*	*No effect*	*Multiple adenomas were statistically significantly higher in men in the intervention group in males/ /People with advanced adenoma might benefit more from selenium*	*No participants with low baseline selenium level were included in this study.*	*44.1%: 42.8%*
400 mg of each Vitamin (C and E) /2years/ (70/67)	*Canada/ (McKeown-Eyssen et al. 1988)*	*Not effective*	*No effect*	*Not reported*	*The placebo used was lactose, which might impact the gut microbiota*	*41.4%: 50.7*
30000 IU vitamin A,1g vitamin C, and 70 mg vitamin E)18 months/ (70/78)	*Italy/ (Roncucci et al. 1993)*	*Significant reduction in adenoma recurrence*	*No effect on adenoma characteristics*	*Not reported*	*Not reported*	*5.7 %: 35.9%*
2mg vitamin A,180 mg vitamin C, 70 mg vitamin E, 200 µg selenium and 30 mg zinc/3years/(164/ 166)	*Italy/ (Bonelli et al. 2013)*	*Significant 39% reduction in adenoma recurrence comparing to the control group*	*Reduction in small and advanced adenoma.*	*Not reported*	*Sample size was smaller than required for 80% power.*	*48.3%: 64.5 %*

Intervention with folic acid.

The search identified three RCTs that studied the effect of folic acid on colorectal adenoma recurrence. **Table 1-6** summarises characteristics and results for folic acid RCTs. 0.5 mg of folic acid used for 3 years in the United Kingdom Colorectal Adenoma Prevention Trial (ukCAP) (119), the 2nd RCT used a dose of 1 mg of folic acid (111). The 3rd study investigated the effect of a high dose of folic acid, 5 mg, on small sample size (49 patients) for up to 3 years (120).

There was no effect of either supplementation of 0.5mg per day of folic acid for three years or 1mg per day for 3 -6.5 years on colorectal adenoma recurrence risk compared with the placebo; (RR: 1.07;95% CI, 0.85-1.34; *p*=.58) and (RR: 0.87; 95% CI: 0.65, 1.16), respectively (111,119). However, when the patient has a low baseline plasma folate, particularly when combined with high alcohol intake, the 1mg dose was effective in lowering the risk of adenoma recurrence (111).

In contrast, 3 years consumption of a higher dose of folic acid, 5mg per day, significantly reduced adenoma recurrence, mean number of .36 (SD=.69) compared with the placebo group 0.82 (SD=1.17); RR of 64% (OR, 2.77; t = -2.26, *p* = .02, 95% CI, .06-0.84; Chi-Square = 11.2, *p* < .005).

Folate doses of 0.5mg or 1mg did not affect adenoma numbers when compared with placebo and had no effect on the development of advanced lesions (RR = .98; 95% CI, 0.68-1.40)(119) and (RR: 1.03; 95% CI: 0.53, 1.98) (111), respectively. On the other hand, there was a significant reduction in the recurrence of advanced adenoma following supplementation of 5mg of folic acid when compared with the placebo group (120). There was a suggestion that folic acid altered adenoma location. It was observed that most adenomas detected after supplementation with 1mg of folic acid were proximal adenomas (111), with the 5mg dose, patients with left side adenomas had a lower recurrence rate than right side adenomas when compared with the placebo group (*p*<.05) (120). Sub analysis suggests that age of the patients may influence the response. After 3 years of intervention with 5mg of folic acid per day, the recurrence risk in patients less than 70 years was significantly lower than in patients >70years (*p*<.005) (120).

Table 1-6. Results for intervention RCT that investigated the effect of folic acid supplementation on the risk of adenoma recurrence.

Nutrient(s)/dose/ Intervention duration/ sample size (Intervention/Placebo)	***Author/year/ country***	***Effect on risk of adenoma recurrence***	***Effect on adenoma characteristics***	***Results of further analysis***	***Notes/ limitations***	***Reoccurrence in Intervention: placebo***
0.5 mg of folic acid/3years/ (215,204)	*UK and Denmark/ (Logan et al. 2008)*	*No effect*	*No effect on the number of adenomas or advanced adenoma*	*Not reported*	*Show that nutrient baseline level may influence the effect of the intervention*	*30.2%: 27.5%*
1mg of folic acid/3 to6.5years/(237/238)	*USA/ (Wu et al. 2009)*	*No effect*	*No effect on the size and being advanced adenoma. Most adenomas reoccurred in the proximal colon*	*The supplement was effective in people with low baseline folic acid, especially with a high intake of alcohol. Marginally significant protection for people with lower BMI*	*Effect of intervention might be influenced by nutrient baseline, antagonists, and BMI.*	*26.1%: 30.2%*
5mg of folic acid/3years/(49/45)	*UK/(Jaszewski et al. 2008)*	*Significant reduction in risk of adenoma recurrence.*	*Significant reduction in advanced adenoma. Lower adenoma recurrence in the left side of the colon*	*No response for the supplement in patients more than 70 years of age.*	*The effect of intervention might be influenced by the age of participants. The study has a small sample size.*	*Twice as high in the placebo group, compared with the intervention group.*

Discussion

Diet is an important lifestyle factor that is known to play a role in CRC incidence. This review has considered the possibility that diet may also contribute towards colorectal adenoma recurrence in people at elevated risk of CRC. In reviewing the literature on the effects of nutrients on colorectal adenoma recurrence, 16 completed RCTs were identified. There was limited evidence that supplementation of a combination of vitamin A, C, and E (with or without selenium and zinc), may reduce the risk of colorectal adenoma recurrence and here was no effect of vitamin D on adenoma recurrence, with or without calcium. Contrary to expectations soluble fibre was observed to have an adverse effect on colorectal adenoma recurrence. However, there were contradictory results related to the role of calcium and diet that contains less fat and more fibre. The folic acid dose was observed to be an important factor with low dose folic acid having no impact, but high dose folic acid conferring apparent protection against adenoma recurrence. Two small studies also reported a protective effect for supplementation with lactulose and with green tea extract.

Factors that may affect the intervention outcome

Further analysis for the data obtained from the included RCTs revealed that factors such as sex, age, the baseline level of some nutrients may modulate the outcome of the intervention. Response to increasing fibre intake, for instance, was protective for women but not for men. Also, the age of the participants when the intervention was introduced modulated the outcome of intervention with folic acid. Patients who were more than 70 years of age did not respond to the folic acid supplements, while a significant effect on adenoma recurrence was observed in younger participants. This suggests that intrinsic biological events, such as impaired nutrient absorption with age or the accumulation of genetic and epigenetic events in the intestinal mucosa may determine response to intervention. The other factor was baseline nutrient status and the effect of antagonists, a small dose of folic acid was more effective in patients with low baseline plasma folic acid, especially, with high alcohol intake. It also appeared that the adverse effect of soluble fibre was stronger in patients with high baseline calcium intake, indicative of a possible nutrient interaction. These secondary analyses also suggested other factors are related to lifestyle (obesity and smoking). Another issue to consider is the timing of the nutritional intervention. For example, although all participants in studies included in this review had a polepoctomy before recruitment and were considered to be free of adenoma, this assumption is based only on the morphological changes of the mucosa detected by colonoscopy, other molecular changes could already

have taken place in areas that appear healthy and response from these tissues to this nutrient might be different from healthy tissue. Finally, some of the studies were for a short period (only 12 months). As mentioned before, the tumorigenesis process of colorectal mucosa is multistage, and it is estimated that years are required to transfer from one stage to another. An intervention period of 12 or 24 months may have no impact on this long-term process.

Potential mechanisms by which nutrient interfere with mutagenic modification progression in the colon and the rectum

Diet is considered to have a role at all stages of colorectal tumorigenesis. It is not the activity of just one nutrient, it is a complex role by nutrients and metabolites that is performed within complex environment. Moreover, the activity of one nutrient might be subject to the existence or absence of another nutrient or chemical compound as was proposed by Vargas and Thompson in 2012 (12). Food and nutrients affect the health and the well-being of the colorectal tract through several mechanisms including reduction of transit time, effect on gut microbiota and influence bile acids' concentration(127). On the cellular level, antioxidants, vitamin D, and folic acid are believed to play a role in inducing or preventing certain genetic and epigenetic changes that are associated with cancer development (128).

For example, dietary fibre is believed to play several roles. Minerals and phytochemicals bound to fibre modify absorption and metabolism of other nutrients and results in new metabolites with anticancer properties, also fibre controls the movement of chyme and food waste through the gastrointestinal tract (127,129). Another mechanism is through its effect on gut microbiota since it provides prebiotic compounds that are fermented by the gut microbiota and produce short-chain fatty acids (SCFA). Butyrate is one of the SCFA that provides a major source of energy and induces anti-cancer properties (induce apoptosis and decreasing angiogenesis) in colonocyte (130). The WCRF/AICR (2018) reported that there is evidence indicate strong probability that wholegrain and food rich in dietary fibre protects against CRC (90). Contrary to expectations, the fibre intervention studies in this review showed no protective effect of high fibre intake on adenoma recurrence, with or without fat intake modification. Chapkin *et al* highlighted the importance of the availability of ω-3 PUFA to achieve the desired effect of fibre. He suggested that to measure the effect of fibre, both the amount and type of fibre and the amount of ω-3 PUFA intake should be evaluated. That is because evidence show that butyrate has a chemotherapeutic value only if PUFA ω 3 is available (131). A combination of ω-3 PUFA with butyrate (or dietary fibres that when metabolised result in high butyrate concentrations in the colon), leads to

genetics and epigenetics modifications (132). An animals study found that fish oil plus pectin (a soluble fibre that is when fermented by the gut microbiota yields butyrate) led to increases in bcl-2 promoter methylation and apoptosis in carcinogen-induced colon tumors, compared to animals consuming corn oil plus cellulose (133)

Two of the RCTs investigated the effect of fibre on colorectal adenoma using a fibre supplement. No information was given about other components in these supplements. They may lack bioactive ingredients available in food rich in fibre, which might have influenced the outcome of these studies.

Due to the effect of folic acid on genetic and epigenetic integrity, there has been considerable interest over recent decades in its role in CRC pathology. Results from epidemiological and intervention studies proposed a different role for folate at different stages of carcinogenesis. It is suggested that folate may support proliferation in pre-existing lesions, while insufficient intake may lead to the initiation of the disease partly through epigenetic modifications (12,134). In the current review of colorectal adenoma recurrence folic acid dose was observed to be an important factor with low dose folic acid having no impact, but high dose of folic acid conferring protection against adenoma recurrence. It was proposed by Mozzan *et al* in 2017, factors such as the variation between subjects in folic acid metabolism that affect its bioavailability, and the assessment methods of folic acid status should be considered when assessing the role of folic acid and comparing the findings obtained from different studies (135).

The suggested mechanism of action of both calcium and lactulose is by reducing the concentration of the toxic secondary bile acids into the colon by two different mechanisms. Calcium conjugates with the bile acids and prevents their fermentation, while lactulose prevents the formation of these substances by increasing faecal acidity. A significant reduction in adenoma recurrence was found by one study after consumption of lactulose supplement, however, it was only one study and the sample size was small (only 61 patients). Only one RCT of the three included in this review indicated a moderate significant reduction in adenoma recurrence result from calcium supplement, however, the WCRF (2018) reported that consumption of dairy products and calcium supplements probably protects against CRC (90). Since participants included in RCT within this review are high-risk group, this may suggest that calcium may be effective in protection in early stages (initiation of cancer).

Epidemiological studies have shown mixed results with regards to the effect of GTE on CRC and the mechanism of action is not clear. The protective effect reported in this systematic review is based on a

single study and further studies are required to confirm or refute this observation. The MIRACLE trial is an ongoing RCT that is taking place in Germany investigating the effect of GTE on colorectal adenoma patients who have undergone polypectomy. This RCT will provide more information about the effect and the mechanism of action of GTE on colorectal adenoma (136).

Potential limitations of included studies

There are some limitations to the RCTs included that may affect our conclusion. Except for one study that was conducted in Korea, the rest of the studies were limited to participants from the USA, Canada, and Europe. Some of the studies had small sample sizes (n=49, 61, and 70) in their treatment arms (112,113,120). Also, one study indicated the use of lactose as a placebo (112) and another included cellulose in its placebo (115) while the other studies did not specify the placebo used (116,121,123). The placebo used in studies related to the colon should be selected carefully. A percentage of adults have lactose intolerance, for example, in this case, the lactose may reach the colon intact and may affect the results by influencing the environment of the colon and hence the outcome of the study. In addition, cellulose manipulates the colon environment and a fixed-dose over a long period may also influence the outcome of the study. Overall, as with any food or supplement intervention study, the background diet and lifestyle may confound the effect.

Limitations for this review

This review has some limitations. Although a comprehensive search was performed in three databases, no hand searching or grey literature search were performed, which may result in the omission of some key publications in this area. No formal tool was used such as, the Cochrane risk of bias tool for randomised trials RoB 2.0 (137) and the Critical Appraisal Skills Programme (CASP) (138) to assess the quality of the included RCTs and the critically appraisal was performed by only one researcher which may limit the critical analysis and interpretation of the results.

To conclude, this review has found little evidence that nutrients modulate adenoma recurrence in the relatively short-term immediate post-polypectomy years, it also revealed some of the factors that may affect the interventions' outcome. There is limited research in this field and the review highlights the necessity for more research by performing more clinical trial to identify the effect of diet on adenoma recurrence and by secondary analysis of existing data from previously performed clinical trials to identify factors that may influence the outcome.

1.2.4.3 Dietary patterns and risk of colorectal tumourigenesis

Dietary pattern is defined as "the quantities, proportions, variety, or combination of different foods, drinks, and nutrients (when available) in diets, and the frequency with which they are habitually consumed" (139). It is essentially a way of considering the effect of the diet, as a whole, on a specific outcome instead of focusing on one nutrient or food. Dietary patterns are not designed to identify the biological or molecular pathways behind the association between diet and the disease; the methods are used as preliminary data exploration that may provide basic knowledge to direct investigators to develop a hypothesis about the association under investigation (140).

There are two different approaches to assess dietary patterns, the a posteriori or data-driven approach and the a priori or pre-defined approach. The data-driven dietary pattern analysis approach describes the current dietary behaviour of the population and ignores all the previous knowledge about the association between the disease under the study and the dietary components. It is measured by applying a statistical technique on the data to condense it to limited factors based on the interactions between nutrients or foods included in the test (141). The a priori or pre-defined approach is an investigator-defined method. It is based on previously defined scores or indices that were developed according to established knowledge and evidence about the role of a specific food group, item or nutrient on a specific outcome. Several scores have been developed such as the Mediterranean diet score (142), the Healthy Eating Index (143) and the Dietary inflammatory Index (DII) (144).

1.2.4.3.1 Dietary patterns and the risk of colorectal adenoma and CRC

A recent summary of systematic reviews and meta-analysis for the role of dietary patterns on CRC risk was published in 2020. The review identified nine studies that summarised the findings from studies that used the data-driven dietary pattern approach (145). Although different studies used different names to identify the extracted dietary patterns by this approach, similarity of the characteristics of these patterns was observed. In general, the statistical methods used (either Principle Component Analysis (PCA) or factor analysis) were able to extract two distinctive groups of dietary patterns, the first was characterised by a high intake of fruits and vegetables and a low intake of red and processed meat and collectively named as "healthy dietary patterns". The 2nd group was characterised by a low intake of fruits and vegetables and a high intake of red and processed meat, sweets, and fatty food and was identified as "less healthy dietary patterns". In general, the risk of CRC and adenoma were lower in

individual following the more “healthy dietary patterns” and higher in individuals following the “less healthy dietary patterns” (145).

Many pattern scores and indices have been developed under the pre-defined dietary pattern approach; one such score is the Dietary Inflammatory Index (DII) (144). DII is a new tool applied to measure the inflammatory potential of the diet. To calculate the adherence to this score, the dietary intake of the studied population is compared with the list of pro and anti-inflammatory foods and nutrients that was created according to previous knowledge on their effect on the systemic inflammatory biomarkers. A high DII score indicates a pro-inflammatory diet and low DII score indicates an anti-inflammatory diet.

The association between CRC and inflammation is supported by the evidence showing that usage of substances with anti-inflammatory activity such as aspirin and the ω-3 PUFA are protective against CRC development and progression (146,147).

Three studies explored the association between DII and CRC and reported that a high DII score (which indicates consumption of a diet with an overall pro-inflammatory effect) is associated with a 12 to 65% higher risk of developing CRC (148–150). One case-control study conducted in Iran explored the association between DII and colorectal adenoma reported that after adjusting for energy, a high DII score (proinflammatory) might be associated with an increase in the risk of colorectal adenoma (151). However, a meta-analysis by Moazzen *et al* pooled 38 studies used to assess the association between dietary indices and the risk of CRC and urged caution with regards to the interpretation of the data. Although Moazzen *et al* reached similar conclusions that higher diet quality had a preventative role in CRC development, the pooled analysis warned that the quality of such studies was poor. They also highlighted that the variation in dietary patterns’ indices, differences in the population and follow-up period lead to inconsistent results that are insufficient to make dietary recommendations (152).

1.2.4.3.2 Dietary patterns and the risk of adenoma recurrence

One small study explored this association using the data-driven dietary pattern analysis approach (153). The study was conducted within the European fibre-calcium intervention trial and included 442 colorectal adenoma patients. At the three-year colonoscopy, adenoma reoccurred in 20.8% of the patients. Three dietary patterns were extracted from baseline dietary intake and were identified as the *Mediterranean, Western and snacks dietary patterns*. The analysis showed that the Mediterranean

dietary pattern was associated with a lower risk of recurrence in females. No association between dietary patterns and adenoma recurrence in males (153).

One study explored the association between the predefined dietary pattern score, measured by the DII method, and the risk of adenoma recurrence in 2017 (154). The study was a pooled analysis study that included data from 1727 patients that were enrolled in Phase three clinical trials aimed to investigate the use of either high-fibre cereal supplement or Ursodeoxycholic acid on the risk of adenoma recurrence. After a follow-up period of 3 years, the study found that the DII score was not associated with the risk of colorectal adenoma recurrence or the characteristics of adenoma (size, location or type) in the case of recurrence (154).

Currently, there is insufficient evidence on the precise contributions of diet at early stages of CRC or on the risk of its recurrence. Consequently, there are no clear dietary guidelines for the prevention of adenoma development or recurrence. Identifying and understanding the dietary behaviour that may contribute to the risk of adenoma recurrence may lead to a better understanding of the association which will be used to frame dietary guidelines to improve CRC prevention strategies.

1.3 Aim and objectives

The main aim of this research project is *"To describe the dietary characteristics of patients newly diagnosed with high-risk colorectal adenoma and to explore the association between diet, dietary patterns and colorectal adenoma profile and the risk of recurrence"*. This aim was achieved using dietary and adenoma information from two trials:

i) The SeAFOod trial: a 12 month 2x2 factorial design RCT of Eicosapentaenoic acid and aspirin in patients with high risk colorectal adenoma

ii) The FACT study: a cross sectional study collected data from healthy participants, colorectal adenoma and CRC patients.

The following objectives were used to achieve this aim:

1. To conduct internal and external validation of the dietary data obtained from the seAFOod trial **(Chapter 3).**
2. To describe the baseline demographic characteristics and dietary intake of colorectal adenoma patients recruited to the seAFOod trial **(Chapter 4).**
3. To define the dietary patterns followed by the patients recruited to the seAFOod trial using two dietary pattern analysis approaches, the data-driven approach and the predefined approach (DII) **(Chapter 5).**
4. To investigate if patients changed their diet in the 12 months after being diagnosed with the index colorectal adenoma by comparing reported dietary intake during the 12 months before diagnosis with dietary intake during the 12 months after diagnosis **(Chapter 6- presented in a manuscript format).**
5. To assess if there is an association between dietary intake at baseline and risk of colorectal adenoma recurrence in patients allocated to the placebo group of the seAFOod trial **(Chapter 7).**
6. To investigate if an association exists between crypt cell proliferation, keratin, endocrine cells and dietary intake using data collected from the FACT study. **(Chapter 8).**
7. To explore if an association exists between crypt cell proliferation, keratin, endocrine cells and adherence to the WCRF/AICR general cancer prevention recommendations **(Chapter 8).**

Chapter 2

Materials and methods

Chapter 2 Materials and methods

The chapter is divided into two main sections; i) the materials section will provide background to the data used in this research, ii) the methods section, will describe the data preparation and analysis procedures.

2.1 Materials (Data source)

Two sets of data were used: i) data obtained from colorectal adenoma patients who participated in the seAFOod polyp prevention trial (126); ii) data obtained from participants to the observation arm of the FACT study (155) after having a colonoscopy examination and were diagnosed with either colorectal adenoma, CRC or free from colorectal neoplasia. The two studies were conducted by different principal investigators, at different times. As **Fig 2-1** shows, each set of data was stored in a different format using different software/document. This section will provide a brief overview of each study, its main aim, inclusion and exclusion criteria and the data collection methods.

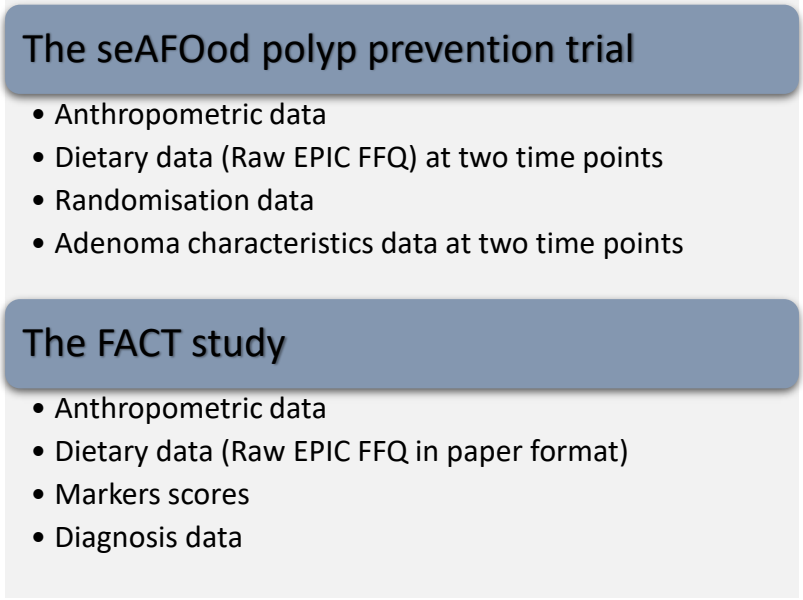


Figure 2-1. A summary of the data provided by the seAFOod trial and the FACT study and included in this research

2.1.1 The seAFOod Polyp Prevention Trial

2.1.1.1 Design of the seAFOod Polyp Prevention Trial

The Systematic Evaluation of Aspirin and Fish Oil Bowel Polyp Prevention Trial (The seAFOod trial) was a multi-centre randomised clinical trial that was launched in England in 2011. The protocol and

design of the study were published in 2013 (156). In summary, the study recruited colorectal adenoma patients during their routine screening colonoscopy from 63 Bowel Cancer Screening Programme (BCSP) centres from around England during the period between November 2011, and June 2016. The patients were classified as at high risk of adenoma recurrence. Ethical approval was obtained from the Trent Research Ethics Committee (10/H0405/90) and the trial registration number is ISRCTN05926847.

The aim of the seAFOod Trial was "*to determine whether the naturally-occurring ω-3 polyunsaturated fatty acid, Eicosapentaenoic fatty acid (ω-3 PUFA EPA) prevents colorectal adenomas, either alone or in combination with aspirin*". Patients eligible for inclusion in the seAFOod trial had five or more small colorectal adenomas or ≥ 3 adenomas with at least one being ≥ 10 mm in diameter. The full list of exclusions are reported elsewhere (156), in summary, patients were excluded from the study if they:

- had a known clinical diagnosis or gene carrier of a hereditary CRC or inflammatory bowel disease,
- had a malignant change in an adenoma,
- were regular user of or have allergy to aspirin or fish oil,
- were unable to comply with the study or enrolled in another interventional clinical trial.

The study was a 2*2 factorial double-blind intervention trial. After diagnosis and removal of adenoma by polypectomy, patients were recruited and randomly allocated into one of the four intervention groups: 2g of EPA plus 300mg of aspirin once daily, 2g of EPA plus aspirin placebo once daily, 2g of EPA placebo plus 300mg of aspirin once daily or 2g of EPA placebo plus 300mg of aspirin placebo once daily. For each participant, the intervention continued from 12 to 15 months.

At enrolment, demographic data, anthropometric measurements and medical history were taken. The dietary intake of the participants was assessed using the EPIC Food Frequency Questionnaires (EPIC FFQ) (157) at two-time points. The first was at diagnosis to assess their dietary intake during the 12 months before recruitment (**visit 1 dietary data**), the 2nd was at the end of the study to assess their dietary intake during the 12 months following the diagnosis (**visit 2 dietary data**). This 2nd dietary assessment was accompanied by a colonoscopy examination to detect adenoma recurrence. Data related to adenoma characteristics (number, size and location) were collected at baseline for all participants and the end of the study from patients with adenoma recurrence (156). **Figure 2-2** summarises the journey of the patients throughout the trial.

Data available from this study for secondary analysis were:

- Anthropometric and demographic characteristics,
- Treatment allocation
- Adenoma number and characteristics at baseline and at exit colonoscopy examinations,
- Dietary intake data at baseline and after 12 to 15 months of recruitment.

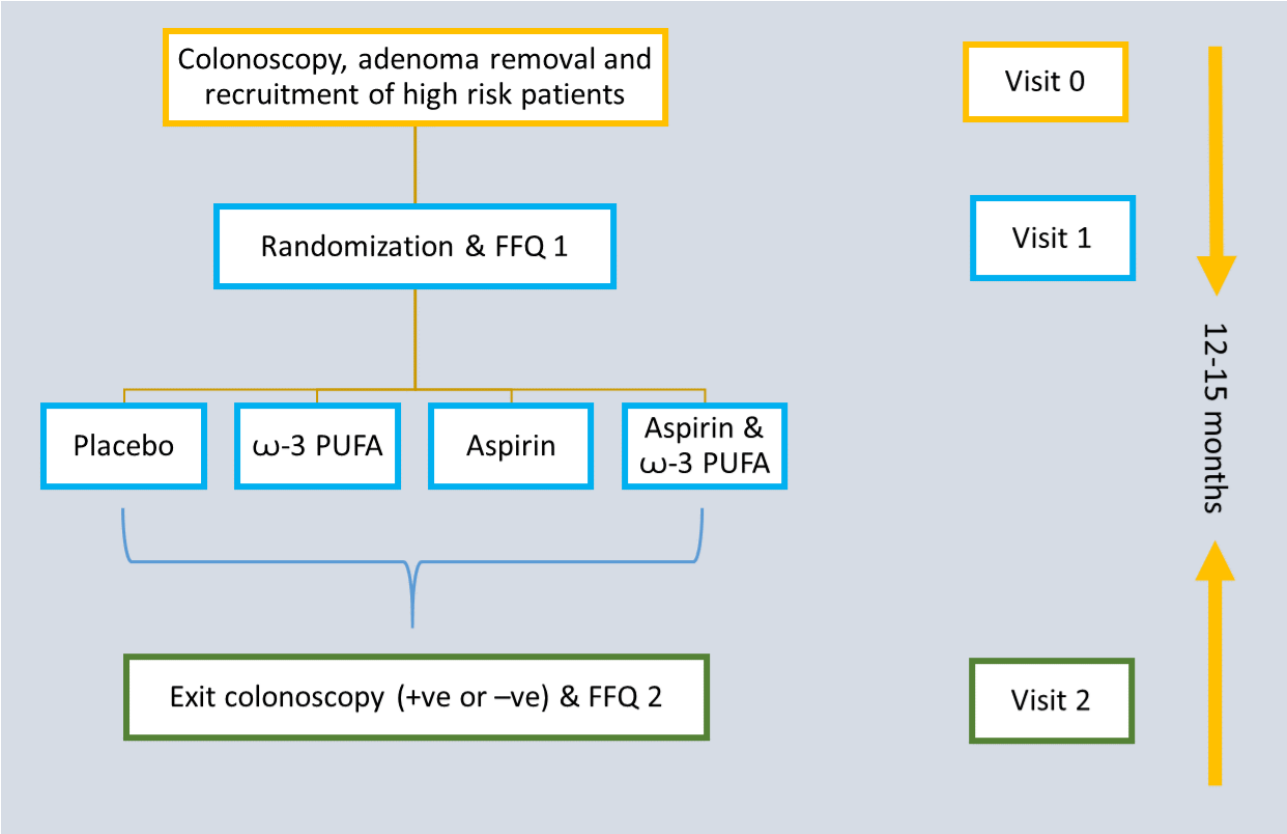


Figure 2-2. Schematic of the seAFOod trial from recruitment to the end of the study.

2.1.1.2 Data provided by the seAFOod Polyp Prevention Trial

Data provided for this thesis were the patients' baseline characteristics, clinical history, anthropometry, food frequency questionnaire responses, randomisation group and adenoma characteristics. The dietary data and the adenoma characteristics data were collected at the beginning of the study and after 12 to 15 months of recruitment. All data, including the FFQ responses, were collated and inputted into a study database by The Nottingham Clinical Trials Unit, University of Nottingham.

Baseline characteristics, clinical history, anthropometric, and FFQ data were received from the Nottingham Clinical Trials unit in December 2017. Data was provided in separate csv files with the

FFQ data collected at visit1 and visit 2 was distributed over three files using a single ID reference number for each patient for visit 1 and visit 2, the number of visits was stated in a separate variable. Therefore, splitting the visit 1 and visit 2 data was not possible using only the ID as a reference. A series of data processing steps (coding, checking, merging and cleaning) were necessary before extraction of dietary data for visit 1 and 2 from the FFQs was possible (Further details given in **Appendix 1**. The raw data was saved in its original form, however, for the purposes of data preparation and extraction a copy of the data was transferred between excel sheet and SPSS files.

Treatment allocation and adenoma characteristics were received from the PI of the SeAFOod trial, Professor Mark Hull in July 2020 . Data was provided in three excel files. The randomisation information was distributed over four columns as shown in **figure 2-3**

randono	treat1	decode1	treat2	decode2
1	B	ASP active	A	EPA active
2	D	ASP placebo	A	EPA active
3	B	ASP active	A	EPA active
4	B	ASP active	C	EPA placebo
5	D	ASP placebo	C	EPA placebo
6	B	ASP active	C	EPA placebo
7	D	ASP placebo	C	EPA placebo
8	D	ASP placebo	A	EPA active

Figure 2-3 Example of randomisation data provided by the seAFOod trial

Two excel csv worksheets contained the adenoma characteristics collected at baseline and at the end of the study. For each adenoma detected, the following information was available

- adenoma location (distal or proximal),
- adenoma number per location
- maximum dimension per location (in mm).

2.1.2 The FACT study.

2.1.2.1 Design of the FACT study

Data used in this analysis was collected from participants recruited for the observational arm of the FACT study (FACT OBS) (155). This was a cross-sectional study that recruited participants from gastroenterology clinics at Sheffield's Northern General Hospital and Royal Hallamshire Hospital during the period between October 2007 and June 2008. The study protocol was published previously (155). Ethical approval for the study was obtained from the North Sheffield Research

Ethics Committee (Reference number: 06/Q2308/93) and the trial registration number is ISRCTN90852168.

The FACT OBS study aimed to explore the association between global protein acetylation and colorectal carcinogenesis by comparing global protein acetylation in colon and rectal mucosal biopsy samples obtained from normal, adenoma and colorectal cancer patients.

The inclusion criteria were males, over 40 years with a BMI between 20 and 29 Kg/m^2 who had a planned colonoscopy. The exclusion criteria were being a female, following a special diet or consuming a high amount of fibre (Non-Starch Polysaccharide (NSP) and Resistant Starch (RS)), smoker, diagnosed with inflammatory bowel disease or type 2 diabetes.

2.1.2.2 Data provided by the FACT study

At enrolment, anthropometric data, demographic data and biopsies were collected. Biopsies were collected from different regions of the colon however data used in this analysis were from biopsies of the mid-sigmoid region only. Two weeks after colonoscopy, dietary intake was self-reported by the participants using the EPIC FFQs (157).

The data available from this study and included in this project were:

- Anthropometry and demographics,
- Diagnosis: healthy, adenoma or cancer,
- Scores for mitosis, cellularity, keratin, Ki67 and Chromogranin A (measured in the biopsies from the mid sigmoid region of the colon),
- Habitual dietary intake data collected by EPIC FFQ that assessed dietary intake over the preceding 12 months.

Dietary data

Dietary data was self-reported using paper form of EPIC FFQs from 98 individuals. As the study used a modified version of EPIC FFQ, a series of modifications were performed on the data to be compatible with the FETA software **(Appendix 2).**

Collection and immunohistochemical analysis of the FACT study biopsies

Biopsies collected from the mid-sigmoid region of the patients' colon were used to measure mitosis, cellularity, keratin, Ki67 and Chromogranin A (CGA). The procedures of biopsy collection and measurements of the indicators were performed by other researchers and methods were published previously. In summary, in healthy participants, samples were collected from the mid sigmoid. In

patients in whom adenoma or cancer was detected, biopsies were collected from three locations: mid sigmoid, contralateral wall to the lesion and from the lesion itself. Only samples collected from the mid-sigmoid region were included in this analysis. Samples were fixed in formalin and embedded in paraffin before sectioning and subsequent immunohistochemically staining. The following measurements were made on the sections:

Mitosis: The procedure used to measure mitosis was published previously (158). In summary, after rehydration and hydrolysis samples were stained with Schiff's reagent and dissected. The number of nuclei was counted in all phases of mitosis through the full crypt to measure the number of mitoses per crypt.

Cellularity: The paraffin embedded tissues obtained from the participants were sectioned in about 5 µm-thick and stained with haematoxylin and eosin. Lengthwise cut crypts were examined using an optical microscope. The number of epithelial cells were counted from the bottom to the top in each hemi-crypt.

Ki67: The details of measuring Ki67 are provided in (158). In summary, heat was used for epitope retrieval, samples were incubated with Ki-67 primary antibodies (Vector Laboratories VP-K 452) and pHH3 and the stain was visualised using Vectastain Universal Elite kit and DAB peroxidase.

Keratin: Immunohistochemical scoring protocol for K8 was developed and used to measure Keratin 8 (159). In summary, antigen retrieval was performed using a high-power microwave, normal horse serum was used as a blocking agent and sample were incubated with the primary antibody (K8 mouse monoclonal M20 from Abcam ab9023) and Biotinylated secondary antibody (anti-mouse IgG from RTU Vectastain. DAB kit (Vector lab- SK-4100) was used for the detection.

Chromogranin A (CgA): A microwave oven was used for antigen retrieval using DAKO, the monoclonal mouse anti-CgA antibody (DAKO) were used. Biotinylated antibodies (Vector Laboratories, Peterborough) were used for staining following the standard horseradish peroxidase procedure and the DAB as the chromogen substrate (Vector laboratories) for visualisation. The scoring was measured by the percentage of positively stained cells. the intensity and percentage of positive or negative CgA stained cells per section were scored per Hemi-crypt (160).

2.2 Methods

This section will introduce the dietary assessment and analysis tools that were used to collect and analyse the dietary intake. It will also summarise the data preparation, analysis and merging procedures and the dietary patterns measurements.

2.2.1 Dietary assessment and analysis tools, an overview.

Both studies used the EPIC food frequency questionnaires (FFQ) to assess the dietary intake of the participants. Dietary data analysis was part of this PhD project and was achieved using the FETA software(161). The two studies used slightly different versions of the FFQ and the FETA software was designed to analyse the version used by the seAFOod trial, therefore, some modifications were made to the FACT study data to make it compatible with the FETA software. This section provides a general description of the EPIC FFQ, and the software used to analyse the FFQ data. The assumptions and modifications performed on the FACT study dietary data will be summarised.

2.2.1.1 The EPIC FFQ dietary assessment tool

The European Prospective Investigation into Cancer and Nutrition Norfolk Food Frequency Questionnaire (EPIC-FFQ) was used by both studies. It is a retrospective method of nutritional assessment on an individual level. The EPIC FFQ is a validated semi-quantitative questionnaire designed to measure food and nutrient intake over the previous 12 months. The form has two main sections. *The first* is a list of 130 food items and beverages obtained from the UK food composition database, McCance and Widdowson's 'The Composition of Foods' 5th edition and its supplementary documents (161). Portion size is provided for each food item based on data obtained from other UK governmental and population surveys (161,162). Participants are asked to record their frequency of consumption of the listed food items and beverages. Nine frequencies are provided for each food item ranging from *never or less than once a month* to *more than six times a day.*

The 2nd section of the EPIC-FFQ allows open-ended handwritten answers. It includes questions related to eating breakfast and breakfast cereals; the type and brand of cereal; type and quantity of milk consumed; the type of fat used in frying and baking; questions about frequency of eating fried food (at home and outside); usage of salt and salt substitute. This section also contains the cross-check questions that inquire about the average weekly intake of fruits, vegetables, meat and fish. The final group of questions relates to the use of nutritional supplements, name, brand, amount and frequency of intake.

2.2.1.2 The Food Frequency Questionnaire European Prospective Investigation into Cancer and Nutrition Tool for Analysis (FETA software)

FETA software is an open-source tool created and maintained by researchers working on EPIC-Norfolk at the University of Cambridge (161). The software is used to process the dietary data collected by the EPIC-FFQ. It converts the frequency of reported consumption to time per day and multiplies it by the amount of the food item in a standard portion. Following that, all information is merged to provide a database for the analysed data (161). The software user has the option to choose the level of the analysis by choosing one of the four output options, with each provides a different level of analysis. The most used output is the 'wide-format' option which provides the average daily intake of food, energy, macro and micronutrients, for each patient and presents the results in a list of 14-food group and 46 nutrients **(Table 2-1)**.

Table 2-1. The food groups, macro and micronutrients included in the FETA output and their units.

Food group	***Unit***	***Macronutrients***	***Unit***	***Micronutrients***	***Unit***
Alcoholic beverages	*g*	*Alcohol*	*g*	*Alpha carotene*	*µg*
Cereals and cereal products	*g*	*Carbohydrate - total*	*g*	*Beta carotene*	*µg*
Eggs and egg dishes	*g*	*Carbohydrate - fructose*	*g*	*Calcium*	*mg*
Fats and oils	*g*	*Carbohydrate - galactose*	*g*	*Carotene - total*	*µg*
Fish and fish products	*g*	*Carbohydrate - glucose*	*g*	*Cholesterol*	*mg*
Fruit	*g*	*Carbohydrate - lactose*	*g*	*Chloride*	*mg*
Meat and meat products	*g*	*Carbohydrate - maltose*	*g*	*Copper*	*mg*
Milk and milk products	*g*	*Carbohydrate - starch*	*g*	*Iron*	*mg*
Non-alcoholic beverage	*g*	*Carbohydrate - sucrose*	*g*	*Total folate*	*µg*
Nuts and seeds	*g*	*Carbohydrate - (total)*	*g*	*Iodine*	*µg*
Potatoes	*g*	*Non-Starch Polysaccharides (NSP)*	*g*	*Potassium*	*mg*
Soups and sauces	*g*	*Fat - total*	*g*	*Energy*	*kcal*
Sugars; preserves and snacks	*g*	*Monounsaturated fatty acids*	*g*	*Energy*	*kJ*
Vegetables	*g*	*Polyunsaturated fatty acids*	*g*	*Magnesium*	*mg*
		Saturated fatty acids	*g*	*Manganese*	*mg*
		Protein	*g*	*Sodium*	*mg*
		Nitrogen	*g*	*Niacin*	*mg*
				Phosphorus	*mg*
				Vitamin A - retinol	*µg*
				Vitamin A - retinol equivalents	*µg*
				Vitamin B2 - riboflavin	*mg*
				Selenium	*µg*
				Vitamin B1 - thiamin	*mg*
				Vitamin B12 - cobalamin	*µg*
				Vitamin B6 - pyridoxine	*mg*
				Vitamin C - ascorbic acid	*mg*
				Vitamin D - ergocalciferol	*µg*
				Vitamin E - alpha tocopherol equivalents	*mg*
				Zinc	*mg*

In this study, the association between dietary intake and colorectal adenoma was based on assessing the dietary intake of foods and nutrients associated with CRC as per the WCRF/AICR recommendations(90). Two of the food groups associated with the disease are not extracted by the "wide format" FETA output, the red and processed meat group and the oily fish group. To extract these food groups, the data was run for a 2nd time in FETA software using the "by ffq line" output

option. This option provides more details about each dietary item consumed. **Table 2-2** shows an example for FETA output when using the "wide-format" (A) and "by ffq line" format (B)

Table 2-2. Example of FETA output (A) using the "Wide-format" option and (B) using the "By ffq line" option

A

1.Wide format

ID	Calcium	Cholesterol	Chloride
	mg	mg	mg
1234567A	1327	412	8157

B

3. By ffq line

ID	Meal ID	Meal portion	Nutrient code	Nutrient quantity
1234567A	1	42.57	8	3
1234567A	1	42.57	12	32
1234567A	1	42.57	13	26

2.2.2 Data preparation

Taken from FETA software website.

As the data was received from each study distributed over many worksheets or documents and in a different format, procedures were conducted to prepare the data and put it in a format that was suitable for merging and further analysis. **Figure 2-3** illustrates a summary of the steps of data preparation conducted before merging the data.

	Original data	Processing	Analysis	Merging
The seAFOod polyp prevention trial	Anthropometric data	Calculate: age, BMI, EEI, BMR		All the data from the seAFOod trial was merged in one SPSS worksheet to be further analysed
	Dietary data (Raw EPIC FFQ) at two time points	Separating visit 1 and 2, cleaning, coding and merging.	Extracting average daily intake using FETA software	
	Randomisation data	Coding and splitting	-	
	Adenoma characteristics data at two time points	Coding, Splitting, merging and calculation		
The FACT study	Anthropometric data	Coding and labelling		All the data from the FACT study was merged in one SPSS worksheet. The baseline data from 533 patients (males) recruited to the seAFOod trial was merged to perform the analysis required in **Ch 8.**
	Dietary data (Raw EPIC FFQ in paper format)	Manual inputting, modification, coding	Extracting average daily intake using FETA software	
	Markers scores	Coding and labelling		
	Diagnosis data	Coding and labelling		

Figure 2-3. A summary of the data preparation steps conducted before merging the data

2.2.2.1 The seAFOod trial data preparation and processing

The main aim of this step was to prepare the dietary data in a format that was suitable for the dietary data analysis software FETA to extract average daily intake of foods and nutrients and then merge it with the baseline and adenoma characteristics' data in SPSS to conduct the statistical analysis. The work required transforming the data between different software and to perform different calculations and analysis. In summary, this was achieved by series of steps, with each step involving calculations, coding and analysis:

- Prepare excel worksheets with the baseline characteristics of patients included in the study.
- Prepare a worksheet with the frequency of consumption in a format that is required by FETA software.
- Categorise the randomisation data
- Extract average daily intake from the FFQ using FETA software.
- Prepare excel worksheets with the adenoma characteristics at baseline and end of the study.
- Merging the three worksheets into a format compatible with SPSS software.

Baseline characteristics data: The age of patients at baseline was calculated using the date of birth and date of consent at enrolment in the study.

The Body Mass Index (BMI): BMI was calculated using data provided for weight and height using excel software using the formula (BMI= weight in Kg /height m^2).

Randomisation data processing: A new variable was created in to reflect the treatment arm.

Adenoma characteristics data: A worksheet was created with visit 1 and 2 data in a format that each case has its data in one row, several steps and calculations were conducted. Details are provided in the **Appendix 3.** In summary excel software was used to separate visit 1 from visit 2 and then separate the adenoma characteristics data obtained from each visit. Variables recoded and remerged using SPSS software. The following variables were calculated from the merged data:

- Total number of adenomas,
- Total number of distal adenomas and total number of proximal adenomas,
- Total sizes of adenomas,
- Total sizes of distal adenomas and total size of proximal adenoma

Dietary data preparation: As **figure 2-4** shows, after separating visit 1 database from visit 2 in the three FFQs files provided, there was a difference in the number of cases in each dataset of the three

FFQ answers for each visit. Ordering the data and allocating one row for all information related to each patient, were performed manually. Once visit 1 and visit 2 data had been separated, the dietary variables from each visit were renamed, coded, reordered to prepare the data for the strict format required by FETA software (161).

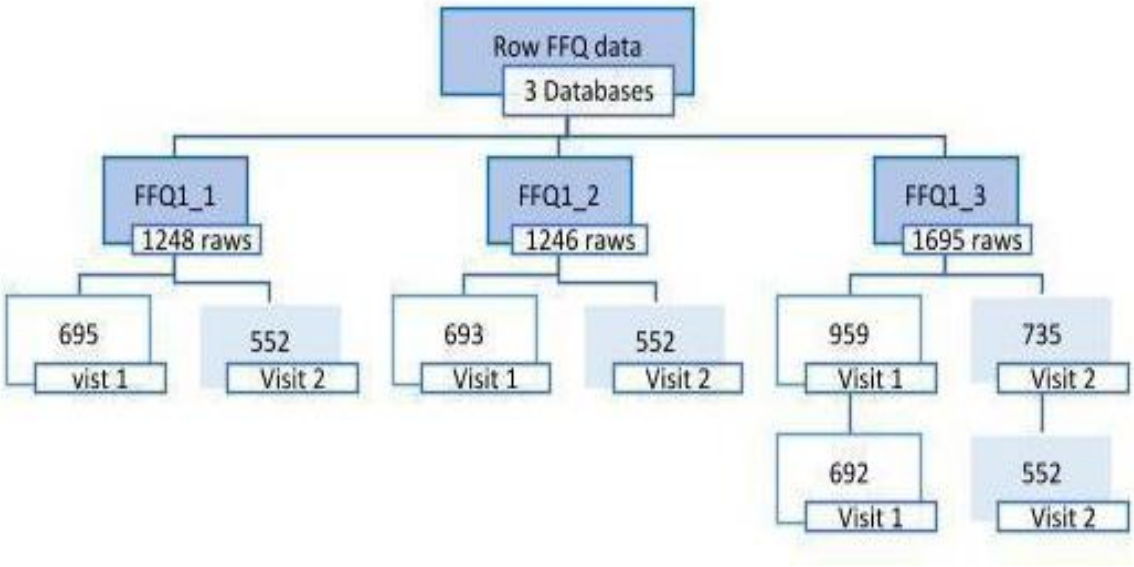


Figure 2-4. Separating and cleaning the seAFOod trial dietary data from visit 1 and visit 2

Before using the 2nd section of the FFQs (which contains handwritten answers), some work was required to deal with human errors during data entry such as spelling mistakes or using different abbreviations for the same item. OpenRefine software was used to help solve this problem, OpenRefine is an open-source desktop application used to clean up and organise data (http://openrefine.org/). This software was useful in correcting all sources of error in handwritten answers, such as spelling mistakes, abbreviations, white spaces. For example, the following answers were provided to indicate that olive oil was used in cooking (olive oil- olive oil. - olive oil based- olive-olive oil used- olive.- olivio- olv- olive oil only- olive oil betrolli). This software can identify similar data and gives the option of choosing one word to replace all the other words in a process called clustering.

The next step was to code the food item into the codes that are identified in the look-up lists provided with the FETA software user documents. This step required a degree of decision making They were used to code for different types and brands of milk, breakfast cereals and fat in a process

known as -free text matching-. In the case that a consumed item was not available on the look-up list, an online search for the ingredients and percentages of nutrients in the consumed item was performed. This was compared to ingredients with the look-up list provided and the nearest food item was chosen.

All the data was then merged into one spreadsheet using the patients' ID numbers as a reference for merging.

Dietary data analysis: The prepared FFQ data for visit 1 and visit 2 were run through the FETA software separately. **Figure 2-5** shows the FETA output sheet where each case has one row and columns contain the average daily intake of all food consumed were grouped into two levels, 14 food groups and 46 macro and micronutrients. FETA output from both visits was coded according to the lists provided by the FETA software user documents to food groups, macro and micronutrients then merged with the main datasheet.

	A	B	C	D	E	F	G	H	I	J	K
1	ID	NUTRIENT_1	NUTRIENT_2	NUTRIENT_5	NUTRIENT_8	NUTRIENT_9	NUTRIENT_11	NUTRIENT_12	NUTRIENT_13	NUTRIENT_15	NUTRIENT_20
2											
3	1001	534.4225	6.39354	1970.96916	530.7111	2331.69758	128.46001	247.5449	2797.29135	0.659442	8.47028
4	1002	94.9921	1.30333	448.44555	366.8236	553.66815	74.41257	137.1982	1323.7822	0.31353	3.91713
5	1003	197.6777	11.44224	1319.16183	1027.006	1649.25739	235.84212	173.9223	3229.2407	0.931481	10.78895
6	1004	533.9392	4.87104	2565.4213	871.9029	2889.2443	274.57485	593.0318	6732.8088	1.913388	18.72607
7	1005	283.6414	35.611	1341.1462	502.4809	1495.9032	104.36314	255.3493	3139.8913	0.853999	9.28516
8	1006	525.0944	69.23962	2126.34415	965.769	2606.82755	267.53996	349.3455	4949.5605	1.202305	11.28956

Figure 2-5. FETA output for EPIC-FFQ analysis. One row was allocated to each patient and the columns contain the amount of energy, nutrients or food group consumed.

Data merging: For the seAFOod trial, the demographic data, the FETA input and output (visit 1 and 2), the adenoma characteristics data (baseline and exit) and the randomisation were all merged in one worksheet using the patients' ID number as a reference for merging in SPSS. The next step was validation of the data by comparing the variables with the results from the seAFOod clinical trial that was published in 2018 (126). The validation results are provided in the validation chapter.

Dietary data Exclusion criteria

Only 692 of the 707 patients recruited provided dietary data at baseline and 552 patient provided dietary data at exit. The cases excluded from the analysis according to the FETA software providers instructions that i) FFQs with more than 10 missing ticks should be excluded from the analysis due to the possible impact on the accuracy of the dietary data, ii) the outliers that are identified as the 0.5% of the lower and the upper values of the distribution of the ratio between the Estimated Energy

Intake (EEI) extracted by the reported dietary intake to the Basal Metabolic Rate (EEI: BMR ratio) (161).

Calculating BMR: BMR was calculated using Henry BMR prediction equation:

$$BMR = weight\ coefficient \times weight\ (kg) + height\ coefficient\ \times\ height\ (m) + constant$$

When the height of a patient was not available, an alternative Henry's equation was used with different coefficients: $BMR\ =\ weight\ coefficient\ x\ weight\ (kg)\ +\ constant$

Weight coefficient, height coefficient and the constant obtained from SACN

Dietary reference values for energy 2011 (163). **Table 2-3** shows a compression between the age band applied in this report and the age band used by Henry's equation (164).

The age band used in this report	***Henry BMR prediction equation age bands***
55-65	*30-60*
>65	*>60*

Table 2-3. Age bands applied in Henry BMR prediction equation this report

Two cases from visit 1 and two cases in visit 2 had no weight data available, however, after excluding the 0.5% of the EEI: BMR ratio, the energy intake of these four cases was within the normal range. Therefore, only eight cases were excluded at this point from visit 1 and 6 cases from visit 2 (**Figure 2-6**)

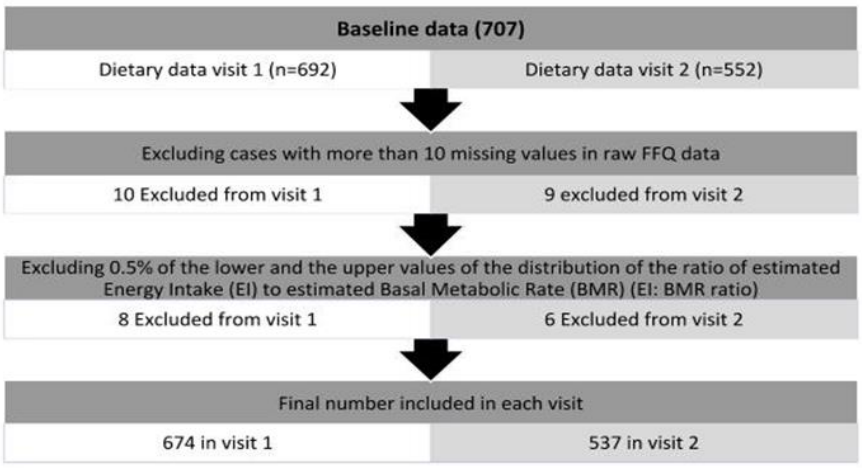


Figure 2-6. Excluded cases from each visit with reasons.

2.2.2.2 The FACT study data preparation

Dietary data preparation FACT: The first step was manual data entry from the 98 EPIC FFQ into one excel sheet, data was then modified to be compatible with the FETA software, details are provided in **Appendix 2**, in summary:

1. The quantitative section of the FFQ was divided into ten food groups, i.e., Meat and fish, Bread and Savory Biscuits, Dairy products and fats, etc.
2. The number of questions and the food items included in each group were compared.
3. The unavailable questions in the modified EPIC FFQ version used by the FACT study were either replaced by a similar question in the original version or an assumption of not consuming the food was taking, for example: one of the questions in the original version of FFQ that was needed by the software but was not included in the FFQ version used by the FACT study was related to the frequency of consumption of roe. To overcome this problem, an extra column was added to the excel sheet with the name "roe" and was coded as (1) for all participants (code 1 is an indicator for zero consumption).
4. An example for replacing a question, a question about the consumption of scones was available in the modified version used by the FACT study and is not used in the original version, and so is not required by FETA software, the answer to this question was allocated to the homemade buns question that is required by FETA, but not asked in the FFQ version used by the FACT study.
5. After data was modified, data preparation was required before running the analysis in FETA software, this included renaming and re-coding (clarified in the seAFOod trial dietary data preparation **Appendix 1**).

Dietary data analysis

As with the seAFOod trial data, the data was run twice using FETA software. The first was to extract the 14 food groups and 46 macro and micronutrients using the "Wide-format" FETA output. The 2nd was to extract oily fish, red and processed meat group using the "By ffq line" option.

Data merging

The FACT study participants' baseline data was merged with the dietary data (FETA input and output) and the biomarkers data. The patients' ID numbers were used as a reference number for merging in SPSS. The final step was merging the data for males from the seAFOod trial with the FACT study date to perform the comparison analysis needed for **Chapter 8.**

2.2.3 Dietary patterns

As mentioned previously, dietary patterns can be measured using two dietary pattern analysis approaches: the data-driven approach ignores all the previous knowledge about the association between diet and the disease under the study and describes the current dietary behaviour of the population. It was conducted on the data provided from only one study (The seAFOod trial data) due to statistical requirements that were met only by this study. The 2nd approach, the predefined dietary pattern approach is based on measuring the degree of adherence to a predefined score that was developed according to the evidence about the role of a specific dietary component of the health outcome. The method used in this research is the Dietary Inflammatory Index (DII) method. This method assesses the inflammatory potential of the diet and was applied to the data provided from both studies.

2.2.3.1 Data-driven dietary pattern approach, using Principal Component Analysis (PCA)

PCA is a statistical technique used to compress the useful information that was scattered in a large set of variables to produce a smaller set of new variables according to the observed linear combination between the original variables. In another words, PCA is used to reduce the dimension of the data. As an example, if 10 variables used in the analysis, the process will produce 10 components. However, the technique helps the researcher to decide about the important components by identifying the variables with the strongest correlation and put them in the first component, and the 2nd strongest correlations in the 2nd component until the weakest correlation in the last (10th) component. This way the data is reduced into a fewer number of components without losing significant information. The technique involves decision making at the step of deciding the number of the component to be used or omitted and in labelling and interpreting the retained components. PCA is widely used to extract the dietary patterns (165)[143]. It identifies the common dietary patterns by measuring the correlation between food groups or items used in the model. SPSS version 26 was used to conduct PCA on the seAFOod trial dietary data to extract the dietary patterns. First of all, the steps involved in the PCA procedure in SPSS will be explained briefly.

Steps involved in PCA

Data standardization: The first step is standardizing the food groups' data to the same scale. The aim of this is that each of the food groups contribute equally to the analysis.

Assessing the suitability of the data: This step involves two measurements: the first is determining if the sample size is suitable for the test, and the 2nd is to assess the correlation and dependency

between the variables. For the sample size, the PCA technique requires at least 10 subjects for each variable and SPSS software run the Kaiser-Meyer-Olkin (KMO) test to check sampling adequacy. KMO with high values (close to 1.0) indicates the adequacy of the sample size and values less than 0.6 indicates data is not suitable to conduct PCA.

For the correlation and dependency, the correlation matrix produced by the test shows the strength and the direction of the correlation between the variables. SPSS uses the Bartlett's test of sphericity to check that the correlations between the variables are adequate to be condensed into a smaller number of components. A non-significant *p*-value means that variables are not related and therefore the data is not suitable for PCA.

Factor extraction: This step is to find the smaller number of components that would explain as much of the variance between the original variables. The following measurements are used to assist in deciding which components to retain:

i) The Kaiser criterion or the (eigenvalue rule): is the amount of variance explained by each extracted factor. The rule is only factors with an eigenvalue of one or more to be retained for further analysis.

ii) Scree test: this is a plot for the eigenvalues of all the factors. It is used to asses in deciding the factors to keep for further analysis. The idea is to find and retain the factors above the point where the curve changes its direction to become horizontal.

Factor rotation and interpretation: At this stage, the variables that are correlated with each other are clustered together into separate components. There is another optional step in the test where the researcher has the option to rotate the components (without changing the original outcomes) to put them in a simple structure that is easier to interpret. There are two methods to rotate the data: oblique rotation which used when the component is correlated, the orthogonal rotation is used when the components are not correlated. When the association between the components is not clear, it is advised to start with the oblique rotation and if the rotated components are not easy to interpret run the orthogonal rotation (166). The software produces two tables to show all the components with eigenvalue more than 1, one table for the loading values before rotation and the other is for the loading value after rotation. The significance of this association is defined by the magnitude and the direction of the loading values. Variables with a loading value of > |0.3| are strongly associated with the component and should be considered in its interpretation. Positive loading values mean positive correlation and vice versa. At the end of this analysis, each individual in the sample will have a

continuous score for retained components, the scores reflect the adherence of that individual to that component.

The final stage is deciding which components to retain and how to label and interpret each one. This step is performed by the researcher and it depends on the knowledge and understanding of the contents of each component.

Measuring data-driven dietary pattern for the seAFOod trial using PCA technique

Dietary data collected at baseline from 674 colorectal adenoma patients recruited to the seAFOod trial was used in this analysis. The 14 food groups extracted by FETA software from the EPIC FFQ collected at baseline were used as the dietary components. SPSS version 26 was used to perform the PCA and to reduce the fourteen groups (**Table 2-4**) into a limited number of dietary patterns that represent the dietary behaviour of this high-risk cohort. The fourteen food groups were chosen because of two reasons, the first is that it covers all the foods consumed by this cohort; the 2nd is that the number of variables (food groups) is suitable for the PCA procedure (more than 10 cases per one variable). At the end of this procedure, each of the dietary patterns extracted will be categorised by high and low intake of food groups included in the model. This procedure will also order the cases according to their association with each of the generated dietary patterns by giving a score for each of the 674 cases included in the analysis for each generated component (or dietary pattern).

Table 2-4. The fourteen food groups extracted by FETA software from dietary data reported in EPIC FFQ and used to extract dietary patterns followed by the seAFOod trial cohort using PCA

Alcoholic beverages	***Milk and milk products***
Cereals and cereal products	*Non-alcoholic beverages*
Eggs and egg dishes	*Nuts and seeds*
Fats and oils	*Potatoes*
Fish and fish products	*Soups and sauces*
Fruit	*Sugars, preserves and snacks*
Meat and meat products	*Vegetables*

PCA procedure:

Data suitability check: To ensure the suitability of the data for PCA both Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were conducted.

Factor extraction: Component extracted based on eigenvalue greater than one and scree plot was also used to aid in deciding about the number of components to retain.

Factor rotation: The factors were rotated using the Oblique oblimin rotation method.

Factors labelling and interpretation: The decision about the number of dietary patterns to retain depends on the eigenvalue, the position on the scree plot and the interpretability of the pattern. Components with food groups' loading factors of $|\geq 0.3|$ will be considered as a significant dietary pattern. Each significant dietary pattern will be labelled according to the type and characteristics of the food groups with significant loading factor.

2.2.3.2 The predefined approach, the Dietary Inflammatory Index (DII) method

An overview

DII is a tool used to measure the inflammatory potential of the diet; it is used to assess an individual's diet on a scale from maximally anti-inflammatory (a negative DII score) to a maximally pro-inflammatory (a positive DII score). It was developed through the evaluation and scoring of nearly 2000 scientific articles that explored the association between diet and the following six inflammatory biomarkers: CRP, tumour necrosis factor and interleukin (IL): IL-1, IL-4, IL-6, IL-10. Forty-five parameters included in its calculation, including foods, nutrients, and other bioactive compounds (**Table 2-5-C**). Nine of the 45 parameters included in the calculating DII has a proinflammatory effect and 36 has an anti-inflammatory effect **(Appendix 4).**

Calculating DII for the data received from the seAFOod and the FACT study:

Table 2-5 shows the 45 food parameters required to calculate the DII score**,** only 25 of them are extracted by the FETA software and were used to calculate the DII for the FACT study **(Table 2-5 A)**. For the seAFOod trial, an extra 5 food parameters were extracted from the raw EPIC FFQ; Garlic, Onions, Green or Black tea and Peppers were extracted from the raw data **(Appendix 5)** and the ω-3 fatty acids were extracted by an external collaborator. **Table 2-5 B** shows the parameters used to calculate the DII for the seAFOod trial. The difference in the number of the parameters used in each study was for validation purpose and will be discussed in detail in the validation **Chapter 3 (Section 3.3.4).**

Table 2-5. Pro and anti-inflammatory foods and nutrients parameters required to measure the DII (A available for the FACT study, B for the seAFOod trial data, and C all 45 parameters required by the original method)

		Pro-inflammatory effect		Anti-inflammatory effect	
C. All food and nutrients parameters used to calculate DII (n=45)	B. Available for the seAFOod trial (n=30)	A. Available for the FACT study (n=25)	B12	Alcohol	Riboflavin
			carbohydrate	B6	Selenium
			Cholesterol	B-carotene	Thiamin
			Energy	Fibre	Vit_A
			Fat	Folic Acid	Vit_C
			Iron	Magnesium	Vit_D
			Protein	MUFA	Vit_E
			SFA	Niacin	Zinc
				PUFA	
				Tea	ω3 Fatty acids
				Pepper	Garlic
				Onions	
			Trans fat	Caffeine	Flavones
				Eugenol	Flavonols
				Ginger	Flavanones
				n-6 Fatty acids	Anthocyanidins
				Saffron	Isoflavones
				Turmeric	Thyme or oregano
				Flavanol-3	Rosemary

The general method for DII calculation used for both studies:

The published method in 2014 (144) was used to calculate the DII for the seAFOod trial and the FACT study participants as following:

1. The average daily intake of the available foods and nutrients included in calculating the DII for each participant were linked to the global database (The global database was developed from data obtained from eleven countries around the world for each of the 45 foods or nutrients).
2. The next step was measuring the Z score for the foods or nutrients available by using the mean and SD of the global intake as follow:

$$Z\ score = \frac{The\ reported\ amount\ of\ food\ or\ nutrient\ consumed - global\ standard\ mean}{Respective\ global\ standard\ deviation}$$

3. To reduce the effect of the right skewing of each dietary factor, the Z score was converted to a percentile score for each patient using SPSS.
4. Multiply the cantered percentile value obtained from the previous step and then subtract 1 from each value.
5. Multiply cantered percentile value for each food parameters by the respective specific inflammatory effect score provided in **Appendix 4.**
6. The overall DII score is then measured by the sum of all the food parameters specific DII score for each individual.

Summary

This chapter provided background about the sources of data included in this research project and described briefly the data preparation steps, the methods used to extract the average daily intake of foods and nutrients and preparing the data for further analysis. The final section showed the dietary patterns' methods used to measure the dietary patterns followed by our samples using two approaches. The following **Chapter 3** will show the different procedures that were conducted to validate the data to ensure its suitability before we use it in further analysis.

Chapter 3

Data verification

Chapter 3 Data verification (Objective 1).

This chapter illustrates the various procedures conducted to validate the data obtained from the seAFOod polyp prevention trial before performing the analysis required to achieve the aim of this thesis. This includes verification for the data preparation methods, validation for reported dietary and alcohol intake and the in-house method used to calculate the Dietary Inflammatory Index (DII).

3.1 Background

The seAFOod trial data

Many factors may affect the accuracy of the data of the seAFOod trial. For example, researchers may make mistakes during data handling and processing and patients could provide inaccurate data when self-reporting their dietary intake. Both sources of errors may affect the accuracy of the results of this project.

Data inaccuracy can result during data preparation, handling and processing. As **Chapter 2** shows, there was considerable manipulation of the data during preparation that could jeopardise the accuracy of the results. Therefore, a validation procedure was required.

Measurement errors of the FFQ are classified into two types: the ***systematic errors***, which lead to inability to detect the association between the dietary intake and the outcome, and ***random errors*** that decrease the precision of the data and lead to incorrect classification for individuals within the measured range of intake. Although measurement errors are inventible in any dietary assessment method, high proportion of errors should be avoided to minimise the negative impact it has on the accuracy of the research results (73).

Several methods are used to assess the type and level of dietary assessment errors, which usually require a 2nd independent measurement to validate the main data. Therefore, the validation method should be ideally considered during the study planning and designing stage. Validation methods range from an expensive and reliable methods such as using biomarkers, to a simple and inexpensive methods such as comparing the means of nutrient intake with others obtained from another source (140). However, since this study is based on a secondary data analysis, the validation of the dietary data was restricted by the available data and therefore the validation was conducted by comparing the results with the results from another studies.

DII calculation

The Dietary Inflammatory Index (DII) is a literature based calculation tool that aims to assess the potential inflammation of the diet. Due to the previous findings suggesting that CRC is associated with proinflammatory diet assessed using DII (151), DII score was the predefined dietary pattern analysis method of choice in this project. To calculate the DII for the participants recruited to the seAFOod trial, we used an in house method following the published method in 2014 (144). Calculating the DII score for the seAFOod trial using the original algorithm data required sharing the data with an external collaborator. This was not possible due to the data-sharing restriction agreements with the seAFOod trial data providers. Therefore, the FACT study dietary data was used to validate the in-house method. The DII was calculated twice, by using the in-house method and using the original algorithm, after that the correlation between the results obtained from the two methods was assessed to validate the accuracy of the in-house method.

3.2 Aim and objectives

The main aim of this chapter is to validate the processed demographic and adenoma characteristics data, the reported diet and alcohol intake, and the in-house method used to calculate the DII before using the data to explore the association between dietary intake and colorectal adenoma development and recurrence.

This aim was achieved through the following objectives:

1. To validate the randomisation, demographic and adenoma characteristics data.
2. To conduct internal and external validation of the FFQ dietary intake data reported during the seAFOod trial and identify the extent of misreporting.
3. To validate alcohol intake reported by the seAFOod trial participants in the EPIC FFQ.
4. To validate the in-house method used to measure the DII score for the seAFOod trial participants.

3.3 Method

This chapter is divided over four sections. Each section uses a different method and data to achieve its objectives **Figure 3-1** shows the objective (yellow) and the methods (blue) for each section.

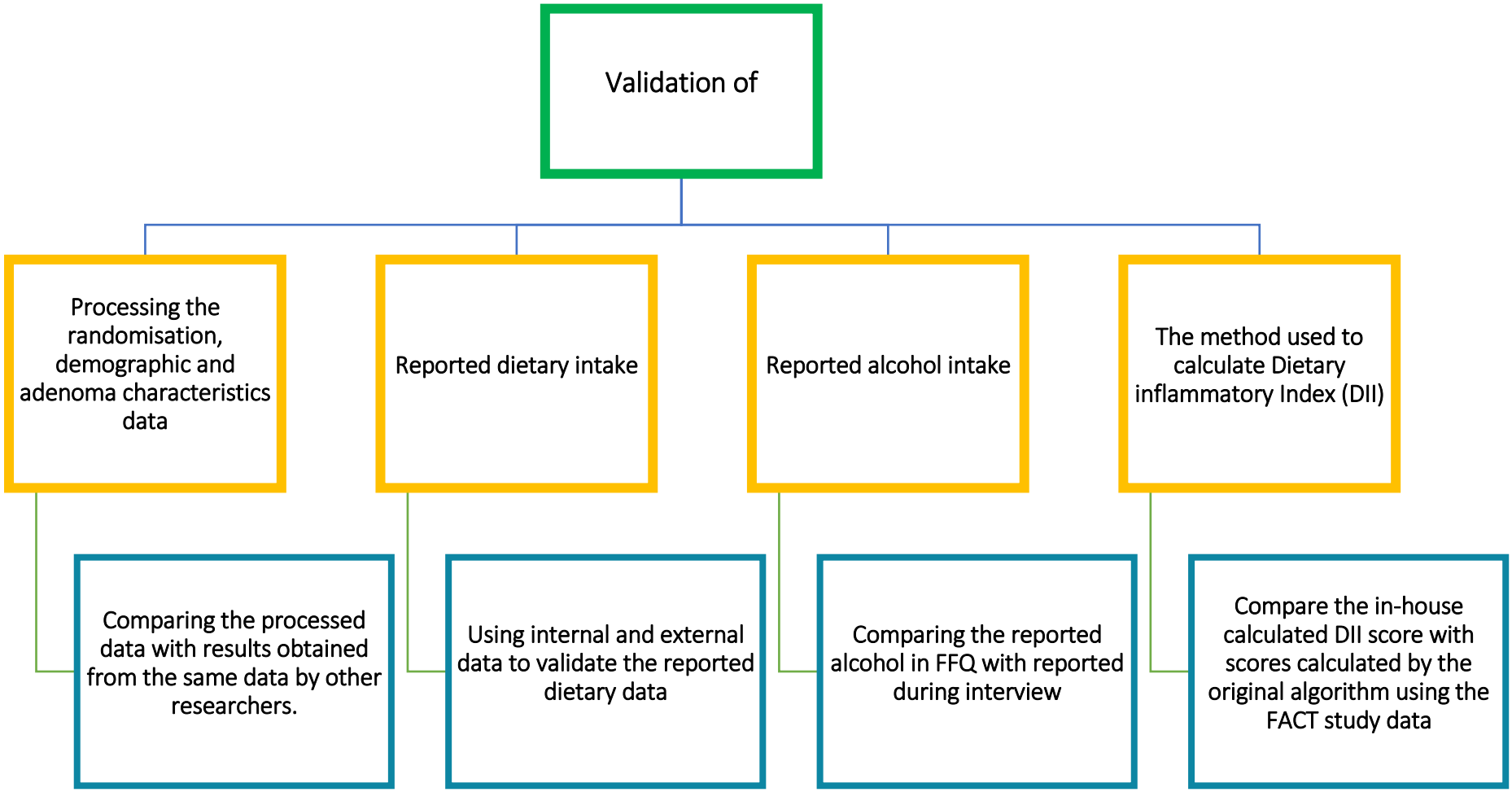


Figure 3-1. Objective of each section of the validation chapter and how it was achieved

3.3.1 Checking the accuracy of the seAFOod trial data (Randomisation , demographics and adenoma characteristics data).

As the methods section in **Chapter 2** shows, many procedures and calculations were performed on the data before merging into one worksheet. In this section, we check the accuracy of the obtained data before performing further analysis.

3.3.1.1 Objective

To check if data processing and calculation procedures performed while preparing the data did not affect the accuracy of the data

3.3.1.2 Method

Validation of the data was performed by the following steps:

a) Measure the distribution of the following variables: age, sex, BMI, adenoma characteristics at baseline and exit among the intervention arms.
b) Compare the results with the figures obtained for the same data by other researchers, and was published previously (126).

3.3.1.3 Results

As **table 3-1** shows, data obtained from the in-house calculation was same as that was published, in terms of the number of patients in each intervention arm, their sex and age. Two differences were detected: 1) the total number of adenoma in the EPA and aspirin intervention arm at baseline, which also affected the total number of adenomas, 2) the number of patients classified as obese in the placebo and EPA arms.

Nottingham clinical trial centre, which is responsible for managing the seAFOod trial data, was contacted and a justification was provided for each detected difference. For the difference in the number of adenomas at baseline, they confirmed that the difference was due to an error in their calculations.

In terms of the difference in the number of obese cases, the difference was due to an anomaly at the data coding step in their calculation process. The three extra obese cases were missing data that were identified by the software as obese cases due to coding error

Table 3-1. A comparison between the results obtained from the in-house calculations and data published in the Lancet paper for the seAFOod trial

	In-house calculations					***Published data***				
Intervention arm	*Placebo*	*EPA*	*Aspirin*	*EPA/Asp*	*Total*	*Placebo*	*EPA*	*Aspirin*	*EPA/Asp*	*Total*
Baseline data										
No. of patients	*176*	*178*	*176*	*177*	*707*	*176*	*178*	*176*	*177*	*707*
Age median (IQR)	*65 (62-69)*	*65 (62-68)*	*65 (62-69)*	*65 (62-69)*	*65 (62-69)*	*65 (62–69)*	*65 (62–68)*	*65 (62–69)*	*66 (62–69)*	*65 (62–69)*
Male n (%)	*139 (79)*	*138 (78)*	*140 (80)*	*146 (82)*	*563(80)*	*139 (79)*	*138 (78)*	*140 (80)*	*146 (82)*	*563 (80)*
Female n (%)	*37 (21)*	*40 (22)*	*36 (20)*	*31 (18)*	*144(20)*	*37 (21)*	*40 (22)*	*36 (20)*	*31 (18)*	*144 (20)*
BMI categories										
Overweight (25–29·9) n (%)	*76 (43.4)*	*77 (43.8)*	*81 (46)*	*77 (43.5)*	*311(44.2)*	*76 (43)*	*77 (43)*	*81 (46)*	*77 (44)*	*311 (44)*
Obese (≥30) * n (%)	*67 (38.3)*	*68 (38.6)*	*71 (40.3)*	*61 (34.5)*	*267(37.9)*	*68 (39)*	*70 (39)*	*71 (40)*	*61 (34)*	*270 (38)*
Adenoma characteristics at baseline										
Total number of adenomas *	*856*	*892*	*927*	*853*	*3528*	*856*	*892*	*927*	*856*	*3531*
>1 proximal adenoma	*141*	*146*	*153*	*144*	*584*	*141*	*146*	*153*	*144*	*584*
Conventional	*812*	*844*	*895*	*809*	*3360*	*812*	*844*	*895*	*809*	*3360*
Tubular or tubulovillous	*807*	*834*	*885*	*803*	*3329*	*807*	*834*	*885*	*803*	*3329*
Villous	*5*	*10*	*10*	*6*	*31*	*5*	*10*	*10*	*6*	*31*
Serrated	*22*	*30*	*18*	*21*	*91*	*22*	*30*	*18*	*21*	*91*
Adenoma characteristics at exit										
No. of patients	*163*	*153*	*163*	*161*	*640*	*163*	*153*	*163*	*161*	*640*
Patients' with≥ 1 adenoma n (%)	*100 (61)*	*97 (63)*	*100 (61)*	*98 (61)*	*395*	*100 (61)*	*97 (63)*	*100 (61)*	*98 (61)*	*395*
No. of adenomas	*231*	*238*	*208*	*166*	*844*	*231*	*238*	*209*	*166*	*844*
Conventional	*220*	*205*	*194*	*155*	*774*	*220*	*205*	*194*	*155*	*774*
Serrated	*8*	*21*	*10*	*4*	*43*	*8*	*21*	*10*	*4*	*43*
Left side	*93*	*98*	*101*	*58*	*305*	*93*	*98*	*101*	*58*	*350*
Right side	*138*	*140*	*107*	*108*	*493*	*138*	*140*	*107*	*108*	*493*

Difference was detected in the total number of **adenomas and the number of cases within the obese BMI category*

3.3.2 Evaluating the level of dietary data misreporting in the EPIC FFQs obtained from the seAFOod trial

3.3.2.1 Aim and objectives:

The aim of this section was to conduct an internal and external validation of the FFQ dietary intake data reported in the seAFOod trial and identify the extent of misreporting. This was achieved through the following objectives:

1. To detect any errors that might occur during dietary data handling and processing.
2. To assess the percentage and characteristics of energy under-reporters (energy-under reporters).
3. To assess misreporting in specific food groups and to explore whether there is an association between energy under-reporting and food groups misreporting.
4. To explore the effect of the measured level of misreporting on data accuracy and the ranking of patients.

3.3.2.2 Materials

Data provided by the seAFOod trial

A brief description of the data provided by the seAFOod trial is provided in **Chapter 2 (Section 2.2.1)** and a full description was previously published in 2013 (156). In summary, 707 colorectal adenoma patients were recruited through the BCSP and the bowel scope screening from 63 centres from around England. Anthropometric data and dietary intake data from the seAFOod trial were used in this section. **Figure 3-2** shows a summary of the aim and objectives of section 2 in this **chapter 3** and the methods used to achieve them

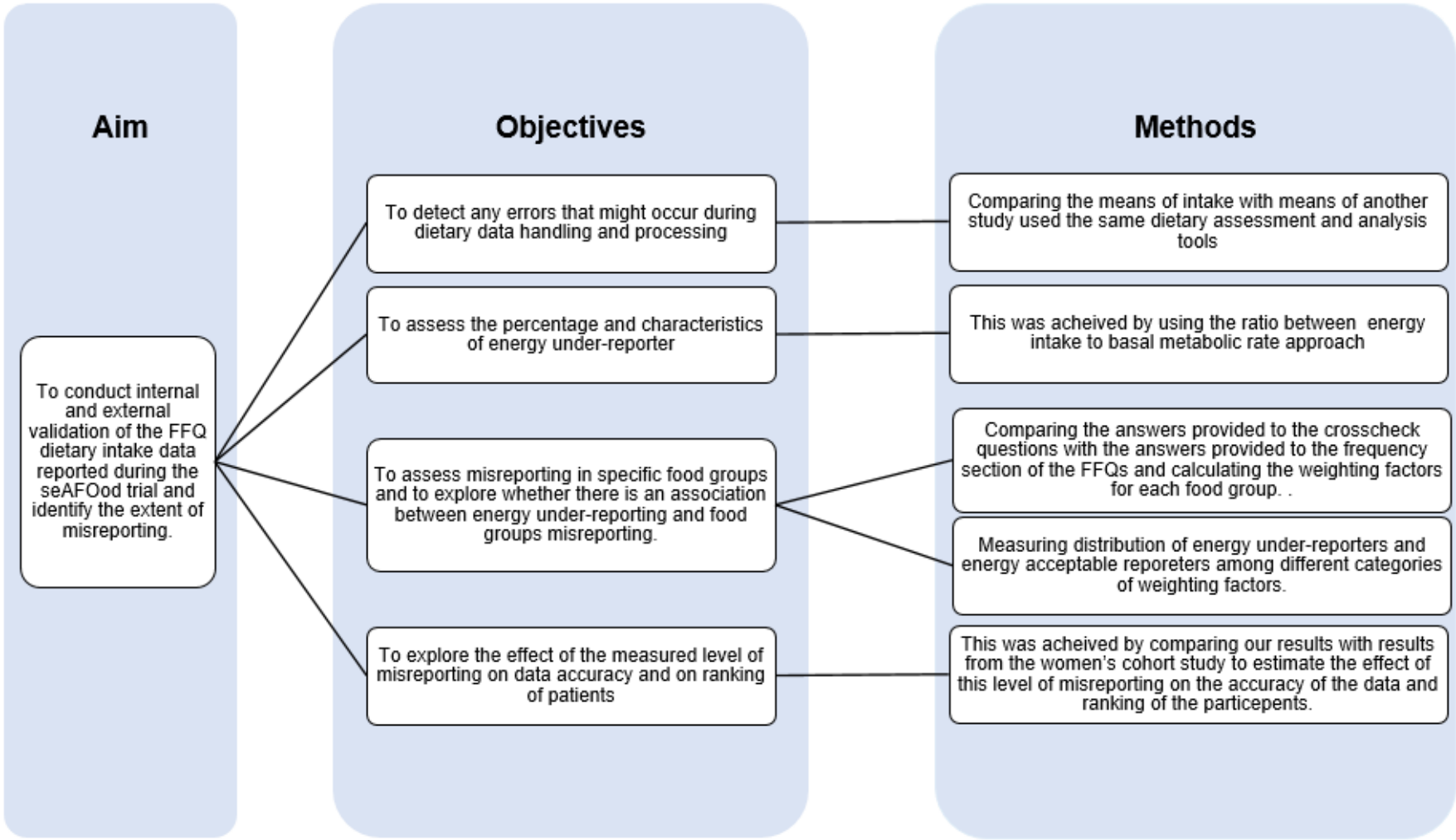


Figure 3-2. A summary of the aim and objectives of section 2 in chapter 3 and the methods used to achieve them

Data used in the comparison

Dietary data reported by the seAFOod trial participants was compared with data from two other studies. The first comparison was made by comparing the FETA output of selected macro and micronutrients with results from the Food4Me study(167). The Food4Me study used the same dietary assessment (EPIC-FFQ) and analysis tools (FETA software). The 2nd external data was used is the Women's Cohort Study (WCS) data (168), which was used to assess the effect of dietary data misreporting on the nutrients intake and ranking of the participants. The WCS used the EPIC FFQ but did not use the FETA software to analyse them.

Data obtained from the Food4Me study

The 1st set of data was taken from a study that aimed to compare the EPIC FFQs with a new online assessment tool (Food4Me FFQ) (167). In the Food4Me study, some modifications were made on the EPIC- FFQ, to assess the dietary intake over the past month instead of past 12 months and the FETA software, was used to analyse the dietary data. Data were obtained from 113 participants from two centres: the University College Dublin and the University of Reading. Fifty-nine percent of the participants in the Food4me study were females, the mean age was 30 (SD=10.2) years and the mean BMI was 23.3 (SD=2.9) Kg/m^2.

Data obtained from the Women's Cohort Study (WCS).

The 2nd external set of data used in this validation chapter was from the Women's Cohort Study (WCS) (168). The data obtained from 6572 women aged 35 to 69 years who responded to a postal EPIC-FFQ. The aim of this study was "*To explore the potential misreporting of specific food groups from food frequency questionnaire (FFQ) data and to examine the effect of using a weighting factor on estimated nutrient intake and ranking of subjects within the cohort according to nutrient intake*". To achieve this aim, the investigators used the answers for the food groups intake reported in the first section of the FFQ (the frequency section) and in the 2nd section (the cross-check question). The details of the method were published in details previously (168). In summary, they calculated the Weighting Factor (WF) for the four food groups (fish, fruit, vegetables and meat) by dividing the amount reported in the cross-check questions over the amount reported in the frequency sections of the FFQs. The measured WFs were used to: i) classify the responders into five categories according to the similarity between their responses to the two type of questions (frequency and cross-check questions) and ii) to assess the effect of the level of food groups' misreporting on the overall nutrients intake. This was by analysing the dietary data before and after applying all and each WF.

3.3.2.3 Methods

Various calculations and analysis were performed to identify the level of misreporting and to predict the effect of this misreporting on the accuracy of the data and the ranking of the individuals within the measured variables, as follow:

3.3.2.3.1 Checking accuracy of dietary data after processing and analysis.

To detect errors that might occur during dietary data handling and processing the means of selected macro and micronutrient intake were compared with data obtained from another study (Food4Me study) that used the same dietary assessment and analysis tools (167).

3.3.2.3.2 Evaluate the percentage and characteristics of energy under-reporters

This was by identifying the energy-under reporters and comparing them with energy-acceptable reporters, in terms of age, sex, BMI and dietary intake.

ENERGY- UNDER REPORTERS

Energy-under reporters s were identified as patients who reported Estimated Energy Intake (EEI) that is lower than their Estimated Energy Requirements (EER). Two values are needed to estimate the EER: the Basal Metabolic Rate (BMR) and the Physical Activity Level (PAL). Henry's formula was used to estimate the BMR for males and females as previously described in **Chapter 2, (Section 2.2.2.1).** As information about PAL was not collected in the seAFOod trial, an assumption for PAL was needed to estimate the EER. Both age and BMI are inversely associated with PAL (169), and the minimum PAL required for sustainable lifestyles is 1.1 (170) . This PAL value was imputed for all the patients, based on the information we have that the majority of this cohort were in older age category (over 94% of them were more than 60 years of age) and 80% of them were overweight or obese, which also suggests that the majority of them were following a sedentary lifestyle. The EER was calculated as follows:

$$EER = BMR \times 1.1$$

The EEI was extracted by FETA software from dietary intake reported in the FFQ. Patients with a self-reported EEI lower than the estimated EER were classified as energy-under reporters and the rest of the patients were considered as energy-acceptable reporters.

3.3.2.3.3 Assessing misreporting in food groups intake and explore whether there is an association between energy under-reporting and food groups misreporting

The 3rd analysis was using the Cross-Check Questions (CCQs) to assess food groups' misreporting. In this section, we used the same approach that was used in the WCS (168). In summary, we measured the WFs for the food groups then inspected the food groups' misreporting level in patients classified as energy-under reporters and energy-acceptable reporters.

Within the EPIC FFQs, two types of questions are used to estimate the consumption of the four food groups: fruits, vegetables, meat and fish. **The first set of questions** asks about the frequency of consumption of several food items that are classified under the same food group category. Example for the food items for the fruit group is presented in **figure 3-3**.

PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
FRUIT **For seasonal fruits marked *, please estimate your average use when the fruit is in season**									
Apples (1 fruit)									
Pears (1 fruit)									
Oranges, satsumas, mandarins (1 fruit)									
Grapefruit (half)									
Bananas (1 fruit)									
Grapes (medium serving)									
Melon (1 slice)									
* Peaches, plums, apricots (1 fruit)									
* Strawberries, raspberries, kiwi fruit (medium serving)									
Tinned fruit (medium serving)									
Dried fruit, eg. raisins, prunes (medium serving)									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Figure 3-3. Example of the frequency section in EPIC FFQ for the fruits' food group

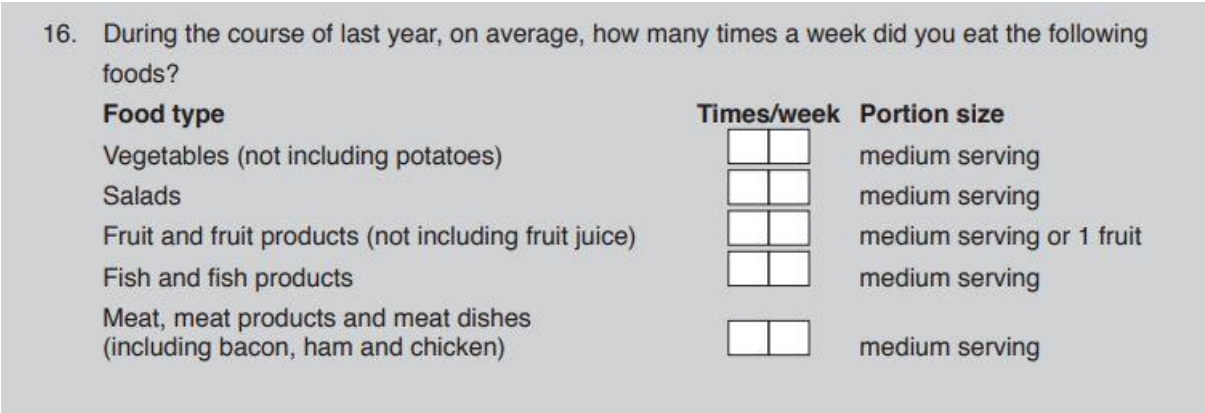
The food items in the frequency section included in each of the food groups used in FETA software is illustrated in **table 3-2**.

Table 3-2. Food items included in each food group in the frequency section of EPIC FFQ

Vegetables (25)		***Meat (12)***	***Fruit (12)***	***Fish (6)***
Garlic	*Spinach*	*Beef*	*Apples*	*Fried fish*
Mushrooms	*Broccoli*	*Burger*	*Pears*	*Fish fingers*
Peppers	*Sprouts*	*Pork*	*Oranges*	*Whitefish*
Beansprouts	*Cabbage*	*Lamb*	*Grapefruit*	*Oily fish*
Green salad	*Peas*	*Chicken*	*Bananas*	*Shellfish*
Watercress	*Green beans*	*Bacon*	*Grapes*	*Roe*
Tomatoes	*Marrow*	*Ham*	*Melons*	

Sweetcorn	*Cauliflower*	*Corned beef*	*Peaches*
Beetroot	*Parsnips*	*Sausages*	*Strawberries*
Coleslaw	*Leeks*	*Liver*	*Tinned fruit*
Beans	*Onions*	*savoury pies*	*Dried fruit*
Lentils	*Tofu*	*lasagne*	*Avocado*
Carrots			

The 2nd section of the EPIC-FFQ contains five cross-check questions (CCQ) one question for each food group **(Fig 3-4).** For analysis purpose, answers from CCQ 1 (vegetables) and2 (salads) were merged under "vegetable group" to reflect the vegetable intake and be used in the analysis.



16. During the course of last year, on average, how many times a week did you eat the following foods?

Food type	Times/week	Portion size
Vegetables (not including potatoes)	☐☐	medium serving
Salads	☐☐	medium serving
Fruit and fruit products (not including fruit juice)	☐☐	medium serving or 1 fruit
Fish and fish products	☐☐	medium serving
Meat, meat products and meat dishes (including bacon, ham and chicken)	☐☐	medium serving

Figure 3-4. The cross-check questions for food groups in the EPIC FFQ

Outliers

Understanding the CCQ by the respondents were examined visually, this was by checking the five answers provided by each patient. If the answers were all unrealistic numbers, the patient was considered not to have understood the questions and was excluded from the analysis. **Table 3-3** illustrate some of the patients that were excluded due to not understanding the CCQs. For fruit and vegetables, a cut-off point of 50 portions per week was used. Any values of more than 50 were considered as a missing value.

Table 3-3. Example for some of the cases who provided unrealistic answers to the CCQ and were excluded from the analysis

Patient ID/food group	***Vegetables****	***Meat****	***Fruit****	***Fish****
49001	*2*	*1*	*49*	*70*
61005	*68*	*34*	*7*	*56*
1004	*72*	*52*	*30*	*52*
8011	*14*	*50*	*12*	*50*
****food groups are provided as number of times consumed per week***				

Measuring portion per week from the frequency section

The purpose of this calculation is to measure the number of the portions of the food groups consumed per week from the responses to the frequency section of the FFQ. Frequencies for each food item in **table 3-4** were converted to the number of times per day.

Table 3-4. Values used to recode frequency of consumption of food items included in the frequency section of the FFQ to obtain the number of portions per day

FFQ category Frequency per day	*Answer in the FFQ*	*New variable: Frequency of consumption per Day*
Never or less than once / month	*1*	*0*
1 – 3 per month	*2*	*.07*
Once a week	*3*	*.14*
2-4 per week	*4*	*.43*
5-6 per week	*5*	*.79*
Once a day	*6*	*1*
2-3 per day	*7*	*2.5*
4-5 per day	*8*	*4.5*
6+ per day	*9*	*6*

After that, four new variables were created in SPSS, one for each food group. This new variable was the total of the food portions of the food items of that food group. The new variables were then multiplied by seven to obtain the number of portions consumed per week of each food group. This calculation was made assuming that patient will consider the same portion size for the same food item when they respond to the frequency question and the CCQs.

Calculating and classifying the Weighting Factor (WF) for each food group

1. The WF for each food group was calculated by dividing the responses to the CCQ *by* the variable obtained from the sum of the frequencies as follows:

$$WF = \frac{Number\ of\ portion\ per\ week\ of\ food\ intake\ from\ responce\ to\ the\ Cross-check\ questions}{Sum\ of\ frequencies\ provided\ in\ the\ first\ section\ of\ the\ frequecy\ questions}$$

2. Each participant had four WFs, one for each food group.
3. The weighting factors were classified into four categories according to the similarity between the responses to the frequency questions and responses to the CCQ. **Table 3-5** shows the range of WF included in each category.
4. Dealing with missing values and zeros when measuring the WFs:
   - If the response for the same food group was zero or missing in one method, while it has a value in the other method, the WF was deleted and considered as a missing value.

- When zero was the answer in both questions, the WF value was considered equal to one. For example, if the answer for fish intake frequency questions were zero and for the CCQ was also zero, the WF was considered as one (the responder was not a fish eater).

Table 3-5. Classifying the weighting factors according to the similarity between responses to the frequency questions and the CCQ.

Actual value of WF	***WF categories***	***Label***
Up to 0.200	*Up to 1/5*	*Much higher than CCQ*
From 0.201-0.666	*From 1/5 to 2/3*	*Higher than CCQ*
From 0.667-1.5	*From 2/3 to3/2*	*Similar to CCQ*
>1.5	*>3/2*	*Lower than CCQ*
CCQ=Cross-Check question		

The WFs' categories classified the participants according to the similarity of their responses to the two methods, after that, an assessment for the association between energy under-reporting

(assessed by EEI lower than the estimated EER) and food groups' misreporting was conducted. This was by assessing the proportion of energy-under reporters and energy-acceptable reporters within each category of food groups WFs.

3.3.2.3.4 Assessing the magnitude of the misreporting by comparing the results with the Women's Cohort Study

Results from an external study, the Women's Cohort Study (WCS) (168) was used to assess the magnitude of food groups' misreporting on the seAFOod trial participants. In the WCS, the frequency section of the FFQ was analysed twice; the first analysis was analysing the data as reported by the participants; the 2nd analysis was analysing the data after applying each and all of the food groups' WFs (fish, fruit, vegetables and meat). The aim of this was to investigate the impact of food group misreporting on the accuracy of nutrient intake and to check whether applying the WFs has an impact on the ranking of the individuals. To assess the magnitude of the misreporting of the seAFOod trial, the proportion of the seAFOod trial participants in each category of the WFs (showed in **table 3-5**) was compared with the proportions reported in the WCS study.

Statistical analysis

SPSS (version 26) was used to perform the descriptive analysis and perform the statistical tests. All data is reported in terms of mean (+/- SD). The strength of the association between different methods of measurements was assessed by the Pearson correlation coefficient. The agreement between CCQ and frequency in measuring specific food group intake was assessed by: Paired sample t-test in the case of normally distributed data, Wilcoxon signed ranks test for not normally distributed

data. Pearson Chi-square test was used to compare categorical data. An independent sample T-test was used to compare dietary intake between and WFs of energy-under reporters and energy-acceptable reporters. *P*-value <.05 was considered statistically significant.

Results

3.3.2.3.5 Accuracy of dietary data preparation and analysis

To check the accuracy of the dietary data of the seAFOod trial, the mean intake of energy and selected nutrients was compared between this data and data published by the Food4Me study (T**able 3-6)**. Mean intake of energy, alcohol, fat, protein and carbohydrate were higher in the seAFOod trial, but when controlled for energy, there was no difference in energy obtained from macronutrients. Micronutrients intake was higher in the seAFOod trial except for total carotene and vitamin C that were higher in the Food4Me study.

Table 3-6. Daily nutrient intakes estimated by EPIC- FFQ for data provided by the seAFOod trial and the Food4Me study

Average nutrient intake per day	***The seAFOod trial n= 674 Mean (SD)***	***Food4Me study n=113 Mean (SD)***
Energy (MJ)	*7.7 (2.44)*	*7.05 (2.02)*
Total Fat (g)	*70 (27.83)*	*66.1 (24.27)*
Total Fat (% TE)	*34 (6.12)*	*34.9 (6.18)*
SFA (g)	*25.9 (11.29)*	*24.9 (10.50)*
SFA (% TE)	*12.6 (3.12)*	*13.13 (3.40)*
MUFA(g)	*25.5 (10.79)*	*23.3 (8.75)*
MUFA (% TE)	*12.4 (2.62)*	*12.3 (2.35)*
PUFA(g)	*12.3(5.45)*	*12.2 (6.33)*
PUFA (% TE)	*6 (1.62)*	*6.4 (2.63)*
Protein (g)	*81.9 (23.42)*	*75.5 (21.82)*
Protein (% TE)	*18.4(3.47)*	*18.3 (4.24)*
Carbohydrate (g)	*207 (77.60)*	*197 (69.81)*
Carbohydrate (% TE)	*45.1 (7.53)*	*46.8 (8.14)*
Total sugars (g)	*103.3(44.81)*	*103.9 (45.78)*
Alcohol (g)	*13.5 (16.24)*	*7.2 (9.10)*
Calcium (mg)	*859.6 (307.75)*	*835 (255.85)*
Total folate (µg)	*286.4 (90.41)*	*250 (68.70)*
Iron (mg)	*11 (3.48)*	*9.7 (2.74)*
Total carotene (µg)	*3235 (1697.4)*	*3512 (2004)*
Riboflavin (mg)	*1.9 (.64)*	*1.74 (.47)*
Thiamine (mg)	*1.45(.46)*	*1.34 (.37)*
Vitamin B6 (mg)	*2.2 (.63)*	*2.03 (.52)*
Vitamin B12 (µg)	*7.2 (4.20)*	*5.9 (2.79)*
Vitamin C (mg)	*103 (48.9)*	*107(55.9)*
Vitamin D (µg)	*3.3 (1.77)*	*2.9 (1.90)*
Vitamin E (mg)	*11.3 (5.21)*	*10.6 (4.63)*
Sodium (mg)	*2647 (935.7)*	*2442 (772.86)*

FFQ: food frequency questionnaire. TE: total energy. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids.

3.3.2.3.6 Proportion of energy under reporters of the patients recruited to the seAFOod trial.

Minimum estimated energy intake from FFQ was 2.4 MJ and the maximum was 17.1 MJ. According to Henry's formula, the minimum BMR was 4.4 MJ and the maximum was 11.4MJ. According to

Goldberg (171) classification, EEI: BMR value of <0.76 is considered as underreporting, ratio of 0.76-1.24 indicated an acceptable range of reporting while ratio of >1.24 is classified as energy over-reporters. When this classification was used for the seAFOod trial participants', we found that 15.4% were energy-under reporters , 51.2% energy-acceptable reporters and 29.5% were classified as energy over-reporters. However, to use this classification, information about the PAL is needed for each individual to measure the EER precisely (172) . As the EER measured for the seAFOod trial participants was a rough estimate due to absence of information about physical activity, the Goldberg classification was not used and patients were categorised into two categories, energy-under reporters with EEI: EER of less than one and energy-acceptable reporters of ratio of ≥1. According to this classification, nearly 58% of the patients reported EEI that was lower than their EER. To assess the magnitude of energy misreporting, the difference between EEI and EER was computed. As figure **3-5 shows**, the mean of the difference was -56.7 (SD=2512.4) KJ, which indicates an overall energy under-reporting.

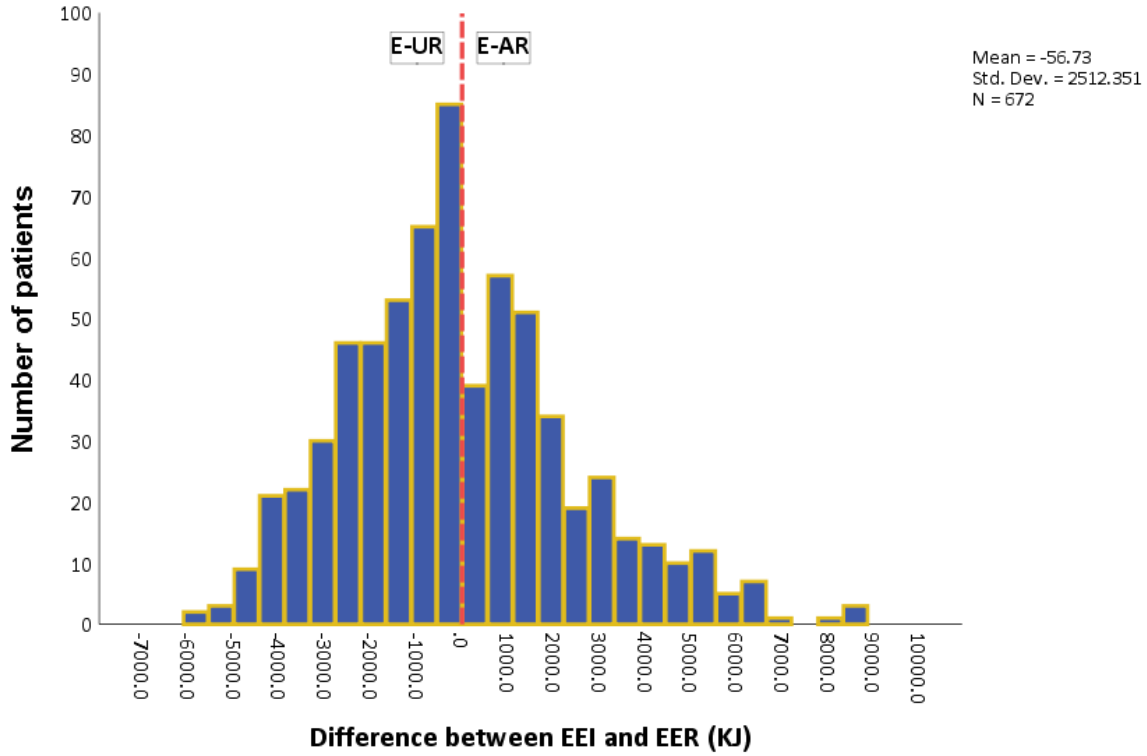


Figure 3-5. Distribution of the patients according to the difference between their EEI and EER

A comparison between energy-under reporters and energy-acceptable reporters in the seAFOod trial indicated that there was no significant difference in their age (p=.454) or sex (p=.27), however, energy-under reporters had a significantly higher BMI (p<.001) **(Table 3-7).**

Table 3-7. Comparing age, sex and BMI of energy-under reporters and energy-acceptable reporters in the seAFOod trial

	Acceptable Reporters	***Under Reporters***	***p (Independent sample t test)***
Age Mean (SD)	*65.4 (4.78)*	*65.1 (4.64)*	*.454*
BMI Mean (SD)	*28.2 (4.89)*	*30.1 (6.23)*	*<.005*
Sex			*p (Pearson Chi-square test)*
Males (n)	*219*	*314*	*.27*
Females (n)	*63*	*73*	

3.3.2.3.7 Using the crosscheck questions to assess misreporting in food groups.

Comparing the results of food group intake from the CCQ and from the frequency questions

Portion consumed per week of fish, fruit meat and vegetables measured by the two methods are provided in **table 3-8**.

Table 3-8. Comparing reported portions of food groups consumed per week in the CCQ and the frequency sections of the FFQ

	Range	***Median***	***Mean***	***SD***	***p****
Fish					
Cross-check Question	*0-10*	*2*	*1.9*	*1.21*	*<.005*
Frequency in main FFQ	*0-21*	*1.96*	*2.6*	*2.03*	
Fruit **					
Cross-check Question	*0-30*	*5*	*5.2*	*4.1*	*<.005***
Frequency in main FFQ	*0-84.5*	*11.5*	*14.1*	*11.8*	
Vegetables					
Cross-check Question	*0-50*	*7*	*7.8*	*4.5*	*<.005*
Frequency in main FFQ	*1.96-138*	*27.4*	*29.4*	*15.5*	
Meat					
Cross-check Question	*0-14*	*5*	*4.6*	*2.1*	*<.005*
Frequency in main FFQ	*0-29.5*	*9.4*	*9.9*	*4.8*	
****Paired sample T test ** Wilcoxon signed ranks test was used to compare fruit intake.***					

Significant differences were detected between the 2 reporting methods for all food groups as shown in **table 3-8.** Pearson correlation identified moderate to weak positive correlations between the 2 methods for all food groups (as shown in **Table 3-9**)

Table 3-9. Correlation between the food groups as reported in the frequency section and the CCQs in the EPIC-FFQ for the seAFOod trial

			Frequency section of FFQ			
			Fish	*Fruits*	*Vegetables*	*Meat*
Cross-check Question	*Fish*	*r*	*.530*	*.176*	*.288***	*-.008*
		p value	*<.001*	*<.001*	*<.001*	*.849*
	Fruits	*r*	*.107***	*.480*	*.207***	*-.054*
		p value	*.009*	*<.001*	*<.001*	*.185*
	Vegetables	*r*	*.141***	*.222***	*.388*	*-.101**
		p value	*<.001*	*<.001*	*<.001*	*.013*
	Meat	*r*	*-.208***	*-.058*	*-.018*	*.284*
		p value	*<.001*	*.154*	*.651*	*<.001*

r= Pearson Correlation coefficient , * p<.05, ** p<.001

The weighting factor for specific food groups and measuring patients' distribution among categories of weighting factor

Table 3-10 shows the number and percentage of participants in each of the five categories of food groups WFs. Only 3.8% of the participants reported similar vegetable intake using both methods of food groups' assessment methods, followed by fruits (22.5%), meat (25.8) and fish (53%).

Table 3-10. Ratio of estimated food groups' intake from CCQ to estimate from frequency section: number (%) of participants in each category

	Much higher than CCQ (WF <1/5)	***Higher than CCQ (WF =1/5 to2/3)***	***Similar to CCQ (WF=2/3to3/2)***	***Lower than CCQ (WF>3/2)***	***Total cases included***
Fish	*3 (.5%)*	*204 (34.9%)*	*312 (53.3%)*	*66 (11.3%)*	*585*
Fruit	*96 (16.6%)*	*338 (58.4%)*	*130 (22.5%)*	*15 (2.6%)*	*579*
Vegetable	*168 (28.1%)*	*405(67.7%)*	*23 (3.8%)*	*2 (.3%)*	*598*
Meat	*37 (6.2%)*	*395 (65.8%)*	*155 (25.8%)*	*13 (2.2)*	*600*

Food groups' misreporting among energy-under reporters and energy-acceptable reporters in the seafood trial

As **table 3-11** shows, means of the WFs of all food groups show that all participants (energy-under reporters and energy-acceptable reporters) under estimated their food group intake in the frequency section of the FFQ. However, the independent sample T test showed that the mean of WFs of energy-under reporters of meat, fruits and vegetables were significantly higher than energy-acceptable reporters s (p<.05), however, the difference was not statistically significant (*p=.*74) in WF of fish intake between the two groups. This indicates that, both energy-under reporters and energy-

acceptable reporters estimated similar consumption of fish when they answered the frequency section and the CCQ section of the FFQs.

Table 3-11. A comparison between weighting factors of energy under reporters and acceptable reporters in the seAFOod trial

	Category of responders according to EEI:EER ratio	*N*	*Mean (SD)*	**p value*
Fish	*Under reporter*	*336*	*.94 (6.31)*	*.74*
	Acceptable reporter	*247*	*.92 (.846)*	
Fruit	*Under reporter*	*340*	*.56 (.392)*	*<.001*
	Acceptable reporter	*237*	*.44 (.297)*	
Vegetables	*Under reporter*	*348*	*.34 (.359)*	*.002*
	Acceptable reporter	*248*	*.27 (.144)*	
Meat	*Under reporter*	*349*	*.62 (.476)*	*<.001*
	Acceptable reporter	*249*	*5 (.262)*	
***Independent sample t test**				

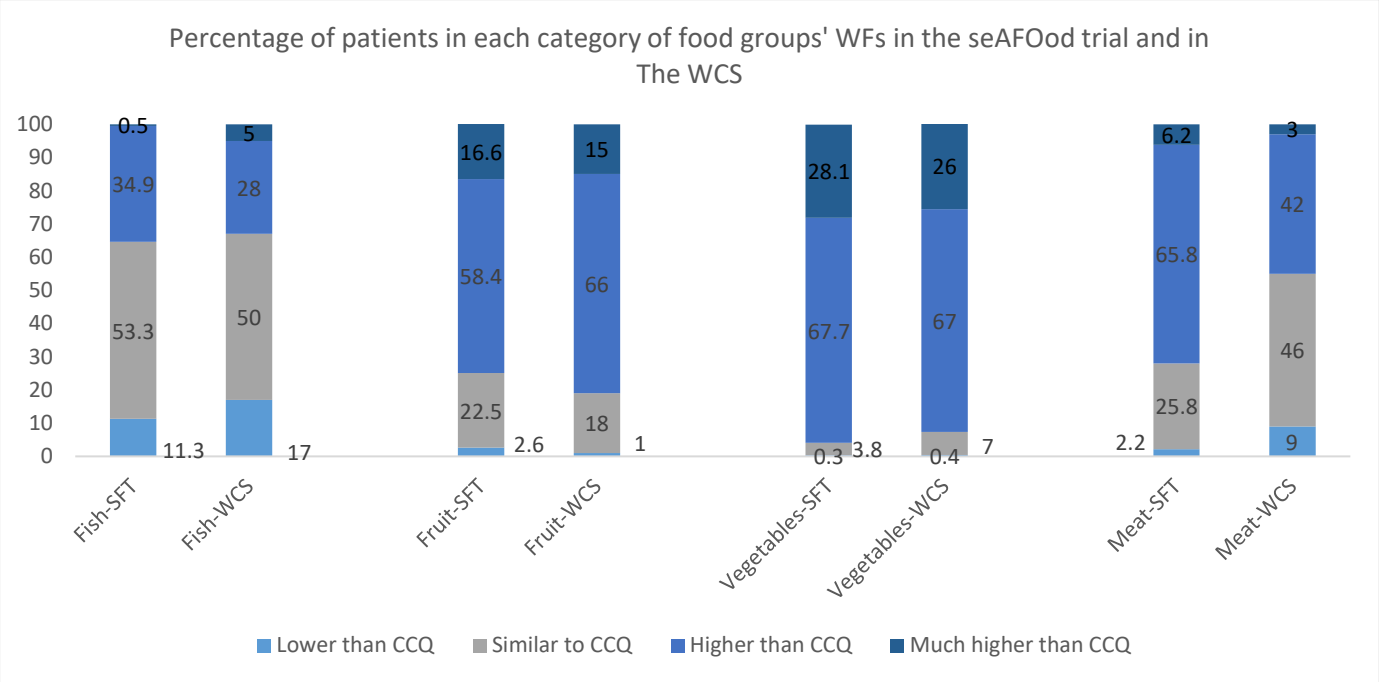
3.3.2.3.8 The magnitude of food groups' misreporting

In this analysis, the distribution of patients among the WFs' categorise was compared with the findings from the WCS. In the WCS, to investigate the impact of food groups' misreporting on the accuracy of the nutrients intake and the ranking of the individuals, the median intake before and after adjusting the analysis for the WFs calculated from the CCQ. As **Table 3-12** shows, adjusted analysis was performed five times, analysis after adjustment for each food group and analysis after adjustment for all food groups. . As **the table** shows, analysing the data after applying each and all of the WFs, had little impact on the median of intake and the ranking of the individuals in the energy and macronutrients intake. However, median intakes and ranking of individual in fibre, vitamin A and C were all affected `after applying the vegetables WF.

Table 3-12. Median intake of selected nutrients, energy and food groups. Standard analysis vs analysis adjusted by WF of each food group and all food groups (Obtained from the Women Cohort Study)

Nutrient	*Standard fruit median n = 5833*	*Weighted fruit median n = 5833*	*Standard vegetable median n = 6314*	*Weighted vegetable median n = 6314*	*Standard meat median n = 6062*	*Weighted meat median n = 6062*	*Standard fish median n = 6211*	*Weighted fish median n = 6211*	*Standard all median n = 5072*	*Weighted all median n = 5072*
Energy (Kcal)	2200	2117	2197	2076	2195	2157	2283	2188	2200	1956
Fat (g)	79	79	79	72	80	77	84	79	79	70
Cho (g)	289	272	289	278	289	288	303	289	290	259
Protein (g)	81	80	81	76	80	78	84	80	81	72
NSP (g)	23.3	21.3	23.2	19.5	23.1	23	24.6	23.2	23.2	17
Vitamin A (µg)	1015	998	1017	703	1018	987	1122	1017	1020	668
Vitamin C (mg)	151	127	151	109	150	149	166	151	151	85
Vitamin E (mg)	7.3	7.0	7.3	5.8	7.3	7.2	7.9	7.3	7.3	5.3
Folate (µg)	349	340	348	280	348	345	363	347	349	268

Figure 3-6 shows the percentage of patients in each category of the WFs in the seAFOod trial and in the WCS. The figure shows the distribution is similar in participants among fish, fruit and vegetables groups' WFs' categories. Regarding meat group, more participants' (75%) overestimated their meat intake in the seAFOod trial than in the WCS (45%).



SFT= seAFOod trial. WCS=Women's Cohort Study

Figure 3-6. The percentage of patients' in the categories of the weighting factor in the seAFOod trial and in the Women Cohort Study

Considering that the distribution of the patients among the WFs of fish, fruit and vegetables were very similar between the seAFOod trial and the WCS, similar effect would be expected from applying these WFs in the seAFOod trial. Regarding the meat WF, since the seAFOod trial participants overestimated their meat intake comparing with the WCS, there is an expectation that applying the meat WF on the seAFOod trial data would have an impact on the median of energy, fat, protein and iron intake.

3.3.3 Validation of reported alcohol intake.

Validation of the alcohol intake reported by the seAFOod trial participants was conducted by comparing the amount of alcohol reported in the frequency section of the EPIC FFQ with the amount reported by the patients during an interview with a research nurse at recruitment.

3.3.3.1 Materials

Mean daily alcohol intake in grams per day was extracted by FETA software from 669 valid EPIC FFQ provided by the patients in visit 1. Data for the same 669 patients for alcohol intake was available in the Case Record Form (CRF) at the beginning of the study. The question was about the number of alcohol units consumed per week and the options given were: none, 1-7units, 8-21units, and 22+ units.

3.3.3.2 Method

1. Calculate the number of alcohol units per week from the data reported in EPIC FFQ, 1 unit contains 8g of alcohol. The following formula was used:

$$Number\ of\ unit\ of\ alcohol\ per\ week\ from\ FETA\ output = \frac{Number\ of\ grams\ conumed\ per\ day\ X\ 7}{8\ gram\ *}$$

2. Same categories and codes were used to label the reported alcohol intake in the CRF and calculated from the frequency section of FFQ (**Table 3-13)** shows the variables and the code used.

Table 3-13. Alcohol intake measured by g/d and unit per week and code used for each category

Number of grams of alcohol per week extracted by FETA from EPIC FFQ	***Number of alcohol units consumed per week reported in the CRF***	***New code used for both methods***
0	*None*	*1*
0.1 to 56	*1-7*	*2*
56.01 to 147	*8-21*	*3*
>147	*22+*	*4*

3. Calculate the difference between the number of units of alcohol reported in the two methods:
   (Difference in alcohol intake $= Units\ of\ alcohol\ reported\ in\ CRF -$
   $Units\ of\ alcohol\ measured\ from\ FFQ$)
   The difference was used to categorise patients into 3 categories as **Table 3-14** shows.

Table 3-14. The difference between estimated alcohol using the two methods and meaning of the results

Results obtained from step 3	***Allocated category (meaning of the result)***
0	*Good reporter (no difference between the two methods)*
+1, +2	*Under reporters in FFQ (reported intake in CRF is higher)*
-1, -2	*Over reporters in FFQ (reported intake in CRF is lower)*

3.3.3.3 Results

As **table 3-15** shows, 60% of the patients reported alcohol intake in FFQ similar to CRF, 21.6% over reported their alcohol intake in the FFQ and 17.6% reported alcohol in the FFQ less than the CRF. Sub analysis revealed that more females than males reported the same alcohol consumption in the two methods (70% vs. 58%) and more males under-reported their alcohol intake in the FFQ than females (20% vs. 10%).

Table 3-15. Number and percentage of patients in each category of alcohol reporting

Category of response	***Number***	***Percentage***
Much higher than CRF	*17*	*2.5*
Higher than CRF	*128*	*19*
Similar response as CRF	*406*	*61*
Lower than CRF	*102*	*15.2*
Much lower than CRF	*16*	*2.4*
Total	*669*	*100*

We also found that people with high consumption of alcohol (> 18g of alcohol per day extracted from FFQ) tend to under-report their alcohol intake in the CRF, on the other hand, people who consumed a lower amount of alcohol (< 8g of alcohol per day extracted from FFQ) tend to over-report their alcohol intake in the CRF.

3.3.4 Dietary Inflammatory Index (DII) validation.

The DII was calculated to measure the potential inflammation of the diet for patients recruited to the seAFOod trial and for the FACT study using the method that was published in 2014 (144). The purpose of this section is to validate the accuracy of the steps used in the calculation and was achieved by collaboration with the DII creators at the University of South Carolina Dr.Shivappa .

3.3.4.1 Objective

To validate the in-house method used to measure the DII for the FACT study and the seAFOod trial.

3.3.4.2 Material and method

1. To achieve this objective, the FACT study data for 98 cases were used.
2. DII score was calculated using 25 food and nutrients parameters shown in **table 2-4C** .
3. The steps used to make the calculations are summarised in **Chapter 2 (2.2.3.2).**
4. Same data (25 foods and nutrients parameters for 98 cases recruited to the FACT study) was sent to the DII creators to calculate the DII using the original algorithm.
5. The next step is to compare the DII scores obtained from the two methods; this was by using an independent sample t-test and Pearson correlation to verify if the two analytical approaches are comparable.

3.3.4.3 Results

Results of the independent sample t-tests show no significant difference between the mean of DII score obtained from the in-house methods (1.01 ±1.6) when compared with the DII score measured by the original method (.69 ± 1.3), p=.141. (**Table 3-16**).

Table 3-16. A comparison between DII score measured by the in-house method with DII score measured by the original algorithm.

	Original method (n=93)	***In-house method (n=93)***		***95% CI***	
			p	*Lower*	*Upper*
Min-Max	*-2.93 - 3.54*	*-3.59 - 4.4*			
Mean (SD)	*.65 (1.3)*	*1 (1.6)*	*.141*	*-.758*	*.108*
Independent sample T-test					

Pearson correlation was conducted to assess the relationship between the DII score calculated by the in-house method and the DII calculated by the original algorithm. There was a strong positive, and significant correlation between the results obtained from the two methods (r = .985, $p < .001$). **Figure 3-7** shows a scatterplot that shows the correlation between DII measured by the original algorithm and DII measured by the in-house method.

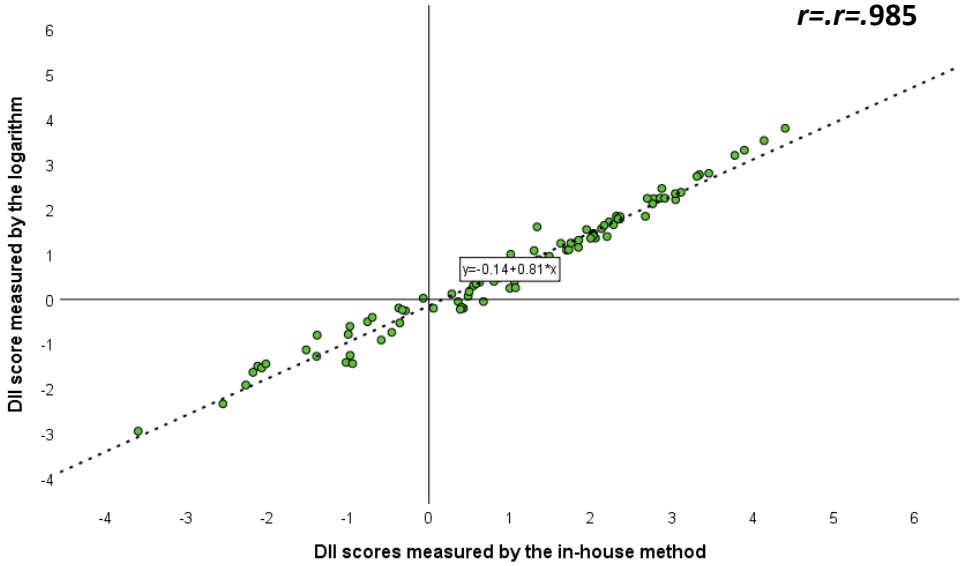


Figure 3-7. A scatterplot that shows the correlation between DII measured by the original method and DII measured by the in-house method

3.4 Data validation discussion

This chapter aimed to check if there were any errors caused to the seAFOod trial data during data handling and processing, to assess misreporting level in dietary and alcohol intake and to validate the in-house method used to calculate the DII score.

3.4.1 Data accuracy

Overall, there is no indication that data preparations and handling procedures had affected the integrity of either dietary, demographics or randomisation data. A small discrepancy was observed between the prepared and published adenoma characteristics data, however, justification was received from Nottingham clinical trial centre for these detected difference as was clarified in the results (**Section 3.3.1.3)**.

Although dietary data from the seAFOod trial will be compared with the data obtained from the NDNS in the next chapter, values of some nutrients are not comparable due to the differences between the methods used to assess and analyse the dietary data and the approaches used to classify nutrients. Therefore, to check the accuracy of dietary data processing, the averages of dietary intake extracted from the seAFOod trial was compared with the dietary data obtained from the FOOD4ME study (167). We found similarity between the results obtained from the two studies that reassured that there were no substantial mistakes in the dietary data handling and processing but some differences were detected that needed further investigation.

The data from the Food4Me study was selected because it was conducted at the time of the seAFOod data collection period, it provided the averages of macro and micronutrients intake assessed by EPIC FFQ and extracted by FETA and it used the same approach to identify the energy under reporters, however, there are some limitations, the first was that the whole set of data was not available. Therefore, no statistical test was performed and the data were checked visually for the means obtained from the two studies. The 2nd limitation was the differences in the demographic characteristics between the two samples recruited to the studies which needed further consideration. The averages of energy, alcohol and most of the nutrients obtained from the FOOD4ME results were lower than the averages obtained from the seAFOod trial. Due to the demographic differences between the two studied samples, this difference is likely to be due to *true differences in the intake* and not due to data processing mistakes.

These differences could be explained by that subjects recruited to the FOOD4Me study were much younger [mean age was 30 years (SD=10.2) *vs.* 65.3 years (SD= 4.6)], more females [59% vs. 20% in the seAFOod trial] and had lower BMI [23.3 (SD=2.9) *vs*. 29.2 (SD 6.1) in the seAFOod trial]. The dietary behaviour of young females with lower BMI is different from older obese males. This was further supported by the findings that after adjusting for energy, no difference in the percentage of energy obtained from macronutrients. Furthermore, the mean intake of sodium by the seAFOod trial participants could indicate higher consumption of processed food and the lower intake of total carotene may indicate a lower consumption of fruits and vegetables. This evidence may suggest a true difference between the dietary behaviour of the two groups.

3.4.2 Dietary data misreporting

in the seafood trial, 58% of the participants were classified as energy-under reporters. following the same approach to identify energy-under reporters in the Food4Me study the proportion of energy-under reporters was 51.3%. Inaccuracy of self-reported diet is associated with factors affecting the participants' response, limitations in the dietary assessment and analysis tools used or due to limitations in the validation method.

Evidence shows that dietary data misreporting is more prevalent among less-educated individuals and in older people (173). Providing accurate data to the FFQ requires the ability to perform complex cognitive tasks of memorizing and averaging of frequency of food and drinks consumed during the previous year (174). The seAFOod trial recruited patients from 63 centres from around England, there is no information about the educational level or the level of numeracy and literacy skills of this cohort, there is a probability that not all the participants were able to understand and provide correct answers to the FFQs. Also, the memory of some patients might be affected by age (patients age was up to 75 years), dementia and other comorbidity. Therefore, it is unlikely that all respondents had the skills or the ability to perform the tasks required to provide correct data.

Evidence suggest that dietary data misreporting is associated with a higher BMI (173). This was also observed in the seAFOod trial, participants who under estimated their energy intake had a significantly higher BMI than patients who were classified as acceptable diet reporters.

Finally, the time and conditions of dietary data collection of the seAFOod trial could influence the psychological status of the patients and the way they responded to the FFQ. As the data was collected after going through the cancer screening process, being newly diagnosed with high-risk colorectal adenoma and while engaging in a clinical trial. All these factors may influence the response of the patients and lead to social desirability bias (175) which result in over-reporting of food that is believed to be healthier. This might be the reason for fruits and vegetable over-reporting observed in this cohort.

Although some of the dietary misreporting may be caused by factors related to respondent ability or bias, errors might also be a consequence of limitations of the assessment and analysis tools used in the study. One of the limitations of EPIC FFQ is that only one portion size is provided to all respondents (176), irrelevant of their age, sex or BMI. In this study, nearly 58% of the patients reported energy intake that is lower than their basal metabolic rate. The seAFOod trial data was collected from around England for males and females age between 55 to75 years and BMI ranges from 18 to 64Kg/m^2. It is not expected that one portion size would cover the needs or the usual eating habits of this cohort. As it was mentioned before, individuals who were classified as energy under reporters had a significantly higher BMI. This could be explained that the conceptualization of the portion size of individuals of high BMI was larger than the portion size measured by the EPIC FFQ and the FETA software.

For all food groups assessed by the two sections of FFQ, means of intake obtained from the frequency section were significantly higher than the means obtained from CCQ questions. A similar trend of over-reporting in the frequency section was also observed in the Woman's Cohort Study (177) The largest difference was observed in the vegetable group. In the seAFOod trial, the average of vegetable reported in the frequency section was nearly four times that was reported in the CCQ section. A probable reason for this discrepancy is that the large number of questions allocated to the vegetables' group in the frequency section (25 question), on the other hand, asking one question in the CCQ to measure the consumption of food group that has many food items, might also lead to under estimation from the respondents.

A comparison of food group misreporting among energy-under reporters and energy-acceptable reporters suggested that there was no association between energy misreporting and food group misreporting. The mean of the WFs indicated that, in all food groups, food groups' misreporting was more prevalent in energy-acceptable reporters. This might suggest that the observed energy

misreporting is associated with the conceptualization of the portion size. These findings may also indicate that energy under-reporting is not directly associated with the ranking of the individual in their food intake.

The final source of error is the method used to classify energy-under reporters. The approach used to identify the energy-under reporters has the advantage that no external reference measure is needed and can be applied for all the participants. However, not having the physical activity level of the participants and applying one value for all of them, has limited the value of the outcome of this measurement and made it more accurate for participants with low physical activity level and who under-report their dietary intake. It was suggested by Nelson (1997) (176) to use an upper cut-off point of estimated energy intake to EER ratio of 2.5 and considering participants who reported values more than this cut-off point as energy over reporters. In this study, the maximum estimated energy intake to EER ratio was 2.3 so no category for over reporters was made.

WFs from the CCQ section could be used to assess the effect of misreporting on the dietary intake overall. Since FETA software was used in this analysis, applying these weighting factors on the data would result in not an integer (a fraction or not a whole number) that would not be acceptable by the software. To adjust for that a further step of modification to the data was required, which will further influence the accuracy of the analysis. Therefore, the data from the Women's Cohort Study was used to predict the effect of this level of misreporting on the results. The comparison suggested that vegetables and meat misreporting may have an impact on the accuracy of macro and micronutrients, which may indicate a need for data adjustment in the case of an association between specific nutrient and an outcome to be measured in the further analysis. The limitations of this comparison are that the data was obtained from females only, the number of food items included in each food group was different, and the analysis tools used were also different in both studies, which might have created some differences in the results. Moreover, likely, the advancement in awareness of healthy eating during the 20 years difference between the two studies had an impact on the responses of the participants.

3.4.3 Validation of alcohol intake

The average alcohol intake reported by the seAFOod trial participants was higher than the intake of the FOOD4ME participants [mean=13.5 (SD=16.24) g vs. mean=7.2 (SD=9.1) g], therefore a further validation

was performed. Alcohol data that was self-reported in the frequency section of the FFQ was validated by the alcohol data provided in the CRF during the interview with the research nurse at the beginning of the study. This validation revealed a modest difference between the amounts of alcohol reported in the two methods. Nearly 95% of the patients reported alcohol intake in the FFQ that is within ±1 category of the units reported in the interview. It is not known which method is more accurate in assessing actual consumption, however, finding this similarity between the two methods may indicate a good reporting level considering the two methods (CRF and FFQ) used different questions, different units and data were collected at different times in different condition. FFQ was self-reported, while data available for the CRF was collected during face to face interview with the health professional in the clinical practice. The findings that heavy drinkers tend to under-report their alcohol consumption was reported previously (178). This might be that heavy drinkers may do not want to admit their drinking behaviour or could be interpreted as a social desirability bias.

Summary

Overall, the analysis in this chapter showed no signs that data processing affected the accuracy and integrity of the data. The in-house method used to calculate the DII provided score with adequate accuracy to rank individuals. The reported dietary data is affected by both individuals' misreporting and FFQ limitations. However, as no signs that the ranking of the individuals was affected, we consider that data is suitable to be used in further analysis with caution in interpreting the result, especially for fruits and vegetables groups.

Chapter 4

Baseline demographic characteristics and dietary intake of colorectal adenoma patients recruited to the seAFOod trial

Chapter 4 Baseline demographic characteristics and dietary intake of colorectal adenoma patients recruited to the seAFOod trial (Objective 2).

Evidence shows that lifestyle factors such as smoking, body fatness and diet are linked to CRC incidence and mortality. The Continuous Update Project (CUP) of the WCRF/AICR continually collects and synthesizes the scientific evidence for a range of cancers including CRC and provides guidelines for reducing the risk of developing cancer (**Fig 1-8**). Overall, the studies that investigated the association between dietary behaviour and early stages of CRC are limited and findings are not conclusive, therefore guidelines to reduce the risk of developing colorectal adenoma are not available.

As previously described in **Chapter 1**, evidence from epidemiological studies suggest that the risk of colorectal adenoma is linked to modifiable risk factors such as body fatness (64), physical inactivity (65), smoking (11), high alcohol consumption (91). Certain dietary behaviours are also associated with the disease, for example, higher risk of colorectal adenoma is associated with high intake of red and processed meat (93) but the high consumption of fruits showed a protective role (100). No association was found between the disease and consumption of milk, dairy products (96), vegetables (99), total energy, fat or protein (179) and for fish, the evidence are weak (94). For dietary fibres, an inverse association was found when fibre was obtained from fruits or cereals but no association with the fibre obtained from vegetables(101). Vitamin C showed a protective role (105), for vitamin D, dietary intake was not associated with the risk of the disease (107) but the risk was significantly lower with higher circulatory levels of 25-hydroxyvitamin D (110). For haem iron, the evidence identified a higher risk of the disease in people with higher body store (102,103).

In this chapter, I will report the demographic characteristics and assess the dietary behaviour of patients diagnosed with high-risk colorectal adenoma using the baseline demographic and dietary data that was collected from patients recruited to the seAFOod trial through the BCSP.

The analysis will begin by describing the number, age, sex, BMI, alcohol and smoking status of all the participants recruited to the seAFOod trial, then, it will investigate the possible bias in the data after excluding cases with incomplete dietary data. This will be followed by a description for the dietary intake on food groups' level, macro and micronutrients levels. Finally, the proportion of participants who met the WCRF/AICR recommendations to reduce the risk of CRC will be assessed. This is

essential to understand the general characteristics of this cohort and to provide an overview of their dietary behaviour during the 12 months before diagnosis.

4.1 Aim and objectives

To describe the demographic characteristics and dietary behaviour of patients newly diagnosed with high-risk colorectal adenoma recruited to the seAFOod trial.

Objectives to achieve this aim are:

1. To describe the demographic characteristics of the seAFOod trial participants.
2. To investigate the possible bias between cases included and excluded from the analysis due to the availability and suitability of dietary data by comparing their demographic characteristics.
3. To describe intake of food groups, energy, macro and micronutrients and compare with the Dietary Reference Value (DRV) for their age and sex.
4. To explore the proportion of patients who achieved the recommended dietary intake of foods and nutrients associated with CRC according to the WCRF/AICR

4.2 Methods

4.2.1 Demographic characteristics of the seAFOod trial participants

Descriptive analysis was performed for age, BMI, smoking status and alcohol consumption using the main dataset generated from the seAFOod trial. Mean and standard deviation used to describe age and BMI. Number and percentage were used to describe the distribution of patients among categories of BMI, smoking status and the number of units of alcohol consumed per week. This description analysis was performed for the whole sample than for males and females separately.

4.2.2 Demographic characteristics of included and excluded cases at baseline

Of the 707 patients recruited to the seAFOod trial, 674 of them (95%) provided dietary data that met the inclusion criteria and were included in further analysis. Both Independent sample T-test and Chi-square test were used to explore if there was a significant difference in demographic characteristics between patients included and excluded from the analysis due to availability and suitability of the dietary data to investigate possible bias in the data.

4.2.3 Average daily intake of food groups, energy, macro and micronutrients during the 12 months before diagnosis.

Dietary intake collected using the EPIC-FFQ (157) was analysed using the FETA software. As previously described in **Chapter 2 (Section 2.2.1.2),** the FETA software extracts the average daily intake of energy, macronutrients, micronutrients and food groups. Analysis for food groups and macronutrients was performed for all the patients and then for males and females separately, analysis for micronutrients was performed for all the samples. In the cases that DRV is different for different age groups, the DRV for over 65 years was chosen, since most of the patients were over 65 years old. In micronutrients analysis, when DRV is different between males and females, the mean of the two value was used in comparing intake with the recommendation. One sample t test was used to compare between the average daily intake and the DRVs (or safe intake-(SI)) according to SACN (163). The proportion of participants who had an average daily intakes below the Lower Reference Nutrient Intake (LRNI) was also calculated. In all analysis *p*-value of .05 was considered significant. For normally distributed data, the average intake reported in terms of mean and standard deviation. For not normally distributed data, median and interquartile range were also reported

4.2.4 The proportion of patients who achieved the recommended dietary intake of foods and nutrients associated with CRC according to the WCRF/AICR.

This section describes the number and percentage of patients who reported dietary intakes of food and nutrients related to CRC within the WCRF/AICR and Public Health England recommendations. This was described for all the cohort and then the Pearson Chi-Square test was used to compare the proportions of males and females who achieved the recommendation. **Table 4-1** shows the food groups that are associated with CRC in the report, the recommended amount used in this analysis and the source of recommendation. As the WCRF/AICR report did not include a specific recommendation for fruit, vegetables and fish intake, the recommendations provided by Public Health England guidelines were used.

Table 4-1. The association between specific food and nutrients and CRC according to the WCRF/AICR report, recommendations for consumption (when available) and source of recommendation

Factor	*WCRF/AICR findings*	*Goal*	*Source of recommendation*
Red and processed meat	*Consumption of processed meat is probably a cause of CRC*	*≤70 gram/day or 500g/week*	*WCRF/AICR report*
Fibre	*Consumption of food rich in fibre probably protects from CRC*	*≥ 30 gram per day**	*WCRF/AICR report*
Fish and fish products	*Limited evidence shows that fish decreases the risk of CRC*	*2 portions per week one of them is oily fish.*	*PHE recommendations*
Fruit and vegetables	*Limited evidence shows that low intake of fruit and non-starchy vegetables increase the risk of CRC*	*≥ 5 portions per day.*	*PHE recommendations*
****30g of AOCA fibre is equal to about 23g of NSP***			

4.3 Results

4.3.1 Demographic characteristics of the seAFOod trial participants

In total, 709 colorectal adenoma patients were recruited to the seAFOod trial through the BCSP from 63 medical centres from around England. Two patients withdrew from the study and 707 provided baseline data and are included in this analysis. As **table 4-2** shows nearly 80% of the cohort were men and the mean age was 65.25 (SD= 4.66) years. Sub analysis showed that 5.4% of them were under 60 years, 37.6% were between 60 and 65 years and 56.8% were between 65 and 74 years. More than 82% had a BMI in the overweight and obese categories. 35.9% of the cases had never smoked cigarettes, about 49.1% were ex-smoker, and only 15% of them were current smokers. 15.6% of the patients did not consume alcohol and 63.4% consumed less than 21 unit of alcohol per week. However, more than 24% of males included in this study consumed more than 22 units of alcohol per week.

Table 4-2. Demographic characteristics of the 707 colorectal adenoma patients recruited for the seAFOod trial

Variable	*All*	*Females*	*Males*
Number (%)	*707*	*144 (20.4)*	*563 (79.6)*
Age years M (SD)	*65.3 (4.66)*	*65.2 (4.5)*	*65.3 (4.7)*
BMI M (SD)	*29.4 (5.6)*	*29.9 (6.9)*	*29.3 (5.2)*
BMI Categories n (% within sex)			
<18.5	*3 (0.4)*	*3 (2.1)*	*0*
18.5-24.5	*123 (17.5)*	*32 (22.5)*	*91 (16.2)*
25 – 29.9	*311 (44.2)*	*43 (30.3)*	*268 (47.7)*
≥30	*267 (37.9)*	*64 (45.1)*	*203 (36.1)*
Smoking status n (% within sex)			
Current Smoker	*106 (15)*	*22 (15.1)*	*84 (14.9)*
Ex-Smoker	*374 (49.1)*	*64 (44.4)*	*283 (50.3)*
Never Smoked	*254 (35.9)*	*58 (40.3)*	*196 (34.8)*
Alcohol units per week n (% within sex)			
None	*110 (15.6)*	*43 (29.9)*	*67 (11.9)*
1-7	*234 (33.1)*	*57 (39.6)*	*177 (31.4)*
8-21	*214 (30.3)*	*33 (22.9)*	*181 (32.1)*
+22	*146 (20.7)*	*10 (6.9)*	*136 (24.2)*
BMI=Body Mass Index			

4.3.2 Compare the demographic characteristics of included and excluded cases at baseline

Thirty-three people needed to be excluded from the dietary analysis for the reasons explained in **Chapter 2 (Section 2.2.2).** As **table 4-3** shows, no significant difference was found in age, BMI or sex between the included and excluded cases, however, there was a significantly higher proportion current smokers in the group that was excluded from analysis. No significant difference was found in the distribution of patients among alcohol consumption categories.

Table 4-3. Comparison of demographic characteristics of included and excluded cases due to dietary data availability and suitability.

Variable	*Included*	*Excluded*	*p*
Number	*674*	*33*	
Sex male n (%)	*536 (79.4)*	*27 (81.8)*	*.749**
Age years M (SD)	*65.3 (4.7)*	*64.7 (3.9)*	*.46$*
BMI M (SD)	*29.4(5.6)*	*29.6 (6)*	*.83$*
Smoking status n (%)			*<.001**
Current Smoker	*98 (14.5)*	*8 (22.9)*	
Ex-Smoker	*334(49.6)*	*13 (37.1)*	
Never Smoked	*242 (35.9)*	*12 (34.3)*	
Alcohol consumption categories in n of units per week (%)			*.903**
None	*106(15.8)*	*4 (12.5)*	
1-7	*223(33.2)*	*11 (34.4)*	
8-21	*205(30.5)*	*9 (28.1)*	
+22	*138(20.5)*	*8 (25)*	
Body Mass Index (BMI), *Pearson Chi-Square, $Independent sample T-test			

4.3.3 Dietary intake of 674 colorectal adenoma patients recruited to the seAFOod trial at baseline estimated using the EPIC FFQ.

Initial inspection of the data distribution revealed that food groups were not normally distributed except the meat and meat products group and the vegetables group. Energy and the majority of macro and micronutrients were normally distributed except for alcohol, Vitamin A retinol, and total carotene. For analysis and presentation, this data was separated into three groups and each group was presented in a separate table as follow:

1 The first table displayed the average daily intake of food groups,
2 The 2nd shows the average daily intake of energy, macronutrients and the percentage of energy from each macronutrient. It also shows the comparison that was made between the intake of the seAFOod cohort and the DRV using a one-sample T-test.
3 The average daily intake of micronutrients and the comparison with DRVs (or safe intake-SI) according to SACN (163) using a one-sample T-test are presented in the 3rd table.
4 This was followed by the fourth table that showed the proportion of patients who did not meet the Lower Reference Nutrient Intake (LRNI) of selected micronutrients.

4.3.3.1 Food groups

The average daily intake of the 16 food groups reported by the 674 patients during the 12 months before diagnosis are provided in **table 4-4**. On average, patients reported consumption of more than 5 portions of fruits and vegetables per day and about two portions of fish per week. The WCRF/AICR guidance is that if you choose to eat meat not to exceed 70g per day, the mean daily intake of red and processed meat suggest that only males exceeded this guideline.

4.3.3.2 Energy and macronutrients

Table 4-5 shows the average daily intake of macronutrients during the 12 months before diagnosis and the percentage of energy obtained from each macronutrient. The percentage of energy obtained from total fat and total carbohydrate was lower than the recommended allowance while energy from protein was higher than the recommended allowance. The mean intake of NSP fibre was significantly lower than the recommended 23g, females and males consumed an average of 15.8 and 15.3 g per day, respectively.

4.3.3.3 Micronutrients

Micronutrients consumption during the 12 months before diagnosis are compared with DRV using a one-sample T-test in **table 4-6.** The analysis revealed that the average daily intake of iron, zinc, sodium, folate, $B_{1,}$ B_2, B_6, B_{12}, niacin and vitamin E all exceeded the recommended DRV, while the average daily intake of selenium and vitamin D was significantly lower than the recommended DRV. No significant difference was observed between the DRV for vitamin A and the average daily intake.

When assessing the proportion of patients who reported intake of selected micronutrients that was below the LRNI, most of the patients met the lower recommendation of riboflavin and folate for their age and sex groups. However, 100% of the females and 99.3% of the males did not meet the recommended 10 µg of vitamin D per day. In addition, 18.8% of females and 39.7% of males reported dietary intake of vitamin A that is below the lower recommendation for their age and sex group. More than 90% of males and females met their LRNI of the included minerals, but 18.1% of the females and 13.6% of the males reported selenium intake that is below the LRNI for their age and sex group **(Table 4-7).**

Table 4-4. Mean (SD) and median (IQR) of daily intake of food groups at baseline in 674 patients recruited to the seAFOod trial

	All (n=674)		***Female(n=138)***		***Male(n=536)***	
Food group	*Mean (SD)*	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*	*Mean (SD)*	*Median (IQR)*
Alcoholic beverages (g)	*242.4 (350.2)*	*125.5 (260.6)*	*90.3 (152.1)*	*47.7 (124.6)*	*281.6 (375.3)*	*146 (265)*
Non-alcoholic beverages(g)	*927.8 (424.6)*	*950 (511.1)*	*911.6 (464.7)*	*941.4 (493)*	*932 (414)*	*952.2 (514.4)*
Meat and meat products(g)	*125.3 (61.2)*	*117.7 (72.6)*	*100.7 (50.4)*	*97.2 (71.8)*	*131.6 (62.1)*	*121 (73.6)*
Red and processed meat (g)	*89.3 (51.2)*	*81 (59)*	*68.8 (38.2)*	*63.8 (58)*	*94.4 (52.8)*	*84.8 (61)*
Milk and milk products(g)	*337.6 (182)*	*309 (261.2)*	*317.5 (182)*	*287.7 (263.1)*	*342.7 (181.8)*	*312.4 (256.7)*
Cereals and cereal products(g)	*203.5 (122.1)*	*177.2 (139.3)*	*181.6 (98.8)*	*158.1 (130.6)*	*209.1 (126.8)*	*184.3 (143.8)*
Potatoes(g)	*93.9 (48.9)*	*89 (59.9)*	*81.9 (47.4)*	*72.2 (62.6)*	*97 (48.9)*	*89 (54.2)*
Fruit(g)	*183.9 (161.6)*	*147.9 (176.1)*	*209.6 (161.7)*	*168.3 (208.4)*	*177.3 (161)*	*142.9 (166.7)*
Vegetables(g)	*252.3 (133.5)*	*233.7 (149.2)*	*273.3 (160.3)*	*244.8 (153)*	*246.9 (125.3)*	*232.6 (151.4)*
Eggs and egg dishes(g)	*20 (16.6)*	*21.5 (14.5)*	*18.3 (16.4)*	*17.5 (14.5)*	*20.5 (16.6)*	*21.5 (14.5)*
Fats and oils(g)	*22.9 (16)*	*17.8 (18.2)*	*18.6 (14.2)*	*14.2 (14.4)*	*24 (16.3)*	*18.9 (18.2)*
Fish and fish products(g)	*43.2 (32)*	*35.5 (31.5)*	*43.9 (29)*	*36.3 (37.6)*	*43 (32.7)*	*35.4 (31.2)*
Oily fish (g)	*14.1 (16.7)*	*8 (16)*	*18.8 (19.4)*	*8 (8)*	*12.9 (15.8)*	*8 (16)*
Nuts and seeds(g)	*5.3 (13.1)*	*2.1 (4.2)*	*7.2 (19.2)*	*2.1 (5.3)*	*4.8 (11)*	*2.1 (4.2)*
Soups and sauces(g)	*56.2 (55.6)*	*41.5 (43.5)*	*57.2 (49.8)*	*43.2 (44.1)*	*56 (57)*	*40.9 (42.8)*
Sugars, preserves and snacks(g)	*41.5 (37.8)*	*32.8 (42.5)*	*35.2 (32.5)*	*26 (35.2)*	*43.2 (39)*	*34.8 (44.2)*

Table 4-5. Mean (SD) daily intake of energy and macronutrients at baseline reported by 674 patients recruited for the seAFOod trial

	All (674)	***Females (138)***			***Males (536)***		
	Mean (SD)	*Mean (SD)*	*DRV*	*p*	*Mean (SD)*	*DRV*	*p*
Energy (MJ/day)	*7.71 (2.4)*	*6.7 (203)*			*7.96 (2.5)*		
*****Alcohol (g/day)***	*13.5 (16.2)*	*7.2 (9.9)*			*15.1 (17.1)*		
% of energy from Alcohol	*5.3 (6.2)*	*3.4 (4.6)*			*5.8 (6.4)*		
Protein (g/day)	*82 (23.5)*	*75.7 (21.3)*	*46.5*	*<.001*	*83.7 (23.8)*	*53.3*	*<.001*
% total energy from protein	*18.4 (3.5)*	*19.3 (3.5)*	*15*	*<.001*	*18.1 (3.4)*	*15*	*<.001*
CHO – total g/day	*208 (78.1)*	*188.5 (68.1)*			*213 (79.8)*		
% total energy from CHO	*45.2 (7.6)*	*46.9 (7.4)*	*50*	*<.001*	*44.7 (7.6)*	*50*	*<.001*
NSP (g/day)	*15.4 (6)*	*15.8 (6.2)*	*23*	*<.001*	*15.3 (6)*	*23*	*<.001*
Fat – total (g/day)	*70.3 (27.9)*	*60.1 (23.6)*			*72.9 (28.4)*		
% total energy from fat	*34 (6.1)*	*33.4 (6)*	*35*	*.002*	*34.2 (6.2)*	*35*	*.003*
MUFA (g/day)	*25.6 (10.8)*	*21.9 (9.7)*			*26.5 (10.9)*		
PUFA (g/day)	*12.3 (5.5)*	*11.1 (5)*			*12.6 (5.5)*		
SFA (g/day)	*26 (11.3)*	*21.6 (9.2)*			*27.2 (11.6)*		

*****Alcohol consumption was not normally distributed median for all 8.6, for females' median =4.7, IQR=10.4, for males, median=9.5, and IQR=16.6.***
Dietary Reference Value (DRV), Mean (M), Standard Deviation (SD), Carbohydrate (CHO), Englyst Fibre- Non- Starch Polysaccharides (NSP), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), Saturated fatty acids (SFA).
Valued compared with the DRV using one-sample T-test

Table 4-6. Average daily intakes of micronutrients for all participants (674) during the 12 months before diagnosis compared with the DRV

	Mean (SD)	***DRV***	***p ****
Calcium mg/day	*864 (308.9)*	*700*	*<.001*
Iron mg/day	*11.1 (3.48)*	*8.7*	*<.001*
Zinc ng/day	*9.25 (2.79)*	*8.25*	*<.001*
Selenium µg/day	*62.5 (23.9)*	*67.5*	*<.001*
Sodium mg/day	*2659 (944)*	*1600*	*<.001*
Total folate µg/day	*287 (92)*	*200*	*<.001*
Vitamin B1- thiamine mg/day	*1.45 (.47)*	*0.85*	*<.001*
Vitamin B2 – riboflavin mg/day	*1.91 (.65)*	*1.2*	*<.001*
Vitamin B6 – pyridoxine mg/day	*2.21 (.63)*	*1.1*	*<.001*
Vitamin B12 – cobalamin µg/day	*7.25 (4.2)*	*1.5*	*<.001*
Niacin mg	*22.52 (7.18)*	*14*	*<.001*
Vitamin A – retinol µg/day	*662 (931.8)*	*650*	*.745*
Vitamin C mg/day	*104 (54.3)*	*40*	*<.001*
Vitamin D µg/day**	*3.26 (1.77)*	*10*	*<.001*
Vitamin E mg/day §	*11.4 (5.23)*	*3.5*	*<.001*

Dietary Reference Value (DRV), Mean (M), Standard Deviation (SD), Vitamin C - ascorbic acid, Vitamin D - ergocalciferol, Vitamin E - alpha-tocopherol equivalents, ** As per SACN 2016, § =Safe intake, *One sample T-test to compare

Table 4-7. The proportion of patients with average daily intakes below the LRNI for selected micronutrients

Micronutrients		*female*	*male*
Vitamin A (LRNI)	*Count*	*26*	*213*
	% within sex	*18.8%*	*39.7%*
Riboflavin (LRNI)	*Count*	*3*	*5*
	% within sex	*2.2%*	*.9%*
Folate (LRNI)	*Count*	*0*	*4*
	% within sex	*0%*	*0.7%*
Vitamin D (RNI)	*Count*	*138*	*532*
	% within sex	*100%*	*99.3%*
Iron (LRNI)	*Count*	*0*	*4*
	% within sex	*0%*	*.7%*
Calcium (LRNI)	*Count*	*6*	*13*
	% within sex	*4.3%*	*2.4%*
Magnesium (LRNI)	*Count*	*0*	*45*
	% within sex	*0%*	*8.4%*
Potassium (LRNI)	*Count*	*7*	*18*
	% within sex	*5.1%*	*3.4%*
Iodine (LRNI)	*Count*	*8*	*31*
	% within sex	*5.8%*	*5.8%*
Selenium (LRNI)	*Count*	*25*	*73*
	% within sex	*18.1%*	*13.6%*
Zinc (LRNI)	*Count*	*0*	*35*
	% within sex	*0%*	*6.5%*
Lower Reference Nutrient Intake (LRNI), Reference Nutrient Intake (RNI)			

4.3.4 Proportion of males and females achieved the recommended dietary intake of foods and nutrients associated with CRC according to the WCRF/AICR for the total sample

As **table 4-8** shows, more than 59% of the patients exceeded the maximum amount of red and processed meat advised per day (WCRF/AICR)(90). Regarding the healthy eating recommendations provided by Public Health England (PHE) (180), for fish, 42.2% achieved the recommended two portions per week but only 14.2% consumed the recommended weekly oily fish portion. Half of participants reported eating the recommended 5 portions of fruits and vegetables/day, but less than 15% achieved the recommended amounts of fibre. Subgroup analysis using Pearson Chi Square to compare the proportion of males and females who achieved the recommendation revealed that significantly higher proportion of females than

males achieved the recommended amount of red and processed meat (57% vs 37%) and of oily fish (25.4 vs 11.4%). No significant difference in the proportion of males and females who achieved the recommendations for total fish, fruits and vegetables or fibre was observed.

Table 4-8. Number (%) of patients reported consumption of the recommended amounts of red meat, processed meat, fish, oily fish, fruits, vegetables and fibre at baseline for the whole group and according to sex

Food group/nutrient goal	***All (674)***	***Female (138)***	***Male (536)***	***p ****
< 70g of red and processed meat per day	*271 (41)*	*75 (57.3)*	*196 (37)*	*<.001*
> 2 portions of fish per week	*286 (42.4)*	*62 (44.9)*	*224 (41.8)*	*.507*
≥ 1 portion of oily fish per week	*96 (14.2)*	*35 (25.4)*	*61 (11.4)*	*<.001*
≥5 portions of fruits and vegetables per day	*336 (50)*	*78 (56.5)*	*258 (48.3)*	*.104*
≥23g/day of NSP	*77 (11.4)*	*17 (12.3)*	*60 (11.6)*	*.764*
**** Pearson Chi square***				

4.4 Discussion

More than 95% of the 707 colorectal patients recruited to the seAFOod trial reported their dietary intake at the baseline, which provides a valuable source of information to explore the dietary behaviour of this high-risk group.

The proportion of males recruited to this study was higher than the proportion of females. This is in line with the previous RCTs for colorectal adenoma patients (112–125) in which the percentage of males recruited ranged from 52.9 to 92%, however, the percentage of colorectal adenoma females recruited to the study performed by Wu *et al* (2009) in the UK was more than males (62.4%) (111). In the UK, evidence shows that adenoma is more prevalent in males (12%) than females (6.2%)(40). Other factor that might partly explain this detected difference is that evidence show that the FOBT is more sensitive in males than females (181), which might lead to inviting more males for the colonoscopy examination than females and therefore more males were available for recruitment for the seAFOod trial.

The prevalence of high BMI observed in this study was previously reported in colorectal adenoma patients (64,182). The evidence collected in the WCRF/AICR CUP report strongly link body fatness with the development of CRC. Two mechanisms are proposed for this association. The first is that large number of adipocytes leads to a chronic low grade systemic inflammation state due to continuous

generation of proinflammatory molecules such as interleukins (183). The 2nd proposed mechanism is the insulin resistant condition that is prevalent in individuals with high body fatness. *In vivo* studies show that high insulin is associated with increase in cellular proliferation and apoptosis reduction in CRC cell lines (184).

The completeness of the data collection was good, with only 3% of the sample not providing useable self-reported FFQ data. This might be explained that the data was collected just after being diagnosed with high-risk colorectal adenoma and during a period of frequent contact with health professionals. Evidence showed that involvement in cancer screening programs could provide a teachable moment for the patients (185). Patients may also consider that completing the FFQ is a sign for their contribution in the RCT they are involved in. When the demographic characteristics of the included and excluded population were considered there was no difference, except that the proportion of smokers was higher in the excluded group. Although the excluded group was a small sample (33 patients) it is worth mentioning that smoking was previously associated with dietary data misreporting (186).

Comparing our results for energy intake with the National Diet and Nutrition Survey (NDNS), revealed that in the seAFOod trial males reported slightly lower energy intake (7.96 KJ) than males in the NDNS (8.16 KJ), while the mean of females reported in the seAFOod trial was higher (6.7 KJ) than females of the NDNS (6.24 KJ) (187). Participants in both the NDNS and the SeAFOod trial failed to report energy intakes meeting the Estimated Average Requirement for this age group, which suggests under-reporting of food intake that is reflected in the estimated energy intake, this was investigated in details in the validation **Chapter 3**. Males and females, on average, consumed 89.9 g of red and processed meat per day, this is higher than the average of reported in the NDNS for people from 65 to 74 which was 73g/day. The percentage of energy obtained from total fat (34%) and from SFA (12.6%) reported by the seAFOod trial participants were similar to that reported by the NDNS participants aged 65 to 74 between 2014 and 2016, which was 34.1% from fat and 13.05% from SFA (187). The average daily intake of NSP fibre extracted from the dietary intake of the seAFOod trial participants was 15.4 (SD=6) g. This is lower than the SACN Carbohydrates and Health report recommendation of 23-24g/day of NSP (188). The reported average intake of adults 64years and over in the UK between 2014-2016 was 18.1 (SD=6.4) g/day. However, the method used to assess the fibre intake in the FETA software and used in this analyses only extracts the NSP which makes about 78% of the AOCA fibre assessed in the NDNS.

Comparing the micronutrients intake with DRVs of this group revealed that in general, patients met the recommended amounts of vitamins and minerals. Regarding micronutrients associated with CRC as per the WCRF/AICR report, dietary iron was more than the recommended amount (11.1 vs 8.7 mg/day). Dietary intake of vitamin C derived from the FFQ was high, however, an overestimation of vitamin C is expected due to the over-reporting detected in fruits and vegetables. Average daily sodium intake was 2659 mg, this exceeded the maximum allowance from the WHO which is daily intake below 2000mg (189). Average of daily intake of vitamin D from food was 3.26 (SD=1.77) µg/day that is lower than the DRV of 10µ per day, but similar to that was reported in the NDNS report for adults 65 years and over (3.32, SD=2.24) µg/day. When the proportion of patients with intake below the LRNI was assessed in **table 4-7,** all the females and 99.3% of males did not meet the LRNI of vitamin D. This indicates a high prevalence of low intake when compared with the NDNS report for people aged 65-74, where the proportion reported was 39% of males and 32% of females did not meet the LRNI. This comparison also indicates that there is a higher prevalence of patients with intake below the LRNI of vitamin A (39.7% and 18.8% vs. 6% and7%), but lower prevalence of selenium low intake (13.6% and18.1% vs. 34% and 57%), in males and females respectively.

However, a limitation for the micronutrients assessment for the seAFOod trial participants is that this analysis does not account for nutritional supplement intake. Therefore, the figures of some of the micronutrients might be underestimated.

These findings were compared with results reported previously for colorectal adenoma patients that were recruited to three studies: the FAB2 study [n=90, 57.8% males, mean age=64.5 (10.8) years, mean BMI=25.9 (4.25) Kg/m^2] (190); the Wheat Bran Fiber Trial (WBF) [n=1304, 67% males, mean age= 65.9 (8.8) years, mean BMI= 27.4 (4.4)Kg/m^2] (191) ; the Polyp Prevention Trial (PPT) [n=1905, 64.5% males, mean age= 61.1 (9.9) years, mean BMI= 27.6 (3.9)Kg/m^2] (192).

The mean daily intake of energy reported by the seAFOod trial (7.71 MJ) was lower than previously reported in colorectal adenoma patients, it was 4% lower than reported by the WBF and the PPT (8.05 and 8.03 MJ) (191), and 25% lower than the FAB2 study (10.9 MJ) (190).

The average daily intake of fat (70g) was similar to that was reported by the WBF (69g) trial but lower than that was reported by the PPT study (76.3g) (192) and by the FAB2 study (87g). Reported fiber intake

(15.4g) was lower than reported by the WBF (21.9g) (191), PPT study (18.6g) (192) and the FAB2 study (24g) (190). Average alcohol intake (13.5g) was higher than that was reported by the PPT study (7.7g) (192) study and the WBF study (7.2g).

The observed differences could be due to difference in demographic characteristics of the seAFOod trial participants. For example, high alcohol consumption could be related to high proportion of males (80%) and low energy intake could be a consequence of energy misreporting of people with high BMI (29.9Kg/m^2) (discussed in details in **Chapter 3**). It should be mentioned that these comparisons have limitations due to differences in dietary assessment and analysis tools used. For example, the FAB2 study used a modified version of EPIC FFQ and used an in-house method to analyse the dietary data (190), the WBF trial used the Arizona Food Frequency Questionnaire to assess dietary intake and used an in-house analysis method (191). Finally, the PPT used a modified version of the Block-National Cancer Institute Food Frequency Questionnaire and used an in-house method for analysis. Using different techniques to assess and analyse dietary intake may limited the comparability of the figures. As each method uses different portion size, follows different food groups' classification method and uses different food composition table to estimate nutrients' content of each food.

Assessment of patients' dietary behaviour of foods associated with CRC revealed a significant difference between the dietary behaviour of males and females. The proportion of females who consumed food that was lower in red and processed meat and higher in oily fish was higher than males. Although the analysis shows that about half the patients consumed five or more portions of fruits and vegetables per day, this figure will not be considered as a true reflection for their intake due to the misreporting level was detected in this data (explained in details in **Chapter 3**).

A study collected lifestyle data from 208 patients attending surveillance colonoscopy using a questionnaire that contained questions about the consumption of red meat and avoiding of processed meat (193). 80% of the patients reported consumption of less than 500g per week (<70g/day) of red meat per week and 9% reported avoidance of all processed meat. This is a higher proportion of participants to report meeting the recommendations for meat intake than was reported by the seAFOod trial participants (80% vs. 41%) however, this difference might be explained by the higher percentage of males in our sample than this study (80% vs 51%).

This analysis is limited by several factors. The FFQ dietary assessment tool used in this research has limitations including reporting errors resulting from either problem in memory or misevaluation of

portion size. Also, patients provided the history of their dietary intake during the process of adenoma investigation; this may also cause a response bias in their answers. As a consequence, the results of this research should be carefully evaluated and values should not be considered as absolute values. Another source of limitation is the difference in sample size between males and females, which may affect our attempt to compare their dietary intake separately. Finally the major limitation is that the lack of the control group without colorectal adenoma limits the interpretation of the results since it was not possible to perform a case control analysis allowing a comparison of the findings with a sample with the same characteristic but free from the disease.

Summary

In this chapter, the demographic characteristics and dietary intake at baseline were explored for patients newly diagnosed with high-risk colorectal adenoma. The analysis showed that this cohort was collected from a homogeneous group that most of them had one or more of the risk factors associated with the risk of colorectal adenoma (age, BMI, and being males). Findings also revealed that their dietary intake lacks several features of the healthy eating guidelines recommended by Public Health England and the WCRF/AICR recommendations for CRC prevention. Overall, diet was high in red, processed meat and iron and low in fibre and vitamin D. Also it revealed that more females followed the recommendations for red and processed meat and oily fish consumption. In the next chapter, to obtain a complete evaluation of the dietary behaviour of this cohort, the dietary behaviour will be explored using the dietary pattern analysis.

Chapter 5

Dietary patterns of colorectal adenoma patients recruited to the seAFOod trial

Chapter 5 Dietary patterns of colorectal adenoma patients recruited to the seAFOod trial (Objective 3).

The analysis was performed on nutrients and at food level in the last **Chapter 4** is important to evaluate the diet quality, however, it is considered as a reductionist approach because it focuses on each dietary component separated from the others and has the limitations that it does not account for the complexity, interaction and correlation between dietary component (194).

Following a holistic approach, by measuring the dietary patterns, has become increasingly used in recent years to explore the association between diet and chronic disease (195) . Dietary pattern analysis methods are essentially a way of considering the effect of the diet as a whole on a specific outcome. It is useful for preliminary data exploration to highlight any existing association between dietary intake and the outcome of interest (140). It is not expected that dietary pattern analysis will provide any biological explanation behind the association revealed, if any. However, a hypothesis can be generated and investigated in further research. An advantage of this approach is that it accounts for nutrients interaction within the consumed diet that might be missed in dietary analysis using the reductionist approach (140). However, the two approaches might be used alternatively or complementary to each other (195).

In 2016, Godos and colleagues published a systematic review and meta-analysis of observational studies that investigated the association between dietary patterns extracted by the data-driven approach and the risk of colorectal adenoma. The analysis revealed that the risk of colorectal adenoma increases in individuals following dietary patterns characterised by high consumption of red and processed meat, refined grains and unhealthy snack (high in salt and sugar). The study also found that the risk decreases in individuals following dietary patterns characterised by high consumption of fruits and vegetables (196).

Two studies investigated the association between the predefined dietary pattern DII and the risk of colorectal adenoma. One cross-sectional study in the USA included 44278 individuals (197) and one case-control study in Iran included 134 colorectal adenoma cases and 240 controls (151). Both studies observed that a proinflammatory diet measured by energy adjusted DII was associated with higher risk of colorectal adenoma.

Previdelli and colleagues (2016) recommended using the two dietary pattern approaches at the same time on the same population to obtain a complementary evaluation to the dietary behaviour (198) .

This chapter explores the dietary behaviour of patients newly diagnosed with high-risk adenoma by describing the dietary patterns measured by the two approaches using the dietary data collected at baseline of the seAFOod trial.

This analysis will begin by describing the dietary patterns of the seAFOod trial cohort generated by the Principal Component Analysis method (PCA) and calculated by the Dietary Inflammatory Index (DII) method. The association between the two dietary patterns will be explored. This analysis will be conducted to explore if there is an association between dietary patterns extracted by two different approaches (data-driven and predefined) and by using different dietary components (as food groups were used to measure data-driven dietary patterns and nutrients were used to calculate the predefined dietary patterns).

The next analysis in this chapter is to explore if there is an association between age, sex, BMI and smoking behaviour of the patients and following a specific dietary pattern. After that, an analysis will be conducted to explore if any of the dietary patterns was able to differentiate between individuals consuming high and low amounts of foods and nutrients associated with CRC. The final analysis in this chapter is to explore if an association exists between following a specific dietary pattern and adenoma characteristics.

5.1 Aim and objectives

To explore dietary patterns of colorectal adenoma using *a posteriori and a priori* approaches, and to investigate the association between those dietary patterns and adenoma characteristics in patients recruited to the seAFOod trial.

The objectives are:

1. To describe the dietary patterns generated by PCA method and calculated by the dietary inflammatory index score (DII).
2. To explore if an association exists between the scores of dietary patterns extracted by PCA method and calculated by DII method.
3. Assess the relationship between dietary patterns , demographic characteristics and intake of foods and nutrients associated with CRC
4. To explore if there is an association between adherence to a specific dietary pattern and adenoma size, number or location.

5.2 Materials (Data)

The data used in this chapter was obtained from the seAFOod trial. It includes the baseline demographic and dietary intake data, dietary patterns' scores extracted by PCA and calculated by and DII method (details provided in **Chapter 2 section 2.2.3)** and the baseline adenoma characteristics data.

5.3 Methods

In summary, the PCA results will be presented and justifications for retaining and labelling the factors will be explained in details. The results from DII calculations will be described and compared with DII scores obtained from the global data. Pearson correlation analysis was performed to assess the relationship between scores of dietary patterns extracted by PCA and calculated by DII method.

The following analysis was conducted to assess if an association exists between following a specific dietary pattern with demographic characteristics and dietary consumption of foods and nutrients associated with CRC, as follow:

1) demographic characteristics: by comparing the age and BMI (using an Independent sample T test) and the proportion of current smokers, males and females (using Chi square test) in quartiles 1 and 4 of the dietary patterns extracted by PCA and measured by DII.

2) the consumption of foods and nutrients associated with CRC in quartiles 1 and 4 of the dietary patterns extracted by PCA and measured by DII (using independent sample T test).

The purpose of this analysis was to explore whether any of the extracted dietary patterns was able to identify a group of patients that met the dietary recommendations associated with CRC reported by WCRF/AICR.

The final analysis was to explore if an association exists between adherence to a dietary pattern and particular adenoma characteristics in this cohort. The analysis was performed using Mann-Whitney U test to investigate if the adenoma size (mm), number and location (proximal, distal colon) is different between the upper and lowest quartile for each dietary pattern. The upper and lower quartiles were examined because linear relationship is one of the assumptions that are required to conduct a regression and the association between the dietary pattern scores and adenoma characteristics data did not meet this assumption.

The methods used to achieve each of the objectives of this chapter are summarised in **figure 5-1**

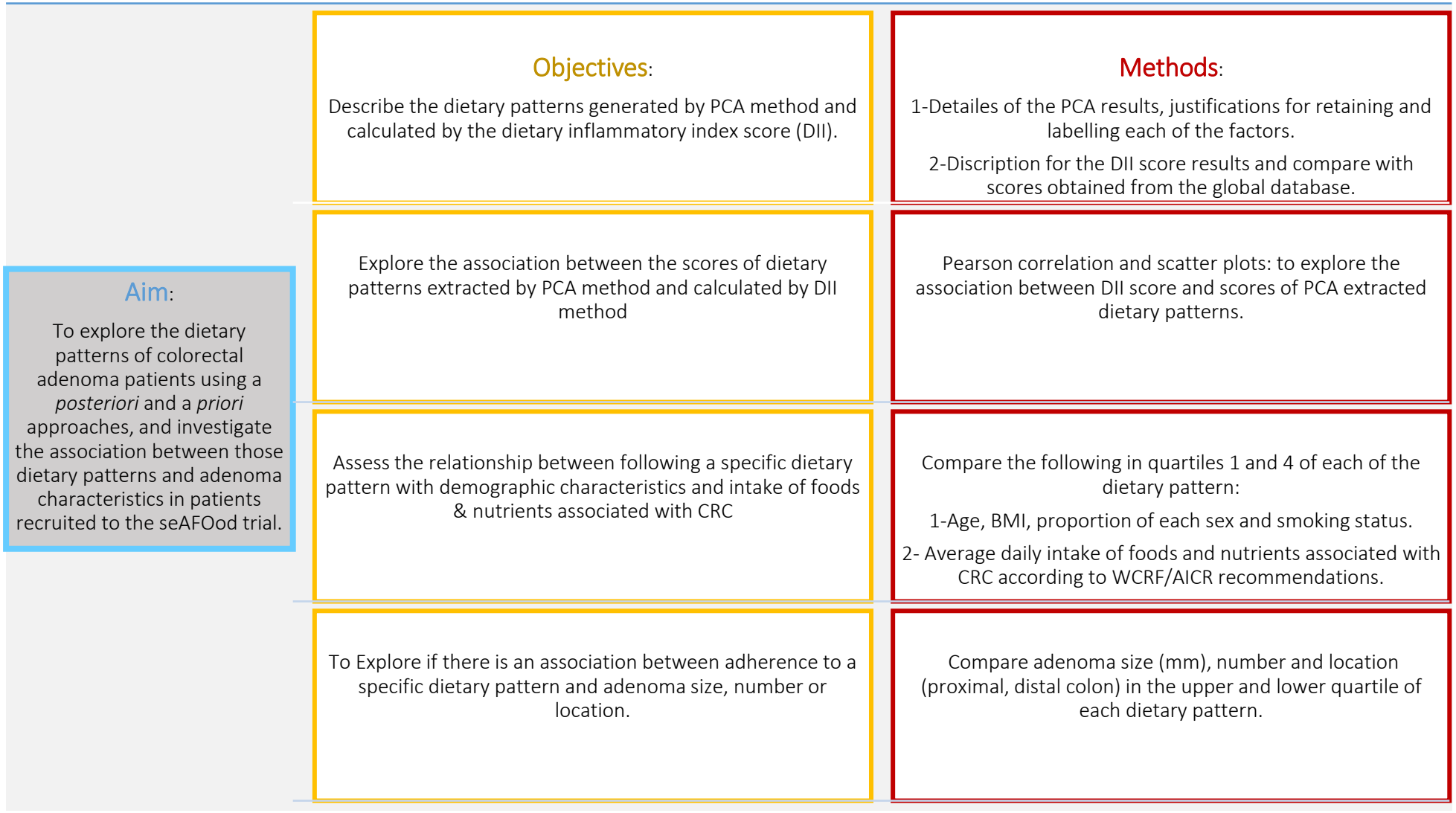


Figure 5-1. A summary for the aim and objectives of chapter 5 and the methods used to achieve them

5.4 Results

Baseline data from 674 (536 males and 138 females) colorectal adenoma patients recruited to the seAFOod trial was available and included in this analysis.

5.4.1 Description of dietary patterns

5.4.1.1 Data driven dietary patterns extracted by PCA.

Fourteen food groups extracted by FETA software from reported dietary intake were included in PCA using SPSS version 26. The KMO value was .677, which indicates that the sample size was suitable to run the PCA. Bartlett's Test of Sphericity was significant (p<.001) indicating that there is an adequate correlation between the food groups that would enable clustering the factors into less number, then each component can be characterised by high or low consumption of food groups.

Scree plot break was after the fourth component (**Figure 5.2**). Oblimin rotation revealed a number of strong loading factor on the first three components; loading factors on the fourth component were not significant, therefore, it was omitted from further analysis. The first three components explained 37.9% of the total variance in food groups' intake and were retained to identify the major dietary patterns followed by this cohort **(Table 5-1).**

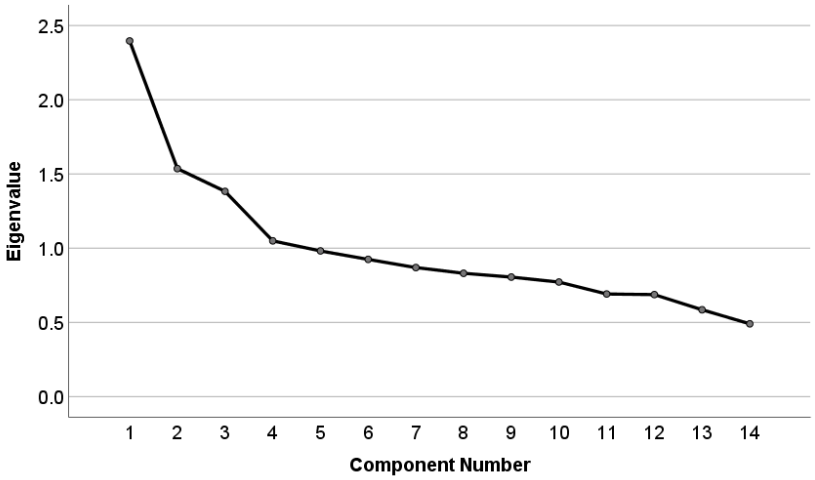


Figure 5-2 Scree plot for PCA of 14 food groups derived from the SeAFOod dataset

Table 5-1. Total Variance explained for PCA extracted component

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	2.396	17.116	17.116	2.396	17.116	17.116	2.090
2	1.536	10.970	28.086	1.536	10.970	28.086	1.897
3	1.383	9.876	37.962	1.383	9.876	37.962	1.300
4	1.049	7.496	45.458	1.049	7.496	45.458	1.352
5	.981	7.010	52.467				
6	.924	6.602	59.070				
7	.869	6.210	65.280				
8	.831	5.934	71.213				
9	.805	5.751	76.964				
10	.772	5.512	82.476				
11	.691	4.936	87.412				
12	.687	4.907	92.319				
13	.585	4.178	96.498				
14	.490	3.502	100				

Table 5-2 shows the un-rotated extracted components, **Table 5-3** shows the extracted component after applying the Oblimin rotation and results shows that:

1- The first component explained 17% of the total variance in food groups' intake. The following food groups: (Fats and oils group, Sugars, preserves and snacks group, Cereals and cereal products group, Milk and milk products group, Non-alcoholic beverages group and Potatoes group) had a moderate to high positive loading factors on this component (>.3). As these food groups are source of high energy, this component was labeled as "**High Energy Dietary Pattern**"

2- The 2nd component explained 10.9% of the total variance in food groups' intake. The following food groups: (Vegetables group, Fish and fish products group, Fruit group and Soups and sauces group) had a moderate to high positive loading factors (>.3). As these food groups are sources of essential nutrients and components of healthy dietary intake, this component was labelled as "**Healthy Dietary Pattern**".

3- The 3rd component explained 9.9% of the total variance of food groups' intake. Alcoholic beverages group and Nuts and seeds group had moderate to high positive loading factors (>.3), while Fruit group, Non-alcoholic beverages group and Milk and milk products group had moderate to high negative loading factors (<-.3). This component was labelled according to the two food groups loaded high as "**Alcohol and Nuts Dietary Pattern**"

Table 5-2. Un-rotated PCA solution: food groups (g) and factor loadings for each Principal Component extracted by PCA of data of 674 colorectal adenoma patients recruited to the seAFOod trial

Food groups	***Un-rotated Components***		
	1 (17.1%)	*2 (10.9%)*	*3 (9.9%)*
Alcoholic beverages group (g/day)	*-.068*	*-.014*	*.586*
Cereals and cereal products group (g/day)	*.616*	*-.088*	*-.151*
Eggs and egg dishes group (g/day)	*.472*	*.037*	*.243*
Fats and oils group (g/day)	*.597*	*-.428*	*-.082*
Fish and fish products group (g/day)	*.319*	*.486*	*.113*
Meat and meat products group (g/day)	*.369*	*-.267*	*.467*
Milk and milk products group (g/day)	*.396*	*-.054*	*-.396*
Non-alcoholic beverages group (g/day)	*.387*	*-.089*	*-.425*
Nuts and seeds group (g/day)	*.049*	*.317*	*-.081*
Potatoes group (g/day)	*.443*	*-.295*	*.386*
Soups and sauces group (g/day)	*.411*	*.306*	*.287*
Sugars, preserves and snacks group (g/day)	*.447*	*-.357*	*-.201*
Fruit group (g/day)	*.323*	*.543*	*-.278*
Vegetables group (g/day)	*.466*	*.567*	*.162*
Extraction Method: Principal Component Analysis.			
All cases included in visit one analysis = Included in visit 1			
4 components extracted.			

Table 5-3. Rotated PCA solution: food groups (g) and factor loadings for each Principal Component extracted by PCA of data of 674 colorectal adenoma patients recruited to the seAFOod trial.

	Rotated Components		
	1 (17.1%)	*2 (10.9%)*	*3 (9.9%)*
Alcoholic beverages group (g/day)	*-.080*	*.060*	*.675*
Cereals and cereal products group (g/day)	*.532*	*.208*	*-.145*
Eggs and egg dishes group (g/day)	*.286*	*.324*	*.220*
Fats and oils group (g/day)	*.751*	*-.089*	*.072*
Fish and fish products group (g/day)	*-.082*	*.605*	*-.032*
Meat and meat products group (g/day)	*.146*	*.115*	*.214*
Milk and milk products group (g/day)	*.435*	*.061*	*-.324*
Non-alcoholic beverages group (g/day)	*.422*	*.029*	*-.393*
Nuts and seeds group (g/day)	*.229*	*.179*	*.383*
Potatoes group (g/day)	*.306*	*.091*	*.249*
Soups and sauces group (g/day)	*-.015*	*.556*	*.067*
Sugars, preserves and snacks group (g/day)	*.686*	*-.149*	*.041*
Fruit group (g/day)	*-.075*	*.581*	*-.475*
Vegetables group (g/day)	*.029*	*.743*	*.079*
Extraction Method: Principal Component Analysis.			
Rotation Method: Oblimin with Kaiser Normalization.			
Food groups with factor loadings ≥0.3 are highlighted in bold			

Table 5-4. Food groups with moderate or strong positive factor loadings (⩾0.3) and with moderate/strong negative factor loadings (⩽-0.3) of the each of the dietary patterns.

Dietary pattern label	***Food groups with moderate or strong positive factor loadings (≥0.3)***	***Food groups with moderate or strong negative factor loadings (≤-0.3)***
High energy dietary pattern	*Fats and oils group* *Sugars, preserves and snacks group* *Cereals and cereal products group* *Milk and milk products group* *Non-alcoholic beverages group* *Potatoes group*	
Healthy dietary pattern	*Vegetables group* *Fish and fish products group* *Fruit group* *Soups and sauces group*	
Alcohol and nuts dietary pattern	*Alcoholic beverages group* *Nuts and seeds group*	*Fruit group* *Non-alcoholic beverages group* *Milk and milk products group*
Foods groups are listed in descending order according to the factor loading value in the rotated solution.		

5.4.1.2 Dietary inflammatory index score (DII)

DII score was calculated according to the method published by the score creator in 2014 (144). The calculation method is provided in **Chapter2 (Section 2.2.3.2)** and the validation analysis for the calculation method is provided in **Chapter 3 (Section 3.3.4)**. From the 45 food parameters used in the original method for DII calculation, 30 were available from the seAFOod trial dietary data and were used in this method. As **table 5-5** shows, the DII score of this cohort ranges from -3.82 to +5.14, while the DII score that was calculated from the global database (which contains data from 11 countries) ranges from -8.87 to +7.98 (144) . Median of DII score calculated from the baseline data of the seAFOod trial was higher than the median of DII score calculated for data obtained from the global database. Of the 674 patients included in this analysis, 157 patients (23.3%) had an antiinflammatory dietary pattern score (≤ zero), while 517 patients (76.7%) had a proinflammatory DII score (> zero).

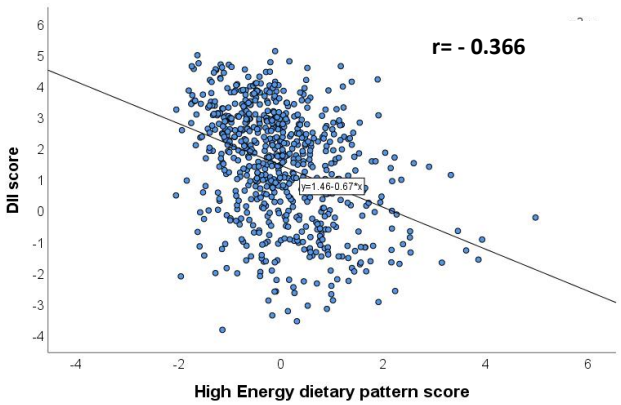
Table 5-5. Description for DII score calculated for baseline data for 674 colorectal adenoma patients recruited to the seAFOod trial.

	DII-score calculated for the seAFOod trial data using 30 food parameters	***DII-score form the global database using 45 food parameters***
Number of cases	*674*	
Mean	*+1.46*	
Median	*+1.75*	*+ .23*
SD	*+1.836*	
Minimum	*-3.82*	*- 8.87*
Maximum	*+5.14*	*+7.98*

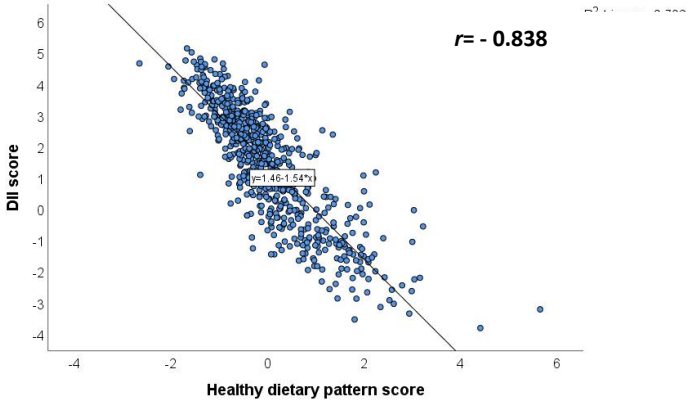
5.4.2 Correlation between the scores of dietary patterns extracted by PCA and measured by DII method

Correlation analysis was used to examine the association between the scores of dietary patterns generated by PCA and calculated by the DII method. Significant negative correlation was found between DII score and the healthy dietary pattern extracted by PCA as Pearson correlation shows (r=-0.838, *p*<.001) (**Figure 5-3- 1B**). Analysis also revealed a significant, but weak negative correlation between DII score and the high-energy dietary pattern (r=-0.366, *p*<.001) (**Figure 5-3- 1A**). No correlation was found between the DII score and alcohol and nuts score (**Figure 5-3- 1C**).

A



B



C

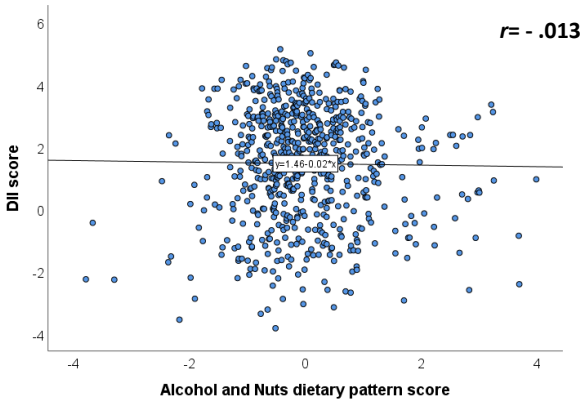


Figure 5-3. Scatter plot to show the correlation between DII score and scores of dietary patterns generated by PCA

r=Pearson correlation

5.4.3 The relationship between following a specific dietary pattern with demographic characteristics and intake of foods and nutrients associated with CRC

No significant difference was detected in the mean of BMI, age or the proportion of current smokers between quartile 1 and 4 of any of the dietary patterns. There is a significant higher proportion of males in quartile 4 when compared with the proportions in quartile 1 in the high energy pattern (27.6% vs 21.8%, $p<.001$) and in the alcohol and nuts pattern (28.5% vs 22.9%, $p<.001$). The proportion of females was significantly higher in quartile 1 when compared with quartile 4 in the high energy pattern (37% vs 14.5%, $p<.001$) and in the alcohol and nuts pattern (28.5% vs 22.9, $p<.001$). No difference in proportions of males or females in quartiles 1 and 4 of the healthy dietary pattern or the DII score (**Table 5-6)**.

Table 5-6. Comparison of the demographic characteristics of patients in quartile 1 and 4 of the dietary patterns

	High energy pattern			**Healthy pattern**			**Alcohol and nuts pattern**			**DII score**		
	Q1	Q4	*p*	Q1	Q4	*p*	Q1	Q4	*p*	Q1	Q4	*p*
No. of patients	168	168		168	168		168	168		168	168	
Dietary pattern score range	*-2.1 to -.69*	*.58 to* 4.98		-2.7 to -.68	.49 to 5.7		-3.8 to -.6	.5 to 3.98		-3.82 to .16	2.91 to 5.14	
Age (Year)	*65 (4.6)*	65.2 (4.9)	.6	65.1 (4.7)	65.9 (4.8)	.1	65.7 (4.6)	65.3 (4.6)	.4	65.6 (4.9)	64.8 (4.9)	.1
BMI (Kg/m2)	*30 (6.5)*	*29 (5.1)*	.07	29.7 (6.3)	29.6 (6.6)	.9	30 (6.3)	29 (5.7)	.1	29.2 (5.6)	29.3 (5.9)	.87
***Current smokers (%)**	30 (30.6)	26 (26.5)	.7	31 (31.6)	19 (19.4)	.4	22 (22.4)	33 (33.7)	.09	17 (17.3)	35 (35.7)	.07
Sex n (%)			<.001			.4			<.001			.8
Male	117 (21.8)	*148 (27.6)*		*133* (24.3)	*130* (24.3)		*123* (22.9)	*153* (28.5)		*133* (24.8)	134 (25)	
Female	*51* (37)	20 (14.5)		38 (27.5)	38 (27.5)		45 (32.6)	15 (10.9)		36 (26.1)	32 (23.2)	

Dietary pattern scores', age and BMI are presented in mean (SD). Sex and smoking status in number and percentage.
*Total number of current smokers is 98 **(**14.4 % of total cases).
Independent sample T test and Chi Square test

Average daily intake of foods and nutrients associated with CRC

This analysis has two purposes, the first is to explore if the dietary patterns' analysis differentiate between patients in their consumption of foods and nutrients associated with CRC. The 2nd is to investigate if any of the dietary patterns was able to extract a group of patients who met the CRC prevention recommendations published in the WCRF/AICR 2018 report (90). **Table 5-7** shows the comparison in average intake of foods and nutrients associated with CRC between quartiles one and four of the dietary patterns using independent sample T test. In the three data driven dietary patterns extracted by PCA: patients allocated in quartile one are the patients with the smallest score, or the least adhered to that dietary pattern; patients allocated in quartile four are the patients with the highest score, or the most adhere to that dietary pattern. For the DII score, patients in quartile 1 are patients consumed antiinflammatory diet and patients in quartile 4 patients consumed the most proinflammatory diet. This section will highlight the dietary behaviour of patients allocated in the eight groups (quartiles one and four of each dietary pattern) in comparison with the WCRF/AICR CRC prevention recommendations. Overall, dietary intake of most of the foods and nutrients associated with CRC was significantly different between quartiles one and four of each dietary patterns. However, dietary intake of each of the eight groups, has either exceeded the recommended amount of one or more of the foods and nutrients associated with high risk of CRC, or failed to meet the recommendation of foods or nutrients associated with lower risk of CRC, as follow:

1- High-energy dietary pattern (Data driven extracted by PCA): The diet of the patients who adhered to this dietary pattern (quartile 4) was high in redand processed meat and iron and low in vitamin D. Diet of patients in the 1st quartile was low in fibre and vitamin D.
2- Healthy dietary pattern (Data driven extracted by PCA): Patients loaded high on this dietary pattern (quartile 4) consumed high amounts of redand processed meat and iron and low amounts of vitamin D. Patients in quartile one of this dietary pattern exceeded the recommendation for red and processed meat, consumed low amounts of fish, oily fish, fruits, vegetables, fibre and vitamin D.
3- Alcohol and nuts dietary pattern (Data driven extracted by PCA): Diet of the patients loaded high on this dietary pattern characterised by high intake of alcohol (>25 units per week), red and processed meat and iron but low in fibre and vitamin D. Patients loaded low on this dietary pattern (quartile 1) consumed diet that was high in iron but low in fibre and vitamin D.
4- DII score (Predefined dietary pattern): Diet of patients with low DII score (antiinflammatory score) exceeded the recommendation for red and processed meat and was low in vitamin D.

Patients with high DII score (proinflammatory diet) exceeded the recommendation for red and processed meat , but did not meet the recommendations for fish, oily fish, fruits, vegetables, fibre and vitamin D.

The mean of vitamin C intake in the 8 groups met the recommended amount, however, as the validation identified over reporting of fruits and vegetables (the main source of vitamin C), it is likely that these values are over estimated.

Findings from this sub analysis are in line with the findings of the strong negative correlation that was found between the score of the healthy dietary pattern generated by the PCA and the DII score. As **table 5-7** shows that the dietary intake of foods and nutrients associated with CRC by patients allocated in the upper quartile of the healthy dietary pattern (adhere more to this dietary pattern) was similar the dietary intake of patients classified as consuming an antiinflammatory diet measured by DII score.

5.4.4 Adenoma number and size in quartiles 1 and four of the dietary patterns

As **table 5-8** shows, Mann-Whitney U test revealed that the total size of distal adenomas in patients loaded high on the healthy dietary pattern (quartile 4) was significantly smaller [mean=19 mm (SD=13 mm)] when compared with the size of distal adenomas in the first quartile [mean=22 mm (SD=15 mm), $p=.01$]. No significant difference in adenoma size or number in patients in quartiles one and four of the high-energy dietary pattern, the alcohol and nuts dietary pattern or the DII score.

Table 5-7. Mean (SD) of average daily intake of foods and nutrients associated with CRC compared in quartiles 1 and 4 of the dietary patterns

Dietary pattern	***High energy pattern***			***Healthy pattern***			***Alcohol and nuts pattern***			***DII score***		
Quartile*	*Q1*	*Q4*		*Q1*	*Q4*		*Q1*	*Q4*		*Q1*	*Q4*	
Score range	*-2.1 to -.69*	*.58 to 4.98*		*-2.7 to -.68*	*.49 to 5.7*		*-3.8 to -.6*	*.5 to 3.98*		*-3.82 to .16*	*2.91 to 5.14*	
No. of patients	*168*	*168*	*p*	*168*	*168*	*p*	*168*	*168*	*p*	*169*	*166*	*p*
Energy	*5.5 (1.3)*	*10.5 (2.1)*	*<.001*	*6.2 (1.8)*	*9.3 (2.5)*	*<.001*	*7.5 (2.4)*	*8.3 (2.6)*	*.004*	*9.6 (2.6)*	*5.8 (1.5)*	*<.001*
Alcohol	*16.2 (17.8)*	*12.1 (16.1)*	*<.001*	*11 (14.8)*	*15 (17.1)*	*.013*	*5 (5.7)*	*29 (21.7)*	*<.001*	*15.4 (17.3)*	*10 (13.7)*	*<.001*
Meat and meat products	*105 (56.6)*	*145 (67.5)*	*.025*	*106 (54.8)*	*135 (67.5)*	*<.001*	*99.7 (53.3)*	*149 (75)*	*<.001*	*137 (64.6)*	*101 (49.7)*	*<.001*
Red and processed meat	*72 (41.2)*	*107 (57.1)*	*<.001*	*76 (44)*	*97 (58.9)*	*<.001*	*71 (42.6)*	*111 (60.1)*	*<.001*	*95 (56.3)*	*75.8 (45.7)*	*<.001*
Fish and fish products	*44 (36.9)*	*45 (31.4)*	*.82*	*23.8 (16.5)*	*70 (44)*	*<.001*	*43.5 (39.2)*	*42 (27.8)*	*.68*	*60.7 (42.6)*	*28.2 (18.4)*	*<.001*
Oily fish	*15.6 (17)*	*15.4 (17.9)*	*.89*	*6 (8.7)*	*27 (22.9)*	*<.001*	*14 (15.3)*	*12.4 (15.5)*	*0.28*	*24.9 (22.5)*	*6.3 (7.6)*	*<.001*
Milk and milk products	*227 (123)*	*450 (193)*	*<.001*	*312 (176.5)*	*381 (192)*	*<.001*	*433 (194)*	*263 (151)*	*<.001*	*397 (189)*	*285 (155)*	*<.001*
Fruit and vegetables	*416 (254)*	*477 (225)*	*.02*	*233 (98)*	*695 (263)*	*<.001*	*545 (298)*	*381 (200)*	*<.001*	*698 (264)*	*244 (112)*	*<.001*
Fibre	*12.6 (5)*	*19.5 (6.2)*	*<.001*	*10 (2.9)*	*21.9 (5.9)*	*<.001*	*17 (6.5)*	*15 (6.2)*	*.005*	*22.9 (5.2)*	*9.7 (2.6)*	*<.001*
Iron	*8.9 (2.5)*	*13.7 (3.3)*	*<.001*	*8.2 (2.2)*	*14 (3.4)*	*<.001*	*10.8 (3.4)*	*11.8 (3.7)*	*.006*	*14.7 (3.2)*	*7.7 (1.8)*	*<.001*
Vitamin C	*95.2 (53.8)*	*117 (48.4)*	*<.001*	*63 (28)*	*155 (67.6)*	*<.001*	*123 (71.6)*	*93.8 (42.3)*	*<.001*	*157.5 (65.4)*	*61 (23.8)*	*<.001*
Vitamin D	*2.6 (1.4)*	*4.3 (1.9)*	*<.001*	*2.3 (1.2)*	*4.7 (2.1)*	*<.001*	*3.1 (1.7)*	*3.2 (1.6)*	*.6*	*4.5 (2.2)*	*2.1 (.9)*	*<.001*

Units of energy is MJ/day, food groups and fibre in g/day, iron and vitamin C in mg/day and vitamin D in µ/day. *In the three patterns generated by PCA, patients allocated in quartile 4 are the patients who strongly adhere to the dietary patter. In the DII score, quartile 4 is for patients with the most proinflammatory diet

Did not reach the recommended amount of the food or nutrients associated with low risk of CRC

Exceeded maximum amount of the foods or nutrients associated with high risk of CRC

Table 5-8. Comparison of baseline adenoma characteristics in quartiles 1 and 4 of DII score and the four dietary patterns extracted by PCA

Dietary pattern	***High energy pattern***			***Healthy pattern***			***Alcohol and nuts pattern***			***DII score***		
Quartile	*Q1*	*Q4*		*Q1*	*Q4*		*Q1*	*Q4*		*Q1*	*Q4*	
N of patients	*168*	*168*		*168*	*168*		*168*	*168*		*168*	*168*	
	M (SD)	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*
Adenoma numbers												
Total	*5 (2.4)*	*5.2 (2.6)*	*.97*	*5 (2.6)*	*4.9 (2.5)*	*.18*	*4.7 (2.1)*	*5.2 (2.5)*	*.36*	*4.9 (2.5)*	*5 (2.4)*	*.6*
Proximal	*2.3(2.3)*	*2.5 (2.2)*	*.42*	*2.3 (2.3)*	*2.5 (2.4)*	*.19*	*2.2 (2.1)*	*2.4 (2.3)*	*.34*	*2.5 (1.8)*	*2.64 (1.8)*	*.36*
Distal	*2.6 (1.7)*	*2.7 (1.9)*	*.55*	*2.7 (1.8)*	*2.4 (1.6)*	*.75*	*2.5 (1.6)*	*2.7 (1.9)*	*.05*	*2.4 (2.1)*	*2.4 (2.4)*	*.53*
Adenoma size (mm)												
Total	*33 (16)*	*32 (17)*	*.36*	*33 (16)*	*31 (15)*	*.15*	*32.1 (13.9)*	*33 (16.6)*	*.98*	*32.3 (17.3)*	*32.3 (14.9)*	*.49*
Proximal	*11 (11)*	*12 (12)*	*.68*	*11 (12)*	*12 (12)*	*.07*	*11.1 (11.3)*	*11.7 (11.9)*	*.52*	*12.3 (12.5)*	*11.7 (13.2)*	*.39*
Distal	*22 (14)*	*20 (14)*	*.13*	*22 (15)*	*19 (13)*	*.02*	*21.1 (14.2)*	*21.3 (14.8)*	*.91*	*19.9 (14.8)*	*20.5 (13.9)*	*.47*

M=Mean, SD= Standard Deviation
Comparison was by conducting Mann-Whitney U test

5.5 Discussion

This research sought to explore the dietary patterns of patients newly diagnosed with high-risk colorectal adenoma using two dietary pattern approaches. The data driven analysis generated three dietary patterns labeled as “high energy”, “healthy” and “alcohol and nuts”, and explained more than 3rd of the variance in dietary intake.

Sex of the patients was the only demographic characteristic that was associated with adherence to the high energy and alcohol and nuts dietary patterns, where the proportion of males in quartile four of both dietary patterns was higher than the proportion of females. These findings are in agreement with the study conducted in Canada by Beaudry *et al* (1998). They investigated the association between dietary patterns generated by factor analysis and sex of the patients (199) and found that males adhered more to the high-energy pattern while females adhered more to the health-conscious’ pattern.

The mean of the DII score for this cohort was +1.46 and ranged from -3.82 to +5.14 with the majority of the patients (76.7%) having a proinflammatory score (DII>0). The DII score obtained from studies used similar number of food parameters (25–30) usually ranges from −5.5 to +5.5 (200). A limitation of this comparison is that it was based on the number of food parameters included in computing the DII score but not based on the direction (pro or anti-inflammatory effect) and magnitude of their inflammatory effect. For example, both ω-3 fatty acids and MUFA has an antiinflammatory effect but the magnitude of ω-3 fatty acids is (-.436) much bigger than the magnitude of MUFA (-.009). On the other hand, both iron and cholesterol has a proinflammatory effect but the effect of cholesterol (+.110) is bigger than the effect of iron (+.032). Therefore, the difference detected between studies used DII score might be due to the different food parameters used in the calculations rather than a difference in the dietary behaviour of the individuals (201).

Two studies explored the association between DII score and colorectal adenoma and reported that a proinflammatory diet assessed by DII is associated with higher risk of colorectal adenoma. The first study was a cross sectional study that was conducted within the screening arm of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial in the USA included data from 44 278 individuals, adenoma was detected in 2655 cases (6% of the whole sample) (197). The 2nd study was a case-control study that was conducted in Iran and included 130 colorectal adenoma patients and 240 controls(151)

both studies used the energy adjusted DII. Computing E-DII scores requires using a specific database of energy-adjusted nutrient scores (200) which we had no access to. In addition, neither of the studies reported the means of the DII, which has limited our ability to compare our findings with these studies.

Dainty and colleagues investigated the association between DII score and the risk of rheumatoid arthritis using data from 141,769 individual enrolled in the UK biobank cohort (202) and found that DII score ranged from -3.88 to +4.22 and the mean was +0.03. Comparing these results with our findings from the seAFOod trial (DII range from -3.82 to +5.14 and mean of +1.46) show that the DII for the seAFOod trial was more proinflammatory. However, this difference could be related to that the sample size of the data included in Dainty study was much larger and more heterogeneous, also the difference in food parameters used calculate the DII in both studies (30 in the seAFOod trial vs. 18 in the UK biobank data).

To our knowledge, this is the first study to compare the DII score with scores of data-driven dietary patterns generated by PCA. The strong negative correlation between DII and the dietary pattern that was labeled as “health dietary pattern” may suggest that DII score could be used not only as an indicator for potential inflammation of the diet but also as an indicator for a healthy dietary pattern. However, more studies are required to verify these findings.

In relation to sub analysis of dietary intake of foods and nutrients associated with CRC in the upper and lower quartile of each dietary patten, overall, the analysis revealed that each of the dietary patterns was able to identify two groups that were significantly different in their dietary intake of most foods and nutrients associated with CRC. This analysis has also justified the labels allocated to each of the data driven dietary patterns. **Table 5-7** shows that quartile 4 in the high-energy dietary pattern was the highest in energy intake, quartile 4 in the alcohol and nuts dietary pattern was the highest in alcohol intake and the highest consumption of fish, oily fish, fruits, vegetables and fibre was observed in quartile four in the healthy dietary pattern.

However, this sub analysis also revealed information about the nature of the sample and the limitations of each of the dietary pattern approaches used.

As **table 5-7** shows the majority of the patients exceeded the daily recommended amount of red and processed meat, but the data driven dietary patterns did not show high loading factor for the meat group on any of the extracted dietary patterns. One might speculate that this was due to the fact that all

the patients consumed high amounts of meat and the PCA procedure was not able to extract a component with a distinctive loading factor for this food group. Which might question the suitability of using PCA method to extract the dietary patterns for such a homogeneous sample.

In relation to the DII score, the sub analysis for food consumed by patients categorised as consuming proinflammatory diet and antiinflammatory diet showed that patients with the lowest DII score (antiinflammatory score) consumed significantly higher amount of fish, oily fish, fruits, vegetables, and fibre, all associated with lower risk of CRC. However, the same group also consumed significantly higher amounts of the red and processed meat group and iron, both associated with higher risk of CRC. These findings raised the question about the suitability of using the DII score to explore the association between diet and colorectal tumorigenesis. The association between diet and colorectal tumorigenesis is complex and is likely to be due to both systemic and local effect. Being designed according to the effect of foods and nutrients on the circulating level of inflammation biomarkers (144), DII score does not account for the local effect of the diet on the intestinal mucosa. As **table5-7,** shows that patients with the most antiinflammatory diet (quartile 1) consumed significantly high amounts of alcohol, red and processed meat and iron. Evidence show that these food items increase the risk of CRC by local mechanisms, an effect that the DII score does not account for.

An example is that iron accumulation in the cells leads to an increase in the oxidative stress through formation of reactive oxygen species (ROS). Which creates a genotoxic environment and may lead to gene mutation and DNA damage that may contribute to colorectal tumorigenesis initiation (203).

Another limitation in using DII score to investigate the association between diet and colorectal tumorigenesis is that evidence shows that alcohol consumption is associated with lower levels of circulating inflammatory biomarkers, therefore it is used as one of the antiinflammatory food parameters in calculating DII score (144). In our analysis, this led to the observation that patients with lowest DII score consumed more alcohol than patients with high DII score. This may limited the use of DII score to investigate this association because of two reasons, the first is that evidence show that high alcohol intake is associated with higher risk of CRC (204) . The 2nd is that, the proposed mechanisms of alcohol in developing and progression of CRC is mainly by direct contact of the mucosa with alcohol and its metabolites acetaldehyde. Evidence show that direct contact may affect the cell structures and cause mucosal damage by disruption of epithelial tight junctions, increased cell proliferation and modulation of

gene expression (205). It is not clear at this point that using the energy adjusted DII would overcome this limitation. More investigations are needed by calculating the normal and energy adjusted DII using the same dataset and conducting sub analysis for foods and nutrients associated with CRC in quartiles 1 and 4 to answer this question.

We found that distal adenomas were significantly smaller in patients followed a healthy dietary pattern. Mehta and colleagues reported a higher risk of colorectal tumor in the distal colon in patients followed western dietary pattern. The study included data from 137,217 participants, 3,260 diagnosed with CRC (206). Several differences between this study and the seAFOod trial may affect this comparison. The sample size, the dietary data assessment and the food groups used in generating the dietary patterns are different. It is not clear if our findings that distal adenomas were significantly smaller in patients followed a healthy dietary pattern are due to multiple comparisons (207) or result from following a specific dietary pattern, therefore, more studies are needed to clarify this.

The results obtained from the dietary patterns are limited by the errors of the dietary data used to extract the dietary patterns, therefore, dietary data misreporting that was identified in **Chapter 3** should be considered when interpreting the results.

Summary

This chapter identified the major dietary patterns followed by the seAFOod trial participants during the 12 months before diagnosis. Data sub analysis showed that the cohort is homogeneous in terms of their age, BMI, and adenoma characteristics (as the size and number were in the inclusion criteria of the study) and it is also homogeneous in their dietary intake. This dietary pattern analysis was not able to extract a group of patients that consumed diet that meet the recommendation for CRC prevention. For example, patients classified as adhere to the healthy dietary pattern and had an antiinflammatory DII score both exceeded the recommended amount of red and processed meat and consumed nearly double their requirements of iron. The analysis also revealed a potential association between the healthy dietary pattern extracted by the PCA and the DII score. However, it also raised the questions about the suitable method of dietary pattern analysis that accounts for the complex association between diet and colorectal tumorigenesis (local and systemic effect).

Chapter 6

Change in the diet in the year following classification as at high-risk for colorectal adenoma recurrence. The seAFOod Trial

Chapter 6 Change in the diet in the year following classification as at high-risk for colorectal adenoma recurrence. The seAFOod Trial (Objective 4).

After describing the dietary behaviour of the seAFOod trial participants during the 12 months before diagnosis in **Chapter 4 and 5**, in this chapter a comparison is made of the dietary intake of the patients during the 12 months after diagnosis with their dietary intake during the 12 months prior to the diagnosis to explore if they changed their dietary intake following diagnosis with high-risk colorectal adenoma. This analysis is important to understand if receiving a diagnosis of high-risk colorectal adenoma influences the dietary behaviour in a population not receiving dietary advice. This Chapter is a manuscript in preparation for submission to the target journal European Journal of Nutrition.

6.1 Abstract

Background:

Diet is a known modifiable risk factor for Colorectal Adenoma (CRA) and Colorectal Cancer (CRC). No dietary or lifestyle guidance is provided to patients during the English BCSP and little is known about dietary behaviour changes following the identification of neoplasia at colonoscopy and polypectomy. This study assessed whether individuals, who were found to have multiple CRAs at BCSP screening colonoscopy and were subsequently recruited to the seAFOod Polyp Prevention Trial (a 2x2 factorial trial of eicosapentaenoic acid and aspirin for CRA prevention), exhibited differences in dietary behaviour at surveillance colonoscopy one year later.

Methods:

This is a secondary analysis of EPIC Food Frequency Questionnaires (FFQ) obtained at the baseline trial visit of the seAFOod trial, just after screening colonoscopy, at which individuals were stratified as 'high risk' based on CRA findings (≥3 CRAs if one > 10 mm, or ≥5 CRAs of any size) and at the time of scheduled surveillance colonoscopy 12 months' later. Trial participants did not receive any dedicated dietary advice or instruction. FETA software was used to extract average daily intake of food groups, energy and nutrients from EPIC Food Frequency Questionnaires (EPIC FFQ). The portion sizes reference used to assess whether patients changed their diet were taken from the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) guidelines on CRC dietary risk factors and

from the Healthy Eating Recommendations provided by Public Health England. The analysis was performed for the whole sample and then sub-analysed by gender.

Results:

Complete dietary data were available for 526 of 709 individuals randomised to the seAFOod Trial. At diagnosis, participants reported a mean (SD) total energy intake of 7.7 (2.42) MJ/day, 18.4% derived from protein, 34.1% from fat and 45.4% from carbohydrate. Patients reported a high intake of red and processed meat 88.5 (50.2) g/day, sodium 2.7 (0.9) g/day, and a low intake of dietary fibre 15.5 (5.9) g/day. At 12 months post-polypectomy, a reduction was detected in mean daily intake of total energy (from 7.7 MJ to 7.5 MJ, $p<.05$) and the percentage of energy obtained from protein (from 18.4% to 17.9%, $p<.01$) There was a small, but significant reduction in the daily intake of red and processed meat (from 88.5 to 77.8g, $p<.018$). Which is about one less portion of red and processed meat per week. This was reflected in an increase in the proportion of patients who met WCRF/AICR CRC recommendations to eat less than 70g/day red and processed meat from 41.8% to 50.4%, (p<.001, McNemar's test). A subgroup analysis revealed that changes in energy and red meat intake were confined to males.

Conclusions:

A modest but potentially beneficial change in diet was observed in the seAFOod trial participants following diagnosis of CRA, in the absence of any specific dietary advice. This is evidence that behaviour may change after colorectal neoplasia is diagnosed. This suggests that cancer screening sessions may represents a teachable moment. However, these findings might be confounded by participating in a clinical trial.

6.2 Introduction

There is strong evidence that Colorectal Cancer (CRC) risk increases with consumption of alcohol, red and processed meat and decreases with consumption of wholegrains, food high in fibre and dairy products, and limited evidence that disease risk increases with high consumption of food containing haem iron, and low fruit vegetable intake (90).

The English Bowel Cancer Screening Program (BCSP) does not include routine dietary advice in the care pathway and it is unknown whether patients modify their diet after CRA diagnosis given lay knowledge of dietary risk factors. Dietary response to CRA diagnosis is difficult to predict: whilst this group is

clinically considered at an elevated risk of developing CRC, patients may conversely interpret polypectomy as being diagnosed “free from cancer” or as licensing continued unhealthy dietary behaviour (208). Qualitative studies found that patients who were diagnosed with intermediate to high-risk CRA through the BCSP in England (209) and in Scotland (210) had little knowledge of the link between lifestyle factors and the risk of developing CRC, patients did not know that diagnosis of CRA places them into higher risk of developing CRC, and did not remember receiving any lifestyle advice during the screening process. Both studies interpreted that the lack of motivation to change their lifestyle after diagnosis was due to lack of knowledge (209,210).

Dietary behaviour change is a dynamic and complex process, however with appropriate planning cancer screening programs could be used to motivate and direct people to improve their dietary knowledge and behaviour (193,211,212). Investigating whether patients change their diet after being classified at high-risk of CRA recurrence may help to determine a successful future dietary intervention strategy. This study aimed to explore the change in dietary intake of foods and nutrients associated with CRC after the identification of ‘high risk’ features for colorectal neoplasia recurrence in patients recruited to the seAFOod polyp prevention trial.

6.3 Method

6.3.1 Participants, data collection and study design

This was a secondary analysis of data collected in the seAFOod polyp prevention trial (126). The seAFOod trial was a randomised double-blind placebo controlled 2x2 factorial trial, investigating the effect of aspirin and/or ω-3 fatty acid (eicosapentaenoic acid-EPA) on metachronous CRA. Patients were recruited to the trial through the Bowel Cancer Screening Program (BCSP) centres from around England from 2011 to 2017. Recruitment was after having a colonoscopy examination, diagnosed with advanced CRA and classified as at high-risk of recurrence. At recruitment, each patient received information about the study design, justifications and the possible personal implications (213), however, no information about the role of diet in the development and progression of the disease was given formally to the patients. The diet was assessed using the European Prospective Investigation into Cancer Food Frequency Questionnaire (EPIC-FFQ) (214) at two time points, after randomisation at visit 1 usually 2 weeks after screening colonoscopy, and at the exit, which was at or after the surveillance colonoscopy. The primary

purpose of assessing the diet in this trial was to assess changes in fish intake during trial participation that could confound the results of a trial of the ω-3 polyunsaturated fatty acid EPA. Height and weight were reported at baseline only and used to measure BMI. Smoking status was self-reported by the patients. Patients' demographic characteristics for the whole study have been previously reported (126).

6.3.2 Data Exclusion criteria

Cases were excluded from this analysis if: the baseline or follow-up FFQ was missing; or if FFQ was incomplete (> 10 items missing). The 0.5% of the lower and the upper values of the distribution of the ratio between the estimated energy intake to basal metabolic rate were considered as outliers (161) and were excluded from the analysis.

6.3.3 Dietary analysis

The FFQ data were analysed using FETA software (161) which yielded the average daily consumption of energy, alcohol, 14 food groups, and 44 macro and micronutrients for each record. In this analysis, the data provided in the Cross-Check Question (CCQ) in the EPIC FFQ was also used. The CCQ section of the EPIC FFQ, which is not included in FETA software analysis, required responders to report average weekly consumption of 'medium serving' portions of: meat, fish, fruit, vegetables and salads. Two approaches were used to assess whether people changed their dietary intake of foods and nutrients:

(i) The mean daily intake during the 12 months before recruitment versus during the 12 months after recruitment (data was extracted from the frequency section of EPIC FFQ using FETA software).
(ii) The 2nd approach was measuring the proportion of patients that reported intake within the guidelines during the 12 months before recruitment versus during the 12 months after recruitment (from both FETA output and the CCQs).

To assess whether patients met the recommended of foods and nutrients associated with CRC, two references were used, the WCRF/AICR report (90) and the PHE Healthy Eating Recommendations.(180). For the foods and nutrients associated with lower risk of CRC (fruits, vegetables, fibre), patients who consumed the recommended amount or more were classified as "achieved the recommendations". For foods and nutrients associated with high risk of CRC, patients who did not exceed the maximum amount allowed were classified as "achieved the recommendations". **Table 6-1** shows the food groups used in this analysis, the source of the data (CCQ section or frequency section) and the estimated portion size

Table 6-1. Measurement of mean daily intake of energy, food groups and nutrients using FETA software and corresponding portion

Factor	*Source of data*	*Measurement of average daily intake*
Energy	*FETA output*	*Mean daily energy intake (MJ/d) calculated from food consumed and reported through the EPIC FFQ.*
Alcohol	*FETA output*	*Mean daily alcohol intake (g/d) calculated from alcoholic drinks reported in the EPIC FFQ.*
Total meat	*CCQ*	*Patients provided an estimation for their average portions consumed per week of meat, meat products and meat dishes at the CCQ section.*
Total meat	*FETA output*	*Mean daily meat intake (g/d) was estimated from the red and processed meat (stated below*) plus chicken or other poultry that were reported in the frequency section of the EPIC FFQ.*
Red and processed meat *	*FETA output*	*Mean of daily intake of red and processed meat (g/d) was extracted from EPIC FFQ. This includes Beef, Beef burger, Pork, Lamb, Bacon, Ham, Sausages, corned beef, savoury pies and Liver. 1portion is estimated to equal to 83g*
Dietary fibre	*FETA output*	*Mean daily dietary intake of fibre (g/d) was extracted by the FETA software reported as Non-Starch Polysaccharides (NSP). As the recommendations by SACN is to consume >30g/day of AOAC, and 1g of AOAC fibre used by SACN for Dietary Reference Values is estimated to be equal to 0.76 g of NSP. The recommended amount of fibre used in this comparison was 22.8g/day***
Milk and dairy products	*FETA output*	*Mean daily intake of milk and dairy products (g/d) was extracted by the FETA software and provided the total in grams per day.*
Fruits and vegetables	*FETA output*	*Fruits and vegetables intake were measured by summation of fruit group with vegetables group in FETA output. The software provides the consumed amount in grams per day and one portion was considered to be equal to 80g*.*
	CCQ	*Patients provided an estimation for their average weekly consumption of salads, fruits and vegetables in 3 questions. The answers for the three questions were merged in one variable. Number of portions was divided by 7 to estimate average daily intake and to measure number and percentage of patients achieved 5 portions of fruit and vegetables per day at the two time points.*
Fish and fish products	*FETA output*	*Average daily intake of fish was extracted from EPIC FFQs with FETA software. This group includes White Fish, Oily Fish, Shellfish and Roe. The average consumption was measured as portions per week and the portion was estimated to be 140g.**
	CCQ	*Patients provided an estimation for their average weekly consumption of fish and fish products at the CCQ section, this was used to estimate the change in intake after adenoma diagnosis.*
Oily fish	*FETA output*	*Average daily intake of oily fish was extracted from EPIC FFQs with FETA software long format output. The average consumption was measured as portions per week and the portion was estimated to be 140g*.*
Vitamin C, D and Iron	*FETA output*	*Average daily intakes were extracted from EPIC FFQ in mg/day for vitamin C and iron and µg/day for vitamin D.*

An estimate for portion sizes for meat, fish, fruits and vegetables were obtained from the Eatwell guidelines published by Public Health England (PHE).
****22.8g is 0.76 of the 30g recommendations.***

Table 6-2. The association between specific food and nutrients and CRC according to WCRF/AICR report and recommendations for consumption when available

Factor	***WCRF/AICR findings***	***Goal to decrease the risk of CRC***
Alcohol	*Consumption of alcohol is a convincing cause of CRC*	*No safe limit*
Energy	*Body fatness increase the risk of CRC.*	*-*
Red and processed meat	*Consumption of processed meat is probably a cause CRC*	*≤70 gram*
Fibre	*Consumption food rich in fibre probably protects from CRC*	*≥ 30 gram per day*
Milk and dairy products	*Consumption of dairy products probably protects from CRC*	*-*
Fish and fish products	*Limited evidence fish decrease the risk of CRC*	*2 portions per week one of them is oily fish.*
Fruit and vegetables	*Limited evidence low intake of fruit and non-starch vegetables increase the risk of CRC*	*≥ 5 portions per day.*
Iron	*Limited evidence food rich in haem iron increases the risk of CRC*	*-*

6.3.4 Statistical analysis

Descriptive analysis and statistical tests were performed using IBM SPSS Statistics for Windows, Version 26. Descriptive data were provided as mean and standard deviation. The difference between the included and excluded cases were tested by the independent sample t-test for continuous variables and Chi-square test for categorical variables. Dietary intake comparisons (at recruitment vs. at exit) were undertaken using the paired sample T-test and Wilcoxon signed-rank test according to the variable distribution. The proportion of patients achieving recommended intake of food and nutrients of interest reported at recruitment and at exit was assessed by McNemar's test. The results were considered significant if the p value was ≤.05.

6.4 Results

Five hundred and twenty-six seAFOod Trial participants had complete dietary data at both time points and were included in the analysis. There were no differences in age, BMI or smoking status between included cases and excluded cases except that there was a higher percentage of females in the excluded group (26.5% versus 18.3%, p <.05).

6.4.1 Baseline demographics

There were 430 (82%) males in the study group. The mean age was 65.3 (SD=4.75) years. The mean BMI was 29.5 (SD=5.69) Kg/m^2 and the majority (>83%) were in the overweight or obese BMI categories. Only 14% were current smokers and 37% never smoked.

6.4.2 Dietary intake

The mean daily intake of energy and CRC-related food groups and nutrients at diagnosis and after 12 months are summarised in **Table 6-3**. Data for food groups, macro and micronutrients reported and extracted by FETA software for this cohort are provided in the Supporting information. (**Appendix 6 and 7**).

At diagnosis, mean daily self-reported energy intake was 7.7 MJ/d, with the percentage of energy derived from fat, protein and carbohydrate being 34.1%, 18.4% and carbohydrate 45.4%, respectively. Mean daily intake of Non Starch Polysaccharide (NSP) fibre was 15.5g and vitamin D from the diet was 3.3µg, both lower than the recommended levels (23g and 10mcg, respectively). Mean daily intake of sodium was 2.7g, which exceeded the maximum allowance (2.4g/d) recommended by the Scientific Advisory Committee on Nutrition (SACN) (215)**.** The mean reported intake of other micronutrients met the PHE recommendations (216)**.** Mean daily intake of red and processed meat was 88 (SD=50.2) g. This exceeds the maximum allowance recommended by WCRF/AICR (<70g/d).

The mean daily intake of alcohol reported in the FFQ and extracted by FETA software was 12.6g (approx. 1.6 units per day). When this amount compared with the number of units of alcohol reported during the interview at the recruitment stage, 94% of the patients reported alcohol intake in the EPIC FFQ that is within ±1 category of the number of units of alcohol reported during the interview.

Table 6-3. Mean (SD) of daily intake of energy, alcohol, food groups and nutrients for 526 colorectal adenoma patients recruited to the seafood trial at diagnosis and after 12 months.

	Factor	All (526)			Females (96)			Males (430)		
		Baseline	At exit	p	Baseline	At exit	p	Baseline	At exit	p
Strong Evidence	Alcohol (g/day)	12.6 (14.8)	12.4 (15.1)	.729	6.5 (9.3)	5.9 (7.8)	.327	14 (15.5)	13.9 (16)	.895
	Energy (MJ/day)	7.7 (2.42)	7.5 (2.52)	.018	6.7 (2.03)	6.8 (1.99)	.703	7.9 (2.45)	7.6 (2.59)	.01
	Red and processed meat (g/day)	88.5 (50.2)	77.8 (48.2)	<.005	67.4 (37.2)	60.3 (48.4)	.228	93 (51.4)	81.5 (47.4)	<.005
	Fibre (g/day)	15.5 (5.9)	15.4 (6.03)	.588	16.6 (6.5)	16.8 (6.5)	.559	15.3 (5.7)	15 (5.9)	.424
	Milk and dairy products (g/day)	341 (182.6)	334 (184.8)	.325	316 (188.4)	325 (182.6)	.457	346 (181)	336 (185.5)	.194
Limited Evidence	Fish and fish products (g/day)	43.2 (32)	43.2 (35)	.992	43.6 (29.4)	43.9 (36.04)	.935	43 (32.6)	43 (35.2)	.963
	Oily fish only (g/day)	14 (16.5)	14.5 (18.1)	.747	18.3 (18.5)	15.4 (17.2)	.11	13 (15.9)	14.4 (18.2)	.251
	Fruit and vegetables (g/day)	439 (228.8)	442 (235.5)	.6	521 (290.9)	548.8 (290)	.170	420.5 (208.5)	418.6 (214.8)	.831
	Iron (mg/day)	11 (3.4)	10.6 (3.4)	.002	10.5 (3.1)	10.1 (3.2)	.129	11.1 (3.4)	10.7 (3.5)	.007
	Vitamin C (mg/day)	105 (54.8)	105 (53.8)	.837	122.8 (81)	124.3 (64.6)	.825	101 (46)	101 (50)	.684
	Vitamin D (mcg/day)	3.3 (1.75)	3.2 (1.9)	0.6	3.2 (1.8)	2.9 (1.7)	.073	3.3 (1.8)	3.3 (1.95)	.871

*According to the variable's distribution both paired sample t-test and Wilcoxon signed-rank test according were used.

Reported mean daily intake of fruits and vegetables at baseline met the 5 portions-a day recommended by PHE (180). Patients reported a consumption of about two portions of fish and fish products per week, however, mean daily intake of oily fish indicated a consumption of approximately 0.7 portion per week, which is lower than the PHE recommendations of ≥1 portion per week (180).

6.4.3 Change in diet following diagnosis

Comparing the mean (SD) of reported dietary intake during the 12 months before the diagnosis with intake during the 12 months after diagnosis revealed a reduction in mean daily intake of energy (from 7.7 (2.42) to 7.5 (2.52) MJ/day), and in red and processed meat (from 88.5 (50.2) to 77.8 (48.2) g/day). Subgroup analysis revealed that this reduction was only significant in males: energy intake reduced from 7.9 (2.45) to 7.6 (2.46) MJ/day, $p<.05$, red and processed meat reduced from 93 (51.4) to 81.5 (47.4) g/day, $p<.01$. No change was detected in the percentage of energy obtained from fat and carbohydrate, however a small (from 18.4% to 17.9%) but significant ($p=.002$) reduction was detected in the percentage of energy from protein. Although a statistically significant change was detected in iron, calcium and zinc intake ($p<.05$), the reduction in mean daily intake was small. No change was detected in intake of other food groups and nutrients as shown in the supporting information (**Appendix 6 and 7).**

6.4.4 Proportion of patients achieving WCRF/AICR recommendations

Table 6-4 shows the number and percentage of patients who reported dietary intakes of food and nutrients related to CRC within the WCRF/AICR and PHE recommendations. This was measured from the FFQ main matrix and from the CCQs' section. Data reported in the main FFQ matrix showed that at diagnosis, nearly 42% of the patients met the recommendation of less than 70g/day of red and processed meat (55.2% of females and 38.8% of males). After diagnosis, there was an 8.6% increase in the number of patients who met this recommendation; (p <.001 McNemar's test), A subgroup analysis revealed that the change was significant for males (p <.001) but not for females (p=.054). For the total meat intake reported in the frequency section of the FFQ showed that at baseline 16.5% of the patients consumed less than 70g of meat per day, this significantly increased to 22.8% ($p<.001$) during the 12 months after diagnosis. Analysing data according to the sex of the patient showed a significant increase in the proportion of males who reduced their total meat intake but not significant for females. Data reported in

the CCQ section indicated a non-significant reduction in the proportion of patients reduced their meat intake, however, this difference could be due to that the question enquired about consumption of all types of meat and not limited to the red and processed meat. The FFQ data showed that before diagnosis more than 50% of the patients reported consumption of the recommended 5 portions of fruits and vegetables per day with no significant change after diagnosis. No change in percentage of people achieved the ≥ 2 portions of fish per week but subgroup analysis revealed that the proportion of females who consumed one portion of oily fish per week decreased from 25% to 15.6%, $p < .05$. The CCQs data revealed over reporting of the fruits and vegetables group in the FFQ main matrix and only 2.3% of the patients reported that they consume, on average, ≥5 portions of fruits and vegetables per day, however, no change in consumption of this food group detected by the two methods. No significant change in proportions of patients consumed the recommended amount of fibre.

Table 6-4. Number and percentage of patients who consumed the recommended amount of red and processed meat, fish and fish products, fruits and vegetables groups and fibre before and after diagnosis.

	All (526)			***Females (96)***			***Males (430)***		
Food group/nutrient	*Baseline*	*At exit*	*p*	*Baseline*	*At exit*	*p*	*Baseline*	*At exit*	*p*
Red and processed meat*	*220 (41.8)*	*265 (50.4)*	*<.001*	*53 (55.2)*	*63 (65.6)*	*.054*	*167 (38.8)*	*202 (47)*	*.001*
Total meat *	*87 (16.5)*	*120 (22.8)*	*<.001*	*28 (29.2)*	*37 (38.5)*	*.122*	*59 (13.7)*	*83 (19.3)*	*.004*
Total meat (CCQ)	*329 (69.7)*	*344 (72.9)*	*.192*	*64 (75.3)*	*68 (80)*	*.454*	*265 (68.5)*	*276 (71.3)*	*.315*
Fish and fish products *	*222 (42.2)*	*219 (41.6)*	*.873*	*41 (42.7)*	*39 (40.6)*	*.832*	*181 (42.1)*	*180 (41.9)*	*1*
Oily fish *	*72 (13.7)*	*75 (14.3)*	*.82*	*24 (25)*	*15 (15.6)*	*.49*	*48 (11.2)*	*60 (14)*	*.156*
Fish and fish products (CCQ)	*264 (50.2)*	*290 (55.1)*	*1*	*45 (46.9)*	*54 (56.3)*	*.804*	*219 (50.9)*	*236 (54.9)*	*1*
Fruits and vegetables *	*266 (50.6)*	*270 (51.3)*	*.782*	*60(62.5)*	*65 (67.7)*	*.359*	*206 (47.9)*	*205 (47.7)*	*1*
Fruits and vegetables (CCQ)	*12 (2.3)*	*9 (1.7)*	*.607*	*5 (5.2)*	*3 (3.1)*	*.678*	*7 (1.6)*	*6 (1.4)*	*1*
Fibre *	*56 (10.6)*	*48 (9.1)*	*.382*	*13 (13.5)*	*13 (13.5)*	*1*	*43 (10)*	*35 (8.1)*	*.332*

CCQ=Cross-Check Questions
Notes: Not all the 526 cases included in this analysis answered the CCQs.
Meat question in the CCQ section includes all meat and not only the red and processed meat
****FETA output***

6.5 Discussion

This is the first study to assess and report the change in dietary intake after CRA removal through a comprehensive dietary assessment. Measurement of diet in previous studies was limited to specific questions about consumption of food groups within lifestyle questionnaires (193). Using the FFQ as a dietary assessment tool at two time points allowed a systematic assessment for the total dietary intake at the two time points, which enabled exploring dietary behaviour before and after the diagnosis and assess whether any changes occurred.

At baseline red and processed meat intake was more than the average intake of 65years and over in the UK reported by the NDNS, an average of 88.5(SD=50.2) g/day vs 63 (SD=43) g/day (217). A study conducted in 2008 in the UK in adults newly diagnosed with CRA reported an intake of 102g/day of red and processed meat (218), which is even higher than the intake in this sample, however, this difference could be due to the use of different dietary assessment and analysis tools. A reduction of about 12g/day, an equivalent to one portion of red and processed meat per week was observed by males, also the analysis revealed that significant increase in the percentage of males that adhered to the WCRF/AICR recommendation for red and processed meat in the 12 months post- diagnosis and recruitment to the trial, in addition a significant reduction was detected in mean daily energy intake in males only. These findings are similar to those published by Cottet *et al.* (2005). The study included 338 colorectal adenoma patients (55% males) that were recruited to the European Cancer Prevention (ECP) Study, which explored the role of calcium and fibre supplementation in the prevention of CRA recurrence. During the 3 years follow-up period, only males changed their dietary intake. The change was observed in total energy, fat, protein, cholesterol and calcium (153).

These findings show that positive changes might be achievable and some of those high risk people may benefit from knowledge based dietary intervention strategy.

Although the FFQ tool appeared to result in a significant over-reporting of fruits and vegetables intake compared with the CCQs, neither analytical approach identified significant change in daily intake of this group at surveillance colonoscopy. A study conducted in 2002 reported that a tailored, simple written message led to increase in intake of fruits and vegetables in patients who attended the cancer screening (219).

While going through the screening and diagnosis process, patients become more interested about knowing the association between lifestyle and health (220). This is described as a "teachable moment", where patients, could spontaneously change their lifestyle to reduce their risk of developing the disease (221). Previous studies suggested that the teachable moment created during cancer screening programs could be used to direct people to make positive changes in their lifestyle (221,222). Providing nutritional advice was not planned as a part of the seAFOod trial, however, there is a possibility that health professionals provided information about the association between diet and CRA as a response to patient's request. Behavioural change is a complex multistage process (223) that is influenced by both internal factors (e.g. age and gender) and external factors (e.g. contact with health professionals) (224). Ostlin *et. al*. (2006) suggested that development of a successful health education intervention strategies require consideration of behavioural differences between males and females during different stages of research: planning, collecting and analysing data (225). Although the high percentage of males recruited to the seAFOod trial may reflects the nature of the disease being more prevalence in males (226–229), it is not clear if the detected gender difference in dietary change was due to difference in behaviours or due to the imbalance in the sample size.

This study has some limitations. It is important to acknowledge that this data were collected from specific risk stratum of colorectal adenoma patients and may not reflect the behaviour of the wider screened population. Data included in this analysis was obtained from patients who were engaged in an interventional clinical trial that included EPA and this may have raised awareness of diet and may have influenced their behaviour. For example, their behaviour might be affected by the long-term engagement with the health care practitioners and by having more information about the disease and the potential effect of the intervention on the disease, or may in itself selected for more change-motivated participants.

Another limitation of this study is the imbalance of the number of males and females included. A review about gender inequality in clinical practice, published in 2020, revealed lack of effort in primary health care intervention studies to reduce gender bias (230). We recommend that future studies would benefit from representative samples of both males and females and a mixed method design to investigate the reasons behind different dietary behaviour.

Other limitations are related to our measurements. FFQ, has limitations including reporting errors resulting from either problem in memory or misevaluation of portion size (231). There is some evidence of underreporting: the majority of the participants were categorised as overweight or obese, yet the mean daily energy intake was 300kJ lower than that for this age group reported in the NDNS (232). Also, the CCQs revealed that fruits and vegetables groups were over-reported in the main FFQ. This may also reflect social desirability bias, that is when people under report food that is advised not to be over consumed for example meat, but at the same time they over report food with more health benefits, such as fruits and vegetables.

Despite the discrepancy in the data obtained from the answers provided to different type of questions measuring the same food group (frequency and CCQ) , same direction of change/no change were obtained using the two methods. Both analysis revealed no change of proportions of people reported meeting the recommended daily intake of fruits and vegetables. Also an increase in the proportion of patients who consumed less meat was observed by the two methods, although it was not significant by the CCQ data, that might be due to the question include all meat and not just the red and processed meat included in the main FFQ. Another limitation is that no qualitative data was collected to identify if the change in dietary intake was intentional or is it unintentional change.

To conclude, this analysis revealed a limited improvement in the diet of some patients after colorectal adenoma diagnosis. However, changes are modest and there is potential for further improvement in this population as a whole as well as opportunity for simple interventions (given the absence of any formal diet advice). Future studies should address how to amplify effects at this life point.

Chapter 7

The association between diet, dietary patterns and colorectal adenoma characteristics and recurrence in the placebo arm of the seAFOod trial

Chapter 7 The association between diet, dietary patterns and colorectal adenoma characteristics and recurrence in the placebo arm of the seAFOod trial (Objective 5).

After describing the baseline dietary intake and the dietary patterns of the patients recruited to the seAFOod trial in **Chapters 4 and 5** and measuring the change in the dietary behaviour in **Chapter 6**, this chapter will explore if dietary intake at baseline is associated with the risk of colorectal adenoma recurrence after 12 months of polypectomy.

Background

Evidence shows that unhealthy data-driven dietary patterns that are characterised by high consumption of red and processed meat are associated with a high risk of colorectal adenoma while dietary patterns high in fruits and vegetables are associated with a lower risk of colorectal adenoma (196,233). In term of the association between dietary patterns and the risk of colorectal adenoma recurrence, one small study explored this association using the data-driven dietary pattern analysis approach (153). The study was conducted within the European fibre-calcium intervention trial and included 442 colorectal adenoma patients. At the three-year colonoscopy, adenoma reoccurred in 20.8% of the patients. PCA Data driven dietary patterns were extracted from dietary data collected at baseline and reported for males and females separately. For females, PCA generated three dietary patterns that were identified as the *Mediterranean, Western and snacks dietary patterns*. The analysis showed that the Mediterranean dietary pattern was associated with a lower risk of recurrence in females. No association between dietary patterns and adenoma recurrence in males was found (153).

One study explored the association between the predefined dietary pattern score, measured by the DII method, and the risk of adenoma recurrence in 2017 (154). The study was a pooled analysis study that included data from 1727 patients that were enrolled in Phase three clinical trials aimed to investigate the use of either high-fibre cereal supplement or Ursodeoxycholic acid on the risk of adenoma recurrence. After a 3 year follow-up period the study found no association between DII score and the risk of colorectal adenoma recurrence or the characteristics of adenoma (size, location or type) in the case of recurrence (154).

Overall, the association between dietary patterns and the risk of colorectal adenoma recurrence is relatively under-explored. This chapter investigates if dietary intake alters the risk of adenoma

recurrence after one year of polypectomy in high-risk adenoma patients recruited to the seAFOod trial through the BCSP. This analysis is one of the objectives of this research needed to achieve the main aim of this research, which is to explore the association between diet, dietary patterns and colorectal adenoma development and recurrence. In this chapter, the analysis is restricted to patients allocated to the placebo arm of the seAFOod trial. It was decided that it was not scientifically valid to consider the association with the dietary patterns in the context of an intervention. Although numbers were significantly reduced as a consequence, it was agreed that the approach should be taken as a 'proof of principle'.

7.1 Aims and objectives

1. To investigate the relationship between baseline dietary intake (nutrient, food groups and dietary patterns) and risk of adenoma recurrence at 12 months post polypectomy.
2. To explore the association between dietary patterns and colorectal adenoma characteristics in the case of recurrence.

The data from the 156 patients allocated to the placebo arm of the seAFOod trial was used to achieve these aims through conducting the following objectives:

1. To compare demographic characteristics, dietary intake of foods and nutrients associated with CRC and dietary patterns' scores in patients with and without adenoma recurrence.
2. To explore the association between dietary patterns' scores and risk of colorectal adenoma recurrence by comparing the scores between the groups with and the group without adenoma recurrence and measuring the probability of change in adenoma risk with change in dietary patterns' scores.
3. To compare the distribution of adenomas in quartiles one and four of each of the four dietary patterns using the adenomas size, number and location.

7.2 Data

The data set used in this chapter was collected from the patients allocated to the placebo arm of the seAFOod trial. Age, sex and BMI data were collected at baseline. The association between dietary patterns (DII and PCA derived) and the risk of colorectal adenoma recurrence used the baseline dietary data only. The adenoma recurrence data and its characteristics in the case of recurrence were obtained

from the exit colonoscopy examination that was performed after 12 months of the index colonoscopy. Only patients with complete baseline dietary data and with exit colonoscopy data were included in the analysis.

7.3 Statistical analysis

Figure 7-1 shows a summary for the analysis methods used to achieve the aims and objectives of this chapter. The analysis began by conducting a comparison in demographic characteristics between patients with and without adenoma recurrence. Independent sample T-test was used to compare means of age and BMI and Chi-square test was used to compare the smoking status and sex of the patients. Both Independent sample T-test and Mann-Whitney test were used to compare the intake of foods and nutrients associated with CRC according to the WCRF/AICR report between cases with and without adenoma recurrence.

The dietary patterns (both predefined and data-driven) for this cohort (156 patients) were measured as part of the total cohort using dietary data collected at baseline. The dietary patterns' scores and quartiles were extracted from the measurements performed for the total sample of 674 patients. The methods used are presented in details in **Chapters 2 and 5.** In summary, The PCA method was used as a data-driven approach, using 14 food groups, and extracted three dietary patterns that were labelled according to the correlation between the foods groups included in the analysis as follow the *'high-energy'* dietary pattern, the *'healthy'* dietary pattern and the '*alcohol and nuts'* dietary pattern. The DII score (144) was measured using 30 food parameters. It was used as one of the predefined dietary pattern methods, it measures the inflammatory potential of the diet where a high DII score indicates consumption of a diet that has an overall proinflammatory effect, while a low score indicates consumption of a diet that has an overall anti-inflammatory effect.

Before exploring the association between the dietary patterns and the risk of adenoma recurrence, the means of the dietary patterns' scores were compared in patients with and without adenoma recurrence using independent sample T, also the data of the dietary patterns' scores was presented for patients with and without adenoma recurrence using the violin plots. The advantage of using the violin plots is that it allows the visualization of all the sample and allows a visual comparison of the distribution of the data of patients with and without adenoma recurrence among the dietary patterns' scores. After that,

multiple logistic regression models were used to calculate odds ratios of adenoma recurrence and 95% confidence intervals. Two logistic regression models were used, the first model included the three dietary patterns extracted by the PCA and the 2nd model included the DII score, both models were adjusted for age, sex and BMI. DII was not included in the first model because one of the assumptions required for the logistic regression is that no correlation should be between the independent variables included in the model. As **Section 5.4.2 in Chapter 5** shows, there was a correlation between the DII score and the healthy dietary pattern extracted by the PCA, therefore separate models were used.

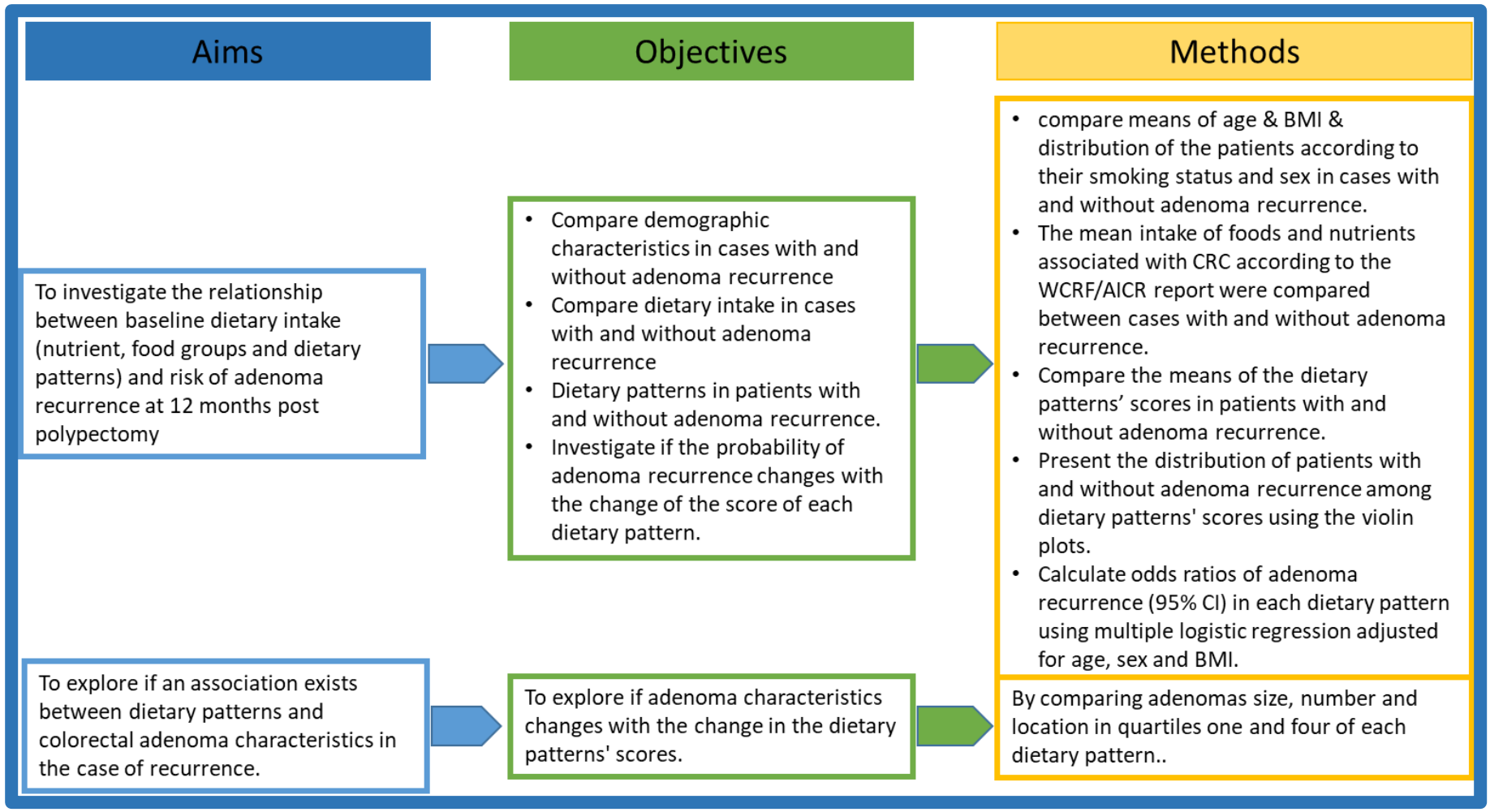


Figure 7-1. Summary for the aims and objectives of chapter 7 and the analysis methods used to achieve each one

The association between dietary patterns and adenoma characteristics was explored by comparing the distribution of adenoma by its size, number and location in quartiles one and four of each of the four dietary patterns, using the Mann-Whitney U test. The allocation of patients in the dietary patterns' quartiles was extracted from the total 674 cohorts.

7.4 Results

Of the 176 patients allocated to the placebo arm, only 156 cases had complete baseline dietary data and exit colonoscopy data and were included in this analysis (seven cases had no complete dietary data and 13 cases had no exit colonoscopy data).

7.4.1 Baseline subject characteristics and average daily intake of foods and nutrients associated with CRC in patients with and without adenoma recurrence

One year after polypectomy, one or more adenomas were detected in 62% of the cases allocated to the placebo arm of the seAFOod trial, 64.5% of the males and 53.1% of the females. Independent sample T-test showed no significant difference in age and BMI of patients who were diagnosed with adenoma after one year of removing index adenoma and in patients free from adenoma recurrence. No significant difference between the two groups was found in sex and smoking status as the Pearson Chi-square test shows in **Table 7-1-A**.

No significant difference was observed in energy intake or in the intake of foods and nutrients associated with CRC according to WCRF/AICR between patients with and without adenoma recurrence (**Table 7-1-B**).

Table 7-1. Baseline characteristics, dietary intake of food and nutrients associated with CRC and the dietary patterns' scores in patients with and without adenoma recurrence

Adenoma recurrence	***No***	***Yes***	*p*
A-Baseline subject characteristics			
Number of cases (%)	*59 (37.8)*	*97 (62.2)*	
Age mean(SD)	*64.7 (4.9)*	*64.9 (4.6)*	*.97*
BMI mean (SD)	*29 (5.3)*	*29.5 (5.03)*	*.76*
****Sex***			*.293*
Male n (%)	*44 (74.6)*	*80 (82.5)*	
Female n (%)	*15 (25.4)*	*17 (17.5)*	
****Smoking status n (%)***			*.307*
Current smokers	*9 (15.3)*	*20 (20.6)*	
Ex-smokers	*30 (50.8)*	*43 (44.3)*	
Never smoked	*20 (33.9)*	*34 (35.1)*	
B-Energy, foods and nutrients associated with CRC Mean (SD)			
Energy (MJ/day)	*7.5 (2.10)*	*7.6 (2.55)*	*.96*
$ Alcohol (g/day)	*14.5 (16.85)*	*13.4 (14.34)*	*.67*
Meat and meat products (g/day)	*131.7 (57.57)*	*125 (62.8)*	*.51*
Red and processed meat (g/day)	*92 (53.75)*	*87.8 (45.37)*	*.61*
$Fish and fish products (g/day)	*38.9 (23.35)*	*37.9 (25.74)*	*.81*
$ Oily fish (g/day)	*13.4 (14.29)*	*11.5 (13.32)*	*.39*
Milk and dairy products (g/day)	*347.4 (201)*	*360.1 (199.3)*	*.69*
Fruit and vegetables (g/day)	*470.2 (280.2)*	*420 (208.7)*	*.21*
Fibre (g/day)	*16.3 (6.46)*	*14.9 (5.98)*	*.17*
Iron (mg/day)	*11.4 (3.22)*	*10.7 (3.27)*	*.22*
Vitamin C (mg/day)	*117.8 (92.54)*	*99.4 (46.96)*	*.10*
Vitamin D (µg/day)	*3.4 (1.6)*	*3 (1.85)*	*.21*

****Pearson Chi-square***
$ Mann Whitney test showed no significant difference in alcohol (p=.851), fish (p=.792) or oily fish (p=.321)
Independent sample T-test was used for other variables

7.4.2 Dietary patterns and risk of adenoma recurrence

Description of dietary patterns' scores in patients with and without adenoma recurrence

As **table 7-2** shows, no significant difference was found between patients with and without adenoma recurrence in scores of the '*high energy dietary pattern', 'healthy dietary pattern', 'alcohol and nuts dietary pattern' and the 'DII scores' as the* independent sample T-test. To show the distribution of the cases among the dietary patterns' scores violin plots were used (**Figure 7-2**). The figure shows the adenoma recurrence data according to dietary pattern score for the 156 patients in the placebo arm of the seAFOod trial. The width of each plot corresponding to the frequency of the cases on a particular dietary pattern's score and the small black boxes show the mean of each dietary pattern score. It was observed from the plots that the distribution of the cases around the scores of the data-driven dietary patterns is similar for cases with and without adenoma recurrence. The DII plot is different for those with and without adenoma – this may suggest that some difference may be observed had the sample size be bigger.

Table 7-2. Dietary patterns' scores in patients with and without adenoma recurrence

Adenoma recurrence	***No***	***Yes***	*p*
Dietary patterns scores Mean (SD)			
High energy dietary pattern	*-.14 (0.90)*	*.01 (1.06)*	*.36*
Healthy dietary pattern	*.09 (0.98)*	*-.09 (1.01)*	*.29*
Alcohol and nuts dietary pattern	*-.04 (1.03)*	*.05 (1.08)*	*.61*
DII dietary pattern	*1.18 (1.80)*	*1.48 (1.98)*	*.33*
Independent sample T-test			

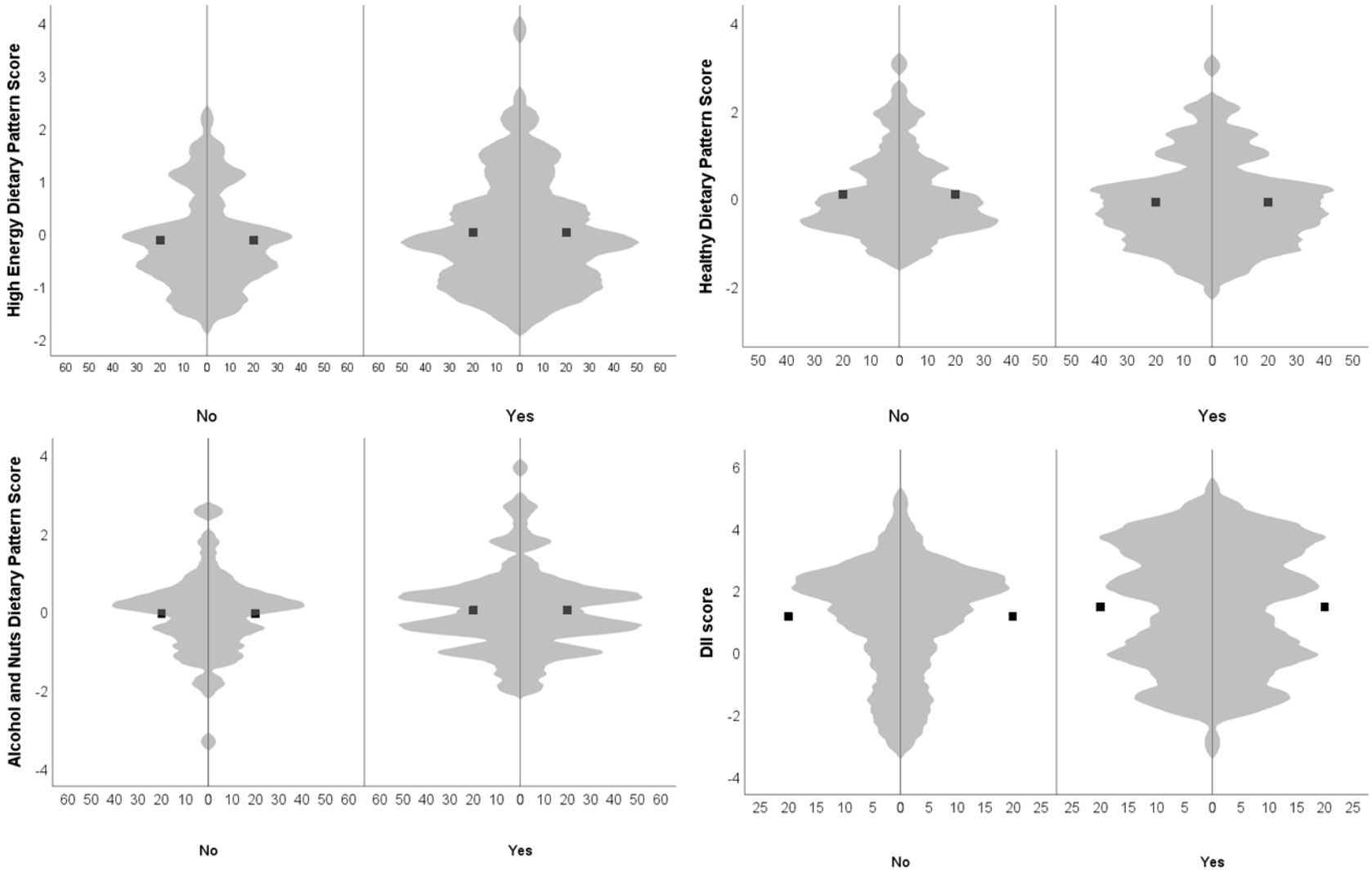


Figure 7-2. Violin plot to show the distribution of cases with (Yes) and cases without (NO) adenoma recurrence among dietary patterns' score

. Small black boxes show the mean for each score

7.4.3 Change in dietary patterns scores and the risk of adenoma recurrence

To investigate if the probability of adenoma recurrence changes with the change of the score of the dietary patterns, two separate logistic models were used one for each dietary pattern approach

Data-driven dietary patterns extracted by PCA model

The first logistic regression model contained the following six independent variables: age, sex, BMI, and the scores of the *high-energy dietary pattern, the healthy dietary pattern and the alcohol and nuts dietary pattern*. The full model containing all predictors was not statistically significant, χ^2 (6, N = 155) = 5.6, *p* = 0.469. The model as a whole explained between 3.6% (Cox and Snell R square) and 4.8% (Nagelkerke R squared) of the risk of adenoma recurrence, and correctly classified 63.2% of the cases. As **table 7-3** shows none of the independent variables included in the analysis made a statistically significant contribution to the model. A sensitivity analysis was performed by testing each dietary pattern score separately while controlling for other factors in the model, and the same pattern of results was found.

Table 7-3. Adjusted Odds Ratio (OR) and (95%CI) for the association between the score of the dietary pattern extracted by PCA and adenoma recurrence.

	B	***S.E.***	***Wald***	***df***	*p*	***OR***	***95%CI***	
							Lower	*Upper*
sex	*.44*	*.43*	*1.07*	*1*	*.30*	*1.55*	*.67*	*3.58*
BMI	*.03*	*.03*	*.63*	*1*	*.43*	*1.03*	*96*	*1.10*
Age	*.00*	*.04*	*.01*	*1*	*.94*	*1.00*	*.93*	*1.08*
High energy dietary pattern score	*.28*	*.19*	*2.13*	*1*	*.15*	*1.33*	*.91*	*1.94*
Healthy dietary pattern score	*-.31*	*.19*	*2.77*	*1*	*.10*	*.73*	*.51*	*1.06*
Alcohol and nuts dietary pattern score	*.08*	*.17*	*.21*	*1*	*.65*	*1.08*	*.78*	*1.49*
Constant	*-.83*	*2.60*	*.10*	*1*	*.75*	*.44*		

Pre-defined dietary pattern measured by DII score model

To assess the impact of the inflammation potential of the diet measured by DII score on the probability of adenoma recurrence a separate logistic regression model was used. The model contained the following four independent variables: age, sex, BMI and DII score. The full model containing all

predictors was not statistically significant, χ^2 (4, N = 155) = 2.8, *p* =.59. The model as a whole explained between 1.8% (Cox and Snell R square) and 2.4% (Nagelkerke R squared) of the risk of adenoma recurrence, and correctly classified 62.6% of the cases. As **table 7-4** shows, no variable of independent variables included in the analysis made a statistically significant contribution to the model.

Table 7-4. Adjusted Odds Ratio (OR) and (95%CI) for the association between the DII score and adenoma recurrence.

	B	***S.E.***	***Wald***	***df***	*p*	***OR***	***95%CI***	
							Lower	*Upper*
sex	*.535*	*.41*	*1.71*	*1*	*.19*	*1.71*	*.77*	*3.82*
BMI	*.012*	*.03*	*.14*	*1*	*.71*	*1.01*	*.95*	*1.08*
Age	*-.002*	*.04*	*.00*	*1*	*.95*	*1.00*	*.93*	*1.07*
DII score	*.083*	*.09*	*.89*	*1*	*.35*	*1.09*	*.92*	*1.29*
Constant	*-.260*	*2.53*	*.01*	*1*	*.92*	*.77*		

7.4.4 Dietary patterns and colorectal adenoma characteristics

As illustrated in **table 7-5**, there was no difference between quartiles 1 and 4 of the four dietary patterns in adenoma number, size or location in the 97 patients diagnosed with adenoma recurrence at the end of the study (Mann Whitney U).

Table 7-5. Adenoma characteristics in quartiles 1 and 4 of DII score and the four dietary patterns extracted by PCA in the placebo group (156 cases) in the case of recurrence at the end of the study

Dietary pattern	***High energy pattern***			***Healthy pattern***			***Alcohol and nuts pattern***			***DII score***		
Quartile	*Q1*	*Q4*		*Q1*	*Q4*		*Q1*	*Q4*		*Q1*	*Q4*	
No. of patients*	*44*	*37*		*40*	*40*		*39*	*38*		*44*	*36*	
No. of patients with adenoma recurrence	*28*	*24*		*29*	*22*		*24*	*25*		*29*	*29*	
% within Adenoma recurrence	*28.9*	*24.7*		*29.9*	*22.7*		*24.7*	*25.8*		*29.9*	*29.9*	
	M (SD)	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*	*M (SD)*	*M (SD)*	*p*
Adenoma numbers												
Total	*1.6 (2.6)*	*1.5 (1.5)*	*.5*	*1.4 (1.2)*	*1 (1.1)*	*.07*	*1.5 (2.7)*	*1.7 (2.2)*	*.53*	*1.3 (1.8)*	*2.2 (1.3)*	*.05*
Proximal	*1.0 (2.6)*	*.7 (1.2)*	*.9*	*.8 (1.3)*	*.4 (.7)*	*.11*	*1.1 (2.7)*	*1 (1.9)*	*.72*	*.7 (1.4)*	*1.5 (.7)*	*.1*
Distal	*.6 (.9)*	*.8 (1)*	*.2*	*.6 (.8)*	*.6 (.8)*	*.84*	*.4 (.8)*	*.7 (1)*	*.18*	*.6 (.8)*	*.7 (.6)*	*.94*
Adenoma size (mm)												
Total	*5.3 (8)*	*5.8 (7.5)*	*.7*	*4.6 (4.8)*	*3.9 (5.6)*	*0.18*	*4.3 (6.6)*	*6.6 (9.5)*	*.44*	*5.5 (8.4)*	*7.5 (5.5)*	*.11*
Proximal	*2.5 (6)*	*2.7 (5.5)*	*1*	*2.7 (4.4)*	*1.3 (3.2)*	*0.1*	*2.9 (6.1)*	*3.2 (6.8)*	*.71*	*2.8 (6.3)*	*4.4 (2.8)*	*.11*
Distal	*2.8 (5.9)*	*3.1 (5.6)*	*0.2*	*1.9 (3.7)*	*2.6 (4.7)*	*0.49*	*1.4 (2.7)*	*3.4 (6.1)*	*.11*	*2.7 (4.8)*	*3 (2.7)*	*.77*
Mann-Whitney U test												

7.5 Discussion

This chapter aimed to investigate if an association exists between dietary patterns and colorectal adenoma recurrence after one year of polypectomy. Data included in this analysis were collected from the 156 colorectal adenoma patients allocated to the placebo arm of the seAFOod trial who had complete dietary data at baseline and attended the trial exit colonoscopy after one year. The analysis showed no significant differences between patients with and without adenoma recurrence in terms of their age, sex, BMI or smoking status. No significant difference between the two groups was observed in their average daily intake of energy, alcohol, foods and nutrients associated with CRC as per the WCRF/AICR report. The diet of patients with and without adenoma recurrence in the placebo arm reflected the dietary intake of the cohort as a whole and was high in red and processed meat (average of 629g/week of red and processed meat vs the < 500g/week recommended by the WCRF/AICR), low in fibre and low in vitamin D. On average, patients from both groups reported consumption of about two units of alcohol per day, about two portions of fish per week, and more than five portions of fruits and vegetables per day.

The adenoma recurrence rate was 62% in the patients allocated to the placebo arm of the seAFOod trial after one year of polypectomy. This is higher than reported in other studies of adenoma recurrence. For example, a meta-analysis included data from patients allocated to the placebo arms of 20 randomised clinical trial for the period from 1988 to 2016; found that the rate of adenoma recurrence after one year of polypectomy was 37% (234). The higher percentage of adenoma recurrence in the patients recruited to the seAFOod trial is possibly due to the difference in inclusion criteria between this study and the studies included in the meta-analysis. For example, the inclusion criteria for one of the studies included in this meta-analysis that explored the role of metformin on adenoma recurrence, the inclusion criteria was diagnosis with single or multiple colorectal adenomas at index colonoscopy. While the inclusion criteria for the seAFOod trial was restricted for patients diagnosed with advanced adenoma (≥5 adenomas or ≥3 adenomas of which at least one is ≥1cm). Diagnosis with advanced adenoma at index colonoscopy was found to be an independent predictor factor for adenoma recurrence (235). Additionally, the Bowel cancer screening programme in England classify patients diagnosed at screening colonoscopy with advanced adenoma as a high-risk group and a surveillance colonoscopy is

recommended after 12 months of diagnosis (236). This indicates that a high rate of recurrence is expected to be high within this short period.

Contrary to the previous studies (237–239), this analysis found no significant difference in recurrence between males and females. This could be due to a small sample size of females recruited to the seAFOod trial.

The results of this analysis did not show any significant association between the risk of adenoma recurrence and following any of the dietary patterns extracted by the data-driven approach. This outcome is contrary to that reported by Cotte *et al.* (2005), who found that the Mediterranean diet was associated with a lower risk of colorectal adenoma recurrence in women after three years of polypectomy (153). However, in the study conducted by Cotte *et al,* the dietary patterns extracted by the PCA method used 50 food groups and the dietary patterns were extracted for males (n=277) and females (n=165), separately. This small number of cases (277 and 165) for the number of food groups (50) violates one of the assumptions of the PCA, since the PCA method requires an allocation of a minimum of 10 cases for each variable (food group in this case). Therefore, a minimum of 500 cases is needed to extract dietary patterns from 50 food groups. This limitation may affect the validity of the results obtained from this study (153). Another difference between this study and the Cotte *et al.* (2005) study is that the follow-up period for their study was longer than it was for the seAFOod trial (one vs. three years). It is not known if during the 12 months following polypectomy if any molecular changes have started in the seAFOod trial patients but without showing any histological changes that could be detected by the colonoscopy examination. A longer follow up period is needed to eliminate this probability.

No significant association was also found between the DII score and the risk of adenoma recurrence or adenoma characteristics. These findings are in agreement with those obtained by Sardo Molmenti *et al* in 2017 (154)(154) . Although, as was discussed in **Chapter 5**, many factors limit comparing the DII scores obtained from two different studies, such as the number, the magnitude and the direction of the inflammatory effect score of food parameters included in the DII calculations. The study calculated the DII score using the baseline dietary data obtained from 1727 colorectal adenoma patients that were enrolled in Phase 3 clinical trials. DII score was calculated using 27 food parameters (compared with 30 food parameters were used to calculate the DII for the seAFOod trial data). Although the means of DII

score for the seAFOod trial showed consumption of a pro-inflammatory diet (mean=1.5, SD=1.84) and the mean DII score for the Sardo Molmenti study showed an average consumption of an antiinflammatory diet (mean= -1.85, SD=1.6). Both studies found no association between the DII scores and adenoma recurrence. These findings may suggest that DII score is not a predictor of adenoma recurrence.

The distribution of the cases with adenoma recurrence and without adenoma recurrence was similar for the dietary patterns extracted by the PCA, but it was different among the DII score (**Figure 7-2**). The figure shows that patients with adenoma recurrence are distributed equally over the anti and proinflammatory DII scores (positive and negative score), while there is a peak for the number of cases with no adenoma recurrence around the +2 DII score (proinflammatory score). The reason for this distribution is not clear but it may suggest that we need to consider the local effect of food parameters included in calculating the DII score (such as alcohol, fibre and iron) on the development and progression of the disease, and not only their systematic effect when investigating the association between colorectal tumorigenesis and the inflammatory potential of diet.

This small proof of principle exploratory analysis suggests that there is no association between diet and colorectal adenoma recurrence. However, aside from the small sample size there are few other reasons why an association may not be detected between dietary patterns and colorectal adenoma recurrence in this particular sample while evidence showed that an association exists between dietary patterns and colorectal adenoma incidence (196).

The first is the timeframe of the study. This data was obtained from a study that was conducted over 12 months. The follow-up period of the prospective studies included in the meta-analysis that showed evidence about the association between dietary patterns and colorectal adenoma development ranged from 3 to 12 years (196). The 12 months' period may not be adequate to explore the association between dietary behaviour and adenoma recurrence, since the time required for the tumour initiation stage is estimated to be longer than that is required for the tumour progression stages (23). The evidence from the literature review conducted at the beginning of this research project (**Chapter 1**) revealed that limited evidence support that indication that nutrients affect adenoma recurrence risk in the immediate post-polypectomy years. As the molecular mechanism of the association between dietary intake and development of adenoma is not understood, the effect of diet on the development of

adenoma may only be evident after a longer time-period. Colorectal adenoma develops as a result of the accumulation of genetic and epigenetic changes that lead to histological and morphological changes over a long period. For example, one of the mechanisms proposed for the role of alcohol in colorectal tumorigenesis is the depletion of tetrahydrofolate (240,241). Tetrahydrofolate is an enzymatic cofactor that is important for synthesizing and maintaining DNA nucleotides, therefore, the depletion leads to DNA damage (242). The time frame required for this process is not known and there is a possibility that these people are going through these molecular or histological changes but not at a stage that is detected visually by a colonoscopy examination.

The 2nd possible factor could be related to the adenoma diagnosis procedure. Although colonoscopy is the best method available for diagnosis for CRC screening, the sensitivity of the procedure to detect advanced colorectal adenomas is estimated to be from 88 to 98% (243), therefore adenoma detected at the exit colonoscopy might be a missed adenoma at index colonoscopy. A multicenter randomised clinical trial included 406 patients and used the 3rd Eye Retroscope to explore the factors that may affect the rate of adenoma detection during colonoscopy found that 25% of adenomas are missed during the index colonoscopy (244). It is not known if the diagnosed adenomas at the exit colonoscopy were newly developed adenomas or adenomas that were missed at the index colonoscopy.

Finally, as the molecular pathway of the association between dietary patterns and colorectal tumour development and progression is not known, it is not known whether dietary patterns are associated with the tumour progression stage but not the tumour initiation stage.

The findings of the present study should be considered in light of some limitations. The major weakness is the small sample size (156 cases). Although the scale of the data collected at baseline in the seAFOod trial provided *the* opportunity to extract the data-driven dietary patterns using the PCA method, using an active substance such as aspirin and EPA has limited our ability to evaluate the association between dietary intake and the risk of the disease using the whole group. The 2nd limitation of this study is the similarity between the subjects included in the study. In addition to the restricted inclusion criteria followed while recruiting the patients in terms of their age and adenoma characteristics, the analysis revealed that the majority of the patients are males and overweight or obese. Moreover, as reported earlier in **Chapter 5** the seAFOod patients appear to have a homogenous dietary pattern and even the patients scoring highly on the healthy eating pattern failed to meet the current recommendations.

Summary

In this chapter, no association was found between baseline dietary intake and the risk of adenoma recurrence in patients allocated to the placebo arm of the seAFOod trial. There are still many unanswered questions about the association between dietary intake and colorectal adenoma recurrence and further studies are needed with a larger number of participants and a longer follow up period to obtain robust results. The results obtained from the analysis of this chapter should not be generalised and this analysis remains a proof of principle analysis.

Chapter 8

The association between diet, adherence to the WCRF/AICR cancer prevention recommendations and scores of mucosal crypt proliferation, keratin and endocrine cells in the FACT study

Chapter 8 The association between diet, adherence to the WCRF/AICR cancer prevention recommendations and scores of mucosal crypt proliferation, keratin and endocrine cells in the FACT study (Objectives 6 and 7)

The WCRF/AICR report has found an association between lifestyle factors including dietary behaviour with the risk of many cancers and provided evidence based recommendations for the public to reduce their risk of cancer (245).

The association between adherence to these recommendations and CRC risk was investigated in the UK Women's Cohort Study. The study included 30963 participants, including 444 CRC cases after a follow up period of 17.4 (SD=.8) years (246). It used an in-house scoring system to measure the adherence to the WCRF/AICR recommendations for cancer prevention and the risk of CRC and no significant association was found.

In 2018 Shams-White and colleagues developed a standardised scoring system that is used to measure the adherence of individuals to these guidelines (247). Studies used this standardised scoring system revealed an association between adherence scores and lower risk of total cancer (248), breast cancer (249), prostate cancer (250) and pancreatic cancer (251). In 2020, a study investigated the association between adherence to the WCRF/AICR cancer prevention recommendations and risk of CRC within the framework of the PREDIMED cohort study. This study used this standardised scoring system on data from 7216 males and females aged between 55 to 80 years. After a follow up period between 4.4 to 7.3 years, 97 CRC cases were detected. The study revealed a significant inverse linear association between adherence to these recommendations and the risk of CRC (62).

As guidelines provided by the WCRF/AICR are based on epidemiological studies, the molecular/or biological mechanisms of these factors in the development and progression of CRC is not fully understood. In **Chapter 7**, no evidence of an association between dietary patterns and colorectal adenoma recurrence was found in the seAFOod trial participants. However, it is not known whether these dietary behaviours are associated with molecular changes that are not detectable by the colonoscopy examination at this stage. Therefore, in this chapter, we explore if adherence to the

WCRF/AICR cancer prevention recommendations affects the crypt cell proliferation, expression of keratin and chromogranin A in the mucosa of the colon.

In this chapter, the data collected from the observational arm of the FACT study (dietary, demographic data and the biomarkers of colonic crypt proliferation, keratin and chromogranin A) were used to explore the association between the biomarkers' scores and (i) dietary intake of foods and nutrients associated with CRC and (ii) adherence to WCRF/AICR cancer prevention recommendations. Identifying this association is essential to understand the association between diet and colorectal adenoma. As adenoma development is, a long time process that starts with molecular and histological changes that are not detected by colonoscopy examination, this analysis may reveal if an association exists between the modifiable risk factors (diet and BMI) and molecular pathways associated with early stages of colorectal tumorigenesis.

This chapter used the standardised system to measure the adherence to the WCRF/AICR cancer prevention recommendations. Then analysis was conducted over four stages by (i) examining the dietary behaviour of this cohort, (ii) describing their adherence to the WCRF/AICR cancer prevention recommendations, (iii) comparing dietary behaviour of this cohort with the dietary behaviours of males recruited to the seAFOod trial (iv) explore if an association exists between adherence to these recommendations and markers of crypt cell proliferation, keratin and endocrine cells.

8.1 Background

Crypt cell proliferation.

The continuous dividing and renewal of the intestinal mucosa is essential and is tightly controlled (21). Higher rates of cell differentiation leads to hyperplasia (252,253) and changes in cell proliferation pattern and uncontrolled apoptosis are considered one of the earliest events in colorectal carcinogenesis (18,19). Evidence shows that expression of genes that control cellular proliferation are usually different between tumor and healthy tissues (20). A study was conducted by Kohoutova and colleagues in 2018 aimed to compare mitosis and apoptosis in epithelial cells measured in normal mucosa and at different stages of colorectal neoplasia (n= 61 cases). The study reported that patients with non-advanced and advanced adenoma had a significantly higher mitosis activity when compared with healthy participants; however, no difference was observed in mitotic activity when the sample from healthy participants were

compared with healthy mucosa obtained from the patients at different stages of neoplasm (254). This study shows that cell proliferation activity is influenced by both the stage of the disease and the location from which the sample was obtained.

Evidence suggest that there is an association between mucosal cell proliferation and diet. In an animal study, starvation and intermittent fasting affected the proliferation rate in the mucosa, even in animals with preserved nutritional status through parenteral nutrition (255). Another animal study showed that heme supplemented diet for 14 days led to mucosal hyper proliferation (82). In humans, Beeken *et al*. (2017) conducted an exploratory study to assess the impact of diet-induced weight loss on CRC biomarkers. The study found that, in patients who achieved a significant weight loss (mean of 13.56% of body weight), a significant reduction in colonocyte proliferation was observed (256). Another study observed an increase in colonic cell proliferation in heavy drinkers (consumed about 100 g of ethanol/day) when compared with controls (consumed about 30 g of ethanol/day) (257).

The mechanisms of the effect of diet on colonocyte proliferation is not well known, however, it was suggested that direct contact between mucosa and food stimulate different gastric hormones that affect the cell proliferation (258). Regarding dietary haem, it is proposed that high haem consumption damages the surface of the epithelium, this injury leads to an increase in cell proliferation and inhibition of apoptosis as a homeostasis mechanism (82).

There are several indicators used to assess cell proliferation activity, the biomarkers measured in participants recruited to the FACT study and available for this analysis are scores for mitosis, cellularity and Ki67. The mitosis score is identified by counting the cells in haematoxylin- and eosin- stained sections, or by using the Feulgen technique to stain the DNA (259). Ki67 is a nuclear protein that is expressed in the cell cycle and its concentration increases in advanced stages of the cell cycle **(Fig 8-1).** It can be measured by staining the paraffin wax embedded tissues with the antigen of Ki67. Details of the biopsies collection and methods of biomarkers' measurements that was performed by other researchers were involved in conducting the FACT study are summarised in **Chapter 2**.

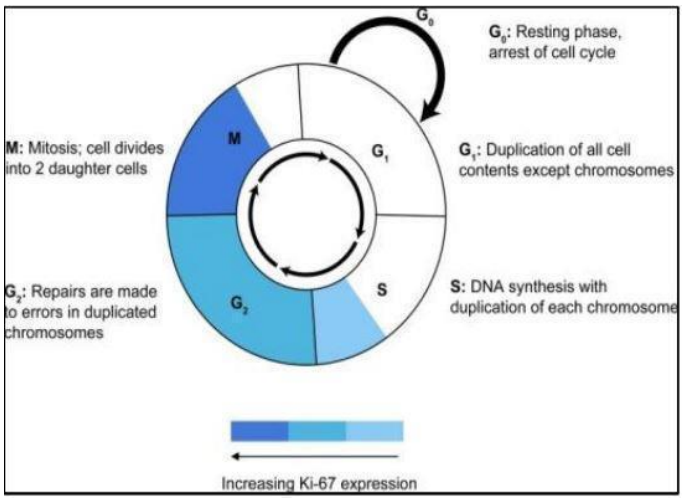


Figure 8-1. Shows the cell cycle and the concentration of Ki67 in different stages
Taken with permission from Mulyawan 2019.

Keratin

Keratins are a group of proteins that are part of the cytoskeleton structure. There are many types of keratin in simple epithelial cells; the primary keratins are K8, K18 and K19. The protein has an essential role in strength and mechanical stability of the cells and for the epithelial tissue (260,261).

There is differential expression of keratins along the colonic crypt. For keratin 8, 18 and 19 expression is similar in different compartments of the crypt. Other keratins such as 7 and 20, the expression in the surface differs from expression at the base of the crypt (262). Evans *et al* (2015) reported a decrease in keratin 8,18 and 19 expression in adenoma patients (263).

There is limited evidence of an association between dietary intake and keratin expression, a cross sectional study included 28 colorectal adenoma patients and 34 healthy individuals was published in 2015 reported that after an increase in dietary fibre for a period of 8 weeks after polypectomy, the keratin expression was restored to normality (263).

Enteroendocrine cells

Enteroendocrine cells (EEC) are hormone producing cells that are scattered through the gastrointestinal tract. Similar to the absorptive cells and the goblet cells, the EEC develop from the stem cells in the base of the crypt, however, they only make about 1% of the mucosal epithelial (264). The ECCs are classified according to the products stored within their secretory vesicles to the Enterochromaffin cells, the L cells

and the D cells. The enterochromaffin are the most abundant type across the gastrointestinal tract (265). An increase in the number of enterochromaffin cells in the intestine of male BALB/c mice that were inoculated with tumor cells has been reported when compared with the control (266).

Hormones secreted by the EEC play an important role in regulation of food intake and glucose homeostasis and production of some of these hormones are affected in individuals with metabolic disease such as type 2 diabetes and obesity (267).

Chromogranin A (CGA) is a soluble glycoprotein, stored and released from the granules of the EEC and is used as a marker for the EEC throughout the GIT (268). CGA is a precursor for bioactive compounds that have a regularity function of glucose balance, lipid metabolism and inflammatory response. Expression of CGA is decreased at the early stages of colorectal cancer, however, similar to mitotic activity, no change was observed in CGA production in normal tissues obtained from cancer patients (269).

8.2 Aim and objectives

The aim of this chapter is to explore if an association exists between dietary intake, adherence to the WCRF/AICR cancer prevention recommendations scores and scores of crypt cell proliferation, keratin and Chromogranin A.

This aim was achieved through the following three objectives:

1. Describe the demographic characteristics and dietary intake of the FACT study participants and compare these characteristics with men recruited to the seAFOod trial.
2. Explore the association between dietary intake of foods and nutrients associated with CRC and levels of crypt cell proliferation, keratin and Chromogranin A.
3. Measure and describe the adherence to the WCRF/AICR recommendations for cancer prevention and explore if an association exists between adherence score and scores of cell proliferation, keratin and endocrine cells.

8.3 Data

In this chapter, we used demographic, dietary and crypt proliferation, keratin and endocrine cells markers' scores data provided for 98 males recruited to the observational arm of the FACT study and the

demographic and dietary data for 533 males recruited to the seAFOod trial. An overview for the FACT study is provided in the material **Chapter 2 (Section 2.1.2).**

8.4 Methods

An overview about the source of the data and the details of data preparation were illustrated in details in Chapter 2. After excluding the data for the cases that did not meet the inclusion criteria, this section will summarises the steps followed to make the calculations and preparations specifically required to achieve the objectives of this chapter. Throughout this analysis, the foods and nutrients associated with the risk of CRC according to WCRF/AICR(90) were used to assess the dietary behaviour. When portion size not recommended in the WCRF/AICR report, the portion sizes were obtained from the PHE Healthy Eating recommendations (180). A summary for the methods used to achieve each of the objectives are presented in **figure 8-2.**

8.4.1 Data Exclusion criteria

The first step is to exclude cases that do not meet the inclusion criteria. That is cases with:

- more than10 missing answers in the original FFQs. As it was advised by the FETA software creators that FFQ with > missing ticks may indicate a misreporting (161).
- reported total energy intake of less than 2092 KJ/day (<500kcal) were considered as under-reporters and excluded from the analysis.

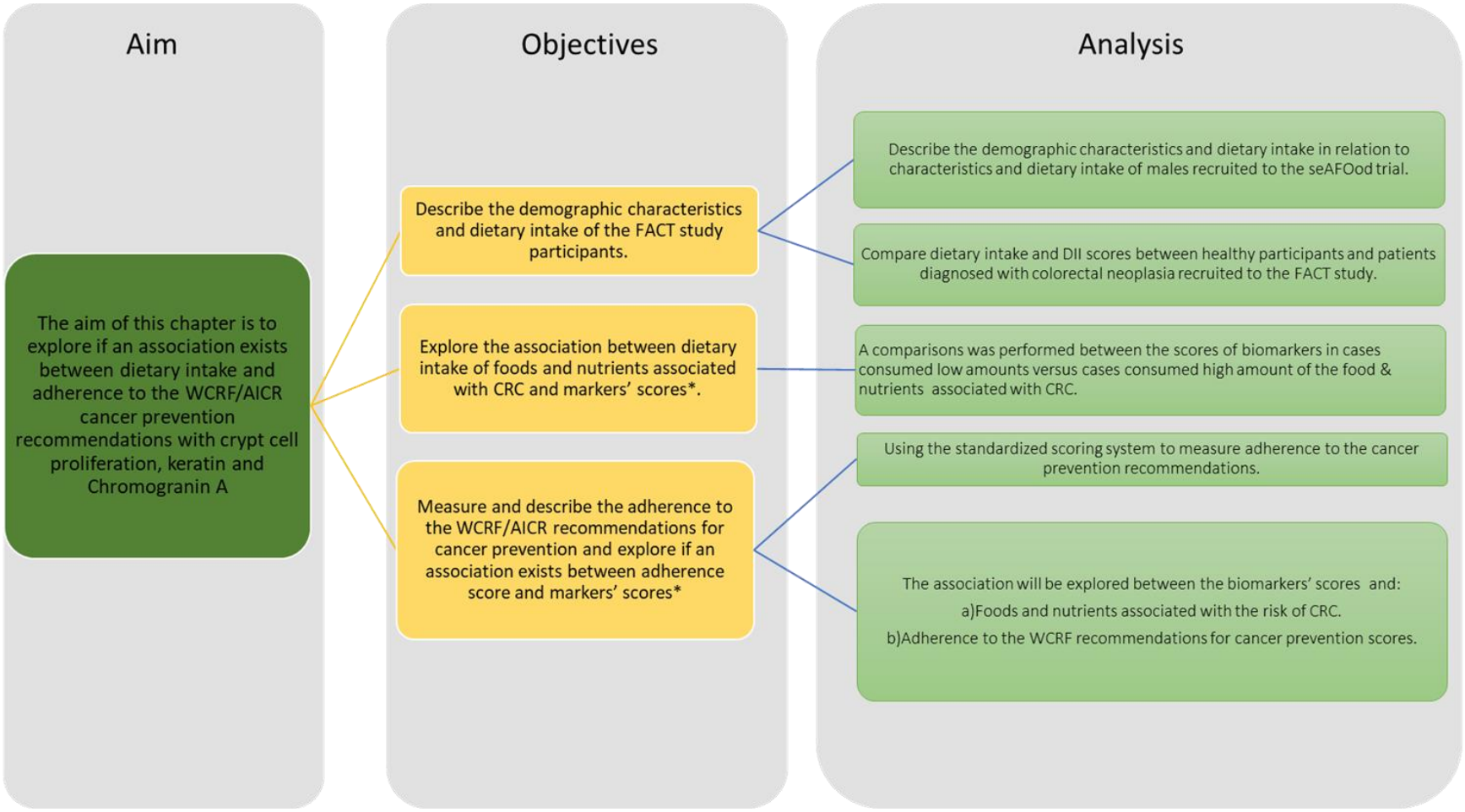


Figure 8-2. A summary for the aims and objectives of chapter 7 and the methods used to achieve objective

8.4.2 Comparing DII of the FACT study participants with males recruited to the seAFOod trial

As the FACT study data was used to validate the in-house method for measuring DII (**Chapter 3, Section 3.3.4)**, the number of food parameters was used to calculate the overall DII was less than the number of food parameters used to calculate the DII for the seAFOod trial. This decision was made after a discussion with the DII creators who agreed to run the calculations using the DII algorithm with the 25 food parameters, rather than 30 food parameters used to measure the DII for the seAFOod trial. Therefore, to be able to compare DII from the two studies, we eliminated DII scores for food parameters used in the seAFOod trial but not in the FACT study (onions, garlic, paper, tea and ω-3 fatty acids) from the seAFOod DII total score before comparing with the DII measured for the FACT study.

8.4.3 Testing the association between dietary intake and the scores of cell proliferation, keratin and endocrine cells

To explore the association between the biomarkers and dietary intake, comparisons was performed between the scores of biomarkers in cases consumed low amounts versus cases consumed high amount of the food or nutrients of interest. To achieve this the following steps were followed:

1. Check distribution of foods and nutrients associated with CRC.
2. Create a new variable for each food or nutrient with two categories according to the distribution:
    i. High intake category: participants with intake of more than the mean or the median of the food or nutrient intake of the whole sample.
    ii. Low intake category: participants with intake of less than the mean or the median of the food or nutrient intake of the whole sample.
3. Compare the scores of the markers in the high and low intake categories using the:
    i. Independent sample t test for normally distributed biomarkers,
    ii. Wilcoxon-Mann Whitney test for not normally distributed biomarkers.

4. Compare the dietary intake of foods and nutrients associated with CRC in participants classified as “high-intake” or “low-intake” with the recommendations using one sample T test.

8.4.4 Assessing adherence to the WCRF/AICR cancer prevention recommendations

The standardised method of adherence to the WCRF/AICR cancer prevention recommendations that was published in 2019 was followed in this measurement (247). As not all the data required by this standardised method was available, some modifications were made to make the data compatible with the standardised method. This section clarifies the original standardised method, description for the steps followed to make the FACT study data compatible with this method, and the approach followed to categorise the total score to either adhere or non-adhere categorise. The original scoring system and summary for the modifications applied to each point, when needed are clarified in **Appendix 8**.

The original (or standardised) published method for measuring adherence score to the WCRF/AICR for cancer prevention recommendations.

The following eight factors were included in the standardised adherence scoring system: (a) maintaining healthy body weight, (b) physical activity, (c) high consumption of diet rich in wholegrains, vegetables, fruit, and beans, (d) low consumption of fast and processed foods, (e) low consumption of red and processed meat, (f) low consumption of sugar-sweetened drinks, (g) low consumption of alcohol, and (h) breastfeeding (optional).

The criteria used to estimate the score was given 1 point for full adherence, 0.5 points for limited adherence, and 0 point when the recommendation was not met. Some recommendations were subdivided, for example both BMI and waist circumferences were used as indicators for maintaining body weight. To keep the optimum score for body weight as 1 point, the points were divided equally between both indicators (0.5 point for adherence, 0.25 point for limited adherence and 0 point for not adhering). This subdividing was also used for (Eat diet rich in wholegrains, fruits and beans factor) to include mean daily intake of fruits and vegetables and mean daily intake of fibre.

Modifications and changes for the standardised system to suit the FACT study data:

Details for the standardised scoring system for adherence to the WCRF/AICR cancer prevention recommendations and modification performed is provided in details in **Appendix 8**. In summary the modifications were as follow:

1. The score of BMI was doubled to 1 as data on waist circumference is not available (this was suggested by the scoring developer when data about one component is not available) (250).

2. The mean daily intake of sodium consumed was used as an indicator for fast and processed food consumption, since data about this food is not extracted by the FETA software. Using sodium as an indicator for this group was previously published (248).
3. Data about physical activity and sugar-sweetened drinks was not available and was excluded from the score.
4. As only males are included in this analysis, breastfeeding score was excluded.

Total score categories

Previous studies (249,250) classified the adherence score into three categories (≤3 points indicate minimal adherence, >3–≤5 points an intermediate adherence and >5 points is a maximum adherence). In this study, data for only five of the recommendations is available, the total scores ranged from zero to 5 points. Where higher scores showing high adherence to the WCRF/AICR recommendations. The score categorised into two categories: a score ≤3 points was considered as a minimum adherence and any score of >3 was considered as adherence.

8.4.5 Statistical analysis

For descriptive analysis mean (SD) and median (IQR) were used for continuous variables and number (percentage) for categorical variables. Kolmogorov–Smirnov test was used to assess the distribution of the data. Independent sample T test and Mann-Whitney U test were used to compare variables depending on the distribution of the data. ANOVA was used to compare DII score between healthy participants, adenoma and cancer patients. Chi square test was used to compare adherence to the WCRF/AICR cancer prevention recommendations in healthy and neoplasm participants. One sample T test was used to compare consumption of foods and nutrients associated with CRC with the recommendations.

8.5 Results

8.5.1 Description of demographic characteristics and dietary intake in relation to males recruited to the seAFOod trial

The mean age (SD) of the 88 males included in this analysis was 65 (10.4) years and the mean (SD) of the BMI was 26.8 (4.4) Kg/m^2. Independent sample T test revealed no significant difference in the age of

participants of the two studies (65.2 Vs. 65.3 years), however, BMI of the adenoma patients recruited to the seAFOod trial was significantly higher than BMI for males recruited to the FACT study (26.8 vs. 29.2 kg/m2, $p<.001$).

The FACT study participants reported a mean daily intake of energy of 8.3 (SD=3.1) MJ, 47% of the energy was derived from carbohydrate, 17% from protein and 34% from fat. On average, participants reported a consumption of 1.5 units of alcohol and more than 5 portions of fruits and vegetables per day. Per week, participants consumed more than 2 portions of fish and 546g of red and processed meat. Diet was low in fiber and vitamin D and was high in sodium.

An independent-samples T-test revealed that the seAFOod participants consumed significantly higher amount of red and processed meat (93g vs. 78g, $p=.012$) while the FACT study participants consumed significantly higher amount of the milk and milk products group (383g vs. 341g, $p= .049$). **Table 8-1** shows a comparison between 88 males recruited to the FACT study and 533 males recruited to the seAFOod trial in their age, BMI and dietary intake

Table 8-1. A comparison between participants recruited to the FACT study and males recruited to the seAFOod trial in age, BMI, DII score, energy, alcohol, foods and nutrients associated with CRC

Study	***FACT (n=88)***		***seAFOod (n= 533)***		
	Mean	*SD*	*Mean*	*SD*	*p**
Age (year)	*65.2*	*10.4*	*65.3*	*4.74*	*.931*
BMI (kg/m2)	*26.8*	*4.4*	*29.2*	*5.44*	*<.001*
Alcohol (g/day)	*12.5*	*15.8*	*15.2*	*17.15*	*.163*
Energy (MJ/day)	*8.3*	*3.1*	*7.95*	*2.47*	*.332*
Red and processed meat (g/day)	*78*	*46.4*	*93*	*52.8*	*.012*
Fibre (g/day)	*15.5*	*5.9*	*15.3*	*5.96*	*.796*
Milk and dairy products (g/day)	*383*	*187*	*341*	*181.2*	*.049*
Fish and fish products (g/day)	*45.4*	*32.3*	*42.7*	*32.4*	*.46*
Fruit and vegetables (g/day)	*447*	*218*	*423*	*227*	*.37*
Iron (mg/day)	*10.6*	*3.8*	*11.2*	*3.54*	*.129*
Vitamin C (mg/day)	*107*	*50.9*	*101*	*48.3*	*.274*
Vitamin D (mcg/day)	*3.6*	*2.47*	*3.3*	*1.75*	*.195*
DII score	*.85*	*1.57*	*.69*	*1.7*	*.419*
****Independent sample T test***					

Comparison between healthy participants and patients diagnosed with colorectal neoplasm

No significant difference was detected in average daily intake of energy, alcohol, foods and nutrients associated with CRC between normal participants and patients with adenoma or cancer recruited to the FACT study **(Table 8-2)**.

Table 8-2. Dietary intake of foods and nutrients associated with the risk of CRC in healthy participants and patients diagnosed with adenoma or cancer recruited to the FACT study.

Diagnosis (N)	***Normal (40)***		***Neoplasm (48)***		
	Mean	*SD*	*Mean*	*SD*	*p*
Alcohol (g/day)	*12*	*17.3*	*13.2*	*14.6*	*.635*
Energy (MJ/day)	*7.8*	*2.95*	*8.7*	*3.17*	*.159*
Red and processed meat (g/day)	*67.8*	*39.6*	*86.5*	*50.2*	*.06*
Fibre (g/day)	*14*	*5.1*	*16.4*	*6.4*	*.103*
Milk and dairy products (g/day)*	*380*	*188.3*	*385*	*188*	*.884*
Fish and fish products (g/day)	*42*	*27.4*	*48.6*	*35.8*	*.32*
Fruit and vegetables (g/day)	*407*	*168.3*	*479*	*249.8*	*.125*
Iron (mg/day)	*10*	*3.4*	*11.1*	*4*	*.154*
Vitamin C (mg/day)	*95*	*44*	*117*	*54.7*	*.052*
Vitamin D (µg/day)	*3.4*	*2.6*	*3.8*	*2.3*	*.389*
Independent sample T test and Mann-Whitney test					

The mean of DII score of the FACT study participants was 0.85 (SD=1.6). A one-way between subject ANOVA was conducted to compare the DII score between normal participants, adenoma and cancer patients revealed no significant difference between the groups [F (2, 85) =1.6, *p*=.2].

8.5.2 The association between dietary intake of foods and nutrients associated with CRC and markers' scores

Table 8-3 shows the mean scores of the biomarkers in participants who consumed high amount and low amounts of the foods and nutrients associated with CRC.

Individuals with high iron intake had a significantly lower Ki67 score (Ki67, percent and number) ($p<.05$). This difference was lost when the analysis performed on normal and adenoma participants separately, which might be due to a smaller sample size used in sub analysis. Mitosis was higher in adenoma patients who consumed higher amount of vitamin C. In adenoma patients only, low consumption of vitamin D was associated high score of mitosis.

High iron intake was significantly associated with increase expression of keratin ($p<.05$). When data was analysed for normal participants and adenoma patients separately, no difference was detected in normal participants but remained significant in adenoma patients. Keratin score was significantly higher in adenoma patients who consumed high amount of alcohol when compared with adenoma patients consumed less amount ($p<.05$).

When data was analysed for all participants, individuals with high fish intake had a significantly lower score of CGA percentage ($p<.05$), subgroup analysis showed that this negative association was only for adenoma patients. A significant reduction in CGA and CGA percentage was associated with high vitamin D intake in the whole sample but after analysing each group separately, no association was found.

No difference was found between any of the biomarkers' scores explored and intake of energy, red and processed meat, fibre, fruits and vegetables, milk and milk products.

Table 8-3. A comparison between biomarkers scores in the FACT study participants who consumed high and low amounts of foods and nutrients associated with the risk of CRC

		Food/nutrient	Alcohol (g/d)			Energy (MJ/d)		
			Low intake	High intake		Low intake	High intake	
		N of cases	**45**	**43**	***p***	**44**	**44**	***p***
		Mean (SD)**	**2.5 (2.3)**	**22.9 (17)**		**6 (1.3)**	**10.6 (2.6)**	
All		**Biomarker*****	**Mean (SD)**	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**	
	ISTT	**Mitosis**	5.7 (3.34)	5.4 (2.74)	.6	5.6 (3.66)	5.5 (2.4)	.8
		ki67	0.4 (.14)	0.3 (.13)	.3	0.37 (.15)	0.3 (.12)	.3
		Ki67%	35.8 (13.42)	34.8(12.97)	.8	37.9 (13.7)	32.4 (11.9)	.1
	MWUT	**Cellularity**	72.9 (10.52)	71.6 (14.87)	.2	74.2 (14.9)	70 (9.5)	.5
		Ki67 Number	27 (11.95)	26.8 (16.47)	.5	29.9 (16.72)	23.6 (9.6)	.2
		KSI	0.9 (.85)	1.3 (.75)	.12	1.1 (.8)	1.3 (.8)	.3
		KCI	.6 (.63)	0.9 (.74)	.07	0.7 (.6)	0.8 (.74)	.36
		KCD	0.6 (.63)	1.1 (.9)	.09	0.7 (.66)	0.9 (.9)	.3
		CGA	1.2(.64)	0.8 (.58)	.005	1 (.64)	0.9 (.6)	.4
		CGA%	2.5 (1.44)	1.8 (1.38)	.006	2.2 (1.5)	2 (1.4)	.6
Normal		**No. of normal cases**	**23**	**17**		**23**	**17**	
	ISTT	**Mitosis**	6.6 (3.58)	6.4 (2.9)	.9	7 (4.03)	6 (2.03)	.35
		ki67	0.38 (.14)	.37 (.14)	.9	0.4 (.2)	0.4 (.1)	.34
		Ki67%	37.6 (12.92)	39.2 (12.6)	.8	40 (14.5)	36 (9.9)	.35
	MWUT	**Cellularity**	73.15 (8.8)	76.4 (17.8)	.9	77.9 (15.5)	70 (7.7)	.1
		Ki67 Number	27.7 (11.1)	32.3 (18.7)	.6	32.4 (17.9)	26 (8.3)	.4
		KSI	0.79 (.72)	1.1 (.68)	.1	0.9 (.76)	0.8 (.7)	.8
		KCI	0.39 (.74)	.6 (.6)	.3	0.5 (.5)	0.5 (.6)	.6
		KCD	0.49 (.55)	.61 (.65)	.7	0.6 (.56)	0.5 (.6)	.6
		CGA	1.3 (.64)	.8 (.25)	.02	1.1 (.5)	1.1 (.6)	1
		CGA%	2.6 (1.44)	1.7 (.53)	.04	2 (1.02)	2.3 (.5)	.9
Adenoma		**No. of adenoma cases**	**16**	**21**		15	**22**	
	ISTT	**Mitosis**	5.1 (3.1)	4.9 (2.53)	.8	4.4 (2.95)	5.4 (2.6)	.3
		ki67	0.34 (.15)	0.3 (.12)	.4	0.3 (.13)	0.3 (.13)	.6
		Ki67%	31.7 (17.9)	29.4 (14.1)	.8	34.7 (14.8)	26.7 (15.9)	.36
	MWUT	**Cellularity**	76.4 (11.73)	70.2 (7.68)	.05	72 (10.7)	73.6 (9.6)	.58
		Ki67 Number	25.1 (16.35)	20.4 (11.49)	.6	26.3 (14.4)	19.4 (12.9)	.3
		KSI	2 (.37)	1.9 (.44)	.4	1.8 (.3)	1.9 (.5)	.3
		KCI	1.4 (.54)	1.6 (.4)	.3	1.7 (.3)	1.4 (.5)	.2
		KCD	1.2 (.47)	1.8 (.5)	.03	1.6 (.3)	1.5 (.7)	.9
		CGA	1 (.64)	0.5 (.38)	.02	0.7 (.5)	0.7 (.5)	.9
		CGA%	2 (1.1)	1.2 (.75)	.02	1.4 (1.03)	1.6 (.9)	.7

*Cases divided according to level of intake: more than the median or less than the median** means are provided in units clarified in the table head. *** Biomarkers are not available for all cases. ISTT=Independent Sample T Test, MWUT= Mann–Whitney U test. KSI=Keratin Surface Intensity, KCI= Keratin Crypt Intensity, KCD=Keratin crypt Density, CGA= Chromogranin A

Table 8 3. A comparison between biomarkers scores in the FACT study participants who consumed high and low amounts of foods and nutrients associated with the risk of CRC, continues

		Food/nutrient	Red and processed meat (g/d)			Iron (mg)		
			Low intake	High intake	*p*	Low intake	High intake	*p*
		N of cases	44	44		44	44	
		Mean (SD)**	43.3 (17.1)	112.6 (40)		7.8 (1.7)	13.5 (3.2)	
All		**Biomarker*****	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
	ISTT	**Mitosis**	5.5 (3.3)	5.5 (2.8)	.90	6 (3.6)	5.1 (2.3)	.20
		ki67	0.4 (0.1)	0.3 (0.1)	.09	0.4 (0.1)	0.3 (0.1)	.03
		Ki67%	37.7 (12.7)	32.9 (13.3)	.16	39.2 (12.9)	30.7 (12.1)	.01
	MWUT	**Cellularity**	72.9 (11.7)	71.4 (14)	.22	74.5 (15)	69.9 (9.5)	.40
		Ki67 Number	28.3 (11.8)	25.5 (16.2)	.12	30.7 (16)	22.4 (9.8)	.04
		KSI	1.2 (0.8)	1 (0.8)	.34	0.9 (0.7)	1.4 (0.8)	.01
		KCI	0.8 (0.7)	0.7 (0.7)	.73	0.6 (0.6)	1 (0.7)	.04
		KCD	0.8 (0.8)	0.8 (0.8)	.90	0.6 (0.6)	1.1 (0.9)	.03
		CGA	1 (0.7)	1 (0.6)	.69	1 (0.6)	1 (0.6)	.73
		CGA%	2.1 (1.4)	2.2 (1.5)	.71	2.1 (1.5)	2.2 (1.4)	.87
Normal		**No. of normal cases**	21	19		24	16	
	ISTT	**Mitosis**	6.6 (3.4)	6.5 (3.1)	.90	7.3 (3.8)	5.6 (2)	.10
		ki67	0.4 (0.1)	0.3 (0.1)	.10	0.4 (0.2)	0.3 (0.1)	.10
		Ki67%	41.5 (10.7)	35.1 (13.9)	.10	40.7 (14.3)	34.9 (9.3)	.10
	MWUT	**Cellularity**	75.3 (9.9)	73.6 (16.5)	.30	78 (15.1)	69.4 (7.6)	.06
		Ki67 Number	31 (9.7)	28.3 (18.5)	.13	32.8 (17.5)	25.2 (8)	.16
		KSI	1 (0.7)	0.8 (0.7)	.53	0.9 (0.7)	1 (0.7)	.44
		KCI	0.5 (0.5)	0.5 (0.6)	.83	0.4 (0.5)	0.6 (0.6)	.56
		KCD	0.5 (0.5)	0.6 (0.6)	.62	0.5 (0.6)	0.6 (0.6)	.48
		CGA	1 (0.5)	1.2 (0.6)	.33	1 (0.5)	1.2 (0.6)	.45
		CGA%	2.1 (1)	2.3 (1.4)	.76	2 (1.1)	2.4 (1.5)	.47
Adenoma		**No. of adenoma cases**	18	19		14	23	
	ISTT	**Mitosis**	4.8 (3)	5.1 (2.5)	.70	4.7 (3.2)	5.2 (2.5)	.60
		ki67	0.3 (0.1)	0.3 (0.1)	.20	0.4 (0.1)	0.3 (0.1)	.09
		Ki67%	32.2 (16.5)	28.3 (15.1)	.60	39.2 (11.2)	24.9 (15.5)	.10
	MWUT	**Cellularity**	74.2 (10.4)	71.2 (9.4)	.42	73.7 (10.9)	72.5 (9.7)	.95
		Ki67 Number	24.9 (14.9)	19.8 (12.5)	.48	30.1 (12.2)	17.8 (12.7)	.08
		KSI	1.9 (0.5)	2 (0.3)	.80	1.6 (0.3)	2.1 (0.4)	.05
		KCI	1.4 (0.5)	1.6 (0.2)	.67	1.6 (0.4)	1.5 (0.5)	.70
		KCD	1.4 (0.5)	1.8 (0.6)	.50	1.5 (0.3)	1.5 (0.7)	.79
		CGA	0.8 (0.7)	0.6 (0.3)	.92	0.8 (0.5)	0.7 (0.5)	.43
		CGA%	1.5 (1.2)	1.4 (0.5)	.54	1.5 (1)	1.5 (0.9)	.73

***Cases divided according to level of intake: more than the median or less than the median** means are provided in units clarified in the table head. *** Biomarkers are not available for all cases. ISTT=Independent Sample T Test, MWUT= Mann–Whitney U test. KSI=Keratin Surface Intensity, KCI= Keratin Crypt Intensity, KCD=Keratin crypt Density, CGA= Chromogranin A**

Table 8 3. A comparison between biomarkers scores in the FACT study participants who consumed high and low amounts of foods and nutrients associated with the risk of CRC, continues

		Food/nutrient	Milk and dairy products (g/d)			Fish and fish products (g/d)		
			Low intake	High intake	*p*	Low intake	High intake	*p*
		N of cases	44	44		44	44	
		Mean (SD)**	233 (87.9)	533 (131)		24 (10.4)	66 (32.7)	
All		**Biomarker*****	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
	ISTT	**Mitosis**	5.5 (3.2)	5.5 (2.9)	.90	5.1 (3.1)	5.9 (2.9)	.30
		ki67	0.4 (0.1)	0.3 (0.1)	.40	0.3 (0.1)	0.4 (0.1)	.80
		Ki67%	38 (15)	32.9 (10.9)	.10	35.4 (12.6)	35.2 (14.1)	.90
	MWUT	**Cellularity**	74.9 (13.6)	70 (11.7)	.20	71.4 (13.2)	73.1 (12.3)	.70
		Ki67 Number	30 (17.2)	24.1 (10)	.30	26.6 (12.6)	27.4 (15.9)	.90
		KSI	1.1 (0.8)	1.2 (0.8)	.65	1.1 (0.8)	1.2 (0.9)	.41
		KCI	0.8 (0.7)	0.8 (0.7)	.91	0.7 (0.7)	0.8 (0.7)	.52
		KCD	0.8 (0.7)	0.9 (0.8)	.61	0.7 (0.8)	0.9 (0.8)	.48
		CGA	1 (0.6)	1 (0.7)	.88	1.1 (0.5)	0.9 (0.7)	.05
		CGA%	2 (1.4)	2.3 (1.5)	.27	2.4 (1.3)	1.9 (1.6)	.01
Normal		**No of normal cases**	20	20		22	18	
	ISTT	**Mitosis**	6.7 (3.3)	6.3 (3.2)	.70	5.9 (3.1)	7.2 (3.4)	.20
		ki67	0.4 (0.1)	0.4 (0.1)	.30	0.4 (0.1)	0.4 (0.1)	.80
		Ki67%	40.4 (14.4)	35.9 (10.3)	.20	38.1 (11.4)	38.4 (14.4)	.90
	MWUT	**Cellularity**	75.9 (15.2)	73.1 (11.2)	.81	74.3 (12.6)	74.8 (14.4)	.77
		Ki67 Number	32.3 (18)	26.6 (9.3)	.56	28.9 (12.3)	30.5 (17.5)	.80
		KSI	0.8 (0.7)	1 (0.7)	.34	0.9 (0.7)	0.9 (0.7)	.96
		KCI	0.4 (0.5)	0.5 (0.6)	.73	0.5 (0.5)	0.5 (0.6)	.66
		KCD	0.4 (0.5)	0.6 (0.7)	.44	0.5 (0.6)	0.6 (0.6)	.97
		CGA	1 (0.4)	1.1 (0.7)	.78	1.1 (0.5)	1 (0.6)	.59
		CGA%	1.9 (0.8)	2.5 (1.5)	.19	2.3 (1.2)	2.1 (1.3)	.31
Adenoma		**No of adenoma cases**	20	17		16	21	
	ISTT	**Mitosis**	4.8 (3.1)	5.1 (2.3)	.70	5.1 (3.6)	4.9 (2)	.90
		ki67	0.3 (0.2)	0.3 (0.1)	.90	0.3 (0.2)	0.3 (0.1)	.90
		Ki67%	33.7 (18.3)	27.6 (13)	.50	33.1 (17.1)	27.3 (13.9)	.50
	MWUT	**Cellularity**	76.2 (9)	69.9 (10.1)	.14	72.7 (11.7)	73.1 (8.8)	.95
		Ki67 Number	27.1 (16.6)	18.7 (10)	.57	25.4 (16.1)	19.3 (10.2)	.57
		KSI	2 (0.5)	1.9 (0.4)	1.00	2 (0.2)	1.9 (0.5)	.74
		KCI	1.8 (0.2)	1.3 (0.5)	.07	1.7 (0.3)	1.4 (0.5)	.36
		KCD	1.7 (0.3)	1.4 (0.7)	.19	1.9 (0.6)	1.4 (0.5)	.27
		CGA	0.7 (0.4)	0.7 (0.6)	1.00	0.9 (0.4)	0.6 (0.6)	.02
		CGA%	1.5 (0.7)	1.5 (1.1)	.86	2 (0.7)	1.3 (1)	.01

*****Cases divided according to level of intake: more than the median or less than the median** means are provided in units clarified in the table head. *** Biomarkers are not available for all cases. ISTT=Independent Sample T Test, MWUT= Mann–Whitney U test. KSI=Keratin Surface Intensity, KCI= Keratin Crypt Intensity, KCD=Keratin crypt Density, CGA= Chromogranin A

Table 8 3. A comparison between biomarkers scores in the FACT study participants who consumed high and low amounts of foods and nutrients associated with the risk of CRC, continues

		Food/nutrient	Vitamin C (mg)			Vitamin D (µg)		
			Low intake	High intake	*p*	Low intake	High intake	*p*
		N of cases	44	44		44	44	
		Mean (SD)**	70 (19.7)	144 (45)		1.9 (.62)	5.3 (2.46)	
All		**Biomarker*****	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
	ISTT	**Mitosis**	5.5 (3.4)	5.5 (2.8)	.9	5.9 (3.3)	5.1 (2.8)	.20
		ki67	0.4 (0.1)	0.3 (0.1)	.7	0.4 (0.1)	0.3 (0.1)	.80
		Ki67%	35.5 (14.7)	35.1 (11.4)	.9	36.3 (13.5)	33.9 (12.6)	.50
	MWUT	**Cellularity**	72.8 (15.2)	71.6 (9.7)	.6	71.7 (12.2)	72.8 (13.5)	.70
		Ki67 Number	27.6 (16.8)	26.2 (10.8)	.8	27.7 (14.7)	25.8 (13.4)	.70
		KSI	1.1 (0.8)	1.2 (0.9)	.6	1 (0.9)	1.3 (0.8)	.19
		KCI	0.7 (0.6)	0.8 (0.8)	.9	0.6 (0.6)	0.9 (0.8)	.17
		KCD	0.7 (0.6)	0.9 (0.9)	.6	0.6 (0.7)	1 (0.9)	.15
		CGA	1 (0.6)	1 (0.7)	.9	1.1 (0.6)	0.8 (0.7)	.01
		CGA%	2 (1.3)	2.3 (1.6)	.8	2.4 (1.3)	1.9 (1.5)	.02
Normal		**No of normal cases**	23	17		23	17	
	ISTT	**Mitosis**	7.2 (4)	5.7 (2)	.1	6.5 (3.4)	6.6 (3.1)	.90
		ki67	0.4 (0.2)	0.4 (0.1)	.3	0.4 (0.1)	0.4 (0.1)	.80
		Ki67%	39.3 (14.8)	37 (9.5)	.6	38.6 (13.7)	37.7 (11.1)	.80
	MWUT	**Cellularity**	76.3 (16.7)	72.2 (5.8)	1.0	73 (11.5)	76.6 (15.5)	.58
		Ki67 Number	31.7 (18.3)	27 (7.8)	.8	29.3 (15.5)	30.1 (13.7)	.63
		KSI	1 (0.7)	0.8 (0.7)	.3	0.9 (0.7)	0.9 (0.7)	.73
		KCI	0.6 (0.5)	0.3 (0.5)	.1	0.5 (0.5)	0.4 (0.6)	.27
		KCD	0.6 (0.5)	0.5 (0.7)	.2	0.5 (0.5)	0.5 (0.7)	.61
		CGA	1 (0.5)	1.2 (0.7)	.7	1.2 (0.5)	0.9 (0.6)	.12
		CGA%	2 (0.9)	2.5 (1.6)	.8	2.3 (1.1)	2 (1.4)	.19
Adenoma		**No of adenoma cases**	16	21		13	24	
	ISTT	**Mitosis**	3.9 (1.5)	5.8 (3.2)	.02	6.3 (3.2)	4.3 (2.2)	.03
		ki67	0.3 (0.1)	0.3 (0.1)	.5	0.3 (0.1)	0.3 (0.1)	.40
		Ki67%	25 (14.3)	33.8 (15.9)	.3	33.8 (17.2)	28.3 (14.8)	.50
	MWUT	**Cellularity**	71.7 (8.2)	73.8 (11.3)	.5	76.8 (9.4)	70.8 (9.8)	.25
		Ki67 Number	18.3 (9.7)	25.2 (15.5)	.5	26.4 (15.9)	20.2 (12.4)	.46
		KSI	1.9 (0.6)	1.9 (0.4)	1.0	2.4 (0.2)	1.8 (0.4)	.01
		KCI	1.4 (0.4)	1.6 (0.5)	.4	1.2 (0.8)	1.6 (0.3)	.39
		KCD	1.4 (0.5)	1.6 (0.6)	.5	1.4 (0.9)	1.6 (0.5)	1.00
		CGA	0.6 (0.5)	0.8 (0.5)	.3	1 (0.8)	0.6 (0.3)	.10
		CGA%	1.3 (1)	1.6 (0.9)	.2	2 (1.4)	1.3 (0.6)	.24

*****Cases divided according to level of intake: more than the median or less than the median** means are provided in units clarified in the table head. *** Biomarkers are not available for all cases. ISTT=Independent Sample T Test, MWUT= Mann–Whitney U test. KSI=Keratin Surface Intensity, KCI= Keratin Crypt Intensity, KCD=Keratin crypt Density, CGA= Chromogranin A

Table 8 3. A comparison between biomarkers scores in the FACT study participants who consumed high and low amounts of foods and nutrients associated with the risk of CRC, continues

		Food/nutrient	DII Score		
			Low intake	High intake	*p*
		N of cases	44	44	
		Mean (SD)**	-0.4 (1.1)	2.1 (.7)	
All		**Biomarker*****			
	ISTT	**Mitosis**	5 (2.5)	5.9 (3.5)	.20
		ki67	0.3 (.12)	0.4 (.15)	.20
		Ki67%	33.2 (10.9)	37 (14.6)	.30
	MWUT	**Cellularity**	69.8 (8.9)	74.6 (15.3)	.20
		Ki67 Number	24.3 (9.8)	29 (16.7)	.40
		KSI	1.3 (.8)	1 (.8)	.15
		KCI	0.9 (.8)	0.6 (.6)	.14
		KCD	1 (.9)	0.6 (.6)	.11
		CGA	1 (.7)	0.9 (.6)	.97
		CGA%	2.3 (1.7)	1.9 (1.1)	.91
Normal		**No of normal cases**	17	23	
	ISTT	**Mitosis**	5.6 (2)	7.3 (3.8)	.10
		ki67	0.3 (.1)	0.4 (.15)	.09
		Ki67%	35.5 (10.3)	40.3 (13.9)	.20
	MWUT	**Cellularity**	69.6 (7.5)	78 (15.4)	.08
		Ki67 Number	25.5 (8.6)	32.6 (17.4)	.24
		KSI	0.9 (.7)	0.9 (.76)	.67
		KCI	0.5 (.6)	0.5 (.47)	.71
		KCD	0.6 (.7)	0.5 (.48)	.57
		CGA	1.2 (.7)	0.9 (.4)	.21
		CGA%	2.7 (1.5)	1.9 (.8)	.08
Adenoma		**No of adenoma cases**	21	16	
	ISTT	**Mitosis**	5 (2.6)	4.9 (2.9)	.90
		ki67	0.3 (.12)	0.3 (.15)	.60
		Ki67%	29.8 (13.4)	31 (18.7)	.80
	MWUT	**Cellularity**	71 (10.2)	75 (9.7)	.46
		Ki67 Number	20.5 (11.5)	25 (16.4)	.67
		KSI	2 (.4)	1.8 (.47)	.42
		KCI	1.5 (.55)	1.5 (.23)	.30
		KCD	1.6 (.7)	1.5 (.22)	.67
		CGA	0.7 (.6)	0.8 (.49)	.35
		CGA%	1.4 (.9)	1.7 (.9)	.17

*****Cases divided according to level of intake: more than the median or less than the median** means are provided in units clarified in the table head. *** Biomarkers are not available for all cases. ISTT=Independent Sample T Test, MWUT= Mann–Whitney U test. KSI=Keratin Surface Intensity, KCI= Keratin Crypt Intensity, KCD=Keratin crypt Density, CGA= Chromogranin A

Compare dietary intake of foods and nutrients associated with CRC with the recommendations in participants classified as "high-intake group" or "low-intake group"

As described in the method section of this chapter, the intake of foods and nutrients associated with CRC in participants of the FACT study were divided into two categories, low intake and high intake. The aim of this classification was to use the two groups to explore if there is an association between intake of these nutrients and scores of the biomarkers. Before conducting this analysis, in this section we explored the average daily intake of the two categories with the recommendations. **Table 8-4** shows the results of one sample T test, which revealed that participants categorised in the high and low fibre intake groups consumed significantly lower amounts of fibre than the recommendations. For other food groups, participants who were categorised in the high intake category, consumed significantly higher than the recommendation and the low intake consumed significantly lower than recommendations.

Table 8-4. Comparing dietary intake of foods and nutrients associated with CRC with the recommendations in participants classified as "high-intake group" or "low-intake group"

Foods or nutrients	***Recommendations***	***Low-intake group***		***High-intake group***	
		mean (SD)	*p*	*mean (SD)*	*p*
Red and processed meat	*<70gm/day*	*43.3 (17.1)*	*<.001*	*112.6 (40.2)*	*<.001*
Fibre (NSP)	*>23gm/day*	*11.2 (2.5)*	*<.001*	*19.8 (5.1)*	*<.001*
Fish and fish products	*>40gm/day*	*24.2 (10.4)*	*<.001*	*66.7 (32.7)*	*<.001*
Fruit and vegetables	*>400 gm/day*	*284.6 (84)*	*<.001*	*608 (189.14)*	*<.001*

8.5.3 Adherence to cancer prevention recommendation

Of the 88 participants included in this scoring, 70 patients (79.5%) did not adhere to the WCRF/AICR recommendations for cancer prevention and scored < 3 points. The other 18 participants (20.5%) had a total score of ≥ 3 with a maximum of 3.75 points. When analysing the data for normal and neoplasm separately, >75% of each group had a score of < 3 points **(Table 8-5)**

Comparing the demographic characteristics and dietary intake in patients who were classified as adhere and or not adhere to the WCRF/AICR cancer prevention recommendation

No difference in age of participants who adhered to the cancer prevention recommendation when compared with the group who did not adhere however, participants in the non-adherence group had a significantly higher BMI (27.7 vs. 23.3 Kg/m^2, p<.001). In terms of dietary intake, a significant difference was detected in intake of fibre, alcohol and red and processed meat but not in other nutrients or in DII scores (**Table 8-5**).

Table 8-5. A comparison between the adherence group and non-adherence group in factors included in measuring the adherence to cancer prevention score and DII score

Adherence to cancer recommendations	*Did not adhere*	*Adhere*	*p*
Number of cases	*70*	*18*	
Score range	*.25 to 2.75*	*3 to 3.75*	
Diagnosis			*.602***
Normal N (%)	*33 (82.5)*	*7 (17.5)*	
Neoplasm N (%)	*37 (77.1)*	*11 (22.9)*	
Age (year)	*65.6 (10.6)*	*63.8 (9.8)*	*.5*
BMI (kg/m²) mean (SD)	*27.7 (4.4)*	*23.3 (1.8)*	*<.001*
Energy (MJ/day)	*8.2 (3.1)*	*8.8 (3.0)*	*.5*
Alcohol (g/day)	*13.9 (17.1)*	*6.9 (7.0)*	*<.05*
Meat and meat products (g/day)	*129.3 (61.2)*	*84.7 (26.6)*	*<.005*
Red and processed meat (g/day)	*86.4 (47.9)*	*45.2 (15.9)*	*<.005*
Fish and fish products (g/day)	*46 (34.6)*	*44 (21.7)*	*.8*
Milk and milk products (g/day)	*361 (180.8)*	*467 (192.7)*	*<.05*
Fruit and vegetables (g/day)	*425 (214.14)*	*531 (220)*	*.07*
Fibre (g/day)	*14.8 (5.79)*	*18.2 (5.8)*	*<.05*
Iron (mg/day)	*10.5 (4.04)*	*10.8 (2.9)*	*.8*
Vitamin C (mg/day)	*103 (47.9)*	*123.3 (59.9)*	*.13*
Vitamin D (µ/day)	*3.5 (2.5)*	*3.9 (2.5)*	*.6*
DII score mean (SD)	*0.94 (0.5)*	*0.5 (1.6)*	*.3*
Independent sample T test. **Chi square test			

Explore if adherence to WCRF/AICR recommendations affects the scores of cell proliferation, keratin and endocrine cells

No significant difference was found in the biomarkers score between participants who adhered or did not adhere to the WCRF/AIRC cancer prevention recommendations. **(Table 8-6).**

Table 8-6 The relationships between adherence to the WCRF/AICR recommendations and the cellular scores

	Marker /Adherence category	***Did not adhere***	***Adhere***	***p***
ISTT	*Mitosis Mean (SD)*	*5.4 (2.9)*	*5.8 (3.5)*	*.60*
	ki67 Mean (SD)	*0.34 (.14)*	*0.37 (.12)*	*.40*
	Ki67 % Mean (SD)	*34.6 (13.5)*	*37.6 (.12)*	*.40*
MWUT	*Cellularity Median (IQR)*	*71.2 (13.5)*	*75.2 (13.3)*	*.15*
	Ki67Number Median (IQR)	*23.45 (15)*	*30.2 (18.5)*	*.12*
	KSI Median (IQR)	*1.2 (1.5)*	*1.5 (1.3)*	*.39*
	KCI Median (IQR)	*0.8 (1.5)*	*0.6 (1)*	*.33*
	KCD Median (IQR)	*0.75 (1.5)*	*0.6 (1)*	*.28*
	CGA Median (IQR)	*0.9 (1)*	*0.65 (.8)*	*.33*
	CGA % Median (IQR)	*1.7 (1.2)*	*1.5 (1.5)*	*.29*
	ISTT=Independent Sample T test. MWUT=Mann-Whitney U test			

8.6 Discussion

CRC is a major public health issue; according to the Cancer Research UK, it is ranked as the 2nd biggest cancer killer in the UK for both males and females(270). Evidence shows that following a healthy lifestyle may prevent about 50% of the cases (90). Currently, available prevention guidelines are based on epidemiological studies and molecular mechanisms behind these findings remain unclear. Identifying the molecular pathways that mediate the association between dietary intake and lifestyle factors with the disease will improve our understanding of the disease development and progression, which may help in developing evidence based prevention guidelines. In this chapter, a secondary data analysis was performed to explore the association between diet, cancer prevention recommendations and markers of cell proliferation, enteroendocrine cells and keratin in the mucosa of the mid sigmoid region of the colon of healthy individuals and patients diagnosed with different stages of colorectal tumourigenesis.

Before conducting this analysis, an internal comparison was performed between healthy individuals and patients diagnosed with a neoplasm. The analysis showed no significant difference in dietary intake or DII score between normal participants and patients diagnosed with adenoma or cancer recruited to the FACT study. This was followed by a comparison between this cohort and the males recruited to the seAFOod trial. We found that the FACT study participants consumed significantly higher amount of milk and dairy products, while the seAFOod participants consumed significantly higher amount of red and processed meat. Dietary intake of participants from both studies was characterised by high consumption of sodium and low consumption of dietary fibre and vitamin D. The BMI of participants in the seAFOod trial was significantly higher, but there was no difference in age. As the difference detected between the two groups in factors that are associated with the development (BMI, red and processed meat) and prevention (milk and dairy products) of CRC, these differences should be considered when comparing the two groups.

Overall, we found that crypt cell proliferation markers were associated with higher consumption of iron vitamin C and low consumption of vitamin D. In adenoma patients, mitosis in the crypt was higher in people with high consumption of vitamin C and lower with high consumption of vitamin D. A significant decrease in Ki67 expression of in participants consumed high iron. These findings are contradicted to the results obtained from an animal study that showed that proliferation of colonic epithelial is higher in haem-fed animals when compared with controls. This experiment used food supplemented with haem

iron (0.5 µmol of haem per gram of food). Two factors may affect this comparison. The first is that the study used haem iron and the value extracted by FETA software is for haem and non-haem iron. The 2nd possible reason is that the total amount of consumed iron is not mentioned in this study. Which might be very large dose when compared with the intake of the FACT study.

The analysis also showed that keratin levels are lower in individuals consuming high amount of vitamin D. These are contradicted to the findings that were reported from an *in vivo* study that was conducted in 2003. The study explored the effect of 1, 25 dihydroxy vitamin D3 on the gene expression profiles in human colon cancer cell lines using oligonucleotide microarrays (271). The study found an increase in expression of keratin-13. However, vitamin D intake was below the recommended 10µ by SACN for most the individuals, moreover, there is no information about the circulatory vitamin D level, which gives an accurate level for the bioavailable concentration.

The majority of the participants had a low adherence score for the WCRF/AICR cancer prevention recommendations (80% had score of <3/5) there was no association between age or diagnosis and the adherence score. However, the group who had higher adherence score had a significantly lower BMI and their reported dietary intake was within the recommendation. Their diet was low in alcohol, red and processed meat and higher in fibre. If the reason behind this low level of adherence to the cancer prevention recommendation was lack of knowledge or lack of motivation, it is not known as no qualitative data was collected from the participants. No association was detected between adherence to the WCRF/AICR and scores of mucosal proliferation, keratin or CGA.

Although previous studies reported a positive association between pro-inflammatory diets, measured by DII, and the risk of colorectal adenoma and CRC (151,272,273), in this analysis we could not find a difference in DII score between healthy participants, adenoma or cancer patients. Moreover, DII score was not associated with the biomarkers of cell proliferation, keratin and CgA. The sample size used in this analysis is small and may not be adequate to detect a significant difference between the diagnosis groups. However, as it was discussed in **Chapter 5,** using the DII score in exploring the association between diet and colorectal health needs more consideration to the method in terms of how many food parameters used to calculate the DII and whether they have an anti or pro-inflammatory effect.

This analysis has some limitations. The small sample size may not be sufficient to test the difference and to obtain a statistically significant result to reflect the true difference. The proportion of subjects described as “normal” were attending the GIT clinic for either other illness in their GIT or maybe had a polypectomy and attending for screening for adenoma recurrence.

Another limitation that may affect our results is that biomarkers may not reflect the actual cellular behaviour since previous study reported that cellular proliferation might be affected by the bowel preparation process before the colonoscopy examinations (274).

The limited data available to score adherence to cancer prevention recommendations may affect the scores and the categorization of the participants. This also has affected the comparability of the results with other studies. This analysis highlighted the need for developing a questionnaire to measure adherence to the WCRF/AICR cancer prevention recommendations. Although a standardised scoring system was developed to measure the adherence to these recommendations, differences between studies in data availability and tools of measurements influence the quality of the scoring and validity of comparing results from different studies.

Overall, more studies are needed to explore the mechanisms involved in the association between dietary intake, lifestyle and different stages of colorectal tumorigenesis

Chapter 9

General discussion and conclusion

Chapter 9 General discussion and conclusion

This research project aimed to describe the dietary intake of patients that were newly diagnosed with high risk colorectal adenoma and to explore the association between dietary intake and the risk of colorectal adenoma recurrence. In each of the results chapters, the findings were discussed in detail including the strengths and limitations of each analysis. This chapter will summarise the main findings of each chapter and discuss the findings in relation to the literature. It will also include the strengths of and limitations that affected this thesis as a whole. This will be followed by recommendations for future research and the possible implications of these findings on public health policy. The final section will provide the conclusion statement of this thesis.

9.1 Summary of main findings

The first step of this research project was to conduct a literature review to identify the gap in the research literature about the association between dietary intake and different stages of colorectal tumorigenesis **(Chapter 1)**. The review found that:

- There is evidence that lifestyle, including diet, may alter the risk of CRC. The WCRF/AICR collected the scientific evidence and produced the guidelines for the policy makers, the health professionals and the public to reduce the burden of CRC.
- There is some evidence about the association between dietary behaviour and colorectal adenoma development, which, in general, are similar to the risk factors of CRC.
- However, limited studies explored the association between diet and the risk of colorectal adenoma recurrence and, currently, no systematic reviews or meta-analysis were conducted in this area of research. Therefore, a systematic approach was followed to summaries the evidence from RCTs that explored the effect of nutritional intervention on the risk of colorectal adenoma recurrence. The conclusion of this review was that there was limited evidence that an intervention with a combination of vitamin A, C, and E and an intervention with high doses of folic acid may reduce the risk of colorectal adenoma recurrence. This review also revealed that the nutritional intervention outcome might differ by sex, age and alcohol intake.

Concerning the association between dietary patterns and the risk of colorectal adenoma recurrence, one study explored the association using the dietary patterns extracted by the PCA method and found a

protective effect for the *Mediterranean* dietary pattern in women only. One study explored the association using the DII score method and found no association between DII score and the risk of recurrence.

Overall, this review found that limited evidence is available in the literature for the association between dietary behaviour and the risk of colorectal adenoma development and recurrence. Therefore, the main aim of this research project was *"To describe the dietary characteristics of patients newly diagnosed with high-risk colorectal adenoma and to explore the association between diet, dietary patterns and colorectal adenoma profile and the risk of recurrence"* This aim was achieved through seven objectives through using two sets of data, details about each set of data are provided in **Chapter 2**.

The first set of data was used to achieve **objectives 1 to 5** stated in **Chapter 1**. Using data from the seAFOod trial, which was an RCT that was conducted through the BCSP and collected data from 707 colorectal adenoma patients, who were classified as at high risk of colorectal adenoma recurrence. Data of dietary intake and adenoma characteristics were collected at two-time points, at diagnosis and 12 months after. Dietary data from the seAFOod trial was not explored before; hence, before using this data the **first objective** of this research was to conduct a series of internal and external comparisons and calculations to validate its accuracy (**Chapter 3)**. The data was of an acceptable level of accuracy but dietary misreporting was detected and therefore a degree of caution is needed when interpreting the results, especially in regards to consumption of fruits and vegetables.

The seAFOod trial data collected at baseline was used to achieve **objectives 2 and 3 in Chapters 4 and 5**. A combination of these objectives was to thoroughly describe and explore the dietary behaviour of this high-risk group using more than one approach as follow:

Objective 2: Describe the demographic characteristics and dietary intake of patients newly diagnosed with colorectal adenoma and classified as at high risk of recurrence **(chapter 4)**.

This analysis included data from 674 patients, 79.4% of them were males, with a mean age of 65.3 (SD=4.7) years and a mean BMI of 29.4 (SD=5.6) Kg/m^2. The analysis revealed 59% of the patients exceeded the maximum amount of red and processed meat recommended by WCRF/AICR (70g/day). Average daily intake of iron was more than the DRV for this age group. However, less than 15% of the patients achieved the recommended daily amounts of fibre and the diet was low in vitamin D. Subgroup

analysis per sex revealed that significantly higher proportion of males exceeded the maximum recommendations of red and processed meat (63% vs 43%) and significantly higher proportion of females consumed the weekly-recommendation of oily fish (25.4 vs 11.4 %).

Objective 3: To explore dietary patterns in patients recruited to the seAFOod trial using two dietary pattern analysis approaches, the data-driven approach and the predefined approach **(Chapter 5).**

In summary, the data driven dietary pattern was conducted using the PCA method including 14 food groups. The analysis extracted three distinct and interpretable components; these components were labelled according to the food items with the highest correlations as "*High-energy" "healthy" and "alcohol and nuts*" dietary patterns.

For the pre-defined dietary patterns' analyses approach, 30 food parameters were used to calculate the inflammatory potential of the diet following the DII method. The score ranged from -3.82 to +5.14 and the mean was +1.46 (SD=1.836), which indicates a proinflammatory score. However, this analysis found no association between following a specific dietary pattern and adenoma characteristics.

Objective 4 to explore if patients modify their diet after being diagnosed with a high risk of colorectal adenoma in the absence of any dietary guidelines **(Chapter 6).**

The dietary data collected at baseline and the exit of the study were used to achieve this objective. The results indicated that males reduced their average daily intake of energy, red and processed meat but no change was detected in dietary intake of females during the year following diagnosis with high-risk colorectal adenoma.

Objective 5: To explore if there is an association between the dietary behaviour at baseline and the risk of colorectal adenoma recurrence **(Chapter 7).**

The dietary patterns extracted using the baseline data and the adenoma recurrence data at 12 months post polypectomy of the 156 participants allocated to the placebo group were used to achieve the 5th objective of this thesis. The analysis showed no significant differences between patients with and without adenoma recurrence in terms of their age, sex, BMI or smoking status. No significant difference between the two groups was observed in their baseline average daily intake of energy, alcohol, foods and nutrients associated with CRC as per the WCRF/AICR report. The results of this analysis did not show any significant association between the risk of adenoma recurrence and following any of the dietary

patterns extracted by the data-driven approach or the predefined approach using the baseline data. No association was found between adenoma characteristics, in the case of recurrence, and dietary intake or dietary patterns.

This 2nd set of data was obtained from the FACT study and was used to achieve the **6th and 7th objectives** stated in **Chapter 1**. The FACT study is a cross-sectional study collected data from 98 individuals (males). Patients had a colonoscopy examination at the gastroenterology clinic and were either classified as normal or with colorectal neoplasia (CRC or adenoma). This study collected dietary data and biopsies from the mid sigmoid region of the colon of each participant. Other researchers used these biopsies to measure the scores for cell proliferation, endocrine cells and keratin.

Objective 6: To explore if an association exists between dietary intake and crypt cell proliferation, keratin or endocrine cells (**Chapter 8**).

The analysis revealed that, high crypt cell proliferation (assessed by mitosis) was observed in adenoma patients who consumed a high amount of vitamin C and in all participants consuming low amount of vitamin D, however, lower crypt cell proliferation (assessed by Ki67) was observed in all participants consuming high amount of iron. Low expression of endocrine cells marker (assessed by CGA) was detected in adenoma patients consuming high amounts of fish. In adenoma patients, high intake of alcohol and iron were associated with a significantly higher expression of keratin.

Objective 7: To explore if an association exists between crypt cell proliferation, keratin, endocrine cells and adherence to the WCRF/AICR general cancer prevention recommendations (**Chapter 8**).

This analysis show no association between adherence to the WCRF/AICR cancer prevention recommendations and markers of cell proliferation, keratin or endocrine cells.

9.2 Discussion of main findings and comparison with the literature.

The results obtained from the seAFOod trial data analysis will be discussed in relation to the findings from the FACT study. However, the comparison performed between the participants recruited to the two studies revealed that males recruited to the seAFOod trial (n=533) had a significantly higher BMI and consumed significantly more amount of red and processed meat when compared with the males recruited to the FACT study (n=88), **(Table 8-1 in Chapter 8).**

Eighty percent of the seAFOod trial participants were classified as overweight or obese, this is higher than the percentage reported in adults in the UK in 2020 (62.8%) and detected in FACT participants (67%). Epidemiological studies associated high BMI with the development of colorectal adenoma and the WCRF/AICR report found 'strong convincing evidence' that body fatness increases the risk of CRC (WCRF Report 2018) (90). Epidemiological studies show that the risk of developing colorectal adenoma is 19% higher with each five units increase in BMI (64). The mechanism linking body fatness with colorectal tumorigenesis is not fully understood but two mechanisms are proposed. The first is related adipocytes expression of proinflammatory molecules, such as C-reactive protein and interleukins and cause chronic systemic low grade inflammation (183). The 2nd mechanism is that an insulin resistant condition is highly prevalent in obese individuals. *In vivo* studies used colorectal cancer cell lines find high insulin level is associated with stimulation of cell proliferation and reduction in apoptosis (184). There was no significant association between the scores of cell proliferation (mitosis, cellularity or Ki67) and BMI classification in the FACT study. An assessment of the indicators related to the proposed mechanisms such as systemic inflammation biomarkers and assessment for insulin resistance may further clarify the role of obesity in the development of colorectal adenoma.

The mean daily intake of alcohol reported by the seAFOod trial participants at baseline was 13.5 (SD=16.2) g. This is similar to that reported by adults aged 64 and more in the UK between 2008 and 2016, as was reported in NDNS results (13.6,SD=18.2)g/day , and similar amount reported by FACT participants, (12.5, SD=15.8)g/day. Alcohol intake increases the risk of CRC based on strong evidence in the WCRF/AICR report. A meta-analysis for the association between alcohol consumption and the risk of colorectal adenoma included data from 25 observational studies revealed that, even with low intake of alcohol (estimated to be <12.5g/day) the risk of adenoma increases by 17% compared with non-drinker (91). Several mechanisms are proposed for this association, locally, the acetaldehyde (a metabolite of ethanol) increases generation of reactive oxygen species and cause mucosal damage and exposure for carcinogens (70). High alcohol intake is also associated with epigenetic modifications that result from tetrahydrofolate depletion that is associated with high alcohol consumption (71). In the FACT study participants, high alcohol intake was associated with low expression of endocrine cells in all participants and was associated with higher keratin crypt density in adenoma patients. This outcome is contrary to that obtained from an animal study that reported an increase in the expression of endocrine cells following high consumption of alcohol (275). Epidemiological evidence suggests that the role of

alcohol in CRC is influenced by the dietary intake of micronutrients with antioxidant activity (retinol, carotene, and vitamins C and E) (276) and the methyl donors (277). Further investigations for the role of alcohol in the development of adenoma may require consideration of these nutrients in the analysis.

At baseline, the mean daily intake of red and processed meat reported by patients recruited to the seAFOod trial was 89.3 (SD=51.2) g/day. This amount exceeded the maximum amount advised by the WCRF/AICR (<70g/day) and was more than the average reported by adults aged 65 and over in the UK during the period from 2010 to 2019, which was 60 (SD=41.8) g/day. Participants recruited to the FACT study reported a significantly lower intake than males recruited to the seAFOod trial (78, SD=46.4 vs. 93 (SD=52.8) g/day, $p < .05$). Observational studies found that the risk of colorectal adenoma is higher in people consuming high amounts of red and processed meat (93) and the link with CRC is strong according to the WCRF/AICR (90). A number of meat components are associated with the development and the progression of colorectal neoplasia, in addition to being rich in nutrients associated with the development of the disease (haem iron, saturated fat and protein), red and processed meat are sources of other carcinogenic chemicals that are used in the preservation of the processed meat and generated during cooking meat at high temperature. One of the mechanisms proposed is the high saturated fat leads to obesity and inflammation through prostaglandin formation (278). High fat intake is also associated with higher quantities of bile acids reaching the colon. At high concentrations, bile acids are metabolised by the microbiota to secondary bile acids, which in high concentrations, are associated with DNA damage and mutation (76,77).

Experimental studies linked heterocyclic amines and polycyclic aromatic hydrocarbons (which are formed during cooking meat at high temperature) and N-nitrosamines compounds (which are added to preserve meat) to the development of CRC (74,75,175). Finally, fermentation of sulphur-containing amino acids in the gut by sulphate-reducing bacteria leads to an increase in levels of the potentially toxic sulphur metabolites (72). A recent observational study reported that long term adherence to dietary patterns associated with sulphur-metabolizing bacteria in stool are associated with higher risk of CRC (88). The other mechanism of meat is being rich in haem iron which will be discussed below.

No association was found between consumption of red and processed meat and markers measured in the FACT study data. A limitation of this analysis is that no information is available about the cooking methods of the meat. To understand the effect of consuming high amount of red and processed meat,

further investigation is needed, for example, measuring the secondary bile acids and the sulphate-reducing bacteria in the stool might highlight some of the mechanisms that link meat consumption with colorectal tumorigenesis.

The mean of iron intake by seAFOod trial patients was 11.1, SD=3.48 mg/day. This was more than the average intake in the UK by adults 64 years and above reported for the period between 2010 and 2019 by the NDNS (9.8, SD=3.3) mg/day and higher than the recommended DRV for this age group (8.7mg/day). The association between iron and the risk of colorectal adenoma was explored using serum ferritin levels as an indicator for the body iron store instead of dietary intake and the association between body iron storage and the risk of adenoma was positive (102,103). On the other hand, a more recent case control study found no association between the risk of colorectal adenoma with dietary iron or serum ferritin (279). The association between dietary haem iron and the risk of developing adenoma was explored in a cohort study that included 17,397 French women, mean age 58.7 (SD=6.8), 1,409 of them developed colorectal adenoma during the follow-up period of (median 5.9, SD=2.4) years. This study found that high haem iron intake is associated with colorectal adenoma risk; however, the association depend on factors such as the site of the adenoma and the ratio between dietary haem iron and dietary antioxidant(104). The mechanism of Iron in colorectal tumorigenesis is through mucosal surface damage and induces cellular proliferation (82) . We observed a lower expression of Ki67 (a cell proliferation marker) and a higher expression in keratin in individuals consumed high amount of iron mg/day compared with low intake (13.4 vs. 7.8) mg. Our findings are not in agreement with the findings from an animal study that found that, feeding male C57BL6/J mice food contains of 0.5 µmol/g haem for 14 days induced colonic cellular hyper proliferation (280). A limitation for the analysing of the association between iron and adenoma and comparing our findings with other studies is that the FETA software extracts all iron from all foods, and for the association with CRC, studies show that the association is with haem iron which is obtained from animal source and not with non-haem iron.

The seAFOod trial participants reported a low consumption of NSP fibre, mean daily intake was =15.4 (SD=6) g/day. These findings are lower than the consumption of NSP fibre in the UK reported by the NDNS for the years 2010 to 2019 (18.3, SD=6.6g/day) , but in agreement with what was reported by the FACT study (15.5, SD=5.9 g/day). The WCRF/AICR reported a probable protective effect for dietary fibre from CRC. For colorectal adenoma, epidemiological studies found an inverse association between fibre intake and the risk of adenoma, that was only significant when the source of fibre were fruits and cereals

but not significant for vegetables' fibre(101,281). These findings highlighted the importance of considering the source of fibre when investigating this association.

Dietary fibre intake of the UK adults and the amount reported by the studies included in this analysis are lower than the recommended 23g/day of NSP (30g of total fibre). This may suggest the prevalence of low consumption of fibre in the UK. Moreover, as the validation analysis **(Chapter 3)** revealed an over-reporting of fruits and vegetables (one of the main sources of dietary fibre) it is highly possible that the actual intake is much lower than this figure.

Several mechanisms are proposed for the protective effect of fibre in colorectal tumorigenesis, however, identification of the type and source of the fibre is important. According to their physicochemical properties, dietary fibres are classified into soluble and insoluble fibres, with each metabolised differently in the colon and has a specific protection mechanism (80). The mechanism proposed for the insoluble fibres (i.e. cellulose) is by increasing the bulk of the stool, dilute the bile acids, reducing the intestinal transit time and minimizing the exposure of the mucosa to the potential mutagens. The soluble dietary fibre (i.e. pectin) is fermented by the gut microbiota forming short-chain fatty acids, such as butyrate, acetate, and propionate. Experimental studies showed that butyrate has an anti-proliferative effect. The different in composition and metabolism of dietary fibre may explain the findings that significant association was found between the risk of colorectal adenoma and fibre is associated with the source of fibre (fruits and cereals or vegetables).

Finally, Chapkin *et al* (2007) identified a synergistic effect between dietary fibre and ω-3 PUFA in the protection from CRC, and recommended assessing the availability of both nutrients when evaluating the effect of fibre (131). An animal study observed an increase in bcl-2 promoter methylation and apoptosis in carcinogen-induced colon tumours in animals after being fed a mixture of fish oil with pectin (soluble fibre) when compared to animals consuming corn oil plus cellulose (insoluble fibre) (133). A combination of ω-3 PUFA and butyrate leads to genetics and epigenetics modifications (132).

We found no association between keratin markers and fibre intake in the FACT study which is contradicted to what was reported by Evans *et al* in 2014 where they observed a reduction in keratin expression after 8 weeks of increasing fibre intake from <12.5 to >20g/day (263). This difference may be explained by several factors. The two groups of the FACT study used in the comparison consumed less than 20g/day, which might be the amount needed to trigger this change. The type of fibre and the

proportion of each fibre consumed by the two groups is not known and finally, the effect of fibre maybe confounded by other confounders that were not considered in the analysis.

Our findings of the low intake of vitamin D at baseline was similar to the findings from the NDNS report and the FACT study (3.26, SD=1.77, 3.3, SD=2.2 and 3.6, SD=2.47) µ/day, respectively. No association was found between dietary vitamin D and adenoma risk in women as per a cohort study that was published in 2005(107), however, a protective role of vitamin D was observed when the association was assessed using the serum concentration of 25-hydroxyvitamin D in meta-analysis that included 16 observational studies (110). The WCRF/AICR report concluded a protective effect of vitamin D on the development of CRC was based on evidence obtained from studies explored the role of dietary, serum level and supplementation of vitamin D (90). Animal studies show that the active form of vitamin D, (1, 25- dihydroxyvitamin D3) has a role in reducing proliferation and inducing apoptosis (282). Also evidence associates vitamin D with improved immunity and reduction in inflammation (282,283). Individuals with low intake of vitamin D in the FACT study had a significantly higher crypt cell proliferation (assessed by mitosis). These results differ from the findings reported by Fedirko and colleagues (2009) who found that a 6 months intervention with (800 IU/20mcg) vitamin D and calcium carbonate (2g) promoted epithelial cell differentiation measured in biopsies obtained from apparently healthy tissues from colorectal adenoma patients. This study also reported no difference in baseline serum 25-OH–vitamin D or 1,25-(OH)2–vitamin D between the groups, but a significant difference was found at the end of the intervention (79). However, the difference observed between studies might be due to using high dose of vitamin D in the intervention when compared with the dietary intake reported by the FACT participants. Because skin sun exposure is more important than dietary intake for the serum levels of 25-dihydroxyvitamin D3 serum level of vitamin D is required to explore the association between vitamin D and colorectal tumorigenesis.

Overall, the observed baseline high prevalence of obesity, high intake of red, processed meat and iron and low intake of fibre and vitamin D may all contributed to the development of colorectal adenoma through various mechanisms. Moreover, the evidence shows that synergistic and antagonist effects of the nutrients (i.e. iron and antioxidants and ω-3 PUFA and soluble fibre) should be considered when assessing the effect of each nutrient on the health condition. The association between diet and colorectal tumorgensis is also affected by the interaction between diet and the gut microbiota. Since

evidence show that macronutrients influence the microbiota balance and an increase in select bacteria, such as sulphur-metabolizing bacteria have been associated with the development of CRC (72).

To describe, broadly, the dietary behaviour of this cohort and how individuals were combining the food groups in their diet, two methods were used to assess the dietary intake following the holistic approach of dietary pattern analysis. Each of the methods was further explored by assessing the consumption of foods and nutrients associated with CRC in quartile 1 and 4. This sub analysis revealed that the diet of patients in quartile 1 and 4 (on the opposite scales of the scores) of each of the dietary patterns extracted by the PCA method, did not meet the recommendations for CRC prevention suggested by WCRF/AICR **(Chapter 5).**

DII score of participants of the seAFOod trial (mean= 1.46, SD=1.84) and the FACT study (mean=0.85, SD=1.57) indicate that the type and amount of foods and nutrients consumed by a large proportion of the participants of both studies, induce a high systemic inflammatory potential. Similar to the data driven method, this analysis failed to extract a group of participants that consumed a diet within the recommendations of WCRF/AICR.

The final holistic method was used in this research project was assessing the score of adherence to the cancer prevention recommendations recommended by WCRF/AICR. This method was applied to the data collected from the FACT study. The score of adherence to the cancer prevention recommendations was able to split the cohort into two groups that were significantly different in their consumption of foods and nutrients associated with CRC. It is understandable that this was because these foods and nutrients are included in the calculation process, but due to the sensitivity of the association between diet and colorectal tumorigenesis (the association result from both the systemic effect of the diet on the serum level, effect on mucosa and microbiota), this observation should be considered when choosing the research method.

Interestingly, a strong negative correlation was observed between the healthy dietary pattern extracted using 14 food groups by the data-driven approach with the inflammation potential of the diet calculated using 30 food parameters by the predefined dietary pattern approach. To our knowledge, this is the first study to conduct a comparison between the data driven dietary pattern (using PCA method) and the predefined dietary pattern using DII score. However, these findings need to be verified using other data.

The analysis revealed that no association between any of the PCA extracted dietary patterns and the risk of colorectal adenoma recurrence, using a model adjustment for age, sex and BMI. This outcome of no association is contrary to that reported by Cottet *et al.* (2005) who found that Mediterranean dietary pattern extracted by PCA reduces the risk of colorectal adenoma recurrence in women (153). However, many factors may affect the validity of this study as discussed in detail in **Chapter 7**.

No significant association was found between the DII score adjusted for age, sex and BMI and the risk of adenoma recurrence. These findings are in agreement with those obtained by Sardo Molmenti *et al* in 2017 (154), who found no association between DII score and the risk of colorectal adenoma recurrence in 1727 patients after a 3 years follow up period.

In this analysis, the direction of the association between the risk of adenoma recurrence and the score of the healthy dietary pattern was negative (-0.31), which was opposing the high energy pattern, alcohol and nuts pattern and the DII score (0.28, 0.08, 0.083). This means that the higher the adherence to the *healthy* dietary pattern, the lower the probability of adenoma recurrence. However, this was not significant (OR=.73, 95% CI=0.51-1.06, *p*=.01). A large sample size is needed to further investigate this observation.

In general, the PCA extracted dietary patterns have the advantages of that they account for the variation in and assesses the overall quality of the diet. This method usually generates unrelated dietary patterns that can be included in the statistical models to assess the association between the dietary pattern and the outcome of interest (284). However, due to the homogenicity of dietary behaviour of the patients recruited to the seAFOod trial, we observed similar unhealthy dietary behaviours that were prevalent in quartile 1 and 4 of each of the extracted dietary patterns. The disadvantages of PCA extracted dietary patterns is that they are prone to subjectivity in selecting the food groups included in the model, in deciding the number of principal components to retain and in labelling the retained factors. Which limited the comparability of the dietary patterns obtained from different studies (284).

For the predefined dietary pattern the advantages, in general, is that they are based on scientific evidence (inflammatory biomarkers and dietary intake in the case of DII). They are easy to understand and use and they are repeatable and comparable across populations (284). However, in in the case of the DII, some limitations were observed. Sub analysis for the DII score revealed a prevalence of unhealthy dietary behaviour in patients who had an antiinflammatory DII score. Another disadvantage of

DII score was observed, is that it uses food parameters that are not usually assessed by the common nutritional assessment and analysis tools. Accordingly, we do not recommend comparing DII scores between different studies. Depending on the availability, studies use different numbers of food parameters with different magnitude (small or large effect) and opposite effect (pro or anti-inflammatory effect). For example, our findings were similar to the findings obtained using data from 141,769 individuals enrolled in the UK biobank cohort (202). DII score ranged from -3.88 to +4.22 and the mean was +0.03 and for the seAFOod trial, DII range from -3.82 to +5.14 and mean of +1.46. Although the score seems similar, the actual diet between the two groups could be significantly difference, because the two studies used different number of food parameters (30 in the seAFOod trial vs. 18 in the UK biobank data). The magnitude (how big is the effect) and the direction (pro or antiinflammatory) of the 12 food parameters used in the seAFOod trial but not used for the UK Biobank data is not accounted for in this comparison. Therefore, if these 12 food parameters have an antiinflammatory effect, they would have lowered the DII mean of the seAFOod trial towards a negative value (antiinflammatory), while not including them in the UK Biobank, leads to not account for their antiinflammatory effect, which will give a false indicator for a more proinflammatory overall DII score.

Although strong scientific evidence linked between lifestyle and the development of CRC, CRC screening programmes do not include any diet or lifestyle advice during its process. In the case of the seAFOod trial, and in the absence of nutritional advice, during the 12 months following diagnosis, a significant reduction was detected in daily intake of energy, red and processed meat in males. Similar findings were observed in the cohort recruited to the European Cancer Prevention study (ECP), which show during the three years following adenoma diagnosis only males reduced their intake of energy, protein, total fat and cholesterol(153).

Disease screening experience provokes interest in the health condition (285), but knowledge of the association between the disease and the risk factors is needed before we expect any positive changes from the patients (210). In 2013, Anderson and colleagues reported that, in the UK, this area of health promotion is underdeveloped and she suggested that cancer control strategy should include lifestyle promotion during cancer screening within its plan, which is important to reduce risk of developing cancer and the risk of other non-communicable diseases (286).

Diagnosis with colorectal adenoma during CRC screening is challenging, because it is received by patients as free from cancer and may motivate them to continue their behaviour. A qualitative study for colorectal adenoma patients to reduce their intake of red and processed meat revealed prevalence of lack of readiness to change in many participants and also it was noted that patients did not know that their adenoma diagnosis increases their risk of developing CRC (209).

A national survey was conducted in 2016 to explore patients' willingness to receive lifestyle advice during CRC screening. It included 308 individuals who participated in flexible sigmoidoscopy bowel screening programmes and found that 81.8% of the participants were willing to receive lifestyle advice around the time of the screening and 85.1 % were willing to receive lifestyle advice if the results were abnormal. However, 8.8% reported that receiving lifestyle advice might discourage them from attending other screening session in the future. Any lifestyle intervention within a screening programme therefore needs to be wary of compromising the uptake of screening. This survey also found that more women reported willingness to receive the lifestyle advice than men (287).

Lifestyle intervention RCTs for patients with colorectal adenoma were assessed in a review included five studies. The review indicated that positive changes in the lifestyle behaviour in patients diagnosed with colorectal adenoma was observed after providing different types of intervention ranged from motivation and goal setting to tailored and individual counselling for a period ranged from 8 months to 4 years (288). A recent meta-analysis assessed the effectiveness of tailored physical activity and dietary interventions amongst adults attending colorectal and breast screening included five RCTs. This meta-analysis found that a tailored lifestyle intervention accompanied by a follow-up support (ranges from 3 to 24 months) led to reduction in body weight, increase in consumption of fruits and vegetables and increased in physical activity (289).

Weather the change observed in the SeAFOod trial (without providing an advice) and the change observed in the RCTs with a tailored intervention would last when the RCTs end is not known. As it was reported that the psychological impact of the disease screening process is only high shortly after the screening and a relapse in behaviour was observed after a short term of follow-up (224).

Overall, there is some evidence that there is a willingness for individuals engaged in CRC screening program to receive lifestyle advice, however, whether providing general advice or a personal advice will be more effective is not known. Lifestyle intervention strategies are influenced by many factors such as

health literacy (290), age, health status, income, body weight, smoking and physical activity (291) and, to be successful, the lifestyle interventions plan should be adaptable to the characteristics of each individual (292). As was seen in the seAFOod trial, the change was only observed in the males, which may suggest that sex of the patient may also impact the cancer screening experience outcome, therefore, a personal tailored intervention maybe more effective.

Because of the complexity of providing a personalised nutrition service, Ben van Ommen and colleagues recommended using a flexible systems biology model that allows for tailoring a dietary recommendations that is suitable for the person's health status, requirement and goals (293). However, many challenges facing the development of personalised nutrition service, one of them, is the unavailability of personalised nutrition digital infrastructure, which is needed for experts to develop an effective system (294). Sean H Adams and colleagues recommended developing a digital interdisciplinary knowledge ecosystem with the required information to help expert to design a tool for providing a personalised nutrition using the available technology (295).

Finally, limited evidence is available about the level of knowledge of the health professionals about the association between diet and CRC. An increase in knowledge and behaviour change skills were observed in a group of CRC Screening Practitioners, after enrolling in a training about "risk reduction, and how to provide a health promotion advice" (296). These findings may indicate that, health professional may need training before they can provide an effective lifestyle advice.

In the seAFOod trial, only sex of the patients was associated with both dietary behaviour at baseline and the observed change in dietary intake during the 12 months following the diagnosis. Overall, the analysis revealed that the proportion of females following a healthier dietary pattern was more than males, females consumed less meat and more fish, fruits and vegetables. These findings are in agreement with what was previously reported, a study found that females in Finland and the Baltic countries consumed more fruits and vegetables than males, while males consumed more meat than females (297).

Healthier dietary intake was observed by the scores of dietary patterns, females scores of the high energy dietary pattern and the alcohol and nuts dietary pattern were lower (which means less adherence to these dietary patterns) However, in terms of change in dietary intake, the analysis revealed that positive change was only detected in males. This observed difference in dietary behaviour between males and females should be further explored and considered when providing health advice.

9.3 Overall strength and limitations of this research project

The large number of high-risk colorectal adenoma patients participants recruited to the seAFOod trial is the key strength of this research project. It allowed for a comprehensive assessment of the dietary intake of this high-risk group using more than one approach. In addition, being recruited from different BCSP from around England, this sample is a representative sample for the dietary intake and lifestyle behaviour of people from different regions of the country.

Another advantage of this study is using three empirical approaches and comparing the ability of each approach to assess the dietary intake of foods and nutrients associated with CRC. The dietary pattern analysis methods (PCA and DII) and the cancer recommendation prevention score in which dietary behaviour indicators are used as essential parameters for its calculation.

As a secondary data analysis project, this research has some advantages such saving time and money in data collection and markers' measurements. The seAFOod trial data was collected and stored in an electronic format. The molecular markers of the FACT study were measured, scored and saved in an electronic format.

Another advantage of being a secondary data analysis project that we were able to cross-link the data obtained from the FACT study with the data obtained from the seAFOod trial. The data from FACT study was used to compare the dietary intake of the two groups. In addition, the availability of scores for cell proliferation, keratin and endocrine cells accompanying the dietary data of the FACT study, provided an opportunity to explore if intake of key nutrients is associated with the cellular and molecular activity of the mucosa. Finally, availability of the FACT study data facilitated the process of validation of the in-house method used to calculate the DII score as it was clarified in **Chapter 3.**

As a secondary data project, this analysis also inherent some disadvantages. As we did not contribute in the research planning, or the data collection stages, some of the information that were needed to answer some of this research questions were not available. We had to make assumptions for the physical activity levels ,for example, to estimate the energy requirement (Chapter 3) and we were not able to assess if the change in dietary intake detected in **Chapter 6** had any effect on the BMI due to not to including body weight measurement at the end of the trial data collection plan. No qualitative data was collected during the study about knowledge of the association between lifestyle and the risk of the

disease. Therefore, the reason behind the detected change in dietary behaviour of males will remain unknown. Another limitation of this research project was not having a control group. Dietary data provided by the seAFOod trial was only collected from high-risk colorectal adenoma patients. This limited our ability to compare the baseline dietary data with a group of people with the same demographic characteristics but with no adenoma to verify any observation. Although their dietary intake was compared with the general population using the data from the NDNS group and the UK biobank, one cannot confirm that the data used in the comparison was obtained from individuals that were free from adenomas.

A further limitation is that the sample size for the adenoma recurrence analysis was small, as the analysis was confined to patients allocated to the placebo arm of the SeAFOod trial only. **(Chapter 7**).

Another limitation is the short follow-up period of only 12 months may not be adequate to explore the association between dietary intake and the risk of colorectal adenoma recurrence. Progression of the healthy mucosa to adenomas is a result of the accumulation of genetic mutations and epigenetic events that may take long time to be detected by colonoscopy examination (23).

The analysis conducted on the seAFOod trial data verified homogeneity of the patients recruited in terms of dietary intake, age, BMI. However, although this may be considered as a strong aspect of the seAFOod trial, it imposed a limitation for this analysis. As we propose that this was the reason behind that the dietary patterns' analysis, using the two approaches, were not able to extract a group of patients that consumed foods and nutrients that met the recommendation of the WCRF/AICR to be assessed in relation to a group that did not meet the recommendation.

Homogeneity was also observed in adenoma characteristics data. As only patients with certain adenoma characteristics were recruited to this study, the analysis was not able to detect any association between dietary intake and either the location, size or number of adenoma.

A further limitation is that the small number of females recruited to the seAFOod trial and no females recruited to the FACT study, although we conducted subgroup analysis based on the sex of the patient however, results from this analysis remain uncertain due to small number of females.

Finally, several factors may limit the accuracy of the dietary assessment. The accuracy of the estimated dietary intake of both studies are influenced by the limitations of the dietary assessment (214) and

analysis (161) tools used. In addition, as the analysis in the validation **Chapter 3** showed that there is a high level of misreporting that affected both energy and intake of some of the food groups. Finally, not including the nutritional supplement that some patients may regularly use in calculating the DII or in assessing the association between micronutrients intake and the disease may affected the accuracy of the analysis of the data obtained from both studies.

There was some general limitations of assessing the association between the cellular activity markers in the FACT study in relation to dietary intake. The sample size was small and it is not clear if it was able to detect the significant of the association. In addition, the dietary intake of participants classified as high intake and low intake was significantly different from the recommendations of the WCRF/AICR for cancer prevention (Either significantly higher or lower than recommendations) **(Table 8-4).**

9.4 Recommendations for future research

The BCSP could be used to conduct a prospective cohort study to assess the association between diet and different stages of colorectal tumorigenesis. Collecting dietary data from both healthy individuals and patients diagnosed with different stages of colorectal adenoma and cancer from a large sample could be used to further examine the association using different approaches.

In addition, this field of research would benefit from developing a qualitative questionnaire to understand the level of public knowledge about the association between lifestyle factors and the development of the disease. This would assist in developing a suitable lifestyle intervention plan to be included within the BCSP.

The seAFOod trial dietary data could be further explored. The data for BMI and dietary intake could be used to measure the adherence of this cohort to the WCRF/AICR cancer prevention recommendations. This score could be used to further verify the findings that this scoring system is able to (when compared with the DII and PCA scores) distinguish between two groups who consumed a diet that is in line with the CRC prevention recommendations. The adherence score could be also calculated using the exit data and be compared with the baseline data to assess if patients changed their behaviour after being diagnosed with high-risk colorectal adenoma.

Using the standardised adherence to cancer prevention recommendation score is an advantage of this research, however, due to limitations in the available data, not all the required factors for the score

were available and some modifications were made to the available data to make it suitable for the calculation (**Chapter 8**). Even so, the results obtained added valuable knowledge to the overall analysis in relation to the different methods that might be used to assess dietary behaviour of this high-risk population. This may highlight the need for developing a questionnaire to assess the adherence to the WCRF/AICR cancer prevention recommendations. It may provide a screening tool for public health assessment, which then be used to highlight the specific lifestyle factors that may need intervention. This questionnaire could be either included with different cancer screening programmes or be used in organizations (schools, universities and companies). An advantage of this tool would be short, quick and easy to analyse when compared with the traditional dietary assessment methods.

In **Chapter 5** the analysis revealed a strong association between the DII score and the healthy dietary pattern extracted by the PCA method. Data analysis research to verify these findings is needed. If these findings are proved, this may indicate that the DII score might be used not only to measure the potential inflammatory of the diet but also as an indicator for the healthy dietary pattern. This might be useful in assessing the healthy dietary pattern when using the data-driven dietary pattern analysis is not possible.

9.5 Implications on public health policy

This analysis revealed a high prevalence of obesity among the patients recruited to the seAFOod trial (80%) and a lesser extent in individuals recruited to the FACT study (67%). This analysis also revealed that the dietary intake of both groups lacks some of the characteristics of a healthy diet. Which indicates that individuals at the stage of BCSP may benefit from a lifestyle intervention plan. It was suggested by Anderson *et al* (2013), that the cancer screening programmes may provide a window for intervention with lifestyle advice, including dietary advice (286). As discussed previously, several methods could be used to introduce lifestyle changing advice, either by providing personalised advice, computer generated personal advice or general advice. However, this could be only achieved by distributing a simple lifestyle message to increase the awareness of possible changes that these people could make to improve their health. The WCRF/AICR has published an infographic format for cancer prevention recommendations **(Figure 1-7).** This one page provides valuable information and achievable recommendations that were based on scientific evidence. These resources could be used to motivate people to improve their lifestyle. Which will not only reduce their risk of cancer but also is beneficial for the prevention of other chronic diseases such as diabetes and cardiovascular diseases.

9.6 Conclusion

Colorectal adenoma patients recruited to the seAFOod trial were characterised by being overweight and obese and their diet during the 12 months before diagnosis was high in alcohol, iron, red and processed meat and low fibre and vitamin D. The data driven dietary pattern analysis identified three dietary patterns: *high-energy, healthy, alcohol and nuts patterns*. A proinflammatory DII score characterised the diet of the majority of the patients. During the 12 months after diagnosis, men significantly reduced their intake of energy and red and processed meat, however, the reason behind this changes are unknown. No association was found between dietary patterns at baseline and the risk of adenoma recurrence at 12 months post polypectomy. The analysis showed no association between dietary patterns and adenoma number or anatomical location. The FACT analysis suggests a lower crypt cell proliferation in participants consumed high amount of iron and high crypt cell proliferation in participants consuming low amount of vitamin D. In adenoma patients, high intake of alcohol and iron were associated with a significantly higher expression of keratin.

Overall, colorectal tumorigenesis is a complex disease and diet is only one of the many factors associated with its development or prevention. This research provides insight into the application of different dietary analysis approaches within the context of diet and colorectal health. However, this analysis suggests the need for prospective longitudinal studies that consider the complex association between diet and colorectal tumorigenesis. Analysis for this relation will require a consideration for the systemic availability of the nutrients, direct effect of nutrients on the mucosa, effect of food on microbiota and finally, consideration of the interaction between nutrients.

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Appendices

Appendix 1: The seAFOod trial dietary data processing

The data provided had only one reference number for each patient, which was used in visit 1 and visit 2 data and there was no specific reference number for each visit for each patient. Also, the FFQ1_3 data set contained multiple rows for each participant, therefore, splitting and merging the visit one and visit two data was not possible using the reference number alone. A series of data processing, checking and cleaning of the databases was therefore required before any extraction of dietary intake data from the FFQs.

The main aim of our method, at this point, was to prepare a database that contains baseline characteristics and nutrient intake of patients in a format that applies to the SPSS software for statistical analysis. The work required transforming the data between different software and formats to be able to perform different calculations and analysis. In summary, this work achieved through the following steps, with each step included numerous calculations, coding and analysis:

- Prepare excel worksheets with the baseline characteristics of patients included in the study.
- Prepare a worksheet with the frequency of consumption of different food items merged into a single database for use with FETA software.
- Merging the two worksheets, exclude the outliers and recode the variables into a format that is applicable with SPSS software for statistical analysis.

Cleaning and reordering

As the figure below shows, after separating visit one database from visit two in the three FFQs files provided, there was a difference in the number of cases in each dataset of the three FFQ answers for each visit:

Visit one data

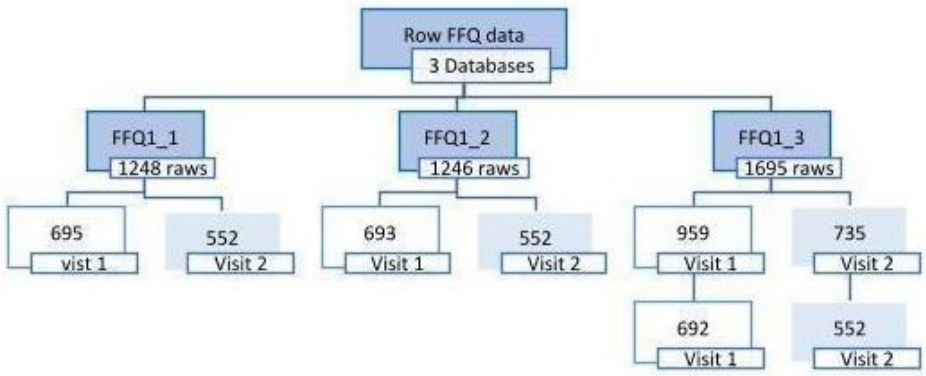
- FFQ1_1 had information spread over 695 rows.
- FFQ1_2 had information spread over 693 rows.
- FFQ1_3 had information spread over 959 rows.

The rows in the first and 2nd FFQs reflected the number of patients with a difference of six cases between them, however, for the 3rd FFQ, information obtained from each patient was distributed, irregularly, over a different number of rows. Ordering the data and allocating one row for all information related to each patient, was performed manually. By the end of this step, the FFQ1_3 had a total of 692 patients in total, which is one case less than the FFQ1_2.

Visit two data

- FFQ1_1 had information spread over 552rows.
- FFQ1_2 had information spread over 552 rows.
- FFQ1_3 had information spread over 735 rows.

The rows in the first and the 2nd FFQs were identical and they show the responses from 552 patients, however for the 3rd FFQ, like the first visit data, information distributed, irregularly, over different number of rows. Similar manual work performed in the previous step to clean and reorder the data. By the end of this step, the FFQ1_3 had a total of 552 patients in total, *which is identical to the other two datasets related to this visit.*



Separating and cleaning dietary intake data from visit 1 and visit 2.

Data entry and cleaning

Once visit 1 and visit 2 data had been separated, the following processes were performed on each visit to prepare the data for the strict format required by FETA software (Cambridge 2013).

1. Renaming and reordering food items to match the sequence required by FETA software.

2. Recoding frequencies of intake in the ***first section of the FFQs*** as required by FETA software. This was achieved by matching answers of frequencies of intake with numbers from (1 to 9). Table 6 shows the frequency of food intake and the code allocated for each frequency, in the case of no frequency entered, (-9) entered to donate missing data.
3. Preparation of the ***2nd section of the FFQs*** required a degree of decision making to assign the most appropriate food code to the handwritten text by using the look-up lists that contained codes for different types and brands of milk, breakfast cereals and fat (see appendix 1-3). This process is known as ***-free text matching***-. In the case that a consumed item was not available on the look-up list, an online search for the ingredients and percentages of nutrients in the consumed item was performed. This was compared to ingredients with the look-up list provided and the nearest food item was chosen. In the case of fats and oils, when more than one type was given, the code of only the first type of fat was used and the other options ware deleted since FETA accepts just one type of fat as an answer for this question. For breakfast cereals, FETA can analyse up to four types, so codes were used accordingly.

Answers provided in EPIC FFQ for the frequency of food consumption

Frequency of food intake	Code allocated
Never or less than once a month	1
1 to 3 times a month	2
Once a week	3
2 to 4 times a week	4
5 to 6 times a week	5
Once a day	6
2 to 3 times a day	7
4 to 5 times per day	8
+6 times a day	9

5. OpenRefine software, which is an open-source desktop application used to clean up and organise data http://openrefine.org/. This software was useful in correction of all sources of error in handwritten answers, such as spelling mistakes, abbreviations, white spaces.

For example, the following answers were provided to indicate that olive oil was used in cooking (olive oil- olive oil. - olive oil based- olive- olive oil used- olive.- olivio- olv- olive oil only- olive oil betrolli). This software can identify similar data and gives the option of choosing one word to replace all the other words in a process called clustering.

6. Merging FFQs from the three spreadsheets into one SPSS spreadsheet according to the reference number allocated to each patient.
7. Data were analysed using FETA software. Figure 4 shows the FETA output sheet where each case has one row and columns contain the amount of each nutrient consumed by each case.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	ID	NUTRIENT_1	NUTRIENT_2	NUTRIENT_5	NUTRIENT_8	NUTRIENT_9	NUTRIENT_11	NUTRIENT_12	NUTRIENT_13	NUTRIENT_15	NUTRIENT_20	NUTRIENT_22	NUTRIENT_55	NUTRIENT_56	NUTRIENT_57	NUTRIENT_58
2																
3	1001	534.4225	6.39354	1970.96916	530.7111	2331.69758	128.46001	247.5449	2797.29135	0.659442	8.47028	6.61396	219.2211	14.09111	0.24696	13.60453
4	1002	94.9921	1.30333	448.44555	366.8236	553.66815	74.41257	137.1982	1323.7822	0.31353	3.91713	3.3391	69.9143	3.07048	0.00441	3.50392
5	1003	197.6777	11.44224	1319.16183	1027.006	1649.25739	235.84212	173.9223	3229.2407	0.831481	10.78895	7.70499	192.0923	17.30395	0.23814	17.72903
6	1004	533.9392	4.87104	2565.4213	871.9029	2889.2443	274.57485	593.0318	6732.8088	1.913388	18.72607	14.44843	280.6494	18.35994	0.78561	18.25752
7	1005	283.6414	35.611	1341.1462	502.4809	1495.9032	104.36314	255.3493	3139.8913	0.853999	9.28516	9.92871	231.6302	2.87553	0.00882	3.53377
8	1006	525.0944	69.23962	2126.34415	965.769	2606.82755	267.53996	349.3455	4949.5605	1.202305	11.28956	11.8466	355.4488	19.50047	0.00441	22.58888
9	1007	534.1395	17.22075	1728.05968	699.38815	2019.43844	148.23549	192.111	2910.9968	0.789924	11.43773	7.95327	186.1395	13.60257	0.00441	14.87055
10	1008	539.9287	5.89178	2015.4819	856.5692	2323.6511	221.35138	201.8387	4178.6946	0.965856	15.74647	9.23772	227.5558	13.68852	0.00441	10.73426
11	1009	525.1977	4.87104	1946.5961	527.0453	2220.3325	125.31597	240.382	2698.8425	0.638425	8.32879	6.56512	155.8924	7.54753	0	8.55762
12	1010	531.0899	10.05766	2049.7484	629.4122	2518.6544	142.72965	144.695	3145.3629	0.565922	8.10808	6.88331	159.2783	11.27703	0	13.20556
13	1011	528.2937	28.32	1901.8428	1156.2544	2236.3884	246.38941	318.5482	5526.9286	1.431704	19.65324	13.28653	329.7179	17.52518	0.00441	15.72448
14	1012	545.6726	7.291	3283.56342	1167.1628	4192.55206	346.9348	505.5145	6271.3954	1.909432	20.11707	14.72738	266.711	35.27365	1.43577	34.1565
15	1013	543.5937	0	2076.83306	515.89025	2378.18058	153.93642	156.625	3153.27555	0.737114	12.52987	6.76952	192.1041	10.86318	0	11.13846
16	1014	528.3127	0	2195.4428	1025.27	2544.3147	168.95203	166.2397	4200.90315	1.187678	14.65127	8.14866	257.1591	10.5923	0.12789	10.67311
17	1015	180.3401	5.75989	1281.3233	693.3118	1516.7052	147.28333	155.3269	3659.4291	0.84393	13.68059	7.72683	228.3558	14.74402	0.12348	10.0089
18	1016	546.7158	0	2131.11376	236.8488	2514.95868	112.60688	197.1254	2353.6878	0.801536	12.71805	7.54025	221.7613	17.43651	0.24696	14.81776
19	1017	566.9046	0.79296	3140.55708	972.94915	3520.31904	174.3137	436.6223	3771.2574	5.406409	14.147	14.6527	369.9412	10.18361	0.1323	13.57709
20	1018	242.5033	2.34717	1888.62191	1522.2806	2251.41893	246.75365	287.9728	4409.3668	0.924899	12.75546	8.60626	232.6212	15.16491	1.50381	16.07892
21	1019	189.1533	0	944.14295	587.3268	1061.26065	156.78186	143.2366	1828.5978	1.124491	9.70102	6.37988	144.3845	17.90683	0	19.83043
22	2002	3054.7349	1.02074	9635.6287	1162.8106	11533.2421	293.83904	449.3717	8094.1484	2.173697	25.84378	15.22262	386.5222	20.78694	0.02709	16.57859
23	2003	601.6491	5.45059	2720.82102	645.7604	3259.70136	243.0962	260.0235	4081.2831	0.90288	13.58293	8.51231	205.4728	20.28902	0.00882	22.27089
24	2004	539.6493	4.87104	3093.5219	686.5619	3824.8158	242.98723	198.3325	3490.862	0.788687	14.11089	8.43309	208.4036	28.09982	0.75852	23.51244
25	2005	535.4654	14.64611	2281.48663	774.2032	2599.73239	196.89603	166.9046	3623.6035	0.835315	14.28694	9.08722	190.3645	20.07708	1.24425	19.16794
26	2006	1009.5434	27.1875	5377.20018	557.3274	6020.41964	136.79565	14.2945	2454.6255	2.293608	32.50526	14.72996	350.8705	35.41647	0.75852	34.56483
27	2008	102.7376	4.87104	758.6315	849.4997	817.0527	232.963	259.6617	4148.5579	1.748023	10.33109	8.79765	213.2826	9.69561	0	13.2209
28	2009	525.2237	8.59125	1964.1602	533.1988	2465.3889	104.10697	87.7735	2013.4163	0.61511	10.98634	6.39956	183.7103	20.19605	1.39356	13.52526
29	2010	581.3515	1.02074	2794.28591	891.47	3187.71313	191.69741	284.8341	4383.0674	0.949549	16.1634	10.13771	251.6622	13.21415	0.25137	13.29788
30	2011	239.8045	1.55421	1795.80223	522.6453	1982.72639	143.41143	233.445	3722.8492	0.716037	10.47698	7.6512	168.9888	13.06856	0.23373	12.64448
31	2012	192.3705	8.76742	1051.90033	552.4011	1232.08839	223.14399	174.262	4581.7178	1.114188	15.93634	9.79227	249.9855	8.10221	0.12348	8.20468
32	2013	534.3413	10.47162	2293.28332	940.4642	2650.86166	163.81615	215.6864	3458.5297	1.2131	12.15234	7.48097	247.4831	9.39509	0.00441	9.87159
33	2014	527.3947	13.46229	2275.44168	630.9195	2995.16694	176.59254	90.1449	2021.2098	1.004208	10.85747	7.39063	220.7034	16.18329	1.39356	13.84424
34	3001	193.6297	10.22074	1794.46282	1613.0941	2354.70866	345.84474	377.0626	5348.2437	1.321547	16.15075	10.30075	253.5676	29.55345	0.16002	29.04363
35	3002	584.5905	2.09629	3057.72295	710.9511	3957.77275	365.22558	257.6863	4088.612	1.226991	22.94077	9.93563	282.0342	60.63013	0	53.61047
36	3003	17.7053	9.0515	503.64892	1159.4042	556.87746	185.99731	102.0201	3023.5997	0.634944	8.85521	7.77813	123.0122	10.9535	0.67725	14.5703
37	3004	179.7894	0	1121.6216	685.6112	1284.2712	151.22799	222.924	2378.0307	0.656483	5.64804	5.75843	112.2974	10.20917	0.25578	12.59077
38	3005	172.9723	9.96986	829.85918	286.19425	1350.15484	184.18127	240.8013	4197.1865	1.072671	15.13479	10.29645	236.5935	14.65116	0	12.91469
39	3006	1077.1628	0	4890.174	1280.903	5576.8533	240.88138	207.7301	4471.503	1.106825	18.37141	9.18156	244.6209	24.97113	1.76841	29.88473
40	3007	555.8353	0.76125	2899.5077	879.3389	3257.441	152.34691	255.3617	4190.2819	0.730384	16.62694	8.24185	275.1739	10.06445	1.44459	10.22007
41	3008	190.521	18.619	1238.2165	978.2055	1721.6387	172.53502	198.4772	3683.4003	1.58165	12.25186	9.97951	233.7358	15.23234	0.24255	14.02976
42	3009	613.7225	6.28217	2607.8109	1073.6544	4136.2027	290.80964	248.0049	3696.4815	1.03065	15.43325	9.22214	238.4063	57.45493	1.39356	47.44712
43	3010	534.7559	27.1875	2733.668	372.1371	3095.5271	182.17596	278.0842	3427.1843	0.958506	12.08635	12.16579	184.9089	15.26852	0.12348	13.34179
44	3011	559.5273	2.06458	6392.88155	1513.892	7301.68575	301.90467	425.7586	6920.5789	2.066349	28.39931	16.90738	443.934	42.50384	4.52025	30.54172
45	3012	105.9872	11.33525	2287.89183	822.8317	2441.55199	185.20089	161.5462	5229.7799	0.949502	23.12155	12.19092	303.5683	26.95756	1.69848	21.83649

Figure 4. FETA output for EPIC-FFQ analysis. One row was allocated to each patient and the columns contain the amount of energy, nutrients or food group consumed.

8. Names of nutrients were recoded according to FETA output list provided. Table 2 shows the food groups, macro and micronutrients included in FETA output.
9. Baseline characteristics and medical history were merged with FETA output to obtain one spreadsheet that contains all the cases and information required for analysis.

Appendix 2: Modifications performed on the FFQ used to assess dietary intake of FACT study participants to be compatible with the FETA software

Food group	No. of questions		Difference	In the new version of EPIC FFQ	In the EPIC FFQ used in FACT study	Action
	old FFQ	new FFQ				
Meat and fish	16	17	One extra in the new FFQ	Roe	Not available	Added the question and answered as 1*.
Bread and Savory Biscuits	7	5	1 different and two extra in the old	Crispbread	Scones, crumpets. Garlic bread Pitta, nan bread	Deleted garlic bread and pitta bread- crispbread answered as 1*- relocate scones
Cereals	5	2				Deleted the 3 questions from fact after extracting the frequency of cereal consumption into one answer and answered the type of cereal from the answer provided in page 16 of the FACT FFQ. Then questions were relocated into the end of the spreadsheet.
Potatoes, rice and pasta	13	10	3 extra in the old		1	Deleted the 3 questions from FACT.
Dairy products and fats	14	19		Low calorie salad cream Salad cream Other dressing French dressing Very low fat spread		Relocated questions 1 to 4 and. Q5 was added and answered as 1*.
Sweets and snacks	12	18		Readymade cake Homemade buns Readymade buns Readymade fruit pies Home baked sponge Readymade sponge Chocolate bars		=1* =scones. HB Buns. 1* 1* 1* 1*
Soups sauces and spreads	15	8			low cal salad cream salad cream other dressing french dressing Tomato based sauce	1,2,3 were relocated. 5,6,7 were deleted.

					Chocolate spread Dips (houmous or cheese)	
drinks	13	14		Coffee decaffeinated		Added and answered as 1*
Fruits	11	11	no			
Vegetables	27	26		Mixed vegetables (frozen or tinned)		Deleted the mixed vegetables.
Type of milk were the same in the two versions						
The following questions are not available in the old version: Did you eat breakfast cereals? Cereal food type? Fat frying?						
Fat baking	NA					Used default fat baking code.
Visible fat	NA					Left empty.
1* indicates assuming no consumption of the food item. NA=Not available						

Appendix 3: Adenoma characteristics data processing

In terms to prepare a worksheet with visit one and visit two data in a format that each cases has its data in one row, the following steps were performed:

1-Separate data obtained from visit 1, visit 2 and the final visit in three worksheets.

Excel software was used to separate the data using the filter command in the visit number variable. Data was separated over three sheets: one for visit 1, the 2nd for the follow up phon call at the middle of the trial and the 3rd was for the exit data.

2-Separate adenoma data at each visit.

This was achieved by using the filter command in excel on the recno variable (recno= adenoma number) Visit 1 has 20 recno variables and visit two has 16 recno variable.

So, visit one was separated into 20 excel worksheets and visit two was separated into 16 excel worksheets.

3-Variables renaming

Renaming the adenoma variables in each sheet. that is 5 variables in 20 sheets in visit one and 5 variables in 16 sheets in visit 2. This was performed as a premerging step so the adenoma characteristics variables are identified for each adenoma in each visit.

4-Merging data for each visit:

The 20 sheets from visit one were imported into 20 SPSS worksheets and the 16 sheets from visit two were imported into 16 SPSS worksheets. This was performed to guarantee data integrity, which is by using the reference number for each patient to merge the data from each visit.

5-Merging data from the two visits:

The data from visit one and visit two were merged in one SPSS sheet using the patients ID numbers as reference numbers for merging.

Calculations

The merged data was imported from SPSS into one excel sheet to calculate new variables.

The new variables are:

Total number of adenomas,

Total number of distal adenomas,

Total number of proximal adenomas,

Total sizes of adenomas,

Total sizes of distal adenomas,

Total sizes of proximal adenomas.

Table below shows the formulas used to calculate adenoma characteristics.

Action required	Formula
Total number of adenomas,	=COUNT(AA2:AQ2)
Total number of distal adenomas, Total number of Proximal adenomas, Size of distal adenomas, Size of Proximal adenomas.	=COUNTIF(AA2:AQ2,"xxx")
For the number and sizes of adenomas with a specific location as distal or proximal, this formula was used	=IF(AQ2="xxx",AQ2)+IF(Y2="xxx",AT2)
xxx= proximal or distal AA2, AQ2,Y2 and AT2 are name of columns in excel with the required variables	

Merging the adenoma data with the randomisation data and the free fatty acids data.

After conducting all the required calculations, data was exported into SPSS files. The randomisation data was merged with adenoma characteristics data, baseline data and dietary data in one SPSS sheet.

Appendix 4: Food parameters list for DII calculations

Food parameter	Weighted number of articles	Raw inflammatory effect score*	Overall inflammatory effect score†	Global daily mean intake‡ (units/d)	SD‡
Alcohol (g)	417	−0·278	−0·278	13·98	3·72
Vitamin B_{12} (μg)	122	0·205	0·106	5·15	2·70
Vitamin B_6 (mg)	227	−0·379	−0·365	1·47	0·74
β-Carotene (μg)	401	−0·584	−0·584	3718	1720
Caffeine (g)	209	−0·124	−0·110	8·05	6·67
Carbohydrate (g)	211	0·109	0·097	272·2	40·0
Cholesterol (mg)	75	0·347	0·110	279·4	51·2
Energy (kcal)	245	0·180	0·180	2056	338
Eugenol (mg)	38	−0·868	−0·140	0·01	0·08
Total fat (g)	443	0·298	0·298	71·4	19·4
Fibre (g)	261	−0·663	−0·663	18·8	4·9
Folic acid (μg)	217	−0·207	−0·190	273·0	70·7
Garlic (g)	277	−0·412	−0·412	4·35	2·90
Ginger (g)	182	−0·588	−0·453	59·0	63·2
Fe (mg)	619	0·032	0·032	13·35	3·71
Mg (mg)	351	−0·484	−0·484	310·1	139·4
MUFA (g)	106	−0·019	−0·009	27·0	6·1
Niacin (mg)	58	−1·000	−0·246	25·90	11·77
n-3 Fatty acids (g)	2588	−0·436	−0·436	1·06	1·06
n-6 Fatty acids (g)	924	−0·159	−0·159	10·80	7·50
Onion (g)	145	−0·490	−0·301	35·9	18·4
Protein (g)	102	0·049	0·021	79·4	13·9
PUFA (g)	4002	−0·337	−0·337	13·88	3·76
Riboflavin (mg)	22	−0·727	−0·068	1·70	0·79
Saffron (g)	33	−1·000	−0·140	0·37	1·78
Saturated fat (g)	205	0·429	0·373	28·6	8·0
Se (μg)	372	−0·191	−0·191	67·0	25·1
Thiamin (mg)	65	−0·354	−0·098	1·70	0·66
Trans fat (g)	125	0·432	0·229	3·15	3·75
Turmeric (mg)	814	−0·785	−0·785	533·6	754·3
Vitamin A (RE)	663	−0·401	−0·401	983·9	518·6
Vitamin C (mg)	733	−0·424	−0·424	118·2	43·46
Vitamin D (μg)	996	−0·446	−0·446	6·26	2·21
Vitamin E (mg)	1495	−0·419	−0·419	8·73	1·49
Zn (mg)	1036	−0·313	−0·313	9·84	2·19
Green/black tea (g)	735	−0·536	−0·536	1·69	1·53
Flavan-3-ol (mg)	521	−0·415	−0·415	95·8	85·9
Flavones (mg)	318	−0·616	−0·616	1·55	0·07
Flavonols (mg)	887	−0·467	−0·467	17·70	6·79
Flavonones (mg)	65	−0·908	−0·250	11·70	3·82
Anthocyanidins (mg)	69	−0·449	−0·131	18·05	21·14
Isoflavones (mg)	484	−0·593	−0·593	1·20	0·20
Pepper (g)	78	−0·397	−0·131	10·00	7·07
Thyme/oregano (mg)	24	−1·000	−0·102	0·33	0·99
Rosemary (mg)	9	−0·333	−0·013	1·00	15·00

Appendix 5: Steps followed to extract food parameters from FFQ data provided by the seAFOod participants' to be used in DII score calculation.

1 Extracting data related to Garlic, Onions, Green/Black tea and Papers from the raw data was by running the analysis with the food line FETA software output, which provides the average consumption of the reported raw data per day.

2 The ω-3 fatty acid was the sum of DHA and EPA fatty acids which were extracted by the software providers from the raw data).

3 Measuring the amount consumed of the four food items (garlic, onions, paper, and tea) from the raw intake as follow:

   A) Recode the frequency of consumption from the raw data according to table xxx xx.

   B) Multiplying the frequency of consumption by the portion size in grams (as was described by FETA software).

   C) For tea, an assumption was made that every 200ml of tea was made using two grams of tea, the amount consumed in grams was measured by creating a new variable as follow:

$$Amount\ of\ tea\ in\ grams = \frac{amount\ of\ tea\ consumed\ in\ mlx2gm}{200ml}$$

Values used to recode the frequency of consumption of food items included in the frequency section of the FFQ to obtain the number of portions per day

FFQ category Frequency per day	Answer in the FFQ	New variable: Frequency of consumption per Day
Never or less than once / month	1	0
1 – 3 per month	2	.07
Once a week	3	.14
2-4 per week	4	.43
5-6 per week	5	.79
Once a day	6	1
2-3 per day	7	2.5
4-5 per day	8	4.5
6+ per day	9	6

Portion size of the four foods included in DII measurements.

Food item	Portion size in grams_* tea in ml
Garlic	5
Onions	34
Tea*	190 ml
Paper	26

Appendix 6: A comparison in food group intake before and after colorectal adenoma diagnosis

	ALL (n=526)			Female (n=96)			Male (n=430)		
Food group in g/day Mean	Baseline	At exit	*p*	Baseline	At exit	*p*	Baseline	At exit	p value
Alcoholic beverages	222.8	216	.557	86.4	70.6	.249	253.2	248.5	.730
Cereals and cereal products	205.4	198	.101	188.8	188.5	.970	209.1	199.7	.088
Eggs and egg dishes	20.2	20.3	.933	18.5	18.8	.806	20.6	20.6	.997
Fats and oils	22.7	22.3	.523	18.2	20.3	.090	23.7	22.7	.208
Fish and fish products	43.2	43.2	.992	43.6	43.9	.935	43.1	43.1	.963
Meat and meat products	124.4	111.1	<.001*	96.9	89.0	.198	130.6	116.0	<.001*
Of which red and processed	88	77.8	<.001*	67.4	60.3	.228	92.96	81.49	<.001*
Milk and milk products	340.7	334.1	.325	316.3	325.3	.457	346.2	336.1	.194
Non-alcoholic beverages	938.8	898.2	.011*	911.4	861.5	.232	944.9	906.3	.025*
Nuts and seeds	5.6	4.3	.010*	8.0	5.5	.093	5.0	4.0	.047*
Potatoes	93.7	96.2	.428	82.4	85.1	.613	96.2	98.6	.504
Soups and sauces	55.7	59.0	.287	59.0	56.3	.593	54.9	59.6	.201
Sugars, preserves and snacks	41.5	38.8	.066	32.8	32.0	.780	43.4	40.3	.063
Fruit group	185.2	192.3	.300	234.8	256.7	.149	174.2	177.9	.623
Veg group	253.6	253.8	.968	286.3	292.2	.587	246.3	245.2	.827

Paired Sample T test was used to measure change in intake of food groups before and after colorectal adenoma diagnosis for the whole participants and for females and males separately.
Mean (M), Data are shown as mean. * indicates statistical difference in dietary intake between baseline and exit data P<.05.

Appendix 7: A comparison in nutrients intake before and after colorectal adenoma diagnosis

	Total (n=526)			Females (n=96)			Males (n=430)		
	Baseline M (SD)	At exit M (SD)	P	Baseline M (SD)	At exit M (SD)	P	Baseline M (SD)	At exit M (SD)	P
Energy (MJ/day)	7.7 (2.4)	7.5(2.5)	0.018*	6.7 (2.03)	6.8(1.99)	0.703	7.9(2.46)	7.6 (2.59)	0.01*
Alcohol (g/day) **	13 (14.8)	12(15.2)	0.729	6.5 (9.3)	5.9 (7.8)	0.327	14 (15.6)	14 (16)	0.895
% of total energy	5 (5.7)	5 (6.2)	0.504	3 (4.1)	2.9 (4.2)	0.796	5 (5.9)	6 (6.4)	0.45
Protein (g/day)	82 (23.2)	78 (24.4)	<0.001*	75 (20.1)	72 (23.4)	0.111	83 (23.7)	79 (24.4)	<0.001*
% of total energy	18.4 (3.5)	17.9 (3.4)	0.002*	19.2 (3.8)	18 (3.6)	0.002*	18 (3.5)	18 (3.4)	0.053
CHO total (g/day)	208 (77.6)	203 (77.7)	0.065	191 (69.1)	193 (61.8)	0.604	212 (79)	205 (80.7)	0.038*
% of total energy	45 (7.3)	46 (7)	0.738	47.5 (7.5)	47.8 (6.6)	0.657	45 (7.3)	45 (7)	0.885
NSP(g/day)	16 (5.9)	15 (6)	0.588	16.6 (6.5)	16.8 (6.5)	0.559	15 (5.7)	15 (5.9)	0.424
Fat total (g/day)	70 (27.9)	69 (28.3)	0.161	60 (24.3)	62.5 (24.3)	0.257	73 (28.2)	70 (29)	0.054
% of total energy	34 (6.1)	34 (5.7)	0.216	33.3 (6.16)	34.3 (5.8)	0.071	34 (6.2)	34 (5.8)	0.578
PUFA (g/day)	12 (5.4)	12 (5.7)	<0.001*	11.3 (4.9)	11.4 (5.6)	<0.001*	13 (5.5)	12 (5.7)	<0.001*
Calcium mg/day	869 (311.7)	838 (308.7)	0.005*	811(325)	807 (291)	0.853	882 (307.5)	845 (312.4)	0.004*
Iron mg/day	11 (3.3)	10.6 (3.44)	0.002*	10.5 (3.08)	10.1 (3.17)	0.129	11.2 (3.41)	10.7 (3.49)	0.007*
Zinc mg/day	9.2 (2.74)	8.7 (2.83)	<0.001*	8.5 (2.38)	8.2 (2.8)	0.143	9.4 (2.79)	8.8 (2.82)	<0.001*
Selenium µg/day	63 (24.5)	60 (23.7)	0.008*	60 (21.3)	58 (23)	0.929	64 (21.1)	61 (23.9)	0.005*
Total folate µg/day	288 (91)	285 (95.6)	0.062	285 (103.6)	285 (101.5)	0.812	288 (88.1)	285 (94.3)	0.06
Vitamin B12 µg/day	7.3 (4.3)	7 (4.2)	0.002*	6.8 (2.97)	6.5 (4.04)	0.029*	7.4 (4.53)	7 (4.3)	0.011*
Vitamin C mg/day	105 (54.6)	105 (53.8)	0.6	123 (80.9)	124 (64.6)	0.073	101(45.9)	101 (50.1)	0.871
Vitamin D µg/day	3.3 (1.75)	3.2 (1.9)	0.073	3.2 (1.77)	2.9 (1.72)	0.609	3.3 (1.75)	3.3 (1.95)	0.038*
Vitamin E mg/day	11.4 (5.13)	11 (5.8)	0.417	10.8 (4.76)	10.9 (5.04)	0.958	11.5 (5.21)	10.9 (5.7)	0.373
Carotene total (equivalents) µg/day	3292 (1680.8)	3341 (1750)	0.005*	3743 (1907)	3737 (1843)	0.853	3191 (1611)	3253 (1718)	0.004*

Paired Sample T test was used to measure change in intake of energy and nutrients before and after CRA diagnosis for the whole participants and for females and males separately.
Mean (M), Standard Deviation (SD), Before Diagnosis (BD), After Diagnosis (AD), Carbohydrate (CHO), Englyst Fibre - Non- Starch Polysaccharides (NSP), Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA), Retinol Equivalent (RE)

Appendix 8: The standardised scoring system for adherence to the WCRF/AICR cancer prevention recommendations and modification performed

2018 WCRF/AICR Recommendations	Operationalization of Recommendations	Original score	Modification	New score
Be a healthy weight	BMI (kg/m2)			
	18.5–24.9	.5	Data about waist circumference is not available, the score in the healthy weight recommendation was split allocated to the BMI only, with a score of 1 was giving for the healthy range, score of .5 was giving for the overweight range and score of 0 for the obese and underweight ranges.	1
	25–29.9	.25		.5
	<18.5 or ≥30	0		.25
	Waist circumference (cm (in))		Data not available	NA
	Men: <94 (<37)	.5		
	Women: <80 (<31.5) 0.5	.25		
	Men: 94–<102 (37–<40)	0		
	Women: 80–<88 (31.5–<35) 0.25	.5		
	Men: _102 (_40)	.25		
	Women: _88 (_35)	0		
Be physically active			Data not available	
Eat diet rich in wholegrains, fruits and beans	Fruits and vegetables (g/day)		Since the FETA software extracts the NSP which make 77% of the dietary fibre, the following changes were applied: Maximum fibre category changed to >23g instead of 30g.The middle range was 11.5g to 23g and the low range was <11.5g	
	_400 0.5	.5		.5
	200–<400 0.25	.25		.25
	<200	0		0
	Total fibre (g/day)			
	≥ 30 0.5	.5		.5
	15–<30 0.25	.25		.25
	<15 0	0		0

Limit consumption of fast food, and other processed food rich in fat, starches and sugars	Percent of total kcal from ultra-processed foods		As data about energy obtained from ultra-processed food was not available, the amount of sodium consumed was used as an indicator for consumption of processed food. This modification was used previously in a study investigated the association between adherence to WCRF recommendation and biomarkers of one of the pathways related to CRC. The new score is that participants who consumed less than the recommended amount of 2.4g/day of sodium had a score of 1 while participants who consumed more than the recommended amount had score of 0.	
	Tertile 1	1		1
	Tertile 2	.5		0
	Tertile 3	.25		
Limit consumption of red and processed meat	Total red meat (g/wk) and processed meat (g/wk)		Since the FETA software extracts the red and processed meat in one group, this score was divided into 2 categories, for less than 500g/w of red and processed meat score of 1 was given, while for more than 500g/w a score of zero was given.3	
	Red meat <500* and processed meat <21	1		1
	Red meat <500 and processed meat 21–<100	.5		-
	Red meat >500 or processed meat _100	0		0
Limit consumption of sugar-sweetened drinks			Data not available	NA
Limit alcohol consumption	Total ethanol (g/day):			
	0	1		1
	>0– ≥ 28 (2 drinks) males and ≥ 14 (1 drink) females	.5		.5
	>28 (2 drinks) males and >14 (1 drink) females	0		0
(Optional) For mothers: breastfeed your baby, if you can			Not applicable	NA

- 500g/ week of meat was calculated as 71.4g/day.